MJM

AN INTERNATIONAL FORUM FOR THE ADVANCEMENT OF MEDICAL SCIENCE BY STUDENTS

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The MJM congratulates the EMGSS on this year's successful conference!

IN THIS ISSUE

The recent SARS outbreaks have shifted the world's attention back to the unwavering menace of infectious disease. Despite the media-hype of newly mutated viruses, we must not forget the many other infectious diseases that threaten the human integrity on an international scale.

Tuberculosis (TB), a disease that was documented as early as 2000 B.C., is a prime example of the struggle between innovative therapies and resilient pathogens. With over 8 million new cases each year, the World Health Organization (WHO) has declared TB a global emergency and epidemic. Today, it is estimated that one-third of the world population are infected with TB and this number is increasing at an alarming rate.

In this issue, the MJM explores the challenges, the progress and our hopes in the war against TB. In a letter to the MJM, Zhang describes how Non-Governmental Organizations (NGOs) such as Médecins Sans Frontières (MSF) provides students an opportunity to battle TB and other infectious diseases in the front line. Hung et al. describe risk factors for TB conversion in the prison population. In focus, Schurr et al. explain the interplay between host genetics and TB while Menzies and Behr provide insight from a clinical perspective.

In other articles, Alex Henri-Bhargava presents his award-winning research into the history of the famed Montreal Neurological Institute, Agostino et al. go hunting in the grocery store for genetically modified foods, and Jean Adams takes on the infamous OSCE from bothsides of the stethoscope.

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South-East
Regional Health Authority
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Sud-Est

Locum and Permanent Positions

The Department of Anesthesia is looking to expand beyond its current full time active staff of ten members. Current caseload is approximately 10,000 per annum. Our facility provides anesthesia to a wide range of surgical subspecialties including neurosurgery, thoracics and major vascular and orthopedic trauma. Local population served is approximately 115,000 with a greater tertiary referral area of approximately 350,000. Services provided outside the Operating Room include: a full operational chronic pain clinic, acute pain, ICU (as part of a separate multidisciplinary intensivist program) and labor and delivery.

Expansion of anesthetic services to a busy MRI and interventional radiology service is also planned. On call is equally distributed amongst all department members (1 in 10). Opportunities for teaching and research are present.

Moncton is one of the fastest growing communities in Atlantic Canada, and boasts a terrific quality of life. Educational opportunities, cultural events and as easy access to outstanding beaches and National Parks are just some of the benefits of the locale.

The Department of Anesthesia requires that its active, senior active and courtesy members have passed the examinations of the Royal College of Physicians and Surgeons of Canada or the examinations of La Corporation professionnelle des médecins du Québec. Anesthetists looking for permanent or locum positions are encouraged to contact us.

Inquiries can be addressed to:
Dr. Kenneth Mitton, Chief of Staff
South East Regional Health Authority, 135 MacBeath Ave., Moncton NB E1C 6Z8
Telephone: (506)857-5532, fax (506)857-5545,
or email medstaff@sehcc.health.nb.ca

GENERAL/ANATOMICAL PATHOLOGIST

Moncton, New Brunswick

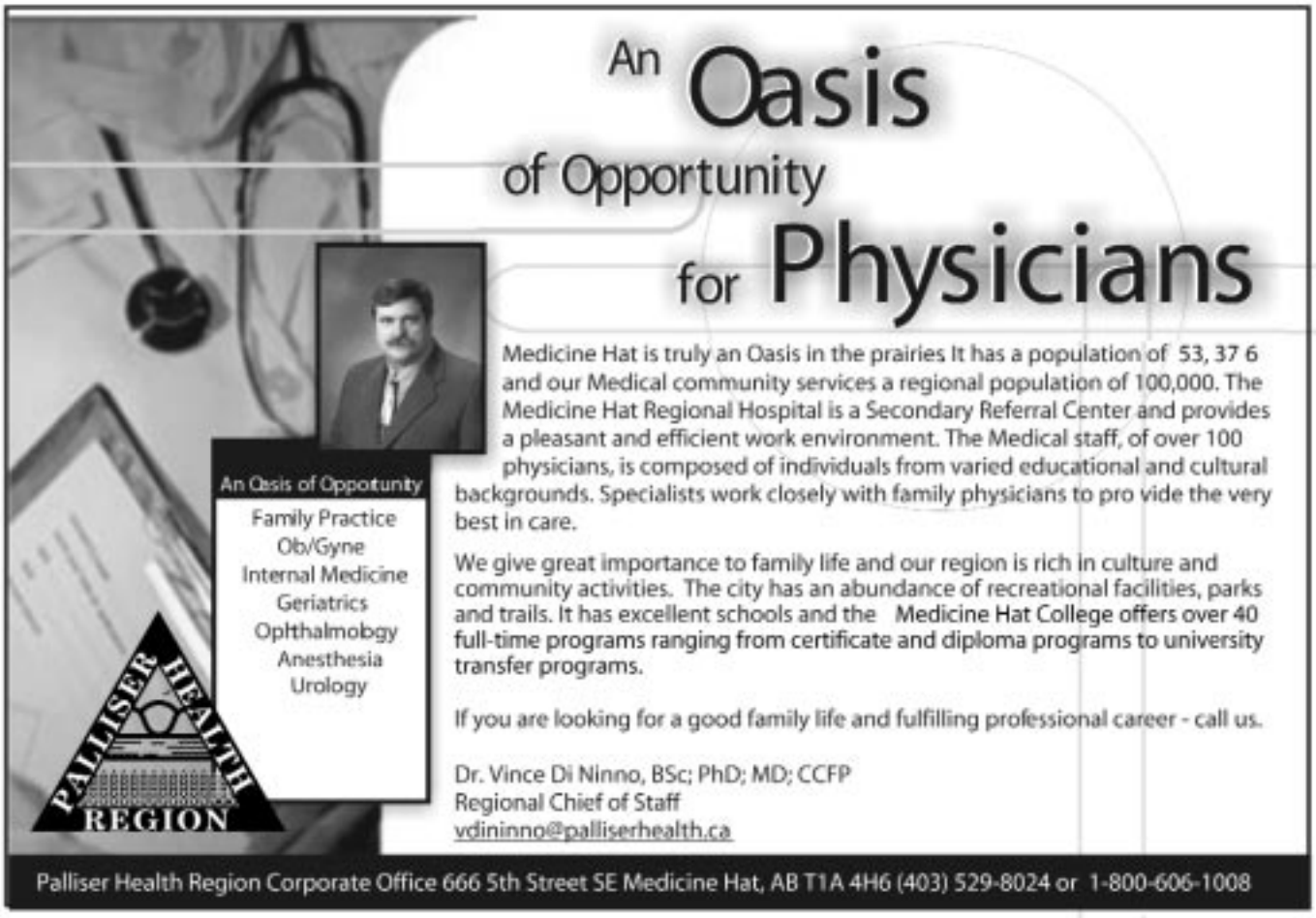
Locum and Permanent Positions
A successful candidate will join a congenial group of laboratory physicians, consisting of anatomical and general pathologists, hematopathologist and microbiologist. We have a full spectrum of surgical pathology, cytology, hematopathology and autopsy in addition to a very active service in chemistry and microbiology in a dynamic laboratory accredited by College of American Pathologists since 1987. This laboratory is in the process of developing a molecular diagnostic section that will play an essential role in the consultative process of hematopathology and infectious diseases. A potential for a departmental directorship on rotational basis exists for an individual with administrative experience or interest.

A temporary locum for flexible time arrangement is also required for year 2003.

The Moncton Hospital is a 400-bed teaching hospital centrally located in the Maritimes. Referral population includes Eastern New Brunswick, Prince Edward Island and North-Western Nova Scotia. The city, with adjoining municipalities, has a population of more than 115,000 and was ranked as one of the best Canadian cities for quality of community life. There is an easy access to warm water beaches of the Northumberland Strait, and also the scenic Bay of Fundy area. Excellent availability of educational opportunities, cultural events and recreational activities in the immediate area.

Department of Pathology and Laboratory Medicine requires one of the following qualifications: a) certification or Fellowship in Pathology of The Royal College of Physicians and Surgeons of Canada or eligibility to write the examination for Fellowship in Pathology; b) certification by The American Board of Pathology; c) certification by la Corporation professionnelle des médecins du Quebec. The salary range is determined by New Brunswick Medical Pay plan, depending on qualification and experience.

Applicants are invited to forward their resume and the names of three referees to:
Dr. M. Kuhn, Director of Laboratories
The Moncton Hospital, 135 MacBeath Ave., Moncton, NB E1C 6Z8
email: makuhn@sehcc.health.nb.ca



EDITORIAL

THE MJM: AN OPPORTUNITY FOR INTERNATIONAL STUDENT COLLABORATION

There is a common saying in today's scientific community: publish or perish. As a result, researchers spend most of their time writing and revising articles for scientific publications. From anaphylaxis to zoology, there is a scientific journal to suit your particular field. Though this at times might seem a bit excessive, it is vitally important. From the monks who illuminated manuscripts while praying in their hilltop monasteries to Gutenberg's industrialization of the written word, the preservation and spread of information has been a great preoccupation. It is said that Isaac Newton only wrote his Principae Mathematica at the insistence of his colleagues who feared most his work would be lost if anything happened to him. Had Newton not acquiesced, the realm of physics might have been set back centuries at his death. The concept of keeping a written record of one's research is central to the scientific process. The free flow of ideas allows collaboration and elaboration between individuals that may not actually ever meet. Furthermore, documenting research and experimental results obviates the need for experiments to be repeated by others. Without such documentation, the sum total of human knowledge might have to be rediscovered with the passing of each generation. Whether in the Great Library of Alexandria or the humble collection dedicated to Sir William Osler, humanity has always realized the necessity to gather knowledge together for all those who wished to learn from it. It is within this philosophical framework that the MJM tries to play its small part.

Being an international peer reviewed student-run medical journal, one of the main goals of the MJM is to introduce novel ideas and interpretations of scientific research from a student's perspective. A medical journal presenting solely student work provides the means for recognizing the high caliber of student research on a larger scale. In fact, the MJM encourages individuals who are relatively new to research to submit their work for publication for the first time, a process that might serve as an important stepping stone and incentive for further research endeavors. For the student editors and publications staff, there are the additional educational benefits associated with the production of the journal and the value of international student collaboration. The present editorial board, which includes students from several countries, including Canada, United States, United Kingdom, Italy, and Israel, has had the unique opportunity to develop and utilize an online forum for editing articles. The mission of the MJM is to facilitate discussions regarding important medical issues and nurture student participation in endeavors that could potentially lead to beneficial outcomes in the medical community. With a mix of original articles, letters to the editor, literature reviews, and our trademark "crossroads" articles that explore the relationship between medicine and humanities, the MJM strives to put together an issue that informs, stimulates, and raises as many questions as it answers.

Aravind Athiviraham, M.D. C.M. (2005), is the seventh Editor-in-Chief of the MJM (picture). His current research is conducted in the Division of Cardiothoracic Surgery, McGill University Health Centre, and compares the postoperative outcomes of off-pump surgical revascularization and conventional coronary artery bypass. **Christopher Labos**, M.D. C.M. (2006), is the Executive Junior Secretary at the MJM. His upcoming research project at the McGill University Health Centre will involve an analysis of the delays to definitive care in trauma patients with a view to suggesting amendments to triage protocols.

A MIDSUMMER DREAM

Summer marks the end of school year for high school and college students in Canada and other places around the world. As the aspiring pupils ready themselves to enter their studies next fall, we should perhaps ponder on how our societies, obsessed with the system of productivity, assessment and selection, often fails to provide what may be just as important to our youngsters- the environment to shape individual talents and the room to dream about the future.

Those who dreamt in the realm of science and medicine wove their reveries into unparalleled historical achievements. Just a century ago, Sir William Osler initiated bedside teaching in medicine, and half a century later, Sir Archie Cochrane introduced the concept of evidence based medicine. Built on these once dreamy ideas, biomedical science and technology have since that time revolutionized medicine and exponentially proliferated the ability of physicians to provide better care to their patients.

From these developments, medical scientists and physicians are each becoming more and more highly specialized. With the potential risk of losing the

broader perspective and of falling into the pitfall of irreconcilable divergence in their viewpoints, the presence of good physician-scientists is crucial to ensure that the knowledge created in one specialty is not lost upon the other. The physician-scientists also take on the important role as teachers, showing students the necessity of applying scientific rigor to both research and patient care. However, the change in practice patterns, the financial burden and the long training have decreased the number of physician-scientists to a sub-optimal level, and the consequences can be felt especially in North America (1).

In a recent article by Francis Collins, Director of the National Human Genome Research Institute, cosigned by a group of highly acclaimed scientists, the future of genomic research was dreamt to be a giant triad consisting of basic sciences, health care and social responsibility (2). The proposal serves as a good blueprint for shaping the future of science and medicine because of its universal applicability. The proposal also identifies six elements that intersect the triad at all levels; two of these elements are education and training, showing again their undeniable importance in supporting the progress of science and medicine.

Yet the simultaneously most important and most difficult aspect of the dream of Collins et al. may be social responsibility. It is a necessity to recruit sufficient resources and to make concurrent advances in ethics, law and social sciences alongside with biomedical science. The power differential between richer and poorer institutions and countries, and the lack of mechanisms to maintain the balance is a serious threat to this future. In the March 7th issue of *Science*, Kofi Annan, United Nations Secretary-General, appealed to all scientists to commit to stop the "acceleration of the disparity between advanced and developing countries, which creates social and economic difficulties at both national and international levels"(3). At the international level, infectious diseases such as AIDS, TB and malaria are WHO declared emergencies that threaten less-industrialized countries, but the current state of resource disequilibrium and power differential prevent the delivery of good medicine and research opportunities to those in need. At the national level, the imbalance may lead to the neglect of important research areas and the creation of heterogeneous health care. To us, students, the inequality in resource distribution may also profoundly impact the quality of education and training, preventing many from pursuing their aspirations at a personal level.

The current state of our education calls for immediate attention. The increase in demand and the lack in supply of physician-scientists have profound implications in maintaining scientific advancement, patient care and teaching; the increasing gap between the affluent and the poor presents as a major obstacle to our common interest. Much solidarity is needed amongst us, the students, to embrace our dreams and our future.

The unique genre of MJM offers the opportunity for all students to communicate their thoughts, to show their concerns, and to demonstrate their efforts, while providing a solid ground for learning and development. The non-profit and peer-review nature of MJM opens an impartial international forum for those willing to share their ideas and passion. As the MJM cherishes the joy of producing issue 7.1, we hope that more dreams will be generated and fulfilled as result of our hard work.

We dream again today. What about you?

REFERENCES

1. Zemlo TR, Garrison HH, Patridge NC, Ley TJ. The Physician-Scientist: Career Issues and Challenges at the Year 2000. The FASEB Journal 14:221-230; 2000.
2. Collins FS, Green ED, Guttmacher AE, Guyer MS. A vision for the future of genomic research. Nature 422, 835 - 847; 2003.
3. Annan K, A Challenge to the World's Scientists, 299(5612):1485; 7 Mar 2003.

Charles C.H. Lin, B.Sc. (Hon.), M.D. C.M. (2005) is the seventh Editor-in-Chief of the MJM. He is interested in the application of functional genomics in physiology and stem cell research, and the social implications of medical practices.

LETTERS TO THE MJM

MORE THAN NEEDLES AND BANDAGES

Dear *MJM*,

I was hesitant at first to join the Médecins Sans Frontières (Doctors Without Borders) volunteer group here at McGill. I thought that I would be committing myself to an organisation whose humanitarian actions were solely medical-related and never crossed over to topics of injustice and human rights violations, which as many people do not realise,is just as crucial, if not more, than needles and bandages. It is an honourable thing to save lives, but it is a crime to do it with indifference. With aspirations of becoming a doctor myself, I was not ready to promote healing with a mouth shut.

Luckily, I soon found out that in addition to providing medical assistance, MSF's main missions are to raise awareness by speaking out, either in private or in public, as witnesses of the plights suffered by populations around the world. As the world's most important independent medical relief organisation, MSF provides assistance in more than 85 countries, in the wake of armed conflicts, civil war, epidemics, chronic refugees' situations, natural disasters and famines, while launching awareness campaigns and publicly denouncing acts that violate humanitarian laws. In fact, it is one of the first non-governmental organisations (NGOs) to have combined medicine with activism. Another important feature is its complete independence from all political, religious and economic influences.

A little more about MSF

Winner of the Nobel Peace Prize in 1999, MSF assists those who have fallen victim to natural or man-made disasters. Its offices are located in 18 countries around the world and its staff is composed almost entirely of volunteers who often work in the most dangerous and remote areas of the world. In a medical crisis situation, within hours, the teams are ready to set off and devote six to twelve months of their lives on a mission. In the field, the teams arrive with equipment specifically designed by the group of logistics to fit the geography and climate of the country, allowing the teams to get right to work (1).

The field teams provide first aid medical assistance, perform surgical operations, restructure health clinics and hospitals, run nutrition and hygiene programs, train local medical personnel and offer mental health care. They also establish long-term programs aimed at treating infectious diseases such as tuberculosis, malaria and AIDS, while directly helping excluded and marginalized groups such as children and ethnic minorities.

MSF also mounts exhibitions, releases publications, and launches campaigns with the aim of raising awareness. An important part of MSF's work consists in addressing any violations of human dignity and humanitarian law encountered by team members on the field, and violations perpetrated or sustained by political actors, by either confronting the responsible actors or putting pressure on them through public denouncement and the mobilization of the international community.

Impartiality and the Media

Most NGOs are not credited with impartiality and neutrality. MSF is one of the few private, non-profit, non-partisan and impartial organisations left. From the beginning of its foundation by a small group of French doctors in 1971, it has continued to distance itself from any political or religious affiliations in order to maintain the neutrality needed to ensure assistance without discrimination on the grounds of race, sex, religious convictions, social stature or political views. Further independence is maintained by primarily turning to public or private donations, and to a limited extent (less than 20%) to international institutions such as the UN, for funding.

Humanitarian aid should be impartial and neutral, and have as sole aim to prevent or relieve suffering according to the victims' needs and interests. However, for the past decade, these principles have slowly declined in the humanitarian world. As the government, the media and the military have become more and more involved, many NGOs have been bought in by those who supply the funds or threatened into submission by authorities (2). "Governments and donors [have] tried to influence belligerents by withholding funding for humanitarian aid, and by providing assistance to particular groups" (3). This type of bias defiles the word "humanitarian", a word that embodies self-devotion to others, a pure form of compassion and altruism, whose very essence is contradicted by any reference to ulterior motives or personal agendas.

An organisation's degree of independence also influences the level of discrimination behind the selection of information it wishes to publicize and the extent to which the organisation will speak out against acts of violence and abuse. Previous biased humanitarian actions in Bosnia, Chechnya, Somalia, Afghanistan and in much of the Arab world, have fuelled feelings of anger among the victims, for whom it has become increasingly difficult to identify friend from foe in conflict situations (4).

The impact of the media on the humanitarian world is very significant. It often dictates the areas in the world that obtain the most help and the level of funding and attention received for a project. Unfortunately, only a small percentage of the populations that find themselves in a situation of danger actually gain the attention of the media and those who are left in the dark receive little sympathy from the outside world.

The media also profoundly influences the organisations themselves. "[S]ome agencies have tried to harmonize both public and private storytelling...There are risks of this outspokenness...that agencies themselves become more enamoured of the politics of moral gesture than of reaching...victims themselves" (5). However, funds are needed to provide assistance, and lobbying for the emotional support of spectators in search of tear-jerking real life drama is where the money's at. Thus awareness campaigns are turned into advertising campaigns, and the bulk of efforts and money is spent in the media rather than in the line of action.

"The agency most determined to get the highest media profile obtains the most funds from donors...[In] doing so, it prioritises the requirements of fund-raising...follows the TV cameras...engages in picturesque and emotive programmes [and] abandons scruples about when to go in and when to leave.... Agencies that are more thoughtful...fail to obtain the same level of public attention, and suffer for it." (6)

Speaking Out

If the sufferings of a population stem from the manipulations of political, military or economic forces, medical assistance alone is not a sufficient humanitarian action. There is no doubt that saving even just one life is an incredible and admirable deed, but a broader remedy must be employed when entire populations are subjected to the consequences of corrupt forces. To provide a long-term sustainable resolution, the problem must be attacked at its source.

If we succeed only in mending the war wounds of a mother and a father, for example, but action is never taken against the movement that is the cause of this war, then later on the children of these parents will in their turn find their own wounds in war, and so will the next generation, and the one after. Do we simply wait and watch in silence for the children to face war and then run to their rescue? It is as absurd as having a leaky pipe with an enormous gap that steadily causes the pipe to crack along its length, and a team of plumbers constantly mending the cracks without giving heed to the gap. Of course the cracks will be repaired, but if the gap is never fixed, the cracks will continue to spread, the pipe will always leak and the problem will never be completely solved. The gap must be filled. We cannot content ourselves with mending the cracks.

Many organisations keep silent because they believe that they have no responsibility or no capacity to influence the oppressive forces. They regard themselves as accountable only for the maintenance of their relief operations, or are afraid that speaking out may jeopardize their freedom to operate in the country. This kind of passive complicity is the easier path, involving fewer risks for the organisation. However, other organisations, acknowledging the power they possess to testify, negotiate and make public statements, hold themselves responsible for speaking out as witnesses of injustice (7).

"[MSF] refuses to accept that silence is a precondition for its operational freedom" (7). MSF teams, known for their "rebellious humanitarianism", were expelled on a few occasions for speaking out: in 1985, after denouncing the Ethiopian government for its diversion of humanitarian aid and the forced migration of its people, a team was forced out of the country; in 1995, the France team was expelled from Rwanda for denouncing the abominable conditions of the prisons and the treatment of the prisoners (8). In these cases, it might appear that speaking out did more harm than good, since the expelled teams were no longer able to provide assistance, but in fact MSF, having 5 operational centres, can take a stand and speak out via one centre, which alone suffers the consequences, while the other centres safely carry on with their work in the country.

Speaking out demands more than passion and conviction. To be heard and believed, one requires facts. To ensure credibility with governments, other international relief organisations and the public audience when issuing statements, MSF takes a scientific approach. The field teams document casualties, conduct intensive surveys and analyse sample groups to gather scientific evidences to support their cases when giving testimony of human rights violations. The studies conducted by MSF in Rwanda, Congo, Sierra Leone and Kosovo, among others, were well reputed and gained much public attention (9).

In the face of horrors, inaction is unacceptable. Being active is hard and risky for the organisations, but remaining passive is costly and dangerous for the victims. After all, is not the right to freedom and respect just as crucial as the right to essential medicines and physical assistance? Upon receiving the Nobel Peace Prize in 1999, MSF put it this way: "Silence has long been confused with neutrality and has been presented as a necessary condition for humanitarian action. From the beginning, MSF was created in opposition to this assumption. We don't know whether words save lives, but we know for sure that silence kills" (10).

The Other Side of Medicine

The issues dealt with not only pertain to MSF as a humanitarian organisation, but also touch on an important and often neglected aspect of medicine in general. Political figures are most influential, but spend most of their time in the public spotlight and little time in direct contact with the actual subjects of their lavish speeches; activist groups are most devoted, and actively promote their cause, but often lack credibility and professional experience. The medical personnel is by far the best placed people to raise the issues of justice, dignity and human rights, not only because of their well-respected social status but also because of their direct contact with victims. The importance of activism in medicine is perhaps not so obvious in Canada, where the majority of population not only has access to medical care but is also protected under strict laws. But it becomes very obvious when working in countries where there is little protection of human rights and little or no medical assistance available, and where health emergencies are often the result of violence, oppression and injustice.

I have often heard medicine being referred to as a "noble" profession. There is nothing noble to be found in the medical field if treating a patient is viewed as merely operating on a machine. If practicing medicine does not go beyond fulfilling the job requirements and earning a good salary, it is no more noble than any other profession. There is a tendency to immediately classify a career in medicine as an admirable one because of the prospect of saving many lives. There is a distinction to be made however, between those who do it with apathy, out of duty or as a routine, and those who truly care for the relief of human suffering, who believe in the high ideals of medical ethics, who are in quest of knowledge and understanding without prejudice, who sacrifice and labour and endure to help others, who are outraged by the sight of any form of abuse of another human being and who take action against it. Therein lies true nobility.

Sincerely,

Sophie Zhang
Faculty of Science
McGill University

REFERENCES

1. MSF Canada. The Work of MSF: Past, Present, and Future. Médicins Sans Frontières / Doctors Without Borders, Canada. www.msf.ca/about. 2003.
2. Parry MS. Phyrric Victories and the Collapse of Humanitarian Principles. The Journal of Humanitarian Assistance. www.jha.ac/articles/a094.htm. 2002.
3. Macrae J, Stevenson F. Legislating for Humanitarian Aid. Humanitarian Practice Network Report. 2002; 21: 33-35.
4. Center for Economic & Social Rights. Violations of the Right to Food by all Parties to the Conflict in Afghanistan. www.cesr.org/Emergency%20Response/righttofoodrapportuer.doc. 2001.
5. Ignatieff M. The Stories We Tell: Television and Humanitarian Aid. In: Moore, ed. Hard Choices: Moral Dilemmas in Humanitarian Intervention. Lanham, Maryland: Rowman & Littlefield. 1998: 291.
6. De Waal A. The End of the Cold War: A New Humanitarian Dispensation, In: Currey, ed. Famine Crimes: Politics and the Disaster Relief Industry in Africa. Bloomington, Indiana: Indiana University Press; 1997: 65.
7. Bouchet-Saulnier F. Between Humanitarian Law and Principles: The Principles and Practices of "Rebellious Humanitarianism." Médicins Sans Frontières / Doctors Without Borders, Canada. www.msf.org/content/page.cfm?articleid=6589C8A5-DC2C-11D4-B2010060084A6370. 2000.
8. MSF France, Chronologie de 1971 à aujourd'hui. www.paris.msf.org/msf/web.nsf/html/459PGX?OpenDocument. 2001.
9. Marschner A. A Scientific Approach to "Témoignage." Médicins Sans Frontières / Doctors Without Borders, Canada. www.msf.org/content/page.cfm?articleid=0F722DE2-BF6A-11D4-852200902789187E. 1999.
10. Melicharova M. Special Agents. Peace Matters. 2002; 38: 23.

FOURTEENTH EUROPEAN STUDENTS CONFERENCE

Dear MJM,

Given, that the MJM and the European Students´ Conference (ESC) share the common goal of encouraging the pursuit and communication of medical research among students, we thought your readers might be interested in learning more about our Conference.

As our name implies, the Conference enjoys a European flavor but we wish to actively encourage Northern American students to also attend, facilitating closer ties and a greater flux of ideas between the two continents.

The 14th European Students Conference will be held from the 4th - 9th of November 2003, at Charité, the medical school of the Humboldt University in Berlin. It offers medical students and young doctors from more than 40 countries a great opportunity to present the findings of their research. Uniquely, professors and specialists will evaluate them. Prizes and scholarships totalling more than 10 000 Euro will be awarded to the best oral and poster presentations.

The fields of neuroscience, pharmacology and infectious diseases are our main focal points at the 14th ESC and we welcome everybody working or researching in this field. Besides that, different

workshops concerning topics in medical education, fundraising for medical projects, working in Europe or alternative medicine will be offered. In addition there will be a forum for interactive discussions between highly regarded scientists and students. Combined with the conference sessions there will be initial skill adoption training courses as chances to get in touch with the practical side of medicine. We will also have an exhibition - the "job contact market", where subject related companies can present themselves or recruit young scientists directly.

One of the main goals of the 14th ESC is anchored in our leitmotif, which expresses our interdisciplinary thinking: For open minded young scientists willing to look beyond.

Our wide variety of cultural and social programme points implies the aim to show Berlin as a European capital with its specific flair and a doorway between east and west. Historical oriented sightseeing tours as well as dinner parties and sport activities will be part of the programme to bring people together.

The 14th ESC has recently extended the agreement with the IFMSA (International Federation of Medical Students´ Associations, ifmsa.org) during the March meeting in Estonia. The IFMSA´s Standing Committee on Research Exchange (SCoRE) recommends that the participants of its research exchange attend at the 14th European Students´ Conference and present their work. If you have done research and are going to attend an exchange programme, why not apply to the 14th ESC?

We enthusiastically welcome students and young doctors from the biomedical sector worldwide to become active as well as passive applicants. The application details and further information are noted down in writing on our website:

esc.charite.de
www.esc-berlin.com

Yours faithfully,

The Organizing Committee
N. Züfle, N. Ledenig, W. Blaum, A. Schuster, A. Gómez-Carrillo, A. Kötter, F. Ufer, S. Herrmann

A MEETING OF THE MINDS

Dear *MJM*,

On February 26, 2003, the Experimental Medicine Graduate Students' Society (EMGSS) proudly hosted the 3rd Annual McGill Biomedical Graduate Conference (AMBGC) at McGill University. The AMBGC is the biggest student-run biomedical symposium at McGill and it was created to strengthen relations among graduate students and to promote awareness of the research being performed throughout the university. The AMBGC gives graduate students the opportunity to hone their presentation skills and to get feedback on their research from a diverse audience. Although only in its third year, the AMBGC has already gained the reputation as being one of the leading biomedical research conferences in the Montreal region. In fact, the 1st AMBGC was selected as the winner of the Forces Avenir Award (Health Category), Québec's most prestigious student award designed to recognize and honor university students.

This year's AMBGC attracted over 250 people and a record high of 102 abstracts were submitted by graduate students from 14 biomedical departments, representing 15 different research institutes. Top-ranked presentations, as selected by a panel of McGill professors, were awarded with cash prizes of up to $1000. The conference was financially supported by 16 sponsors, whose donations totaled over $17,000. This year's keynote lecture was given by Dr. Lawrence Rosenberg of McGill. Dr. Rosenberg's lecture focused on the discovery of INGAP, a naturally-derived peptide which stimulates the regeneration of pancreatic islet cells and is being touted as a potential cure for diabetes. Unfortunately, due to a personal emergency, Dr. Rosenberg's co-speaker, Dr. Aaron I. Vinik of Eastern Virginia Medical School, could not attend.

Overall, the 2003 AMBGC provided an outstanding forum for biomedical graduate students to share some of the exciting research currently being performed at McGill. The EMGSS would like to thank all of the sponsors, graduate students and judges who participated in this event. For more information and for pictures from the 3rd AMBGC, please visit the conference website (1).

Sincerely,

Randy Levitt and Sabrina Perri
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REFERENCES

1. EMGSS. 3rd Annual McGill Biomedical Graduate Conference. http://www.medcor.mcgill.ca/EXPMED/AMBGC

RESEARCH LETTER

TRANSIENT INHIBITION OF SMAD1/SMAD4 TRANSLATION USING ANTISENSE OLIGODEOXYNUCLEOTIDES IN VITRO

Dear MJM,

During normal fetal development, the ureteric bud (UB) branches out of the mesonephros (primitive fetal kidney) and undergoes branching morphogenesis to give rise to the ureter, renal pelvis, calyxes, and collecting tubules of the mature kidney [1]. Molecular pathways that regulate this branching are either inhibitory or stimulatory, and both are essential for a normal growth pattern [2]. In 40% of end stage paediatric renal disease, the UB fails to develop appropriately, most probably due to defects in these pathways [3].

The inhibitory pathway of the Bone Morphogenetic Protein-2 (BMP-2) belongs to the TGF-β superfamily. Gupta et al. have shown that BMP-2 is a very potent inhibitor of renal branching morphogenesis, both in cell culture models and in cultured murine embryonic kidney explants [5, 6]. BMP-2 exerts its effects via intracellular messengers known as SMAD proteins (or MAD-H proteins, the human homologues of the drosophila Mothers Against Decapentaplegic gene). Activation of the BMP-2 receptor phosphorylates SMAD1(R-SMAD), which binds SMAD4 (Co-SMAD), and translocates to the nucleus where it is postulated to regulate gene expression [5, 11 Fig.1]. It is true that BMP-2 inhibits tubule formation and branching, but it is still unknown whether SMAD proteins are essential for this action [5].

Previous work has shown that SMAD1 and SMAD4 are expressed in developing kidneys, but their function is still unknown [12]. Since they are downstream effectors of BMP-2, we hypothesized that SMADs mediate inhibitory signals for UB development, such that blockade of either one will promote branching morphogenesis. In simpler terms, in a kidney that is not branching very well, we hypothesize that the inhibition of the SMAD1 and/or SMAD4 proteins will promote branching morphogenesis and allow the kidney to develop normally.

In order to block SMAD1 and SMAD4 translation, we opted to use Antisense Oligodeoxynucleotides (oligos) purchased from Biognostik Canada, Montreal, Quebec. These oligos are DNA sequences complimentary to the SMAD mRNA, and it is postulated that they can bind to it and inhibit protein translation. These phosphorothioated second generation molecules are considered DNAase resistant, minimally toxic, and independent of lipid agents that facilitate cell entry. Cell culture wise, the mouse inner medullary collecting duct-3 (mIMCD-3) cell line was used [5]. This is an excellent model for the study of UB development because it originates from the UB, expresses

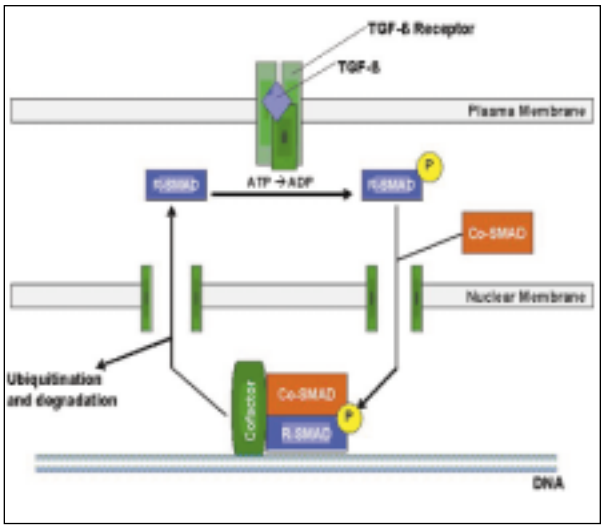

Figure 1. The signaling cascade of the TGF-β family via the R-SMAD (SMAD-1) and the Co-SMAD (SMAD-4)

SMAD1 and SMAD4, and develops 3D tubular structures when cultured on a type I collagen matrix. The cells were cultured in 6-well plates in 1ml of DMEM (5% FCS and 1% P/S). Seeding concentration was 87,000 cells/ml and cells were given 24 hrs to attach properly. The antisense and control oligos for SMAD1 and SMAD4 were added at t=24hrs and refreshed with the media every 24 hrs. Cells were harvested at t=120 hrs for Western immunoblot analysis as per the technique used by Chen et al. [5]. Preliminary dose response assays using fluorescent microscopy had shown that the optimal oligo concentration for uptake with minimum toxicity was in the vicinity of 3μM-5μM. Both these concentrations were used to conduct the antisense experiments described above.(Fig. 2).Two different oligo sequences were used against each of SMAD1 and SMAD4. Hence there were four sets of experiments taking place, each set comprising a unique anti-SMAD sequence with its scrambled and sense control sequences.

Western analysis was performed to measure SMAD concentrations after treatment with the oligos. Some of the gels showed a slight decrease in cytoplasmic SMAD protein, but none of the gels showed any remarkable attenuation of SMAD expression (Fig. 3). Each set was repeated five times and the results were perfectly reproducible. There could be multiple reasons for the failed downregulation of the SMAD proteins. Failed entry into the cell could be ruled out because all immunofluorescence microscopy clearly shows that the FITC-oligos were present in the cytoplasm (Fig. 2). Likewise, it is not very likely that the oligos were being degraded intracellularly because they are DNAase resistant sequences. Moreover, the observed toxicity at 18μM hints to the fact that the oligos are present and active within the cells. One probable reason is a problem

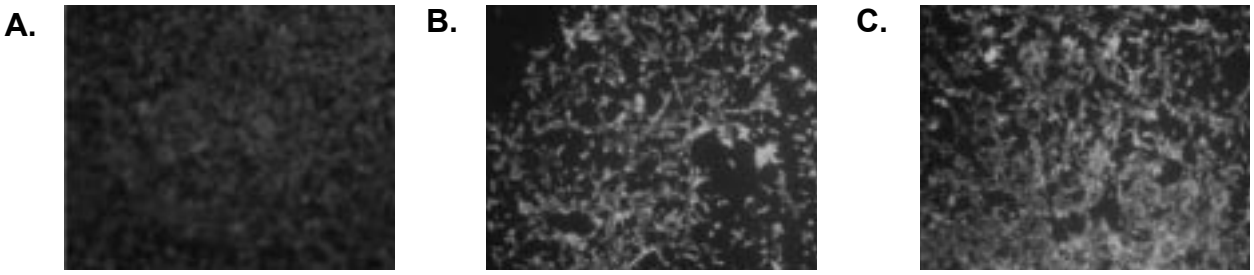

Figure 2. mIMCD-3 cells cultured on 8-well NUNC Labtek II Chamber microslides as visualized under fluorescence microscopy after the addition of FITC labeled oligos. A. Control well shows residual fluorescence. B. 1.0µM FITC-oligo. C. 3.0µM FITC-oligo shows maximal uptake with no notable toxicity. Uptake was shown without toxicity up to 18.0µM of oligo.

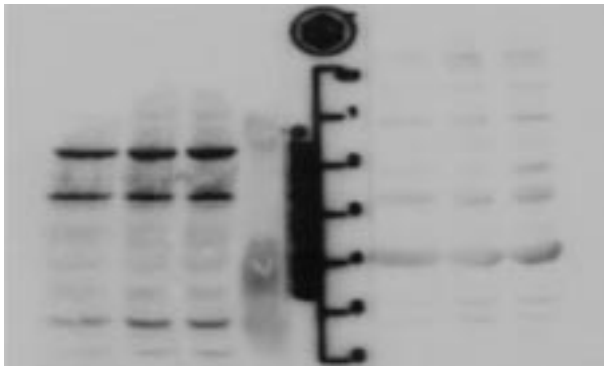

Fig. 3. Western blot analysis using Anti-SMAD1 antibodies after treatment with anti-SMAD1 oligos shows no difference in SMAD-1 levels between the Control (Ctrl), Antisense treated (AS), and Scrambled treated (SC) wells.

with antisense binding Oligonucleotide manufacturers report that there is a 1/10 to 1/8 chance for the anti-sense sequence being active in inhibiting mRNA translation. This low precision is attributed to tertiary folding and steric hindrance between the antisense DNA and the target mRNA. A possible solution for this problem would be to keep on trying different sequences until one eventually succeeds in blocking SMAD1/4 translation.

Although we could not knock-out the desired proteins, this study represents progress in antisense SMAD experiments. We were able to show that that the antisense oligos used in this experiment are indeed accessible to kidney cells, and constitute a promising means of knocking out SMAD1/4, given the right DNA sequence. We have determined the optimal uptake concentrations for these oligos as well as the borderline toxic concentrations. We have also been capable of devising improved cell culture protocols that lead to optimal growth of the mIMCD-3 cell line.

In short, this project has set the practical basis for working with antisense oligos in an mIMCD-3 system. Future work may be aided by the findings of this project in terms of cell culture regiments, dosage requirements, and oligodeoxynucleotide sequences.

Eventually, once appropriate anti-sense sequence is established, we will be able to determine the role of SMAD1 and SMAD4 in renal branching morphogenesis. From there, various studies may be devised to investigate possible *in utero* therapy for congenital renal defects.

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REFERENCES

1. De Vries, L., Zheng, B., Fischer, T., Elenko, E., and Farquhar, M. (2000) The Regulator of G-protein signalling family. Annu. Rev. Pharmacol. Toxicol. 40, 235-271.
2. Panetta, R., Guo, Y., Magder, S. and Greenwood, M. T. (1999) Regulators of G-protein signalling (RGS) 1 and 16 are induced in response to bacterial lipopolysacchiride and stimulate c-fos promoter expression. Biochem. Biophys. Res. Commun. 259, 550-556.
3. Grant, S. L., Lassegue, B., Griendling, K. K., Fukai, M. U., Lyons, P. R. and Alexander, R. W. (2000) Specific regulation of RGS2 messenger RNA by Angiotensin II in cultured vascular smooth muscle cells. Molec. Pharmacology, 57, 460-467.
4. Fong, C. W., Zhang, Y., Neo, S. Y., and Lin, S. C. (2000) Specific induction of RGS16 mRNA by protein kinase C in CEM leukaemia cells is mediated via tumour necrosis factor a in a calcium-sensitive manner. Biochem. J., 352, 747-753.
5. Chen, C., Zheng, B., Han, J., and Lin, S.-C. (1997) Characterization of a novel mammalian RGS protein that binds to Ga proteins and inhibits pheromone signalling in yeast. J. Biol. Chem. 272, 8679-8885.
6. Tamirisa, P., Blumer, K. J., Muslin, A. J. (1998) RGS4 Inhibits G-protein Signaling in Cardiomyocytes. Circulation, 99, 441-447.
7. Limbird, L. E., and Vaughan, D. E. (1999) Augmenting b receptors in the heart: Short-term gains offset by long term pains? Proc. Natl. Acad. Sci. 96, 7125-7127.
8. Zhang, S., Watson, N., Zahner, J., Rottman, J. N., Blumer, K. J., and Muslin, A. J. (1998) RGS3 and RGS4 are G-protein inhibitors in the heart. J. Mol. Cell Cardiol. 30, 269-276.
9. Owen, V. J., Burton, B. J., Mullen, A. J., Birks, E. J., Barton, P., and Yacoub, M. H., (2001) Expression of RGS3, RGS4 and Gi

alpha 2 in acutely failing donor hearts and end-stage heart failure. European Heart Journal, 22, 1025-1020.
10. Kong, Janice (2001) Master of Science (MSc) Thesis, McGill University.
11. Rogers, J. H., Tsirka, A., Kovacs, A., Blumer, J. K., Dorn, G. W., Muslin, A. J. (2001) RGS4 reduces contractile dysfunction and hypertrophic gene induction in Gaq overexpressing mice. J. Mol. Cell Cardiol. 33, 209-218.
12. http://www.ncbi.nlm.nih.gov/Genbank/index.html
13. Greenwood M.T., Hukovic, N., Kumar, U., Panetta, R., Hjorth, S.A., Srikant, C.B., Patel, Y.C. (`997) Ligand binding pocket of the human somatostatin receptor 5: mutational analysis of the extracellular domains. Mol. Pharmacol. 52, 807-814.
14. Greenwood, M.T., Guo, Y., Kumar, U., Beausejours, S., Hussain, S.N. (1997) Distribution of protein inhibitor of neuronal nitric oxide synthase in rat brain. Biochem. Biophys. Res. Commun. 238, 617-621.
15. Mumberg, D., Muller, R., and Funk, M. (1994) Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res. 22, 5767-5768.
16. Dohlman, H. G., Song, J., Ma, D., Courchesne, W. E., and Thorner, J. (1996) SSt2, a Negative regulator of pheromone signalling in the yeast Saccharomyces cerevisiae: expression, localization, and genetic interaction and physical association with Gpa1 (the G-protein a subunit) Mol. Cell. Biol. 16, 5194-5209.
17. Gietz, D., St Jean, A., Woods, R. A. , Schiestl, R. H. (1992) Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. 20, 1425.
18. Druey, K. M., Blumer, K. J., Kang, V. H., Kehrl, J. H. (1996) Inhibition of G-protein-mediated MAP kinase activation by a new mammalian gene family. Nature. 379, 742-746.

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COMMENTARIES

OBSERVING OBJECTIVE, STRUCTURE, CLINICAL EXAMINATIONS (OSCE)

Objective, structure, clinical examinations (OSCE) are increasingly used to examine clinical skills in both undergraduate and postgraduate students in the UK (1). As suggested by their name, the OSCEs are intended to reduce subjectivity amongst examiners by having predefined marking. This objectivity of marking as well as the structure of the examination, where all students are asked to answer the same questions and perform the same procedures, is intended to make OSCEs 'fairer' than other types of clinical examinations, such as short and long cases (2).

I have now taken part in six OSCEs - four as a clinical medical student and two as a 'sim', or simulated patient. My first experience on the other side of the couch was as a young woman who wanted to start taking the contraceptive pill. Fourth year medical students had to determine whether or not I was an appropriate candidate for the pill and explain to me how to take it and what to do if I missed one. More recently, third year dental students were asked to perform an extra- and intra oral examination on me. As a postgraduate student trying to get by on something similar to the minimum wage, the incentive of £9 an hour to do almost nothing is irresistible. Although it makes for a rather mind, or mouth, numbing morning, being a sim patient can have its interesting aspects.

In terms of advice for students, I learned two key things. Firstly, remembering to introduce yourself can make the difference between passing and failing (3). In both exams, specific marks were allotted for introducing yourself and confirming the patient's identity. Secondly, communication skills (3). In the medical exam, there were three marks assigned to 'overall impression'. These weren't awarded for knowledge but for confidence, demeanor and ability to put the patient at ease and treat with respect.

In addition, I also have a number of reflections on the whole process of OSCEs. To begin with, I feel a small sense of injustice that I never had the chance to take part in an OSCE as a sim patient before, rather than after, taking part as a student. Having the opportunity to observe a number of individuals perform exactly the same task gives you a very good sense of what works and what doesn't. Everyone has to develop a clinical style that they feel comfortable with. Being on the receiving end of a number of different approaches to the same problem is a great way of identifying just what aspects you might want to incorporate into your own style.

OSCEs could, therefore, be used as a learning experience for one group of students at the same time as an examination procedure for another. Undergraduate, like postgraduate students, are a willing and constant source of labor. Disclosure of exam contents from student sim patients should be of no concern since (4) it is easy enough to find out what appeared in last year's OSCE by simply asking someone who took it.

Next, there were one or two dental students who managed to hurt me. Not hurting the patient is a golden rule of exams, and clinical medicine in general. However, the OSCEs that I have taken part in had no way of assessing distress caused to patients. It would have taken minimal training and time to ask me to mark each student on their gentleness and include this at the bottom of the examiner's mark sheet. In fact, there are numerous examples in the literature, particularly from the USA, where sim patients are trained to do some or all of the marking at OSCE stations (5-7). This not only allows the patients to comment on 'how it was for them', but can also remove the need for a separate examiner altogether if sim patients are trained to perform all the marking. In turn, this can make OSCEs cheaper and easier to run, as fewer clinical staff are required.

Lastly, the OSCEs that I have taken part in as a medical student have provided no real opportunity for feedback on my performance. Whilst one of the main aims of exams, particularly in the later stages of the course, is to confirm that students will make safe practitioners (8,9), failing to provide feedback beyond an overall mark is a wasted opportunity. The General Medical Council has recently emphasized the need for formative (where students are given feedback on their performance), as well as summative (where students are graded on their performance) assessments in medical education (10). One of the identified benefits of OSCEs are the opportunities they give for formative assessment and feedback (2).

OSCEs are an opportunity for each individual student to be observed by experts in a number of different clinical encounters. It makes sense to make use of this unique opportunity to provide all students with an assessment of their performance that goes beyond pass or fail. Again, there are examples of this in the literature, particularly from the USA, where students are given feedback either after the exam or as part of each OSCE station (2,5). One possible way of providing individualized feedback would be to ask examiners to write, or dictate, a few sentences on each student between stations. These notes could then be transcribed, collated and returned to students with their

marks. Such a procedure would not be particularly onerous and would allow students to learn from the experience of the OSCE, as well as the preparation beforehand.

OSCEs were first trialed in 1975 (11) and despite varied responses from both staff and students (12-16), they look set to stay with us. Although they are undoubtedly an improvement on the old 'long cases' in terms of fairness (1), the current use of OSCEs in the UK seems to present a number of missed opportunities in terms of both education and efficiency.

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REFERENCES

1. Wass V, Jones R, Van der Vleuten C. Standarised or real patients to test clinical competence? The long case revisited. Medical Education 2001;35:321-325.
2. Carraccio C, Englander R. The objective structured clinical examination: a step in the direction of competancy-based evaluation. Archives of Pediatrics and Adolescent Medicine 2000;154(7):736-741.
3. Wass V. Getting through OSCEs. Student BMJ 2000;8(9):361-362.
4. Varma S. Should candidates be given their marked up papers back after examinations - the case for. Student BMJ 1999;7(11):456.
5. Duerson M, Romrell L, Stevens C. Impacting faculty teaching and student performance: nine years' experience with the objective structured clinical examination. Teaching and Learning in Medicine 2000;12(4):176-182.
6. Merrick H, Nowacek G, Boyer J, Robertson J. Comparison of the objective structured clinical examination with the performance of third-year medical students in surgery. American Journal of Surgery 2000;179:286-288.
7. Mavis B. Does studying for an objective structured clinical examination make a difference. Medical Education 2000;34:808-812.
8. Weetman A. Should candidates be given their marked up papers back after examinations - the case against. Student BMJ 1999;7(11):457.
9. Kaufman D. Assessing medical students: hit or miss. Student BMJ 2001;9(3):87-88.
10. Tomorrow's Doctors: Recommendations on Undergraduate Medical Education. July 2002. http://www.gmc-uk.org/global_sections/sitemap_frameset.htm
11. Harden R, Stevenson M, Wilson Downie W, Wilson G. Assessment of clinical competence using objective structured examination. British Medical Journal 1975;1:447-451.
12. Kearney P. OSCEs don't work. Student BMJ 2000;8(3):123.
13. Green T. OSCEs are useful to assess particular skills. Student BMJ 2000;8(4).
14. Morris C. Objective structured clinical examination. Student BMJ 2001;9(7):303.
15. Mottram V. Objective structured clinical examination. Student BMJ 2000;8(2):82.
16. Phillips S. OSCE oscars. Student BMJ 2000;8(4):168.

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IRB REFORM IN NORTH AMERICA: CHALLENGES AND OPPORTUNITIES

Clinical research is necessary to advance medical knowledge and to test new drugs or devices. It is therefore vital to society, and ethically imperative, that clinical studies be performed if patients are to have access to safe and effective treatments. However, clinical research by its very nature involves risk. Subjects who volunteer in clinical studies may receive no benefit; in fact, they may be seriously harmed or even die as a result of their participation. Indeed, research-related injuries and deaths, though relatively few in number (1), have become the subject of much controversy in recent years (2). Thus, protection of human research subjects must be given the highest priority by researchers, their institutions, and the government and regulatory bodies charged with overseeing the clinical research process. Central to this process is the institutional review board (IRB) in the United States (U.S.), or research ethics board as it is known in Canada. (For the purpose of this paper, the term "IRB" will be used to refer to both American and Canadian boards.) The IRB has frequently been referred to as the "first line of defense" in research subject protection (3), yet the specific roles and responsibilities of this board and its members are not clear (4). Defining these roles and responsibilities is more important now than ever before, for several reasons.

THE ISSUES:

IRBs in crisis

First, biomedical science is advancing at an unprecedented rate, and the number of clinical studies is

increasing exponentially (5). This situation can only add to the strain on already over-burdened IRBs (6). Numerous articles in recent literature have made reference to the "crisis" in American IRBs (7,8), being unable to cope with the sheer volume of protocols they are asked to review, and the "pressure-cooker atmosphere" (9) IRB members must contend with as they struggle to balance the interests of subjects with those of the researchers. A report commissioned by the Law Commission of Canada indicates that Canadian IRBs are not faring any better (10). The U.S. Office of Human Research Protection (OHRP; the agency responsible for oversight of IRBs in the U.S.) concluded in a 1998 report that IRBs review "too much, too quickly, with too little expertise" (6), and that the entire system was "in jeopardy" (6). Though there is no equivalent to the OHRP in Canada, Canadian IRBs have not escaped criticism (11,12) or calls for reform (10,13). Clearly, IRBs in North America are overwhelmed under the current system. It is not reasonable to expect these same IRBs to handle double or triple their current workload without a significant reduction in the quality of their reviews. If IRBs are to continue to be relied upon as the primary safeguard of clinical trial volunteers, a clear mandate for their roles and responsibilities must be established. In defining these roles and responsibilities, it may be possible to lessen the current burden on IRBs by delegating certain responsibilities to other committees or regulatory bodies. Alternatively, recognition of the many obligations of the board and its members could result in provisions for increased support to give the members the resources they need to do their job properly.

Liability and the IRB member

The second reason for the impetus to define the IRB's specific roles and responsibilities is the alarming, albeit not new, threat of personal legal liability of IRB members. The U.S. National Commission for the Protection of Human Subjects Report and Recommendations: Institutional Review Boards, published in 1978, clearly states that IRB members may be held personally liable "for malpractice or negligence in discharging their IRB functions" (14). Angela Holder, a legal expert on human subject research, was quick to respond in her 1979 article "Liability and the IRB Member: The Legal Aspects", that such a thing would never actually happen (15). Times have changed. In 2001, a lawsuit filed on behalf of subjects who had participated in a study of a melanoma vaccine at the University of Oklahoma named twelve IRB members as defendants (16). The 130-page complaint contained many allegations, including inadequate procedures for the manufacturing and safety testing of the vaccine, failure to adhere to the protocol inclusion/exclusion criteria, incomplete informed consent documents, and a failure by the IRB to meet its federal regulatory obligations (16). The individual IRB members were accused of negligence in their duties (16). This precedent-setting case sent shockwaves through IRBs across North America.

IRB members are, for the most part, volunteers who commit a tremendous amount of time and energy to the onerous task of reviewing the hundreds of protocols that pour into their institutions each year. Their work is often not respected by researchers, who tend to view the ethics review process as "a bureaucratic pain in the neck" (3). IRB members face a great deal of pressure from researchers, sponsors, institutions, and even the public to push through protocols at a rate that cannot possibly be consistent with a meticulous and thoughtful consideration of all the ethical issues at stake. Though the IRB has the power to require revisions to protocols, "exercise of this power does not enhance a committee's popularity within its institution" (17). In addition, IRB members must make difficult decisions about increasingly complex protocols that do not fall neatly under any guidelines currently in use (17). These decisions, which often come down to judgment calls, require "a fair exercise of intelligence and discretion on the part of IRB members" (18). As one article describes it, "the quality of an IRB's work depends to an inordinate degree on the conscience and commitment of its volunteer members" (19). The idea that individual IRB members could be held legally liable for these decisions is disturbing. This threat of legal action may result in IRBs rejecting more protocols than they approve, or reviews so painstakingly thorough that the review process effectively grinds to a halt (20). It will undoubtedly serve as a deterrent to future IRB members, at a time when it is already difficult to fill these positions (20,21). However, no one would suggest that IRB members be exempt from accountability. Certainly, even without a proper set of guidelines, one would expect IRB members to carry out their duties in a conscientious manner. But knowing precisely what they will be held accountable for, and what protections are in place for them, will be essential if these individuals are to be expected to continue this important work without fear of litigation.

The IRB in the public eye

Finally, for clinical trials to proceed and potentially life-saving treatments to reach patients who need them, researchers need the public's trust. Donors, funding agencies, government, and most importantly, clinical study volunteers and their families, all want assurance

that a solid system of checks and balances is in place to protect research subjects. Without the public's trust, the system cannot function. Unfortunately, this trust has been eroded in recent years by high-profile incidents involving the tragic deaths of research subjects, and the allegations of misconduct that followed. As Dennis De Rosia, chairman of the Association of Clinical Research Professionals, said in an interview, "Each time you have one of these incidents, there's a rash of publicity, and it gets harder to recruit volunteers" (22). Most notable among these are the cases of 18 year-old Jesse Gelsinger, who died while participating in a gene therapy trial at the University of Pennsylvania (23), and 24 year-old Ellen Roche, a healthy volunteer who died after inhaling hexamethonium as part of an asthma study at Johns Hopkins (24). Both of these cases drew international attention when failures of the research subject protection system were uncovered. Since 1998, the OHRP has suspended or restricted research at over a dozen institutions due to IRB inadequacies, including such prestigious institutions as Duke (25) and Johns Hopkins (26). In both Canada and the U.S., research related deaths and injuries have landed IRBs or their sponsoring institutions in court (27,28). Subsequent investigations have often directed much of their criticism at the IRB involved (20). The reports themselves often reflect expectations of the IRB that are simply unwarranted. As an example, the report of the external review committee for the Johns Hopkins incident faults the IRB for not having a pharmacologist on their board (29). Yet none of the current guidelines for IRB membership contain any reference to a requirement for the presence of pharmacologist (30), nor is it obvious that "rigorous pharmacological review" (29) is the responsibility of the IRB (31). Discrepancies such as these serve as excellent examples to illustrate why the IRB and its members must be given a specific framework within which to act. The public's faith in the system is flagging (2,22); a clear set of responsibilities for the IRB would put an end to finger-pointing that further damages that faith.

COPING STRATEGIES AND SOLUTIONS:

Commercial IRBs

Several mechanisms have arisen over the past few years as means to cope with the over-burdening of local IRBs. As a result of the significant increase in industry funded research, commercial or "for-hire" IRBs were established. Commercial IRBs now exist in both Canada and the U.S., though official information on the number of these boards operating in either country is lacking (18). These IRBs primarily review research to take place at research centers affiliated with pharmaceutical companies, at contract research organizations, or in private clinics by medical professionals not associated with academic research institutions (32).

Review through these IRBs is faster and arguably better and more consistent than that obtained through traditional IRBs, given the stipulations these boards may hold regarding the education and expertise of their members and the fact that there is less turnover in their membership. Using a commercial IRB for a multi-center trial may also save time and money by allowing researchers to forgo multiple, redundant reviews of the same protocol at each individual site (32). However, serious concerns have arisen regarding the inherent conflicts of interest that exist within commercial IRBs (18,32). Whether the commercial IRB is one set up by a pharmaceutical company to review research on its own products, or an independent review board on contract to review research taking place elsewhere, in both cases the IRB is a for-profit enterprise. Academic IRBs have also been criticized for conflicts of interest, as IRB members may be motivated by their desire for career advancement, future opportunities for collaboration, or even maintaining friendships with colleagues when reviewing protocols (33). However, because academic IRBs are non-profit institutions, these concerns - at least in terms of financial conflicts of interest - are less obvious. Further problems with commercial IRBs arise when a researcher who has a protocol rejected by one IRB simply takes it to another. The researcher has no obligation to inform an IRB of previous submissions of the same protocol, nor does an IRB have access to any other IRB's review. The problem of "IRB shopping" is a serious one, and one that did not exist when the traditional IRB was the only channel through which a researcher could have their protocols reviewed. Similarly, independent IRBs are not required to monitor the research they have approved, as is the case for traditional IRBs. Independent IRBs may also lack the familiarity with local research conditions and culture that traditional IRBs have. Thus, while the commercial IRB fills an important niche in the context of the current ethics review system, it is not without problems, nor is it a replacement for the traditional IRB. In fact, because there are no clear rules and regulations for IRB review, oversight and accountability, commercial IRBs are no better equipped than traditional IRBs to meet the challenges posed by the present system.

Central IRBs

A second development in recent years is the creation of the central IRB (CIRB) for multi-center trials. This CIRB could perform a detailed review of the science and experimental design of a protocol for multi-center trials, eliminating the need for the IRB at each

individual site to repeat this process (34). The local IRBs could then expedite their review of a protocol approved by the CIRB, focusing their attention on local issues the protocol may present (such as institutional policies, or language differences that may require changes to the consent form), rather than needlessly duplicating the review of the scientific aspects of the protocol (35). An added advantage of the CIRB would be in managing the ongoing monitoring of these trials. Safety reports and annual study reports from multi-center trials comprise a significant proportion of the workload of local IRBs (7). A centralized approach to monitoring these trials would not only free up more of the local IRBs time and resources to put toward other responsibilities (such as review of local studies), but may in fact result in more effective review of adverse events. The CIRB would be reviewing reports of adverse events from all individual sites in the context of the trial as a whole, a comprehensive view that most local IRBs do not have. Furthermore, the CIRB would have access to reports by data and safety monitoring boards (DSMBs) and information from the sponsor that is not available to local IRBs, but which could be crucial in making decisions with respect to monitoring. The CIRB approach is currently in the pilot phase at the National Cancer Institute in the U.S. (34). This pilot model was established in order to increase patient access to National Cancer Institute-supported trials. With sixteen members, all cancer experts, from both academic and community organizations across the U.S., the CIRB initially served 22 local institutions (34). Results so far have been promising, and plans are presently underway to expand to serve 100 (34). However, challenges still remain in terms of the division of responsibilities, both between the local and CIRB and between the CIRB and other bodies (such as the DSMB). Another potential complication is that local IRBs may also want to continue to conduct complete reviews of protocols even after CIRB approval if they are concerned about being held accountable for the decision to approve. This is another instance where defining responsibilities for IRBs would facilitate quicker reviews and more effective collaboration between partners in the review process.

Delegating responsibilities

A third way in which the research ethics review system is attempting to deal with its ever-increasing workload is by rethinking how the IRB manages its many obligations. By most accounts, monitoring is the function that IRBs perform most poorly. Canadian (11) and American (36) reports indicate that few IRBs conduct any monitoring beyond reviewing annual study reports, the bare minimum requirement in both countries (14,37). Papers in recent literature have proposed that monitoring should be delegated to a separate body, particularly for multi-center trials (7,34). Both the Office of Human Research Protection and the Food and Drug Administration in the U.S. are encouraging greater use of DSMBs, and the National Institutes of Health now require an independent DSMB for all Phase III trials (1). Others suggest that certain aspects of monitoring, such as consent monitoring (determining whether research subjects understand the risks and benefits of the research they are being asked to participate in) be delegated to a subcommittee of the IRB, or an intermediary (38). While at present these proposals are just that - proposals - the idea of delegating some of the IRB's responsibilities is attractive. Many IRBs already assign some aspects of the review process, such as the review of contracts between clinical investigators and sponsors, or the assessment of statistical power of clinical trials, to individuals who are not members of the IRB. While the danger exists that adding several subcommittees or consulting bodies will increase the time and red tape involved in reviewing protocols, the reassurance that all the functions of the IRB are being fulfilled by persons with the expertise to perform them properly makes the additional layer of bureaucracy worthwhile. The IRB can then concentrate its time and efforts on thorough primary reviews and oversight of local studies.

THE FUTURE:

In considering any changes to the current system, it must be kept in mind that the IRB's primary purpose is the protection of research subjects. This mandate cannot be achieved without a formal regulatory framework within which the IRB can operate. Such a system would need to establish standards harmonizing national and international laws and policies. A single set of clear guidelines is required with respect to conflicts of interest, division of duties, and accountability. The IRBs need greater support from government and their institutions, both in terms of funding and staff, as well as training and ongoing education for IRB members and clinical investigators.

How close is this major overhaul to becoming a reality? It may be closer than it seems. Last fall, an Institute of Medicine committee delivered its recommendations for improving research subject protection in the U.S. (39). A central theme throughout the report is easing the strain on IRBs by reducing their workload and increasing their resources. Among the recommendations are calls for a national registry to track research participants, as well as the creation of a CIRB. The report also contains a plan to separate the IRB's functions into three committees: one to evaluate

the scientific merit of a protocol, one to assess potential conflicts of interest, and a third to integrate all the information, consider other issues and make a decision. The Institute of Medicine plan also proposes a no-fault compensation system for subjects who are harmed as a result of their participation in research, thereby avoiding litigation. It remains to be seen whether these changes will be implemented, and whether Canada will adopt similar strategies. If our governments, granting agencies and institutions recognize the value of clinical research and the independent ethics review process that must accompany it to our society, then they must also recognize that this important issue needs to be addressed, and deserves our immediate attention.

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REFERENCES

1. Office of Inspector General. Protecting Human Research Subjects: Status of Recommendations. Washington, D.C. Department of Health and Human Services, 2000.
2. Drennan KB. Have the ultimate benefits of clinical trials been maligned beyond repair? DDT 6(12): 597-599; 2001.
3. Lemonick MD, Goldstein A. At your own risk. Time 159(16): 40-49; 2002.
4. Hirtle M, Lemmons T, Sprumont D. A comparative analysis of research ethics review mechanisms and the ICH Good Clinical Practice guideline. Eur J Health Law 7: 265-292; 2000.
5. No author listed. Anticipating a clinical investigator shortfall. Centerwatch 8(April): 1,5-13; 2001.
6. Office of Inspector General. Institutional Review Boards: A Time for Reform. Washington, D.C. U.S. Department of Health and Human Services, 1998.
7. Burman WJ, Reves RR, Cohn DL et al. Breaking the camel's back: multicenter clinical trials and local institutional review boards. Ann Intern Med 134: 152-157; 2001.
8. Levine RJ. The crisis in institutional review boards. Ann Intern Med 134: 161-163; 2001.
9. Phillips DF. Institutional review boards under stress: will they explode or will they change? JAMA 276: 1623-1625; 1996.
10. McDonald M. The governance of health research involving human subjects. Law Commission of Canada. Ottawa, Ontario, 2000.
11. Weijer C, Shapiro S, Fuks A et al. Monitoring clinical research: an obligation unfulfilled. CMAJ 152(12): 1973-80; 1995.
12. Thorne, D. Researchers put patients at risk. Edmonton Journal. September 28: B3; 2002.
13. Bevan J. Toward the regulation of research ethics boards. Can J Anesth. 49(9): 900-906; 2002.
14. National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. Institutional Review Boards: Report and Recommendations of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. Federal Register 43(30 November): 56186, 1978.
15. Holder AR. Liability and the IRB member: the legal aspects. IRB 1(3): 7-8; 1979.
16. Robertson v. McGee, No. 01CV00G0H(M) (N.D. Okla. Filed January 29, 2001.)
17. Freedman B and Glass KC. Weiss v. Solomon: a case study in institutional responsibility for clinical research. Law Med Health Care. 18(4): 395-403; 1990.
18. Lemmens T, Freedman B. Ethics review for sale? Conflicts of interest and commercial research review boards. Milbank Q 78(4): 547-84; 2000.
19. Edgar H and Rothman DJ. The institutional review board and beyond: future challenges to the ethics of human experimentation. Milbank Q. 73(4): 489-506; 1995.
20. Anderlik MR, Elster N. Lawsuits against IRBs: accountability or incongruity? J Law Med Ethics 29(2): 220-228; 2001.
21. Foubister V. Clinical trial patients sue IRB members. Amednews.com. February 26; 2001.
22. Abate T. Special report: experiments on humans. San Francisco Chronicle. August 5; 2002.
23. Stolberg SG. The biotech death of Jesse Gelsinger. N Y Times Mag Nov 28: 136-140, 149-150; 1999.
24. Smaglik P. Asthma study death spurs inquiry. Nature 138(5): 36; 2001.
25. Shamp J. Agency halts federally funded Duke research on new patients. Herald-Sun (Durham NC). May 12: A1; 1999.
26. Kolata G. U.S. suspends human research at Johns Hopkins after death. New York Times. July 20: A1, A18; 2001.
27. Weiss v. Solomon. (1989). R.J.Q. 731.
28. Kus v. Sherman Hospital. (1995). 268 Ill App 3d 771,644 NE2d1214,1216.
29. Cassel C, Stock MC, Wood A, Zapol W, Hellman S. Report of Johns Hopkins University External Review Committee, Aug. 8. 2001.
30. Protection of Human Subjects, 45 C.F.R. 56, IRB membership, § 107. (4-1-00 Edition).
31. Protection of Human Subjects, 45 C.F.R. 56, IRB functions and operations, § 108. (4-1-00 Edition).
32. Office of Inspector General. Institutional Review Boards: The Emergence of Independent Boards. Washington, D.C.: U.S. Department of Health and Human Services, 1998.
33. Cho MK, Billings P. Conflict of interest and institutional review boards. J Investig Med 45(4): 154-9; 1997.
34. Christian MC, Goldberg JL. A central institutional review board for multi-institutional trials. N Engl J Med 346(18): 1405-1408; 2002.
35. Stair TO, Reed CR, Radeos MS et al. Variation in institutional review board responses to a standard protocol for a multicenter clinical trial. Acad Emerg Med 8:636-641; 2001.
36. Gordon B, Prentice E. Continuing Review of Research Involving Human Subjects: Approach to the Problem and Remaining Areas of Concern. IRB 19(2): 8-11; 1997.
37. Medical Research Council of Canada, Natural Sciences and Engineering Research Council of Canada, and Social Sciences and Humanities Research Council of Canada. Tri-council policy statement: ethical conduct for research involving humans. Ottawa: Minister of Supply and Services, 1998.
38. Pilon S. When less is more - a new look at research monitoring. BMC News and Views 1:6; 2000.
39. Institute of Medicine. Responsible Research: A Systems Approach to Protecting Research Participants. The National Academies, Washington D.C., October 3, 2002.

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EMERGING PATTERNS IN THE RESISTANCE TO THE MEDICALIZATION OF BIRTH IN NORTH AMERICA

Childbirth is one of the most ubiquitous experiences of human life. It is an integral ritual of the life cycle. Nevertheless, different cultures have evolved strikingly varied practices surrounding childbirth. As such, one might argue that in any society, “the way a woman gives birth and the kind of care given to her point as sharply as an arrowhead to the key values in the culture” (1).

Within the last century, the process of childbirth has increasingly taken place within the realm of medicine: in a hospital, attended by a physician. This medicalization of childbirth is especially evident in Western societies with an established technomedical infrastructure such as in "non-Indigenous" North America and societies where the medicalization of birth has been imposed such as in Inuit communities. This paper will explore women's concerns regarding the medicalization of childbirth, as illustrated by the example of the Inuit women in Canada.

In North America, the ritual of birth was once the dominion of women, but has since been incorporated into the practice of medicine, causing a shift of "authoritative knowledge" from older women and midwives to trained physicians. The increasing reliance of medicine on technology within North American society has permeated the realm of childbirth. In so doing, the framework of empirical science has been introduced into the birthing process, perpetuating the values of a technologically-oriented society. One might argue that "[b]y making the naturally transformative process of birth into a cultural rite of passage, a society can ensure that its basic values will be transmitted" (2). Many feel as though the process of childbirth is being "sterilized" and its cultural and emotional content diminished.

Many "non-Indigenous" North American women feel as though they are being alienated from the birthing process and have lost control of their own bodies. As such, women are increasingly seeking alternatives to hospital deliveries (3). There has been a recent and marked resurgence of homebirths and reliance on midwives. Midwifery allows women to play a larger role in their deliveries, and returns the process of childbirth to the home: roughly half of all midwife-attended births are performed in the home (4). Until 1992, midwifery was illegal in Canada when Ontario became the first province to legalize midwifery. Since then, four other provinces including Quebec have followed suit and midwifery is becoming an increasingly accepted alternative to hospital childbirths (3). In 2000, 4% of Ontario births were attended by midwives and by the year 2004, the percentage is estimated to rise to 10% (4).

The experience of Inuit women in the last few decades provides an even more salient example of the institutionalization of childbirth and its separation from community life. In traditional Inuit culture, an expectant woman was followed throughout her pregnancy and assisted in childbirth by a midwife and several women helpers (5). Traditional midwives received no official training, but were taught how to deliver by older women. Hence, the skills associated with midwifery were passed on from generation to generation. The knowledge of midwives was considered indispensable and midwives were accorded much respect within the community. In fact, the ability to assist in childbirth endowed women with a sense of pride and empowerment in a predominantly patriarchal society (6). In addition to an honoured position within society, a special relationship existed between the children and the midwives who delivered them. An Inuit boy gave the midwife who delivered him his first catch, and a girl, the first item she sowed (5). For the Nuu-chah-nulth people of British Columbia, the term midwife translates as "she who can do everything." Hence, depriving these women of assisting in childbirth is tantamount to removing them from their influential and honoured position in society (7).

In addition to the reliance on midwives and women helpers, several other aspects of traditional Inuit childbirth are worth mentioning. Unlike current Western practices, such as those outlined in the 2002 National Guidelines for the Childbearing Years (8), an expectant woman was to remain highly active throughout her pregnancy, performing all her daily chores until the advent of labour. It was thought that such activity would give the woman the strength needed to deliver (5). Women would give birth in rapidly constructed snow houses in either a squatting or kneeling position with the midwife behind them (6). The midwife would often enlist the help of the pregnant woman's husband and female relatives, thus involving the whole family in the process of childbirth (5).

The traditional Inuit process of childbirth, as described above, was practiced without scrutiny until the 1960s. During the 1960s, so-called Nursing Stations were set up throughout the Canadian North and deliveries were relegated to officially-trained, licensed midwives within these stations. Although traditional Inuit midwives were legally excluded from these deliveries, these Nursing Stations nevertheless permitted women to deliver within their communities, surrounded by their families. Since the 1980s, however, due mostly to a shortage of professionally trained midwives, deliveries must be performed within

hospitals in cities far removed from the Northern communities. Today, Inuit women are "evacuated" three weeks before their due date and sent to hospitals in cities, such as Winnipeg, where obstetricians perform their deliveries. Hence, within three generations, Inuit women have experienced a transition from birth performed by a traditional midwife in the context of the home and family, to birth performed by a professional midwife in a Nursing Station within their community and presently, to birth performed by a physician in a hospital far removed from their community (5,6).

Since the practice of sending women to distant hospitals began, Inuit women have repeatedly expressed their dissatisfaction with the current system. In fact, since its inception in 1984, a primary concern of the Inuit Women's Association has been the issue of childbirth (5,9). The concerns about the system in place centre around five main issues: 1. the women sent to distant hospitals experience loneliness and alienation, 2. the family is removed from the process of childbirth, 3. the separation of the expectant woman from her husband and children causes undue strain on their relationship, 4. the skills and knowledge of traditional Inuit midwives are being eradicated, and 5. Inuit children are receiving out-of-territory birth certificates. Inuit women feel as though their control over childbirth has been usurped by the government and that they have now become dependent on health care services. The status previously endowed by the knowledge of childbirth on midwives and women in general has been perturbed by the forced reliance on the expertise of faraway physicians.

According to John O'Neil and Patricia A. Kaufert (6), the practice of sending Inuit women to distant hospitals is a manifestation of "internal colonialism." Although a discussion of Inuit childbirth as an extension of colonialism is beyond the scope of this paper, this idea serves to illustrate the clash of cultures at hand. The imposition of the Western culture's birthing practices on the Inuit community has left many Inuit feeling robbed of yet another aspect of their culture and heritage. The Western culture which tends to dissociate childbirth and illness, in general, from community life is in sharp contrast to the Inuit culture which views childbirth and illness as being integral and continuous aspects of community life (6).

As the current situation remains unfavourable to the majority of Inuit women, a compromise of some sort must be reached. Such a compromise was articulated at a workshop held in the Inuit community of Keewatin in 1988 and relies on a cooperation between traditional midwives and obstetricians such that high-risk deliveries would be performed by obstetricians and low-risk deliveries by traditional midwives. Although the death of a newborn was traditionally viewed as "meant to be," Inuit women overwhelmingly approve of seeking the expertise of an obstetrician for high-risk deliveries, but wish to be given the choice to have their births performed by traditional midwives in the case of low-risk deliveries. A spokeswoman for the Inuit Women's Association spoke of a system that will "look into the past to find the elements that can be adapted to contemporary conditions to ensure that the knowledge and experience of the elder midwives is retained as part of Inuit heritage" (5). Others, however, are proponents of a more confrontational approach. Women in the community of Puvirnituq have returned to performing traditional-style births and have reclaimed their dominion over the childbirth process (10). These women are doing so illegally. When Quebec legalized midwifery in 1999, Quebec law failed to recognize training programmes other than the one offered at the Université du Québec in Trois-Rivières. The women training at the Innuulitsivik Health Centre in Puvirnituq are unable to apply for a midwifery license. Negotiations are under way between the provincial Ministry of Health and the Inuit community, and offer promise that the traditional apprenticeship model of training midwives will be officially recognized (7).

It is likely that in the years to come, the trend towards the deinstitutionalization of childbirth will be amplified, though technology's value in reducing infant and maternal mortality will undoubtedly ensure that it remains an integral component of childbirth. However, "[t]he issue is not whether technology is good or bad in and of itself, but under what circumstances should it be used, when does it augment the quality of life of those who use it, when does it detract from that quality, and, perhaps, most importantly, who has the power to decide what is appropriate use" (11). A better understanding of these issues and a discussion of the needs and concerns of those involved will likely enhance the quality, safety and diversity of tomorrow's childbirth practices.

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REFERENCES

1. Kitzinger S. Women as Mothers: How They See Themselves in Different Cultures. New York, NY: Vintage Books, 1980.
2. Davis-Floyd RE. Birth as an American Rite of Passage in Childbirth in America: Anthropological Perspectives. In: Michaelson KL, editor. South Hadley, MA: Bergin and Garvey Publishers, 1988.
3. Davis-Floyd RE, Sargent C. Introduction: The Anthropology of Birth Childbirth in Childbirth and Authoritative Knowledge: Cross-Cultural Perspectives. In: Davis-Floyd, RE, Sargent CF, editors. Berkeley, CA: University of California Press, 1997.

4. Holliday T. The re-emergence of Canadian midwifery: A profession dedicated to normal birth. 2001 www.birthinternational.com
5. O'Neil JD, Gilbert P. Childbirth in the Canadian North: Epidemiological, Clinical and Cultural Perspectives. Winnipeg, MB: University of Manitoba, 1990.
6. O'Neil JD, Kaufert P. The Politics of Obstetric Care: The Inuit Experience in Births and Power: Social Change and the Politics of Reproduction. In: Handwerker W. Boulder, CO: Westview Press, 1990.
7. Carrol D, Benoit C. Aboriginal midwifery in Canada: Blending traditional and modern forms. CWHN Network 4:5-10; 2001.
8. Nutrition for a Healthy Pregnancy- National Guidelines for the Childbearing Years, Health Canada, 2002.
9. Inuit Women's Association. 1984-1985 Resolutions. http://www.pauktuutit.on.ca/resolutions/84-85.html
10. Daviss BA. Heeding Warnings from the Canary, the Whale, and the Inuit. A Framework for Analyzing Competing Types of Knowledge about Childbirth in Childbirth and Authoritative Knowledge: Cross-Cultural Perspectives. In: Davis-Floyd, RE, Sargent CF, editors. Berkeley, CA: University of California Press, 1997.
11. Michaelson KL. Birth Place/Birth Style in Childbirth in America: Anthropological Perspectives. In: Michaelson KL. South Hadley, MA: Bergin and Garvey Publishers, 1988.

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SUFFER THE LITTLE CHILDREN

Doctors and patients have come to expect a cure for most every illness and condition. Unfortunately for both groups, this is not always possible. When faced with the prospect of the imminent death of a patient, doctors may exhibit curative attitudes and perform interventions which may not be in the best interest of the patient. Referral to palliative care may become the only appropriate avenue of treatment, yet it may never be offered. Nowhere is this seen more clearly than in the treatment of a dying child. The lack of healing services provided to terminally ill children is alarming. This dearth of pediatric palliative care stems from the history of palliative care, societal attitudes about dying children, the current standard of medical education, the limited experience of pediatricians with death, and the issues of required parental consent. These factors cumulatively affect the interactions between doctors, parents and children faced with a terminal illness.

The World Health Organization has defined pediatric palliative care as "the active total care of patients whose disease is not responsive to curative treatment. Control of pain, of other symptoms and of psychological, social and spiritual problems is paramount. The goal of palliative care is achievement of the best quality of life for patients and families (1)." Contemporary society is obsessed with curative treatment. The last one hundred years have seen a dramatic increase in medical technology. Many once-fatal diseases are now mere inconveniences and science has given doctors incredible tools to eradicate illness and death. With respect to children, world mortality rates have been declining steadily. The chance of a newborn dying before its fifth birthday is seven percent, down from 25 percent in 1950 (2). This decrease is due largely to advances like pre-natal care, antibiotics, immunizations, and surgical repair of anomalies.

Before the many scientific advances, a physician's role was fundamentally different. Without the many curative measures that we have today, death was much more common and different skills were in demand. Because of their inability to cure, doctors used a palliative approach to comfort and ease the burden of death on patients and their families. Today the curing role has superceded this healing role because science has given us the opportunity to do so.

The ability to cure has changed the focus of medicine. With all the life-saving measures that exist, it is difficult for many to believe that nothing curative can be done. While the ability to prolong life may be possible, it is questionable whether or not it is advisable. In medicine, curing has been associated with life while palliative care has been linked with death. One reason why these associations exist: it seems to be easier for physicians and family to accept that a person died because heroic measures failed.

This direct association of palliative care with death makes its implementation an uncomfortable decision when children are concerned. Dying children defy the natural order (3). While elderly individuals and their families may be more open to palliative care, parents and doctors of children seem to be reluctant to implement it. Those who have lived a long life are more apt to accept its final, inevitable, conclusion. Since they hold out no hope for a permanent cure, palliative care

can be a desirable option for terminally ill adults. The Institute of Medicine states that a "decent or good death is: free from avoidable distress and suffering for patients, families, and caregivers; in general accord with patients' and families' wishes; and is reasonably consistent with clinical, cultural, and ethical standards (4)." Achieving this is the main goal of palliative care.

However, this goal of palliative care is not envisioned in pediatric situations. One reason is lack of pediatric palliative care training and education. Training programs in the United States, like those offered by the American Academy for Hospice and Palliative Care, focus primarily on adults. There are no established national standards for pediatric palliative care curriculum (5). As a result, only 10 percent of pediatric oncologists have had formal courses and only 2.2 percent have undergone a clinical rotation in palliative care (6). In addition, undergraduate medical education in the United States has focused predominantly on palliative care for adult and geriatric patients (7). Therefore, the established adult model of seeking a cure, then palliating until death, is being applied to children. This model does not necessarily fit the situation experienced by caregivers of children. In what has been called the Persephone syndrome, a course of disease that is fraught with rebound-relapse episodes may lead family and other care providers to be more persistent in pursuing aggressive therapy for prolonged periods of time in hope of yet another astonishing recovery (8). Because the adult model is cure or palliate, these children never receive palliative treatment. They are perceived as being perpetually in the cure stage, despite terminal illness. The logical conclusion would indicate to use both curative and palliative treatments, but, because of current educational practices, the combination of potentially curative and palliative medicine currently escapes us (9). Even the World Health Organization's definition of pediatric palliative care as quoted earlier in this paper seems to artificially separate curative and palliative efforts.

Further complicating the issue, pediatricians are relatively inexperienced with death. On average, a general pediatrician will come into contact with 3 children per year who will die (10). This limited exposure has two major effects: first, pediatricians lack experience dealing with the complex emotions related to death; and second, they report feeling a sense of guilt about their inability to cure. As a result, a common reaction is to separate themselves emotionally and physically from the dying children and their families (11).

Although adults involved in the medical care of family members want dying parents not to suffer, they expect their children to outlive them. When families are faced with the terminal illness of a child, they are presented with many challenges, some of which the medical system imposes upon them. They receive an overwhelming amount of technically complicated information from which they must make difficult decisions about the fate of their child. They may feel that an incorrect choice will cause undue suffering or death. As a result, families in these situations prefer to be guided by their practitioners (12).

Unfortunately, this creates a situation where pediatricians may influence parental attitudes and behavior. The feeling that the inability to cure a child is a personal failure, combined with a lack of education in pediatric palliative care, makes pediatricians reluctant to provide palliative care as an option. Even those pediatricians that do receive adult palliative care training are reluctant to turn care over to another clinician because of their strong relationship with the patient and his or her family (13). This makes the family see only one course: cure or nothing. This is unfortunate, because the decision of when to provide end-of-life care is paramount. Delay causes difficulties in tailoring treatment and exacerbates feelings of vulnerability and helplessness in the family and patient (14). Because of the paradoxical issues previously discussed, doctors are waiting for the family to decide to stop the curing process while the family is expecting the doctor to inform them when curative treatments should be abandoned. As a result, neither party actively initiates the discussion of terminal care. This can lead to prolonged suffering. "The need to do everything is a powerful force. This is relevant when interventions focused on cure are no longer reasonable and may well be harmful" (15).

In a Wisconsin study of dying children whose parents had received a palliative care consultation, they found a significant decrease in the administration of blood tests, central lines, feeding tubes, gastrostomy tubes, endotracheal tubes, x-rays, and paralytic medications compared to children whose parents had not (16). This study suggests that once palliative care is introduced as an option, invasive medical procedures with limited benefit are refused.

Misconceptions about children needing protection from pain, assumptions about their ability to understand, or the thought that they are too young to be affected continue today (17). In one study of dying hospitalized children in Edmonton between January 1996 and June 1998, only one child among 77 was documented as being specifically told that they were dying (18). Though legally unable to give informed consent, the "assent" of sick children may be weighed heavily by parents and doctors in deciding a course of treatment. When children are not informed that they are

dying, they cannot make any direct contributions to their care. Because the parents and the child are not both directly included, they have no opportunity to work together in deciding the best course of action (19). Therefore the patient's best interests may not be met.

The combination of these factors contribute to the lack of palliative care options offered for pediatric cases. Parents are dependent on the pediatrician to help make choices about end of life care. The pediatrician is not trained in offering such options, and the child is essentially uninvolved in the process. Since parents are not presented with the option to provide palliative care, they see stopping curative treatment as stopping all treatment for their child.

There is some academic work being done in the area. Some schools, like The Johns Hopkins Children's center, offer a one day seminar to residents (20). Several articles have been written recently (21,22,23) which show that awareness of the problem is growing. The issues at hand are: does medical intervention really lead to an improved quality of life for children? Are the necessary facilities and training available to residents and students to assist them in this aspect? Can doctors make better use of facilities and training that already exist? These are issues that must remain in the spotlight. Only by focusing on them will palliative services for children become more utilized in the future.

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REFERENCES

1. World Health Organization. Cancer Pain Relief and Palliative care in Children. World Health Organization, Geneva 1998.
2. WHO/67 press release 12 October 2000. http://www.who.int/inf_pr_2000/en/pr2000_67.html.
3. Hilden JM, Himelstein BP, Freyer DR, Friebert S, Kane JR: End-of-Life Care: Special Issues in Pediatric Oncology. In Foley KM, Gelbrand H eds. Improving Palliative Care for Cancer. National Academy Press, Washington, 2001. pp 161-198.
4. Institute of Medicine. Approaching Death: Improving Care at the End of Life. Washington, DC: National Academy Press; 1997.
5. Hilden, Himelstein et al., 2001.
6. Hilden JM, Emmanuel EJ, Fairclough DL, Link MP, Foley KL, Clarridge BC, Schnipper LE, Mayer RJ: Attitudes and practices among paediatric oncologist regarding end of life care: results of a 1998 American Society of Pediatric Oncology survey. Journal Clinical Oncology 2001;19:205-212.
7. Billings JA, Block S. Palliative care in undergraduate medical education. JAMA 1997; 278:733-738.
8. Feudtner C, Christakis DA, Zimmerman FJ, Muldoon JH, Neff JM, Koepsell TD: Characteristics of deaths occurring in children's hospitals: implications for supportive care services. Pediatrics 2002; 109:887-893.
9. Hilden, Himmelstein et al., 2001.
10. Sahler OJE, Frager G, Levetown M, Cohn FG, Lipson MA: Medical education about end-of-life care in the pediatric setting: principles, challenges and opportunities. Pediatrics 2000;105:575-584.
11. Sahler et al., 2000.
12. Frager G: Paediatric palliative care: building the model bridging the gaps. Journal of Palliative Care 1996;12:9-12.
13. Hynson, JL and Sawyer, SM. Annotation: Paediatric palliative care; Distinctive needs and emerging issues. Journal Paediatrics Child Health 2001;37:323-325.
14. Frager 1996.
15. Penny NP, Frager G: Refractory symptoms and terminal sedation: ethical issues and practical management. Journal of Palliative Care 1996;12:40-45.
16. Pierucci RL, Kirby RS, Leuthner SR: End-of-life care for neonates and infants: the experience and effects of a palliative care consultation service. Pediatrics 2001;108:653-660.
17. Pettle SA, Britten CM: Talking with children about death and dying. Child: Care, Health and Development 1995;21:395-404.
18. McCallum DE, Byrne P, Bruera E: How children die in hospital. Journal of Pain and Symptom Management 2000;20:417-423.
19. Liben S: Pediatric palliative care: obstacles to overcome. Journal of Palliative Care 1996;12:24-28.
20. Serwint JR, Rutherford LE, Hutton N, Rowe PC, Barker S, Adamo G: "I learned that no death is routine": description of a death and bereavement seminar for pediatrics residents. Academic Medicine. 77(4):278-84, Apr 2002.
21. Dangel T: The status of pediatric palliative care in Europe. Journal of Pain & Symptom Management. 24(2):160-5, Aug 2002.
22. Beardsmore S, Fitzmaurice N: Palliative care in paediatric oncology. European Journal of Cancer. 38(14):1900-7; 1908-10, Sep 2002.
23. Shah R, Ting T, Taylor P, Glover J: The increasing need for pediatric palliative care. West Virginia Medical Journal. 98(3):104-7, May-Jun 2002.

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A MORE OBJECTIVE APPROACH FOR SELECTING THE JOURNAL TO WHICH ONE SUBMITS A MANUSCRIPT

Abstract

There is considerable variability in the methods authors use to identify journals for manuscript submission, and this process may be difficult for some junior (and even some more senior) investigators. Given the wide range of potential journal-candidates for any article being submitted, we believed that a grid-instrument might help authors explicitly set journal selection priorities that reflect realities, hopes, and expectations. Our model is based on five main steps for authors. Step one: authors identify individualized criteria for submission. We produced a research- and pragmatically-based model list of criteria and subcriteria, and subjected it to a modified Delphi process with participants that ranged from junior investigators to seasoned editors. Our proposed list for authors' initial consideration includes: journal prestige, likelihood of manuscript acceptance, quality of review, rapidity of turnaround, and intangibles. Step two: authors weight criteria by importance/priority for that particular author for that particular manuscript. Step three: select potential candidate journals. Step four: score each criterion on a grid for each candidate journal. Step five: calculate scores, and rank journals. All five steps are explained in greater detail in our full model description, and examples of model grid implementation are provided.

This method is rapid and can be engaging, and could make the submission process more efficient and effective. It should be particularly useful for those authors with little experience or external guidance, and for more senior authors who wish to make their journal selection process more explicit.

A five-step analytic model

Authors' motives and methods for choosing a goal journal for manuscript submission vary(1). Such decisions are probably typically made implicitly and informally, with a view to achieving the highest possible prestige, commensurate to the quality of the proposed manuscript(1). Manuscript submission is accompanied by much uncertainty on how the material will be judged(2). Considering the vast array of existing journals, and hence the wide range of potential journals (and editorial reactions) for any article being submitted, it might be beneficial to identify a methodological instrument that helps authors explicitly and parsimoniously set journal selection priorities, in keeping with their own expectations. Such quantitative priority setting would enable authors to both structure and refine their thinking about this often subjective topic.

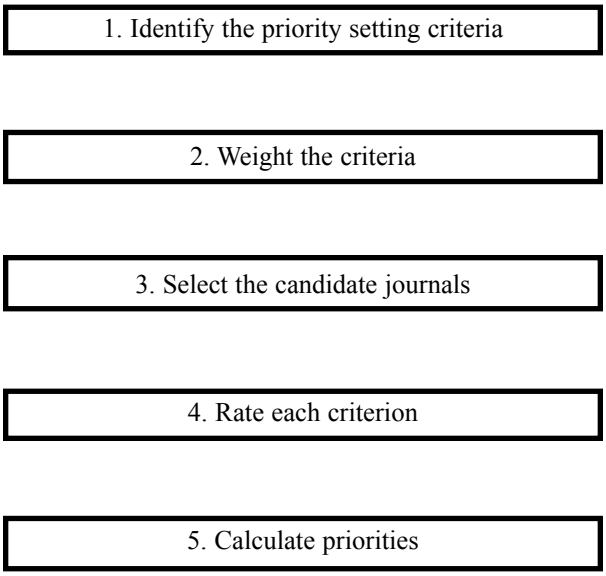

Figure 1. Overview of the journal selection priority-setting process

The model is based on five main steps (Figure 1) that are outlined below.

Step 1: Identifying the main priority-setting criteria.

The first step is to select which criteria to consider in deciding where to submit an article. Our model is outlined below, but authors should modify this model with their own procedures and considerations. To create our list, we produced a provisional list of criteria and subcriteria taken from prior work (1) and guided pragmatically. The list was submitted to 16 colleagues from various nationalities (Italy, Australia, USA, Russia, UK) and healthcare domains, who had in common experience as authors in national and international peer-reviewed journals. Many of these individuals had also served as reviewers and editors, and some were the junior faculty who are potential users of this method. Using the received observations, we constructed a final list, and propose including the following five criteria (Table 1): "Prestige", "Likelihood of acceptance", "Quality of review", "Rapidity of turnaround" and "Intangibles".

Step 2: Weighting the criteria

In addition to selecting criteria, weighting the value of each assigned criterion also drives the outcome. In using our proposed method, authors would need to attentively assess the quality of their work, the rapidity with which they desire publication versus their need for prestigious publications, etc., in accordance with the

Table 1. List of proposed priority-setting criteria

Criterion	Definition
Prestige	The importance attributed to a particular journal.
Likelihood of acceptance	Perception of the probability of a certain article being published in a certain journal
Quality of review	Perception of the fairness of the reviewer process and that, whatever the outcome, there will be a benefit in terms of useful suggestions.
Rapidity of turnaround	Speed with which the editor of a journal passes judgement on an article
Intangibles	Perception of other mainly subjective advantages, such as preference for a certain journal, the need to diversify one's production, acquaintance with an editor, etc.

criteria and subcriteria listed in step 1. The author should weight each criterion on a scale of 0 to 10, conforming with the above considerations. The criterion deemed most important by the author is assigned a value of 10 and the other criteria are rated accordingly. The scale is a rational scale, and the importance of the various criteria are represented proportionally, i.e. a value of 4 corresponds to an importance rating half the value of 8 and twice the value of 2. Decimal fractions of the points on the scale can also be used. A weight of 0 signifies that zero importance is attributed to that particular criterion for that particular manuscript. If there is more than one author, scoring could be conducted via a simplified two-round delphi method in which authors iteratively discuss (either electronically or through other media) and achieve consensus on their reasons for assigning particular weights. Scoring likely should be independent in the first round, but each author may revise his/her ratings on being informed (perhaps in aggregate, anonymous form) of the other authors' scores. A final determination could be made in a second round, with a decision created using consensus, a mean of authors' ratings, or other criteria.

Step 3: Selecting the candidate journals

The choice of candidate journals chiefly depends on an assessment of the manuscript and its prospects, the authors' personal objectives, and the objectives of the institutions for which the researchers work. The task is to reduce the extensive array of journals published throughout the world to a list of desirable candidates for subsequent ranking. A list may be generated from the authors' familiarity (keeping files on experiences with journals may help), colleagues' knowledge, a physical search in a local library, or via the Internet. One easily accessible source, for instance, is that of the journals listed by the main databases (3) or leading Impact Factor (IF) calculating companies (4), which often provide a service online and/or the option of receiving a free sample issue.

Step 4: Rating each criterion

GENERAL CONSIDERATIONS

Most criteria proposed in this article require substantial subjective assessment, with only rapidity of turnaround and prestige (through using a journal's IF or Immediacy Index or readership composition) being more heavily based on objective elements. Each criterion's score should be represented on a scale of 0 to 10, identifying for each criterion the highest-ranking journal and giving it a rating of 10. Other journals will then be given a score directly or inversely proportional to that of the best journal. For example, if three journals (a,b,c) typically present a turnaround of 2 (a), 4 (b) and 10 weeks (c), the score given to the "Turnaround rapidity" criterion will be 10 for journal (a), which has the greatest rapidity. The score for journal b will be calculated by solving for x: a score of x is to a score of 10 as the inverse of 4 weeks is to the inverse of 2 weeks (i.e. x:10=1/4:1/2; or x=5) and the score for journal c by the formula x:10=1/10:1/2 (i.e. 2). Some readers may find it easier to consider score calculation in the following way, solving for x: for journal b, a score of x (for journal b) is to a score of 10 (for journal a) as (using the inverse proportion, since a higher number of weeks is a worse outcome) 2 weeks is to 4 weeks (i.e. x/10=2/4; or x=5) and the score for journal c by the formula x/10=2/10 (i.e. 2). Where there is more than one author, the delphi methodology described in step 2 may be used.

CRITERION 1: PRESTIGE

Definition: The importance attributed to a particular journal.

Specific considerations: A journal's prestige generally depends on reputation, reliability, circulation size, availability, and news coverage. There are somewhat objective methods for the measurement of prestige that rely mainly on citations in scientific journals; while these have been challenged and require critical, cautious adoption, they form a useful guide. The main methods of this kind are the Impact Factor (IF), measuring the

frequency with which the "average article" in a journal has been cited in a particular year after publication, and the Immediacy Index, which considers citations made during the publication year of the quoted items. Another objective subcriterion is journal circulation, which can be measured by number of copies and readership estimates. The author may also be interested in impact via mass media and hence the level of attention paid to journals by the main press agencies. And prestige must be set in the context of specialty, particular objectives, and geography. For example, a laboratorian may prefer to publish a certain work in a journal from his/her own field rather than in a general medicine journal, even if the latter has a higher IF. Similarly, a European author might prefer to publish a work in a good national peer-reviewed journal than in a more renowned international one, bearing in mind the English-language barriers experienced in some countries and the author's desire for use/recognition of the work in their home country. In sum, the "prestige" criteria consider the relationship between perceived journal quality and appeal, and the ensuing personal and/or institutional benefits.

Instructions: A subjective assessment must be made for each journal on a scale of 0 to 10 that considers such factors as reputation, reliability, circulation, availability, media coverage, and IF. The journal considered to be most prestigious should be assigned a score of 10, and the other journals proportionally lower scores. Where consideration is limited to just the IF, we are faced with an objective criterion. In this case, the journal with the highest IF will be given a score of 10 and the other journals a proportionally lower score in relation to their respective IFs. Let's suppose, for example, that there are three candidate journals: BMJ, Annals of Internal Medicine and New England Journal of Medicine, for which the IFs in 2000 were 5.331, 9.833 and 29.512, respectively. In this case, the New England Journal of Medicine will be assigned a criterion score of 10, BMJ a score of 2 (by approximation), derived by calculating the proportion 29.512:10=5.331:x, and the Annals of Internal Medicine a score of 3 (by approximation), derived by calculating the proportion 29.512:10=9.833:x.

Data sources: IF values are published annually by the Journal of Citation Reports (JCR) of the Institute for Scientific Information (ISI). The report may be purchased from the ISI, or may be consulted at many medical libraries. As for subjective assessment, subcriteria might include prestige assigned to a journal by (a) colleagues at one's institution, (b) colleagues outside one's institution in one's field, (c) other colleagues outside one's discipline, (d) one's Chair, (e) the promotions committee at one's institution, or (f) lay people to whom one will talk about one's work.

CRITERION 2: LIKELIHOOD OF ACCEPTANCE

Definition: Perception of the probability of a certain article being published in a certain journal

Specific considerations: This crucial point requires both an impartial view of one's paper (a skilled task) and a very good and current knowledge of the rejection rate, how the target journal is managed, its mission, and the sequence of publications on the same topic. Considerations should include appropriateness of style/manuscript type and content, ability to apply results to the journal's audience, a past record with the target journal, and personal acquaintance with the Editor. Generally speaking, the difficulty in being accepted increases as a journal's prestige increases. Achievement of personal objectives thus demands careful weighting of all criteria.

Instructions: A subjective assessment must be made for each journal on a scale of 0 to 10 that takes account of factors such as those above described. The journal considered to be most advantageous must be assigned a score of 10 and the other journals proportionally lower scores.

Data sources: This is a criterion with many subjective and even unknowable aspects, but it is helpful to know the aims, target and rejection rate of potential journals; these can often be gleaned from the instructions for authors, which are usually available on the Web.

CRITERION 3: QUALITY OF REVIEW

Definition: Perception of the quality of the review process and that, whatever the outcome, there will be a benefit in terms of useful suggestions.

Specific considerations: The helpfulness of the suggestions contained in the reviewed manuscript is linked to the ability of the editorial staff and pool of referees used by the journal, and concerns both the text and statistics/tables. Quality revisions make for good working relations, opportunities for professional growth, and improved articles. This criterion may warrant higher esteem than some authors might accord it, particularly for first submissions.

Instructions: As with the prior criteria, a subjective assessment must be made for each journal on a scale of 0 to 10 that takes account of factors such as those described above, and the journal considered to be most advantageous should be assigned a score of 10 and the other journals proportionally lower scores.

Data sources: This is a subjective criterion chiefly based on one's own, or one's colleagues' experiences, perceptions, and expectations.

CRITERION 4: RAPIDITY OF TURNAROUND

Definition: Speed with which the editor of a journal passes judgement on an article.

Table 2. Example of Priority Scores calculated for three journals (a,b,c) before submitting an article.

		Journal (a)		**Journal (b)**		**Journal (c)**	
Criterion	Criterion weight (W)	Criterion Score (S)	WS	Criterion Score (S)	WS	Criterion Score (S)	WS
Prestige	10	10	100	8	80	3	30
Likelihood of acceptance	7	5	35	8	56	10	70
Quality of review	4	9	36	10	40	1	4
Turnaround rapidity	5	10 (2 weeks)	50	2 (10 weeks)	10	5 (4 weeks)	25
Intangibles	2	5	10	10	20	2	4
Total Score			231		206		133

Specific considerations: Another critical parameter is the speed with which the journal reviews manuscripts and gives authors decisions. Many journals seem to give increasing attention to this factor, thereby shortening response times, in some cases taking advantage of communication by fax or entirely electronic communications. Some hard copy and on-line journals offer the interesting opportunity of following the revision process via Internet. Waiting times of 6 months and over, which still unfortunately occur, are becoming even less acceptable, with the result that many authors are becoming more able to act on a preference for journals that provide rapid reviews and decisions.

Instructions: Whenever possible, an objective assessment must be made for each journal on a scale of 0 to 10. The journal considered to be most rapid must be assigned a score of 10 and the other journals proportionally lower scores. If data are not available, this criterion cannot be applied.

Data sources: The rapidity of turnaround is sometimes indicated by the journal in the section of the Information for Authors that outlines their peer review process. In other cases, this information is based on one's own experience or that of colleagues, and can occasionally be inferred from acknowledgement letters.

CRITERION 5: INTANGIBLES

Definition: Perception of other mainly subjective advantages, such as an aesthetic preference for a certain journal, acquaintance with an editor, etc.

Specific considerations: Lastly, there are less concrete factors that explicitly consider personal taste. These elements include personal preference for a certain journal, its editorial and graphical style, a liking for the editor or a member of the editorial board, or the desire to diversify the journals in which one publishes.

Instructions: A subjective assessment must be made for each journal on a scale of 0 to 10 that takes account of factors such as those above described. The journal considered to be most advantageous must be assigned a score of 10, and the other journals proportionally lower scores.

Data sources: It is a subjective criterion.

Step 5: Calculating priorities

The final choice of journal is made at the end of the process, using a simple mathematical priority-calculating formula. After weighting and rating the criteria, the total score can be calculated for each journal included in the author's list of candidates. Practically speaking, each criterion score is adjusted by the weight given to each criterion.

Let us consider a finite number of candidate journals (a, b, c, ...) based on a finite number of criteria (1,2,3,4,5) which are given criterion weights (W1, W2, W3, W4, W5); these criteria and their weights are the same for all the candidate journals. Each journal also receives criterion scores (S1, S2, S3, S4, S5) which are calculated for each criterion for each alternative journal.

The formula to calculate the priority score for journal

(a) would therefore be as follows: Priority score for journal (a) = [(criterion weight of criterion 1) x (criterion score attributed to journal a for criterion 1)] + [(criterion weight of criterion 2) x (criterion score attributed to journal a for criterion 2)] + [(criterion weight of criterion 3) x (criterion score attributed to journal a for criterion 3)] + [(criterion weight of criterion 4) x (criterion score attributed to journal a for criterion 4)] + [(criterion weight of criterion 5) x (criterion score attributed to journal a for criterion 5)] = [W1 x S(1a)] + [W2 x S(2a)] + [W3 x S(3a)] + [W4 x S(4a)] + [W5 x S(5a)].

Each of the 5 criteria receives a score based on its merits vs. those of the other candidate journals (see Table 2). Let's suppose for journal (a) that the maximum criterion score is given to "Prestige" (i.e. a score of 10), a score of 5 to "Likelihood of acceptance", a score of 5 to "Intangibles", a score of 9 to "Quality of review", and a score of 10 to "Rapidity of turnaround". Each of these scores is multiplied by the weight previously attributed to each criterion (for this example, let's assign: "Prestige"=10; "Likelihood of acceptance"=7, "Intangibles"=2; "Quality of review"=4; "Rapidity of turnaround"=5). Hence, the total score for journal (a) becomes: WS=(10x10) + (7x5) + (2x5) + (4x9) + (5x10) = 100+35+10+36+50 = 231. Priority scores are then calculated for the other candidate journals in the list (b, c, d, and e), which are then ranked, and the journal with the highest score gets priority of submission.

Given this high score, an author may choose to first submit the paper to journal (a). If the paper is rejected by journal (a), the author, preferably after revision based on reviews, may submit the article to journal (b), which presents the score immediately below. In the event of rejection by (b), the author may behave in two ways. S/he may decide to submit the manuscript to journal (c) or, since journal (c)'s ranking is well below that of journal (a) (231) and (b) (206), may opt to repeat the assessment process with additional candidate journals. These candidate journals for reassessment (c, d, e) must be considered according to the first-time assessment procedure, as described above.

Comment on the model

The proposed method is drawn in part from the Donaldson & Sox model for priority setting for the Office of Health Technology Assessment of the Agency for Healthcare Research and Quality, designed to determine which of the countless available health care technologies should be subject to systematic yearly assessment (5). Our model considerably simplifies theirs, particularly mathematically, and is (to the best of our knowledge) the only published method for objective determination of journal selection methodology.

This novel approach to journal selection is a quantitative model. It is suggested because of its explicitness, and its ability to bring together different concepts and units of measurement in the same scale. It clarifies selection criteria, acknowledging their definitions, main characteristics, importance, and subjectivity. Subjective criteria may still prevail; while this method does not provide objective standards, it begins to pragmatically and explicitly outline the subjective and objective criteria for choosing the journal to which one submits a manuscript for publication. While some might find it rigid, time consuming, or overly quantitative, the method could be useful both for experienced authors who wish to make more explicit the criteria they use for deciding manuscript destinations, and for more junior authors whose lack of experience might benefit from a rational guide.

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REFERENCES

1. Frank E. Authors' Criteria for Selecting Journals. JAMA 1994; 272:163-164.
2. Hojat M, Gonnella JS, Caelleigh AS. Impartial Judgment by the "Gatekeepers" of Science: Fallibility and Accountability in the Peer Review Process. Adv Health Sci Educ Theory Pract 2003;8:75-96.
3. http//www.isinet.com/isi/
4. http//www.science.komm.at
5. Donaldson MS, Sox HC. Setting Priorities for Health Technology Assessment: A Model Process. Washington, DC: National Academy Press, 1992.

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ORIGINAL ARTICLE

Risk Factors for Tuberculosis Conversion in a State Prison

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ABSTRACT A case-control study determined the risk factors for latent tuberculosis (TB) conversion among Oregon Department of Correction (ODOC) inmates from July 2000 - July 2001. The first objective was to identity the converters. These were inmates who tested negative for the Purified Protein Derivative (PPD) skin test on entry and subsequently tested positive on annual testing. The second objective was determining the risk factors for conversion by comparing the converters with randomly selected controls. The Correctional Information System (CIS) and Mental Health databases were accessed to obtain health and demographic information. With ninety-nine percent of PPD positive inmates on anti-tuberculosis medications, nearly all male inmates who tested positive from July 00-01 (n = 307) were identified through the ODOC pharmacy records. A medical chart review (276 of 307 or 90%) separated the converters (n = 72) from the reactors who tested positive on entry (n = 123) and the prior positives on medications (n = 81). The conversion rate was 5.0 per 1,000 person-years. Differences between the cases (converters) and controls were analyzed using multivariate logistic regression. The converters were 6 times more likely to be Latino ($p < .005$) vs. Caucasian, over 19 times less likely to live in medium vs. minimum ($p < .001$) or maximum vs. minimum ($p < .001$) security prisons, and over 5 times less likely to live in a medium vs. low ($.012 < p < .031$) or high vs. low ($.002 < p < .007$) density prison. They had 1.4-1.5 times fewer PPD skin tests ($.002 < p < .009$) and lived in 1.5-1.7 times fewer prisons ($.005 < p < .017$). Age, education, county of incarceration, number of incarcerations, and number of visitors were not found to be significant variables. The results revealed a low conversion rate compared to other U.S. prisons. Prison health officials should consider performing two-step skin testing in order to distinguish the booster phenomenon from intramural conversion.

INTRODUCTION

Worldwide, tuberculosis (TB) is the second leading cause of death from a single infectious agent (1). One-third of the world population is infected with Mycobacterium tuberculosis and causes were multi-factorial: decreased funding for tuberculosis surveillance, increased immigration from areas of high TB prevalence, the unfortunate rise in HIV/AIDS, and major outbreaks in congregate settings such as prisons. Prisons had three times the rate of pulmonary TB than the general population and in some New York, New Jersey, and California prisons, the incarcerated were 6-11 times more likely to develop active TB than the non-incarcerated (3-5).

In response to the outbreaks, the Centers for Disease Control (CDC) developed guidelines for correctional facilities in 1989 and again in 1995 (6-7). In the first guideline, the increased risk for active TB due to co-infection with HIV was highlighted. In the second guideline, the same principles of surveillance, namely screening, containment, and assessment, were emphasized. The basic principles revolved around yearly PPD skin testing, treatment with prophylactic

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medications, containment of active cases, and periodic assessments through incidence studies such as this one. Based on these recommendations, the ODOC instituted yearly PPD skin testing in 1990.

With increased surveillance of high-risk populations such as inmates, immigrants, minorities, and the immuno-suppressed, the incidence of active TB in the U.S. decreased from 9.8 cases per 100,000 in 1993 (n = 25,287) to 5.2 cases per 100,000 in 2002 (n = 15,078). (8) In Oregon, the incidence was even lower, with a rate of 3.2 per 100,000 in 2002 (n = 111) (9). Only a few active cases were found in the Oregon Department of Corrections (ODOC). From 1995-2001, there was only one case in each of 1997, 1998, and 2001 (9).

The decrease in incidence ushered a new paradigm with regards to TB control. In May 2000, the Institute of Medicine (IOM) issued a report entitled, "Ending Neglect: The Elimination of Tuberculosis in the United States" (10). The report detailed the multi-factorial strategies necessary to prevent TB resurgence and decisively eradicate TB in the U.S.. Eradication was defined as < 0.1 case per 100,000 person-years. The basic principles revolved around surveillance, applied research, prevention and control, and infrastructure. A shift from active to latent TB screening was emphasized. The focus was on preventing active TB by detecting and treating latent TB. By doing so, the reservoir of Mycobacterium tuberculosis could be essentially eliminated.

In the ODOC, health officials wished to know the extent of TB transmission and the risk factors for latent TB conversion. The known risk factors in other prisons and jails were the following: 1) exposure to an active case, 2) increased crowdedness, 3) increased duration of stay, 4) being housed in multiple institutions, and 5) being incarcerated multiple times (11-13). With this information, they could increase the frequency of skin testing in the high-risk inmates. In addition, the baseline conversion rate could be established and the possible reasons for conversion explored. Of note, the booster phenomenon has been known to cause initial false negative tests. A 'booster' is a person with TB whose immune system is unable to elicit a positive response until the second skin test. They are erroneously mistaken for converters.

METHODS

Study Population

The ODOC consisted of twelve institutions and one intake center during the study period from July 00-01 (14). The inmate population ranged from n = 166 at the Oregon Women's Correctional Center to n = 2,794 at the Snake River Correctional Institution. Men comprised 95% (n = 9,746) of the inmate population and women 5% (n = 573). Three-quarters of the inmates were Caucasian, 11% Latino, 11% Black, and 3% other. One-third of the inmates were between the ages of 18-30, almost half between 31-45 and one-fifth between 45 and 70. Due to the demographic preponderance of men, women were excluded from the study.

Confidentiality

Institutional Review Board (IRB) approval was obtained and inmate names stripped from the records. A unique identifier was used and all the data presented in aggregate form.

Case Selection

Pharmacy records revealed that 307 men were on anti-TB medications during the study period. This captured 99% of men with a recent or past positive skin test. Ninety percent of their medical charts containing skin test data were reviewed (n = 276) and the men separated into converters (n = 72), reactors (n = 123), and prior positives on medications (n = 81). Converters were inmates who tested negative at entry and positive on annual testing. Some of them were positive before the one-year period (n = 23). Reactors were inmates who tested positive at entry, and priors on medications were positive before entry. Ten percent of the records (n = 31) were not reviewed and accounted for a potential of 8 missing converters.

Control Selection

A database manager at the ODOC randomly selected 305 male inmates from all 12 institutions who were never on anti-TB medications. All of these inmates resided in the ODOC from July 00- July 01. Seventy-seven percent (n = 234) of the controls were verified through a medical chart review.

Demographic Information

The demographic variables seen in table 1 were collected from two sources. All the variables were derived from the Correctional Information System (CIS) database except for "drug abuse potential" that came from the mental health computer database. Inmate psychiatric assessments provided the data for that variable.

Data Analysis

The conversion rate was calculated by dividing the number of converters from the estimated inmate-years during the study period.

For the case-control study, univariate analysis was performed with the use of chi-square for categorical

Table 1. Demographic characteristics of the cases and verified controls.

Characteristics	Cases (n=72)	Verified Controls (n=234)
Age in Years	33 (11)	38 (11)
20-29 - no. (%)	20 (27.8)	53 (22.7)
30-39	23 (31.9)	83 (35.5)
40-49	18 (25.0)	62 (26.5)
50-59	9 (12.5)	29 (12.4)
60-69	0 (0)	6 (2.6)
70-79	2 (2.8)	1 (.43)
Race, no. (%)		
Caucasian	42 (58.3)	186 (79.5)
Latino	22 (30.6)	16 (6.8
African-American	5 (6.9)	23 (9.8)
Other	3 (4.2)	9 (3.8)
Citizenship, no. (%)		
United States	57 (79.2)	229 (97.9)
Mexico	12 (16.7)	4 (1.7)
Other	3 (4.2)	1 (0.4)
Birthplace, no. (%)		
Oregon	11 (15.3)	77 (32.9)
Other place	61 (84.7)	157 (67.1)
Birthplace, no. (%)		
United States	40 (55.6)	220 (94.0)
Non-U.S.	32 (44.4)	14 (6.0)
County of Incarceration, no. (%)		
AOC District 1*	1 (1.4)	8 (3.4)
AOC District 2*	6 (7.8)	7 (3.0)
AOC District 3*	3 (8.3)	4 (1.7)
AOC District 4*	8 (4.2)	27 (11.5)
AOC District 5*	6 (8.3)	26 (11.1)
AOC District 6*	12 (16.7)	37 (15.8)
AOC District 7*	4 (5.6)	8 (3.4)
AOC District 8*	26 (36.1)	92 (39.3)
Unknown	6 (8.3)	25 (10.7)
Education, no. (%)		
Un-testable	0	7 (3.0)
Obtained GED*	20 (27.8)	90 (38.5)
No GED*	52 (72.2)	137 (58.5)
Location of the main institution of Incarceration, no. (%)		
AOC District 1*	44 (61.1)	138 (59.0)
AOC District 2*	0	0
AOC District 3*	0	0
AOC District 4*	3 (4.2)	1 (0.4)
AOC District 5*	0	0
AOC District 6*	17 (23.6)	87 (37.2)
AOC District 7*	0	2 (0.9)
AOC District 8 *	1 (1.4)	3 (1.3)
Unknown	7 (9.7)	3 (1.3)
Level of Security, no. (%)		
Maximum	8 (11.1)	58 (24.8)
Medium	6 (8.3)	154 (65.8)
Minimum	51 (70.8)	19 (8.1)
Unknown	7 (9.7)	3 (1.3)
Institutional Density, no. (%)		
High*	38 (52.8)	180 (76.9)
Medium*	22 (30.6)	46 (19.7)
Low*	5 (6.9)	5 (2.1)
Unknown	7 (9.7)	3 (1.3)
Drug abuse potential, no. (%)		
High*	34 (47.2)	104 (44.4)
Low*	28 (38.9)	123 (52.6)
Unknown	10 (13.9)	7 (3.0)
Prior number of incarcerations- mean (SD)	92 (2.25)	1.20 (2.28)
Number of visits in one year- mean (SD)	10 (18)	20 (37)
Number of visitors in one year- mean (SD)	21 (36)	35 (61)
Number of PPD skin tests- mean (SD)	2.7 (1.2)	4.4 (2.3)
Duration of incarceration prior to conversion or July 2001 in days- mean (SD)	609 (539)	1278 (1071)
Number of institutions inhabited- mean (SD)	2.5 (1.1)	2.4 (1.5)
Number of relocations to other prisons- mean (SD)	1.6 (1.1)	1.7 (1.9)

variables and the student t-test for continuous variables. Multivariate analysis was performed with logistic regression. Pearson's correlation was used to choose variables for the logistic model. Variables that were heavily correlated were grouped together and only the most significant ones entered into the main effects model. The best models were presented with Odd Ratios (OR) and 95% confidence intervals.

RESULTS

Demographic Characteristics of Cases and Verified Controls

Thirty percent of the cases (n = 22) were Latino, while only seven percent (n = 16) of the controls were of the Latin race. More of the cases had Mexican citizenship (n = 12 or 17% versus n = 4 or 1.7% for the controls) and were born in a non-U.S. country (n = 32 or 44% vs. n = 14 or 6%). The cases were more likely to live in minimum security prisons (n = 51 or 71% vs. n = 19 or 8%), and less likely to live in high density prisons (n = 38 or 53% vs. n = 180 or 77%). They had fewer PPD skin tests (2.7 vs. 4.4) but lived in more institutions (2.5 vs. 2.4). The other variables are presented in Table 1.

Conversion Rate

The conversion rate was 5.0 per 1,000 person-years. Forty-nine of the seventy-two converters were positive from July 00 - July 01. The estimated person-years were 9,746 inmate-years.

Validity of the Controls

With all the demographic information available on the controls, the verified control sample (n = 234) was compared to the entire control sample (n = 305). There was no statistically significant difference between the two samples (chi-square, $p < .36$ to .99; t-test, $p < .35$ to .73).

Univariate Analysis

The cases were more likely to be non-white ($p < .001$), to have foreign citizenship ($p < .001$), and to be born outside of Oregon ($p < .001$) or in a foreign country ($p < .004$). They tended to live in different districts ($p < .048$), different security prisons ($p < .001$), and different density institutions ($p < .003$) than the controls. They received fewer visits ($p < .02$), fewer PPD skin tests ($p < .001$), and had a shorter duration of stay ($p < .001$).

Correlation Analysis

Race, citizenship, and birthplace were positively correlated ($p < .001$-.005). Many Latinos were Mexican citizens born in Mexico. The level of security was positively correlated with the institutional density ($p < .001$). Self-evidently correlated were the number of visitors and visits ($p < .001$). The number of PPD skin tests and duration of residence were positively correlated as well ($p < .001$). Inmates with longer residences had more annual PPD skin tests. However, the duration of residence & number of PPD skin tests were negatively correlated with the number of institutions lived in ($p < .001$). It appears that inmates who enter the prison system move around multiples times initially before settling down in one location.

Multivariate Analysis

Sixteen models were tested based on variations of the correlated variables. If race was entered, birthplace and citizenship were left out. If security was used, then density was removed. The three models containing the greatest number of significant risk factors are displayed in Table 2.

Based on the three models, the cases were 6 times more likely to be Latino, 10 times more likely to be born outside the U.S., and 13 times more likely to have Mexican citizenship. They were 71-77 times less likely to live in medium vs. minimum security prisons and 19-23 times less likely to live in maximum vs. minimum security prisons. The cases had 1.4-1.5 times fewer PPD skin tests and lived in 1.5-1.7 times fewer prisons. On average, the cases lived in more institutions (n = 2.51 vs. 2.43), but a greater proportion of cases (88% vs. 78%) lived in 3 or fewer institutions, accounting for the trend.

Based on the other thirteen models where only three variables were significant, the cases were 2-3 times more likely to be born outside of Oregon, 5-7 times less likely to live in medium vs. low density prisons, and 6-11 times less likely to live in high vs. low density prisons.

Table 2. Best logistic regression models

Model One	**OR**	**95%CI**	**P-Value**
Birthplace			
(non-U.S. vs. U.S.)	9.87	3.06 - 31.8	.001
Security			
(Med vs. Min)	.014	.005 - .045	.001
(Max vs. Min)	.052	.017 - .158	.001
# PPD skin tests	.696	.529 - .915	.009
Number of institutions	.639	.443 - .922	.017
Model Two	**OR**	**95% CI**	**P-Value**
Race			
(Latino vs. White)	5.98	1.70 - 21.1	.005
(Black vs. White)	.748	.157 - 3.56	.716
(Other vs. White)	1.74	.172 - 17.5	.640
Security			
(Med vs. Min)	.013	.004 - .040	.001
(Max vs. Min)	.049	.017 - .142	.001
# PPD skin tests	.649	.492 - .856	.002
Number of institutions	.585	.401 - .851	.005
Model Three	**OR**	**95% CI**	**P-Value**
Citizenship			
(Mexican vs. U.S.)	13.0	1.77 - 95.1	.012
(Other vs. U.S.)	7.28	.327 - 162	.210
Security			
(Med vs. Min)	.014	.005 - .042	.001
(Max vs. Min)	7.28	.327 - 162	.001
# PPD skin tests	.650	.490 - .861	.003
Number of institutions	.587	.406 - .850	.005

DISCUSSION

The conversion rate in the ODOC was very low compared to other prisons during the epidemic from 1985-1992. Prisons with intramural conversion had average rates between 39 and 67 per 1,000 person-years (11, 15-16). In the Oregon prisons, the known risk factors for intramural conversion were not seen. The

converters lived in prisons with fewer inmates and stayed for shorter durations of time. Their hypothetical exposure to TB was lower compared to the controls in the Oregon prisons.

There are only a few possibilities that can explain the initial negative skin test seen in the 49 converters from July 00 - 01: 1) anergy, 2) incubating disease at admission, 3) intramural transmission, and 4) the booster phenomenon. Anergy is a state of depressed immune response to multiple antigens, while the booster phenomenon is a transient decreased immune response to the antigen in the PPD skin test. The anergic individual is immuno-suppressed, but the 'booster' is often immuno-competent and simply needs the first skin test to 'boost' the immune response to the PPD antigen.

In this study, anergy was not a possible explanation since the converters tested positive on subsequent skin tests. Furthermore, over 60% of the conversions occurred on the second skin test, suggesting the boosting phenomenon. Second, incubating disease at admission is possible, but unlikely to differentially affect Latino men. This would affect all inmates equally. Third, there has only been one active case of TB diagnosed in the Oregon prisons from 2000-2001. It is possible, but very unlikely that this single inmate or a few undiagnosed inmates preferentially infected the Latino males who lived in different institutions. Therefore, intramural conversion seems less likely than the last alternative- the booster phenomenon. The high percentage of conversions on the second skin test and lack of another plausible explanation argue in favor of the booster phenomenon.

The risk factors for boosting have not been studied in the prison population according to the author's literature searches. Research on health care workers, school children, and young adults showed older age, previous BCG vaccination, and sensitivity to atypical Mycobacterium to be risk factors (17-23). Older age decreases the immune response to the skin test antigen, and previous vaccination or sensitivity to atypical Mycobacterium elicits a weaker response respectively. The converters were young to middle-age and only 11% were fifty or older. Regarding the BCG, Mexico does not give these vaccinations. Previous sensitivity to atypical Mycobacterium is a possible explanation.

In the Maryland prisons, the rate of boosting was 1% (24). The health officials there did not think it was cost-effective to initiate two-step skin testing. The question is whether to implement two-step skin testing in the Oregon Department of Corrections. By testing inmates twice, the booster phenomenon can be evaluated. Two negative tests suggest the absence of infection, while a negative followed by a positive test suggests the booster phenomenon. A boosting study would reveal whether Latino men are specifically at risk for conversion. It would give a definitive answer to true vs. false conversion.

At the maximum, the rate of boosting was .46 per 100 inmates, or less than half a percent. It would take 200 extra skin tests to discover one booster in the Oregon prisons. More than one thousand inmates would have to be tested to perform an adequate study. It does not appear cost-effective to test the general inmate population, but testing a subset of Latino men would be both practical and feasible.

Regarding validity, misclassification from the eight potential converters could not change the results. Eight additional 'dummy' inmates were coded in the opposite direction of the results. The Odd Ratios were reduced but remained significant. In addition, ten percent of the data set was double-checked. If > 5% of the data reviewed was inaccurate, the entire variable was recoded again.

Overall, the case-control study was efficient and unique. It used existing data to determine the risk factors for conversion. Only three other prison systems have done this in the U.S.. (11, 15-16) More importantly, a computerized TB registry and the aforementioned boosting study may soon be implemented. Conversion rates can be followed yearly without the need to perform site visits, and a subset of men may be skin-tested twice in the future.

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REFERENCES

1. Centers for Disease Control. World TB Day 2003- Global Fact Sheet. The Impact of Tuberculosis Worldwide.
2. CDC Core Curriculum on Tuberculosis. What the Clinician Should Know. 4th edition, 2000.
3. Braun MM, Truman, BI, Maguire B. Increasing incidence of tuberculosis in a prison inmate population: association with HIV infection. Journal of American Medical Association, 261: 393-7, 1989.
4. Tuberculosis Control Program. Annual Report, 1994. Trenton, NJ: New Jersey Department of Health, 1995.
5. CDC. Probable transmission of multi-drug-resistant tuberculosis in a correctional facility - California. Morbidity & Mortality Weekly Report, 42: 48-51, 1993.
6. Prevention and Control of Tuberculosis in Correctional Institutions: Recommendations of the Advisory Committee for the Elimination of Tuberculosis. Morbidity & Mortality Weekly Report, 38(18): 313-325, 1989.
7. CDC: Controlling TB in Correctional Facilities. Atlanta, GA: U.S. Department of Health and Human Services, 1995.

8. CDC. Reported Tuberculosis in the United States, 2002. Atlanta, GA: U.S. Department of Health and Human Services, 2003.
9. Oregon 2002 Community TB Profile. Oregon TB Control. HIV/STD/TB Program. Dept. of Health Services, 2003.
10. Ending Neglect: The Elimination of Tuberculosis in the United States. Lawrence Geiter, ed. Committee for the Elimination of Tuberculosis in the United States. Division of Health Promotion and Disease Prevention. 292, pages, 2000.
11. MacIntyre CR, Kendig N, Kummer L, Birago, S, Graham N. Impact of Tuberculosis Control Measures and Crowding on the Incidence of Tuberculous Infection in Maryland Prisons. Clinical Infectious Diseases, 24: 1060-70, 1997.
12. March F, Coll P, Guerrero RA, Busquets E, Cayla JA, Prats G. Predictors of tuberculosis transmission in prisons: an analysis using conventional and molecular methods. Acquired Immune-deficiency Syndrome, 14: 525-535, 2000.
13. Bellin EY, Fletcher DD, Safyer SM. Association of Tuberculosis Infection With Increased Time in or Admission to the New York City Jail System. Journal of American Medical Association, Vol. 269, No. 17, 1993.
14. Oregon Department of Corrections Statistics, 2000-2001.
15. Spencer SS, Morton AR. Tuberculosis Surveillance in a State Prison. American Journal of Public Health, 79(4): 507-9, 1989.
16. Johnsen, C. Tuberculosis contact investigation: Two years experience in New York City correctional facilities. American Journal of Infection Control, 21: 1-4, 1993.
17. Hoft DF, Tennant JM. Persistence and Boosting of Bacille Calmette-Guerin-Induced Delayed-Type Hypersensitivity. Annals of Internal Medicine, 131: 32-36, 1999.
18. Menzies R, Vissandjee B, Rocher I, Germain, Y. Annals of Internal Medicine, 120: 190-98, 1994.

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ORIGINAL ARTICLE

Sub-clinical levels of attention deficit-hyperactivity disorder are associated with tobacco consumption in male but not in female smokers

Rebecca L. Douglas, Sean P. Barrett*, Neil T. Hanley and Robert O. Pihl

ABSTRACT An abundance of evidence has demonstrated an association between symptoms of attention deficit hyperactivity disorder (ADHD) and tobacco consumption. However, previous research has focused solely on populations meeting full diagnostic criteria for ADHD, despite evidence suggesting that symptoms below diagnostic threshold can be associated with impairment. Furthermore, the role of gender in the relationship between ADHD symptoms and tobacco consumption has not been determined. To examine the relationship between ADHD symptoms, tobacco use, and gender in a non-clinical population, symptoms of inattention, hyperactivity and impulsivity were assessed in 230 undergraduate students (22 male and 45 female smokers, and 66 male and 97 female nonsmokers). Overall, relative to nonsmokers, the smoking subjects reported significantly higher levels of inattention and hyperactivity. In male smokers, both inattentive and hyperactive/impulsive symptoms were positively associated with the number of cigarettes smoked daily. This relationship did not hold for female smokers, for whom no association was found between symptoms and nicotine consumption. Findings imply that even sub-clinical levels of inattention and hyperactivity/impulsivity are related to indices of tobacco use in males, and support previous research suggesting that significant gender differences may exist in tobacco smoking motives. Results also have potential implications for tobacco cessation programs, which may require more individual tailoring.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a pervasive psychiatric disorder, characterized by symptoms of inattention and/or hyperactivity-impulsivity. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) currently recognizes three subtypes of ADHD: (i) a hyperactive-impulsive type, characterized by excessive motor activity and a difficulty in delaying responses, (ii) an inattentive type, in which individuals display a failure to pay close attention to detail or to sustain attention, and (iii) a combined type that includes features of both hyperactivity-impulsivity and inattention (1). Although traditionally perceived as a form of childhood pathology, it is now widely recognized that ADHD often persists into adulthood (2-4). In order for an individual to receive a diagnosis of ADHD, six out of nine criteria for inattention and/or hyperactivity/impulsivity must be met (1). Individuals presenting with fewer than six criteria do not receive a diagnosis of ADHD, regardless of the degree of

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impairment (1). As no physiological significance has been attached to the cutoff criteria (5,6), the diagnostic threshold for ADHD is potentially arbitrary. In fact, problematic levels of disruptive behavior associated with hyperactivity and impulsivity seem to occur even when the symptom count does not meet the diagnostic threshold (7,6), indicating that the impairment associated with the disorder is not categorical. Although evidence suggests that significant dysfunction can be associated with sub-threshold levels of ADHD symptomatology (6,7), little is known about how 'non-clinically significant' levels of hyperactivity and/or inattention relate to other forms of pathology. Because ADHD has been associated with a range of substance use problems, such as marijuana, cocaine, and other stimulant drug use disorders (8), as well as conduct problems, mood disorders, anxiety disorders, and learning disabilities (2, 3), it remains possible that the presence of even sub-clinical symptoms may increase an individual's risk for certain adverse outcomes, including substance use and other psychiatric disorders.

An abundance of evidence suggests a link between the presence of ADHD symptoms and tobacco usage. ADHD in childhood has been associated with significantly younger age of first tobacco use (9) and younger age of commencement of daily tobacco consumption (10). In addition, a greater proportion of adults with ADHD display tobacco dependence relative to controls (11, 10). The relationship between tobacco consumption and symptoms of inattention and hyperactivity/impulsivity may be partially or completely explained by attempts to self-medicate symptoms of ADHD. Nicotine has been demonstrated to ameliorate both inattention and hyperactive/impulsive behavior (12, 13). Nicotine has also been demonstrated to induce cognitive improvements, mostly in attentional areas, in normal nonsmoking adults as well as those with impairments (13). Even low doses of nicotine have been shown to significantly reduce errors of omission on attentional tasks, without increasing errors of commission, thus indicating that the effects are not simply due to increased responding (13). These effects do not appear to diminish with chronic administration (13). Significant improvements in memory performance, specifically working memory, have also been shown with nicotine administration over long periods of time (13).

The various effects of nicotine on attention and behaviours related to hyperactivity/impulsivity may be linked to nicotine's effect on dopamine transmission. Nicotine indirectly facilitates dopamine transmission, through stimulation of the nicotinic acetylcholine receptors located on dopamine containing neurons (14, 15). Studies of smokers have shown that nicotine seems to inhibit monoamine oxidase-B (MAO-B), thus increasing the availability of dopamine in the brain by preventing breakdown (11). Nicotine is also believed to exert its effect on working memory and on regulation of impulsive behaviors by interacting with the dopaminergic systems and receptors in the brain (11, 13).

Because ADHD symptoms are thought to result from dopamine dysfunction particularly in frontal and striatal regions (2), it seems possible that individuals who display greater inattention or hyperactivity/impulsivity may use tobacco in an attempt to address these symptoms. Previous research has in fact suggested that tobacco may be used for self-medication in clinical ADHD populations (10), and as sub-threshold symptoms of inattention and hyperactivity/impulsivity also result in impairment (6,7), there may be motivation for individuals, even in non-clinical populations, to self-medicate.

There is some evidence to suggest that gender differences may exist in the relationship between smoking and ADHD symptoms. It has been suggested that females may be less sensitive to nicotine's effects (17); the lower efficacy of nicotine replacement therapy found among females in smoking cessation programs suggests a decreased sensitivity in females to the negative reinforcement effects of nicotine (17). Males also appear to be more likely to self-administer nicotine (17). The findings of gender specific nicotine effects raise the possibility of differences in likelihood to self-medicate ADHD symptoms with tobacco.

To date, research that links tobacco smoking with symptoms of ADHD has focused on participants whose symptoms meet the full diagnostic criteria for ADHD. Although such symptoms fall on a continuum (5), with concomitant dysfunction even at levels below the diagnostic threshold (6,7), the degree to which sub-clinical levels of either inattention or hyperactivity/impulsivity may be related to tobacco smoking behavior is not known. Furthermore, although there appears to be significant gender differences in the manifestation of hyperactive/impulsive and inattentive symptoms (16), previous studies examining the link between ADHD and tobacco smoking have either used male participants exclusively (9) or have failed to analyze male and female participants separately (10). Thus, the relationship between inattention and/or hyperactivity/impulsivity symptoms and tobacco consumption remains unknown in females.

The purpose of this study was to examine the relationship between inattention, hyperactivity/impulsivity, and tobacco consumption in a non-clinical population, and to determine how this relationship may differ with respect to gender.

METHOD

Two hundred forty-four undergraduate university students volunteered to participate in the study. Potential participants were excluded on the basis of past or present ADHD or other psychiatric diagnoses, however the use of illicit drugs was not an exclusion criterion. In total, 14 participants were eliminated for not meeting the study's inclusion criteria. Volunteers were approached at various locations at the McGill University campus and were asked to complete anonymous questionnaires. The questionnaire consisted of an eighteen-item checklist for ADHD symptoms, as defined by the DSM-IV (1), consisting of nine items each for inattention and hyperactivity/impulsivity. Because the DSM suggests an assessment of childhood symptoms in adults with ADHD (1) a nineteenth item asked participants to rate their level of childhood hyperactivity. Similar self-report check-lists are frequently used in clinical practice for the assessment of ADHD in adults (e.g. 18, 19) and this method of ascertaining current and childhood symptoms of ADHD is widely considered to be reliable and valid in both clinical (18, 19) and non-clinical populations (20). Participants were asked to indicate the degree to which each item applied to them using visual analogue scales. Based on their self-reported endorsement of symptoms, each participant was assigned a total composite ADHD score, as well as inattention and hyperactivity /impulsivity sub-scores, based on DSM-IV ADHD diagnostic criteria (1). While possible scores ranged from zero to ten on all item scales, with higher scores reflecting greater symptomatology, scores were converted to z-scores before calculating composite scores in order to avoid excessive influence of items with a limited range of responses. Therefore, means reported for overall symptoms and the two sub-scores appear as z-scores. In addition to completing the ADHD self-report scales, participants also reported on their lifetime use of tobacco. Participants were classified as current smoker, never smoker, or former smoker. In order to control for the potential confounding factor of smoking cessation, 15 former smokers were eliminated from the analysis. In addition to reporting their smoking status, smokers also reported the average number of cigarettes typically consumed daily. All participants were blinded to the specific hypotheses of the study, but the investigators were not.

Pearson bivariate correlations were performed in order to determine the relationship between measures of tobacco consumption and ADHD symptomatology, while independent t-tests were performed to examine potential effects of gender and smoking status.

RESULTS

Symptoms of inattention and hyperactivity or impulsivity were assessed in a non-clinical sample of 67 smokers (67% female) and 148 nonsmokers (60% female) with a mean age of 21.57 years (SD = 2.55, range 18 to 30). Males and females did not significantly differ in their current smoking status (26.8% vs. 33.8% classified as current smokers; 2 (1, N=215)=1.15, p=.760, NS) , nor did male and female smokers differ in their average number of cigarettes smoked per day (males M = 5.86, SD = 8.27; females M = 4.46, SD = 7.17; t (65) = .65, p = .479, NS).

The analyses revealed significant differences between smokers and nonsmokers on the measures of overall symptoms as well as inattention and hyperactivity/impulsivity separately. Current smokers reported greater overall ADHD symptoms (M = .09, SD = .44) than did nonsmokers (M = -.07, SD = .37; t (211) = -2.74, p = .007). Smokers rated themselves as experiencing higher symptoms of inattention (M = .11 , SD = .51) than nonsmokers (M = -.08, SD = .49; t (212) = -2.29, p = .023) as well as having higher hyperactivity/impulsivity (smokers M = .08, SD = .49; nonsmokers M = -.07, SD = .45; t (140) = -2.17, p = .031).

Within male and female smokers combined, only symptoms of hyperactivity/impulsivity were related to level of tobacco consumption (r(67) = .254, p =.038). However, when analyzed by gender, it was determined that the relationship found between smoking and level of ADHD symptoms differed between males and females. While daily consumption was strongly associated with overall symptoms (r(22) = .595, p = .003), inattention (r(22) = .483, p =.023) and hyperactivity/impulsivity (r(22) = .554, p =.007) in males, there were no significant relationships between smoking and ADHD symptoms in females (ps > .100, NS).

Finally, the relationship between the degree of childhood hyperactivity and current cigarette consumption was assessed. With male smokers, there was a modest but non-significant association between childhood hyperactivity and current daily consumption (r(22) =.391, p = .07), whereas this association was not observed in female smokers (r(45) =.007, p = .961, NS).

DISCUSSION

Based on previous findings of increased tobacco use in clinical ADHD populations (e.g. 9) and the recognition that ADHD symptoms fall on a continuum (5, 6) with impairment occurring at sub-diagnostic levels (6,7), it was hypothesized that tobacco use would be positively associated with inattention and hyperactivity/impulsivity in a non-clinical sample.

Indeed, significant differences in levels of both inattention and hyperactivity/impulsivity were found between smokers and nonsmokers, with smokers displaying much greater levels of each sub-score. Amongst smokers, daily consumption was positively associated with both inattention and hyperactivity/impulsivity in males. Interestingly however, no significant relationships were found among female participants.

In an attempt to discern the direction of the relationship between ADHD symptoms and tobacco consumption, temporal order was addressed. Although not significant, an association was identified between the reported level of childhood hyperactivity and current tobacco consumption in male smokers. No parallel association was found with female smokers.

The relationship between smoking and symptoms of inattention and hyperactivity/impulsivity found in males may be due to an attempt to self-medicate symptoms of ADHD. Males with more ADHD symptoms may have higher nicotine consumption due to nicotine's positive effects on attention and symptoms of hyperactivity/impulsivity via its indirect effects on the dopaminergic system.

The dramatic gender differences found in the relationships between tobacco consumption and ADHD symptomatology add a further layer of complexity. One possibility for these observed differences is that males alone self-medicate with tobacco. This possibility is supported by the findings that suggest gender differences in the motivation for tobacco consumption and maintenance (17). The current findings are consistent with the evidence that females may be less sensitive to nicotine's effects (16). Given the gender specific effects of nicotine and the proposed gender differences in smoking motives, women may be less likely to self-medicate with tobacco for ADHD symptomatology, as it is the nicotine component in tobacco that is suggested to influence symptoms of ADHD.

There are several implications for the findings of this study. First, the finding that smoking is associated with inattention and hyperactivity/impulsivity even in a non-clinical population supports previous research that has found functional impairment in those below the diagnostic threshold of ADHD symptomatology (6,7). Second, there is support for previous research reporting significant gender differences in tobacco smoking motives. Both these findings have implications for the prevention and treatment of tobacco consumption for those with ADHD symptoms. Individual differences, for example in levels of inattention and hyperactivity/impulsivity, may be factors that need to be considered in designing programs for both prevention and treatment of tobacco consumption. If smoking functions to self-medicate the ADHD symptoms of some individuals, then employing other methods to medicate or manage these symptoms may facilitate smoking cessation. Furthermore, cessation programs may need to be tailored by gender in order to target the appropriate smoking motives.

Future research is needed to further examine this phenomenon of self-medication with tobacco in those with clinical, as well as non-clinical, ADHD symptomatology, as well as to examine possible gender differences in tobacco usage and the effects on symptoms within this population.

REFERENCES

1. American Psychiatric Association. Diagnostic and StatisticalManual of Mental Disorders, 4th ed.. Washington, DC: American Psychiatric Association, 1994.
2. Biederman J. Attention-Deficit/Hyperactivity Disorder: A Life Span Perspective. Journal of Clinical Psychiatry, 59: 4-16; 1998.
3. Troller JN. Attention Deficit Hyperactivity Disorder in Adults: Conceptual and Clinical Issues. Medical Journal of Australia, 171: 421-425; 1999.
4. Wender PH. Attention-Deficit Hyperactivity Disorder in Adults. The Psychiatric Clinics of North America, 21: 761-774; 1998.
5. Levy F, Hay D, McStephen M, Wood C, Waldman I. Attention-Deficit Hyperactivity Disorder: A Category or a Continuum? Genetic Analysis of a Large-scale Twin Study. Journal of American Academy of Child and Adolescent Psychiatry, 36: 737-744; 1997.
6. Neuman RJ, Todd RD, Heath AC, et al.. Evaluation of ADHD Typology in Three Contrasting Samples: A Latent Class Approach. Journal of the American Academy of Child and Adolescent Psychiatry, 38, 25-33; 1999.
7. Hudziak J, Heath A, Madden P et al. The Latent Class and Factor Analysis of DSM-IV ADHD: A Twin Study of Female Adolescents. Journal of the American Academy of Child and Adolescent Psychiatry, 37: 848-857; 1998.
8. Biederman J, Wilens T, Mick E, Milberger S, Spencer TJ, Faraone SV. Psychoactive Substance Use Disorders in Adults with Attention Deficit Hyperactivity Disorder (ADHD): Effects of ADHD and Psychiatric Comorbidity. American Journal of Psychiatry, 152: 1652-1658; 1995.
9. Milberger S, Biederman J, Faraone SV, Chen L, Jones J. ADHD is Associated With Early Initiation of Cigarette Smoking in Children and Adolescents. Journal of the American Academy of Child and Adolescent Psychiatry, 36: 37-44; 1997.
10. Lambert NM. & Hartsough CS. Prospective Study of Tobacco Smoking and Substance Disorders Among Samples of ADHD and Non-ADHD Participants. Journal of Learning Disabilities, 31: 533-544; 1998.
11. Hornig M. Addressing Comorbidity in Adults with Attention-Deficit/Hyperactivity Disorder. Journal of Clinical Psychiatry, 59: 69-75; 1998.
12. Koelega HS. Stimulant Drugs and Vigilance Performance: A Review. Psychopharmacology, 111:1-16; 1993.
13. Levin ED & Rezvani AH. Development of Nicotinic Drug Therapy for Cognitive Disorders. European Journal of Pharmacology, 393: 141-146; 2000.
14. Wonnacott S, Kaiser S, Mogg A, Soliakor l, Jones IW.

Presynaptic Nicotinic Receptors Modulating Dopamine Release in the Rat Striatum. European Journal of Pharmacology, 393: 51-58; 2002.
15. Yu H, Matsubayashi H, Amano T, Cai J, Sasa M. Activation by Nicotine of Striatal Neurons Receiving Excitatory Input from the Substantia Nigra Via Dopamine Release. Brain Research, 872:223-226; 2000.
16. Lahey BB, Applegate B, McBurnett K et al. DSM-IV Field Trials for Attention Deficit Hyperactivity disorder in Children and Adolescents. American Journal of Psychiatry, 151: 1673-1685; 1994.
17. Perkins KA, Donny E, Caggiula AR. Sex Differences in Nicotine Effects and Self-Administration: A Review of Human and Animal Evidence. Nicotine & Tobacco Research, 1: 301-315; 1999.
18. O'Donnell JP, McCann KK, Pluth S. Assessing Adult ADHD Using a Self-Report Symptom Checklist. Psychological Reports. Vol 88: 871-881; 2001.
19. Murphy P, Schachar R. Use of Self-Ratings in the Assessment of Symptoms of Attention Deficit Hyperactivity Disorder in Adults. American Journal of Psychiatry. Vol 157: 1156-1159; 2000.
20. Mehringer, AM, Downey KK, Schuh LM, Pomerleau CS, Snedecor SM, Schubiner H. The Assessment of Hyperactivity and Attention: Development and Preliminary Validation of a Brief Self-Assessment of Adult ADHD. Journal of Attention Disorders. Vol 5: 223-231; 2002.

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ORIGINAL ARTICLE

Detection of Genetically Modified Protein in Soy-containing Foods

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ABSTRACT The use of genetically modified organisms (GMO) in food is a highly public and controversial issue. We have used an enzyme-linked immunosorbent assay (ELISA) to detect the genetically modified protein 5-enol-pyruvylshikimate-3-phosphate (EPSP) synthase within soy-containing food products from the grocery store. We found that EPSP synthase is detectable in 3 out of 5 food products tested. Of specific interest, we found contamination levels of EPSP synthase (0.36%) in Heinz' Pablum Soya Cereal, which is currently deemed to be free of genetic modifications by the company as well as by Greenpeace Canada. These results demonstrate that genetically modified organisms are present in foods commonly available for human consumption and that the widespread use of this technology may make it difficult to ensure that any given product is free of all traces of genetically modified protein.

INTRODUCTION

The use of genetically modified organisms (GMOs) in food has been a lightning rod for disagreement across the globe. From violent demonstrations at trade meetings to African nations rejecting GMO food aid during famine, to the embracement of GMO food by American agricultural policy, this is an issue upon which few people are neutral (1, 2). To the opponents of genetically modified food, such modifications are at best untested and at worst dangerous to health and the environment (3). Worse, they are troublesome in spirit: "frankenfoods" are created by transferring genes between unrelated organisms, making them viscerally unpopular to many. On the other hand, the proponents of GMOs in food argue that such genetic modifications are no worse than the many other additives we already use and that GMO technology has the potential to increase crop yields, decrease pesticide use, increase food's nutritive value, and reclaim land that is currently unusable for agriculture (4, 5). From the global perspective of overpopulation and malnutrition, proponents of GMO food argue that a new "green revolution" is upon us and that millions of lives are at stake if we ignore such technologies (6). Confounding the question is a lack of trust: governments, corporations, and the public all lack trust in each other to ensure that food production is both efficient and safe. Hence, subsidiary arguments such as those about the mandatory labeling of GMO food have come to prominence in the public arena (7, 8).

However, the biggest reason for the polarization of the GMO food issue is ignorance, which is in part due to the paucity of facts available. Due to the relative youth of the technology, no long-term studies pertaining to the effects of GMO foods on population health or environmental sustainability have been performed. Moreover, since there are no regulations requiring the assessment of short-term risk in large populations, it is difficult to determine if GMO food is correlated to disease or if low incidence effects on health or ecology have occurred. Perhaps as a consequence of this factual void, the public's attitude on GMO food remains quite labile: a recent survey showed that 25% of Americans feel that GMO foods are unsafe, 29% feel that they are safe, and the rest are unsure either way. However, when supplied the statement that GMO foods are already

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prevalent in grocery stores, the number of respondents who believed that GMO foods are safe increased to 48% (9). Furthermore, it seems as though the public's opinion on GMO food is highly dependent upon its depiction in the media (10). Together these studies suggest that a small amount of factual information, responsibly presented, can give a context to this debate that is highly influential.

The most common GMO in food on the market today is genetically modified soy, with 75-80% of American soy crops grown from genetically modified seeds (11); Roundup Ready Soybeans engineered by the Monsanto corporation are the most common variety. These soybeans are genetically modified to withstand the effects of Roundup herbicide; weeds and other undesirable plants are eradicated while the Roundup Ready Soybeans are unaffected. Roundup herbicide contains glyphosate, which inhibits the essential plant enzyme 5-enol-pyruvylshikimate-3-phosphate synthase (EPSP synthase), causing growth suppression and death. Roundup ready soybeans contain the bacterial variant of EPSP synthase, which is not affected by glyphosate, meaning that growth suppression caused by Roundup herbicide is rescued in the recombinant soybeans. In principle this results in greater herbicidal selectivity and increased crop yields. It should be noted that the bacterial variant of EPSP synthase is quite divergent from the plant homologue, with the two enzymes sharing only 26% amino acid homology (12).

The GMO food debate is highly coloured by cultural, social, and economic interests. We have made a scientific enquiry in order to help fill the factual void that exists in current public knowledge. To determine the prevalence of GMO foods in our shared food supply and in order to assess the controversial claims of relative health safety or danger posed by advocates and detractors of GMO foods, we tested common soy-containing grocery store foods for genetically modified soy components.

MATERIALS AND METHODS

Foods were purchased from prominent grocery and health food stores in Montreal, Canada. Foods were tested by ELISA using the GMO4 Soya Test Kit (Strategic Diagnostics Inc.) as per the manufacturer's instructions for toasted meals (13). A nearly identical kit (Strategic Diagnostics, Inc.; not available at the time of our study) has now been validated by the Institute for Health and Consumer Protection at the Joint Research Centre of the European Commission. This kit uses identical reagents except for slightly different protein standards. The quantitative protein standards used in this study were protein isolates containing 0%, 0.3%, 1.25%, or 2.5% EPSP synthase. Foods were selected on the basis of being sold in a powdered form and having soy as an ingredient on the label, whether as soy or as an oil derived from soy. Each experiment was conducted three times. Since different optical density readers were used for each trial, the absolute optical density had some variation. To weigh all three trials equally, values were normalized by expressing optical density as a proportion of the background reading for that trial. A standard curve was constructed using the normalized optical density readings of known bacterial EPSP synthase protein standards at 0%, 0.3%, 1.25 %, and 2.5%, followed by the use of linear regression (Figure 1). Abundance was quantified by comparing the normalized optical density readings of food samples to the standard curve.

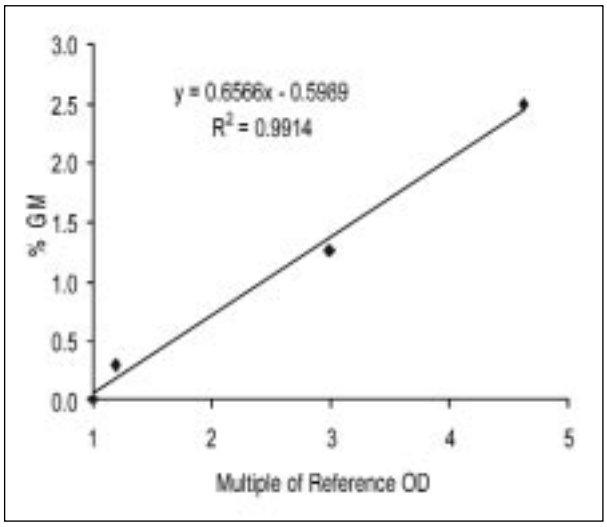

Figure 1. A standard curve of optical density (OD) for bacterial EPSP synthase was created using protein standards containing a known percentage of the protein. See materials and methods for details.

RESULTS

Five foods were tested in total: three foods (Heinz Pablum Soya Cereal for infants, Red Mill whole grain soy flour, and soybean protein powder for bodybuilders) contained whole soybean components, while the other two contained soy lecithin or oils only (President's Choice Organic apple-cinnamon muffin mix and Knorr pasta sauce mix). An ELISA test kit using antibodies against bacterial EPSP synthase was used to detect the prevalence of genetically modified soy protein in these products. Heinz Pablum Soya Cereal was found to contain 0.36% bacterial EPSP synthase, Red Mill soy flour 1.29%, and bodybuilding soy protein 1.07%, while no significant level was detected in the soy oil-based products (Figure 2).

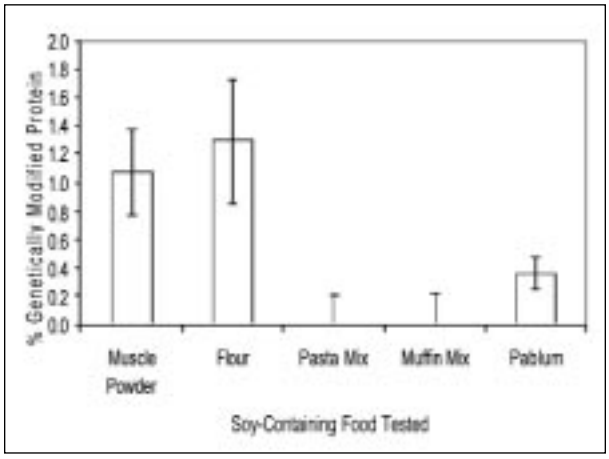

Figure 2. Soy-containing grocery store foods have detectable levels of bacterial EPSP synthase. Quantitative ELISA detected protein levels of bacterial EPSP synthase between 0 and 1.3% in five foods obtained from grocery stores. Error bars represent a 95% confidence interval. Results represent a normalized average of six assays for each food.

DISCUSSION

Using ELISA we have determined that the bacterial protein EPSP synthase is detectable in soy-containing foods consumed by the Canadian population. Three out of five soy-containing products tested contained detectable levels of the protein. Of the two products where genetically modified protein was not detected, soy was only a minor component of the food, being represented in the oil (sauce mix) or lecithin (muffin mix) only. It is possible that these products also contained genetic modifications, but were not detected by the assay due to their low abundance at the protein level. Unexpectedly, Pablum Soya Cereal contained detectable levels of genetically modified protein (0.36%) despite claims by Heinz that their infant foods are GMO free. We attribute this detection to contamination of GMO free soybeans by Roundup Ready soybeans somewhere in the production of the infant cereal. To put the level detected into perspective, the European Parliament recently voted to make the protein threshold of classifying a food as "genetically modified" at 0.5% and above (14). Although foods such as the Pablum Soya Cereal are by this definition GMO free, we have determined that they still contain detectable levels of GMO protein. Unfortunately the classification between "genetically modified" and "contaminated" is a political distinction and not a scientific one, since there is no certainty as to whether there are any health effects of long-term exposure and if these effects depend on the quantity of ingestion. Such a labeling system might be misleading to consumers who truly wish to ingest foods that are free of GMOs, but is preferable for companies that are evidently unable to prevent GMO "contamination."

At the present time there is no evidence to suggest that bacterial EPSP synthase is in any way harmful to health. High dosage acute administration of the bacterial EPSP synthase in mice does not result in noticeable toxicity, and simulated mammalian gastric contents cause rapid degradation of the protein (15, 16). Moreover, the bacterial EPSP synthase does not appear to act as an allergen any differently than the plant version. For instance, examination of IgE-binding proteins in soy does not reveal any difference between the genetically modified and wild type strains (17). It is on the basis of such results that Roundup Ready soybeans have been approved for use in many countries around the world and have found their way into our grocery stores. Furthermore, there has not been a single confirmed clinical report of toxicity or allergenicity to genetically modified soy. However, in the absence of studies monitoring large populations and with nothing known about the long term, many are wary of equating a lack of known harm with general safety. Indeed, several theoretical risks remain (18). Our present results suggest that genetically modified protein is detectable in widely available grocery store foods, but a caveat of our study is our relatively small sample size. An exhaustive study of a much wider range of food products and types of genetically modified proteins would give a better idea as to the prevalence and abundance of genetically modified protein in grocery store foods. Nevertheless, our ability to detect bacterial EPSP synthase in widely available grocery store foods suggests that, at least in the short term, the risk posed to human health is probably low.

It is problematic to generalize to all GMO foods any determinations concerning genetically modified soy. While GMO foods have realized several economic and yield gains, especially in developing countries (19), there is no a priori safety inherent to GMO foods. Each GMO food expresses a different protein, each of which will affect the environment and health in a different way. From a health and scientific point of view, legislators should not expend their efforts on determining thresholds for food labeling or setting up trade barriers to such foods, but rather should be ensuring that these novel foods are as safe as possible. While the uncertainty inherent to science challenges this goal, especially in the long term, other industries such as pharmaceuticals have devised ways to ensure the relative safety of their products. Once safety is ensured, it is then up to society to decide whether such foods are ethical, desirable, or irreconcilable. Given the potential of GMO food technology to increase food yields across the globe, it is important that science work hand-in-hand with society to make sure that the decision is an informed one. Finding GMO foods

surreptitiously spread through the grocery store aisles without societal consultation, as we have found, does not engender the use of reason in the public domain.

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REFERENCES

1. Adam D. Hostilities resume over future of GM crops. Nature. 419:327; 2002.
2. Bohannon J. Zambia rejects GM food on scientist's advice. Science. 298(5596):1153-1154; 2002.
3. Flothmann S, van Aken J. Of maize and men. Is the endorsement of GM crops science or politics? EMBO Reports. 2(8):644-7; 2001.
4. Huang J, Pray C, Rozelle S. Enhancing the crops to feed the poor. Nature. 418(6898):678-84; 2002.
5. Zimmermann MB, Hurrell RF. Improving iron, zinc, and vitamin A nutrition through plant biotechnology. Current Opinion in Biotechnology. 13(2):142-145; 2002.
6. Trewavas, A. and Leaver, C. Is opposition to GM crops science or politics? An investigation into the arguments that GM crops pose a particular threat to the environment. EMBO Reports. 2, 455-459; 2001.
7. Saegusa A. Japan may require labels on genetic food. Nature. 395:628; 1998.
8. Haslberger AG. Monitoring and labeling for genetically modified products. Science. 287: 431-432; 2000.
9. Lok C. Americans perplexed by GM food. Nature. 410:501; 2001.
10. Frewer LJ, Miles S, Marsh R. The media and genetically modified foods: evidence in support of social amplification of risk. Risk Analysis. 22(4): 701-11; 2002.
11. National Agricultural Statistics Service, United States Food and Drug Administration Prospective Plantings Report March 31, 2003 http://usda.mannlib.cornell.edu/usda/usda.html
12. Padgette SR, Taylor NB, Nida DL, et al. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. The Journal of Nutrition. 126(3): 702-16; 1996.
13. Protocol is available at http://www.sdix.com/PDF/Products/7099999%20v2.5.pdf
14. Butler D. Europe gets tough on labeling genetically modified foodstuffs. Nature. 418:114; 2002.
15. Harrison LA, Bailey MR, Naylor MW et al. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. The Journal of Nutrition. X126(3):728-40; 1996.
16. Chang HS, Kim NH, Park MJ et al. The 5-enolpyruvylshikimate-3-phosphate synthase of glyphosate-tolerant soybean expressed in Escherichia coli shows no severe allergenicity. Molecules and Cells 15(1):20-6; 2003.
17. Burks AW, Fuchs RL. Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties. Journal of Allergy and Clinical Immunology. 96(6 Pt 1):1008-10; 1995.
18. Alexander TW, Sharma R, Okine EK et al. Impact of feed processing and mixed ruminal culture on the fate of recombinant EPSP synthase and endogenous canola plant DNA. FEMS Microbiology Letters 2002 214(2):263-9; 2002.
19. Qaim, M. and Zilberman, D. Yield effects of genetically modified crops in developing countries. Science. 299(5608):900-2; 2003.

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ORIGINAL ARTICLE

The Epidemiology Study in Multiple Sclerosis - Relevance to Natural History

J Scott Sloka, MD, PhD*

ABSTRACT Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system white matter that has been extensively studied using the epidemiological approach, and yet an etiology for the disease remains elusive. This paper presents a review of past publications that have made suggestions toward the design of epidemiological studies in MS. A formal search strategy is described, and a short summary of these papers is provided. A natural history of MS based on previous studies is proposed as a framework for describing future directions in the neuroepidemiology of the disease, and categorization based on the clinical forms of MS is described. Within the context of a proposed natural history, suggestions are made on the use of sub-regionalization in cluster studies across different domains, as well as on the use of specific reference points in a patient's lifetime in the analysis of clusters.

INTRODUCTION

Multiple sclerosis (MS) is a chronic, demyelinating disease of the central nervous system white matter that may cause paralysis, sensory disturbances, incoordination, visual impairment, and alterations in bowel, bladder, and sexual function(1). The precise etiology of MS has not been elucidated; however, many observations have been made that suggest both genetic susceptibility and environmental factors play a role(2). In the search for an environmental contribution to the etiology of MS, numerous epidemiological studies have been conducted, and a large body of literature based on the results of these studies has been published (1,3). Many hypotheses of exogenous causes of MS have been explored (4), including exposure to viruses (5,6), organic solvents (7), diet (8), and soil type (9). Intriguingly, many purported associations remain controversial, and studies have shown conflicting results on the contribution of viral causes to etiology (1), conflicting genetic susceptibilities in similar populations to different categories of MS (10,11), evidence that the pathology may not follow clinical observations (12,13), and large temporal gradients in disease populations (either related to changes in environmental factors or to diagnostic coverage) (14,15). Consistent study design in future comparative studies may help to explain these differences and add to the significance of previous research.

The role of the epidemiological study in the search for etiology is well-documented (16). In fact, much of the evidence for both genetic susceptibility and environmental triggers in MS has been elucidated from epidemiological studies (1,2). Historically, variations in incidence and prevalence have been related to temporal and geographical gradients and other variations in risk, in order to generate hypotheses towards the causation of disease. The epidemiological approach has not yielded all the answers, but it holds great merit and much potential to further contribute to the knowledge of disease etiology (16). Although many epidemiological studies have been published, few recent papers have been dedicated to suggesting study methodologies in order to elucidate new epidemiological information, especially in the context of a natural history (16-27).

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Background

An epidemiological study of MS in Newfoundland is currently being conducted in order to both update the results of a previous study (28) and to explore further hypotheses toward the etiology of MS. A survey of previous epidemiological studies was conducted using MEDLINE in order to gain insight from other authors' past experiences. Search terms used were the following: multiple sclerosis and (epidemiology or epidemiological). Altogether, 1791 citations were found for all available years and a review through the abstracts and titles from 1990 to 2001 yielded references that were used to discover the recent trends and suggestions for the design and administration of epidemiological studies for MS (16-27). Although older studies were indeed reviewed, only references to recent papers were included in this summary since an understanding of recent trends in the neuroepidemiology of MS was sought. Furthermore, the historical context of older reviews was captured in the newer reviews. Several recent papers that presented results of epidemiological studies were also surveyed for methodology, data analysis, and concluding suggestions towards the design of both their study and studies conducted by others (27, 29-38). Papers that discussed the epidemiology of MS in the context of the natural history of the disease were particularly sought, but none was found that presented suggestions in the context of a formally presented natural history.

To summarize the existing suggestions of others, new epidemiological insights into MS beget new directions for the study of MS. Problems of current study design do exist but can be overcome. For example, very little of a patient's residential history is captured on consultation sheets, and so other means of information gathering are required (such as questionnaires), and their respective limitations must be accepted (such as response rate). However, it is possible to gather such information using questionnaires (29), and methodologies for optimizing the amount of information that one can elicit from a questionnaire have been suggested by others (21), so lack of patient information should not be a limiting factor. As well, methods to correct for known deficits in data have been proposed (19,37), as have suggestions on the logistics of conducting a study (36). Papers have suggested that age, gender, and race should be collected while performing epidemiological studies and adjustments for these variables made (18); methods to minimize bias while performing ecological studies have also been made (18). Others have discussed methods of eliciting information from time and space analyses (20,22,39) and still others have discussed epidemiological methodologies in the context of genetic research (20,38).

Since the design of epidemiological studies in MS has not been suggested in the context of a proposed natural history for the disease, this paper first presents such a proposal and then makes suggestions towards future study designs. First, a general discussion on latency and induction periods is presented as an introduction to the concepts of formalizing a natural history. The section that follows describes results of several observational studies on MS pertaining to genetic susceptibility, environmental contributions, and disease heterogeneity. The final section formally ties the observations of these studies together and makes suggestions towards future directions in cluster analysis as related to natural history.

NATURAL HISTORY

Current theories on the natural history of a disease have implications on the design of epidemiological studies. In fact, the natural history of a disease may shape the type of data that can be drawn from a study. For example, the time course of the disease affects the feasibility of a study in diseases with sub-clinical periods. Diseases with either long time courses or long latency periods between exposure and disease are both more expensive to study and more susceptible to loss from follow-up. Importantly, however, knowledge of the current thinking on the natural history of a disease also guides one to develop hypotheses in order to supplement or refute current theories.

The concepts of latency and induction period are related to the sub-clinical and clinical progression of MS and are important in the early stages of the disease. Therefore, observations made at these stages contribute to knowledge of the etiology of MS.

Induction Period and Latency

The induction period is defined as the period of time from causation until disease initiation (40) (Figure 1). The beginning of the induction period is the time at which the earliest component cause influences the etiologic mechanism (40). In the case of a genetic susceptibility, the induction period begins at conception. In people who are genetically predisposed to a disease that is environmentally triggered (possibly type I diabetes, MS, atherosclerotic heart disease), the concept of an environmentally-specific induction period may be defined as the period from the first environmental exposure until disease initiation (Figure 1).

It is possible to have multiple dependent or independent environmental exposures (41). These can either be the same environmental pathogen or different pathogens altogether. Initial environmental exposures precede a final exposure that concludes the disease

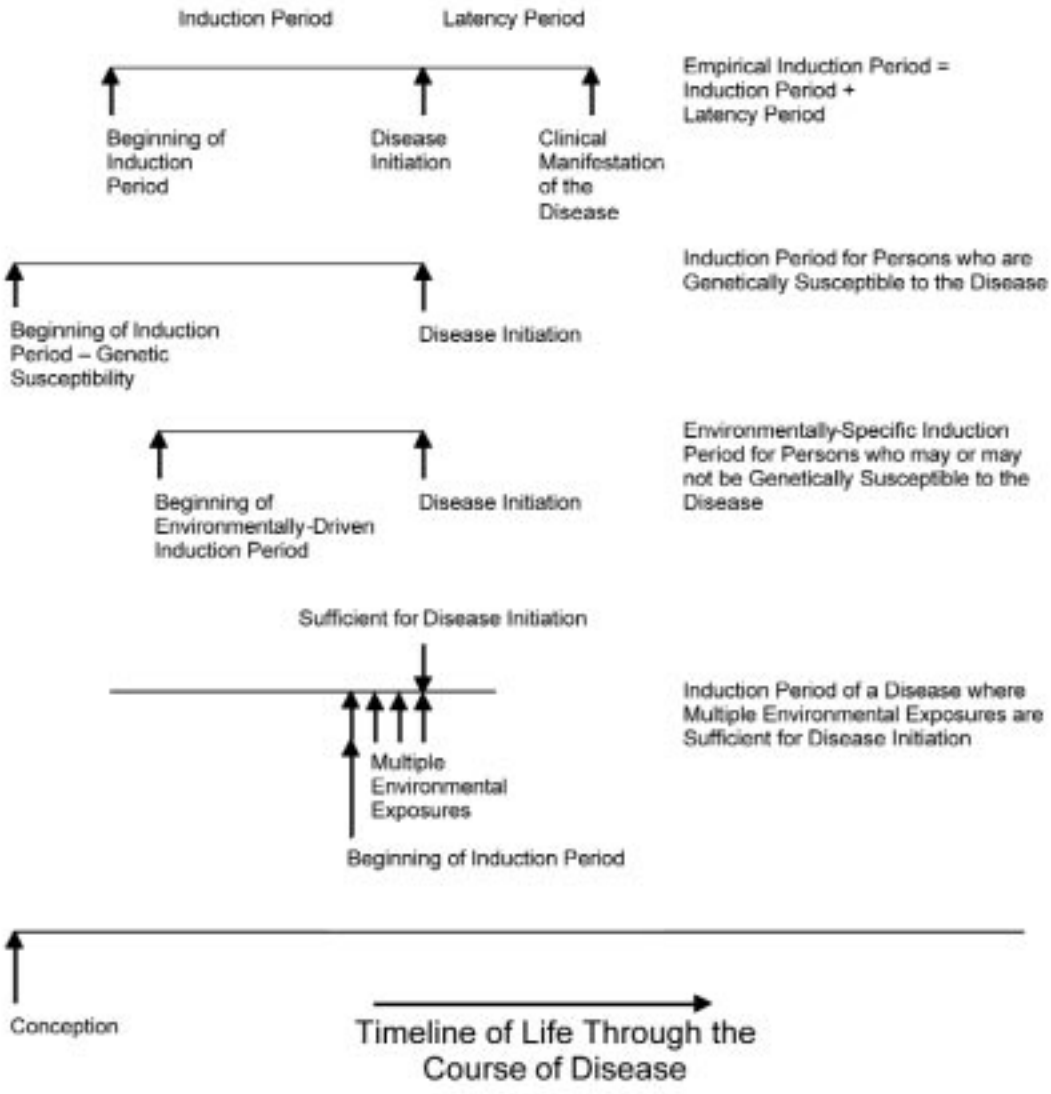

Figure 1. Definition of Latency Period, Induction Period, Empirical Induction Period and Environmentally-Specific Induction Period

initiation process - the final exposure (genetic mutation or environmental exposure) is sufficient to initiate the disease process (Figure 1).

The disease may clinically manifest itself immediately after the final environmental exposure (e.g. bacterial toxins leading to gastrointestinal disease), or it may take decades to manifest itself (e.g. diethylstilbesterol exposure in utero leading to vaginal cancer), depending on the natural history of the disease. This time interval between the initiation of disease and the time at which the disease is first detected, either by the individual or clinician, is called the latency period (40). In other words, this latency period is the time during which the disease is initiated but "hidden" and is typified by diseases such as type I diabetes and MS. In practice, it is sometimes difficult to separate the induction period and the latency period, and so the sum of these two periods is generally used and is defined as the empirical induction period (EIP) (40) (Figure 1).

Therefore, the epidemiological contribution to quantifying the early stages of the natural history of a disease may include the following: the elucidation of the precise pathogen(s), the discovery of the number of exposures required for sufficient cause, the induction period length, the latency period length, and the effects of differing genetic susceptibilities on all of these variables.

GENETIC AND ENVIRONMENTAL CONTRIBUTIONS

As was previously stated, both environmental factors and genetic susceptibility are felt to contribute to disease induction (1,2). This section presents the results

of epidemiological studies specific to both the genetic and environmental contribution to disease. Additionally, disease heterogeneity is discussed since different clinical categories of MS have different clinical presentations and pathologies (12,13) with different natural histories.

Genetic Contribution

Concordance rates among monozygotic twins with MS is approximately 31-40% whereas the concordance rates among fraternal twins and non-twin siblings is approximately 3-5% (42,43) - this demonstrates an approximate six to eight times increase in relative risk. As well, prevalence rates among non-biological siblings adopted into a family are similar to prevalence rates of MS found in the general population and significantly less than for biological relatives, suggesting a significant genetic component to the familial aggregation of MS (42). Genetic susceptibility has also been shown to vary with several genetic markers (44-47), especially the human leukocyte antigen (HLA)-DR2 antigen on chromosome 6 (44).

The mode of transmission is complex and sporadic in most cases (2). The susceptibility itself and the timing of disease initiation may depend on the particular combination of disease susceptibility markers in the individual, thus having an effect on the length of the EIP. As well, the beginning of the EIP may be as early as conception for those with a genetic predisposition (Figure 1). Therefore, a variation in the EIP should be accounted for in any model of the natural history of diseases with a continuum of multiple susceptibility markers. In populations with longer EIPs, there is a greater risk of confounding factors such as migration and co-morbidity that complicate study results. Longer EIPs also increase the chance that the empirical induction period has a greater variability (40). Therefore, the genetic makeup of the population under study should be accounted for in any model of natural history.

Environmental Contribution

A geographical distribution highlighting increased MS prevalence with higher latitudes provides evidence of an environmental contribution (48). However counter to this correlation with latitude, large variations in prevalence among geographically close regions with similar latitudes have been noted in places such as Sicily and Malta (49,50). This may suggest that locally-specific etiologies (either environmental or genetic) contribute to disease pathogenesis.

Migrant studies also contribute to proof of an environmental component. The results of multiple migrant studies in MS (51-57) suggest that people who migrate before adolescence acquire the incidence rates of the region to which they have migrated. In contrast, people that migrate to a region after adolescence retain the incidence rate of the region from which they grew up (58). This compelling evidence is fairly consistent for migration from areas of high risk to areas of low risk and suggests that there is a part of the disease process that depends on geographical location, possibly involving an environmental pathogen. However, the evidence is also fairly consistent that migration from areas of low risk to areas of high risk is not associated with a substantial change in risk (58). This diminishes the strength of conclusions that can be drawn from all migrant studies but does permit the observation that both geography and age play some as-yet undetermined role in the natural history of the disease.

Studies of the age at which people migrate have shown that a general age range might be important in the natural history of the disease in terms of susceptibility to an environmental pathogen. Many studies on age-at-migration suggest that either a general age range (51-57) or a "critical age" at migration alters the risk of disease, and this critical age tends to be close to 15 (52-54, 57) (e.g. populations migrating before the age of 15 from high to low risk regions acquire the lower risk of susceptibility). The implication of these studies is that the risk of acquiring MS may be largely determined by the age of 15 years. However, these studies are based on very small population sizes (58). In other studies from Australia (59) and the US (56), a relation between the age of migration and the change in risk of acquiring MS has been suggested, and still others have suggested that the critical age is not 15 but exists sometime within the latter part of the first two decades of life (57,58).

One study that stands out is an analysis of MS incidence data from an "outbreak" of MS on the Faroe Islands related to the stationing of British troops there during World War II (41). The author analyzed the data and considered both an age at exposure and a latency period of the disease. A model was developed using a critical age of puberty for a sufficient cause exposure (13 years old for females and 14 years old for males), and an average latency period could be consistently estimated from this age to be 5 years for females and 6.3 years for males until the manifestation of disease symptoms. The biological plausibility of these results is supported by the physiological changes occurring at puberty, with the idea that some unknown factor that changes the susceptibility occurs at that point in life and may be either hormonal or developmental in nature. The observations in this study have problems of low power. However, others have calculated latency periods and found ranges of 9 years (60), 9-12 years (61), and 8-

Table 1. Epidemiological characteristics of different clinical forms of MS. There is a large variation in reported values. Therefore, ranges are given where possible and significant comparisons within single studies were used. These differences in epidemiological characteristics suggest that different natural histories should be considered for each clinical form. PPMS tends to have a later age of onset and a smaller male-to-female ratio

Clinical Classification	Clinical Course	Age of Onset (years)	Male to Female Ratio	Percentage of MS Patients
Relapsing Remitting MS (RRMS)	MS with acute exacerbations and full or nearly full remission to baseline	29.2 (66) 30.7+/-8.4 (67)	Not a universal difference with PPMS (68)	63-92%
Secondary Progressive MS (SPMS)	MS that was initially RRMS but now retains progressively additive deficits with each exacerbation	50% of RRMS after 10 yrs, 90% after 25 yrs (69)	1:3.2 (67)	50% of RRMS after 10 yrs, 90% after 25 yrs (69)
Primary Progressive MS (PPMS)	MS that has had a progressive course from onset	35.9+/-9.9 (67)	1:1.7 (67)	8-37% (70-72)

14 years (60), and these results are compelling enough to consider the concept of a critical age and latency period. Yet difficulties comparing the data of migration studies arise due to the lack of uniform diagnostic criteria, uncertainty about the latency period, deficiencies in case finding and follow-up, and enormous variability in the clinical presentation and course of the disease (19).

Heterogeneity of Disease

To further complicate matters epidemiologically, MS may in fact be a collection of heterogeneous disorders by epidemiological, pathological, and diagnostic parameters and may therefore be a spectrum of disease instead of one disease entity (62,63). Based on an international survey of MS experts, MS has been clinically classified as relapsing-remitting (RRMS), secondary progressive (SPMS), and primary progressive (PPMS) disease (see Table 1 for a description of clinical course and characteristics)64. This survey was based on observations of the clinical presentation and course of MS. Another category, progressive remitting (PRMS), was included in the survey paper; however others have suggested that PRMS is, in fact, a variant of PPMS (65).

Differences in the epidemiological characteristics of disease categories suggest that each clinical form has a separable natural history. Further clinical, diagnostic, and immunological evidence for categorizing PPMS as a separate entity from RRMS and SPMS includes lack of clinical attacks (70), fewer lesions on MRI (70), higher in vitro migration (73), different epidemiology (62), differences in immune cell products (73), less inflammation on necroscopy70, and differences in HLA prevalences (74-76). Additionally, it has been reported that SPMS, although an apparent continuation from RRMS, has significant differences from RRMS and PPMS including differences on diagnostic imaging (77).

Given that genetic, pathologic, and clinical differences exist among RRMS, SPMS, and PPMS, one might be tempted to categorize MS based on these distinctions. However, new evidence suggests that the spectrum of disease may also be delineated along pathophysiological boundaries, and these newly-considered boundaries may or may not correlate with the clinical/genetic boundaries suggested above (63). Observations suggest that one form of MS may be characterized by inflammation directed against myelin while another form of MS may be due to progressive axonal degeneration (12,78). Whether the pathophysiological categorization of MS correlates with the clinical/genetic categorization of MS remains to be established. However, the clinical categorization of MS should at least be considered during the design of epidemiological studies since each clinical entity appears to have a different natural history as exemplified by differences in age of onset, male-to-female ratio, and the difference in likelihood of presenting after age 40 (67).

A PROPOSED NATURAL HISTORY

A proposed natural history (Figure 2) would begin with genetic factors that contribute to an individual's susceptibility from conception. As described above, multiple genetic factors contribute to susceptibility, and different combinations of these factors within individuals may affect the length of the induction period, the number of environmental exposures required for sufficient cause, and the clinical progression of disease.

The environmental contribution to disease initiation may occur either in utero or after birth. This contribution may include different factors (such as

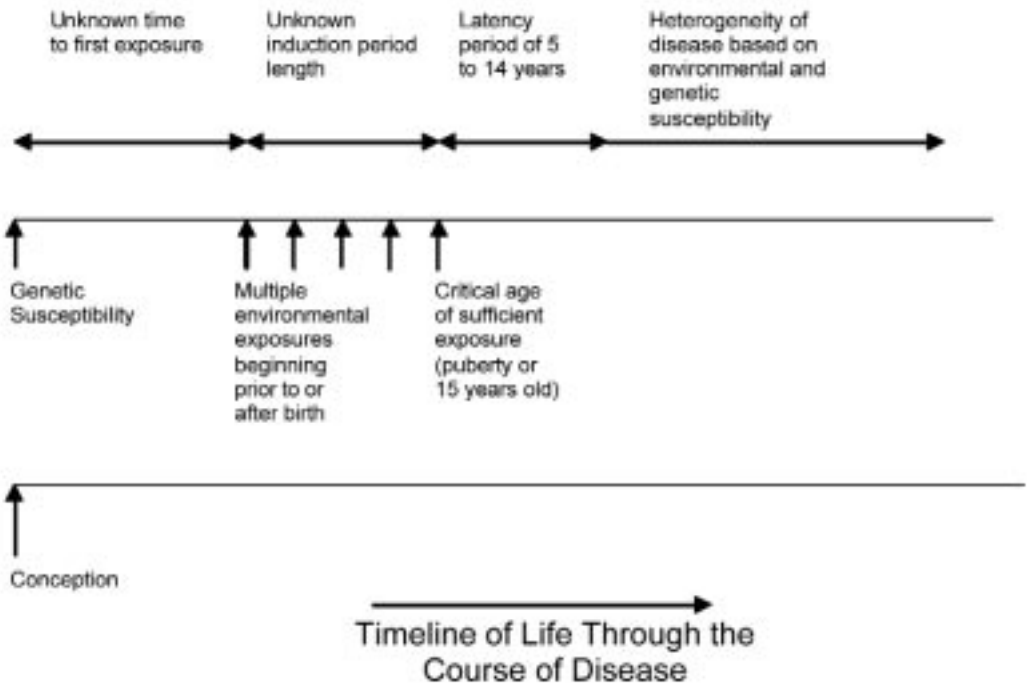

Figure 2. A proposed natural history for MS including genetic factors that affect an individual's susceptibility from birth, multiple environmental exposures, a critical age of sufficient exposure, unknown induction period length, an estimated latency period, and a heterogeneous disease presentation.

exposure to viruses (5,6)), and multiple exposures may be necessary, again depending on the genetic susceptibility of the individual. Therefore, both genetic and environmental variability contributes to a highly variable EIP.

An individual may have different susceptibility to disease initiation depending on a critical age of susceptibility or their pubertal status (as highlighted above in the migration studies). Following this critical age, (highly variable) latency periods have been estimated (see environmental contributions above).

Finally, different clinical categories of MS have been described, each with a different clinical course. There are different ages of onset, different rates of progression, and different male-to-female ratios among the categories, highlighting different natural histories for each.

SUGGESTIONS FOR FUTURE EPIDEMIOLOGICAL STUDIES

Now that a framework for the natural history of MS has been proposed, proposals on the epidemiological search for an etiology may be made in the context of this framework. A body of evidence suggests that clusters of MS exist in both space and time (79-84). In searching for clusters of disease in time and space, which may in turn lead researchers to possible environmental or genetic contributions to disease induction, a study of a large geographical region may be regionalized for the comparison of subregions. The subregionalization is normally selected to reflect differences in subregions in some respect (soil type, type of industry). These subregions need to be large enough to guarantee sufficient statistical power for their comparison (85). It is preferable to have a migrationally stable population for cluster studies so that exposures in space can be correlated without inter-subregional travel diluting the conclusions that can be drawn on exposure versus effect.

Methods to "re-assign" the incidences and/or prevalences have been suggested by several authors (19,86). Figure 3 shows two representations of four geographical subregions. The diagram on the left depicts a hypothetical prevalence map with a uniformly constant prevalence across all subregions. In this example, the prevalence is assigned to the subregion of residence at the time of diagnosis. For the representation at the right, the prevalence is recalculated for an assignment of residence at the time of first attack (which may be different than the residence at the time of diagnosis) indicating, in this hypothetical representation, that a person living in the upper left subregion at the time of diagnosis actually had their first attack while residing in the lower right subregion. If a sufficient number of people had attacks in a given subregion that is significantly more than in other

regions, a cluster in that region would be found and further searching would be necessary to elicit a cause. Incidence remapping is also applied in this fashion.

Thus, time and space clusters may be "hidden" within data sets and may be elucidated by giving a new time reference point for the incidence and/or prevalence, given that these reassignments correlate with biologically plausible periods in the natural history of the disease. This type of reassignment can distill space-time clusters that would not be possible if the natural history of the disease had not been considered.

It is possible to reassign prevalences and/or incidences to dates of first attack if dates and locations of residency are known. It is also possible to reassign prevalences and/or incidences to increments of years prior to first attack, with the age of first attack being the reference. This reassignment process is designed to "simulate", or readjust to, a series of hypothetical latency and/or induction periods of differing length. Problems with this new reassignment method include a dilution of effect across time (a dilution of effect can occur in a case-control study between two groups if the latency period is not chosen correctly (40)).

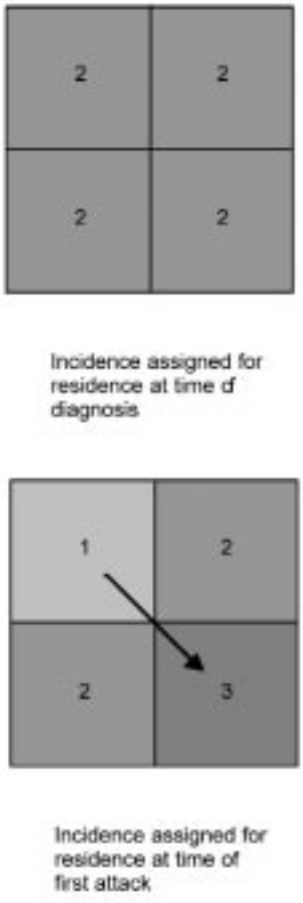

Figure 3. Hypothetical incidence maps showing reassignment of incidences based on subregion of residence at first attack

Reassignment can also be based on an estimated critical age for susceptibility, such as a fixed age (e.g. 15 years old) or the individual's age at puberty. If the natural history of a disease includes a time period where a sufficiently long latency period exists, an estimated latency period preceding the critical age can also be used to look for clustering. This again necessitates commenting on the residential stability of the population under study. If a population is largely stable from the time of birth to the critical age, then a consistent exposure history might be hypothesized. If, however, the population is more transient, then any conclusions based on exposure are considerably weakened.

Finally, reassignment can be referred to the time of birth or subsequent years thereafter. There is some evidence to support the relation of MS to the acquisition of measles (87), a childhood illness that generally occurs prior to most of the "critical age" estimates. A possible reassignment time might be the mean age for the contraction of measles. The time of birth can also be used as the reference time for incidence/prevalence reassignment because this represents a significant change in the environment of the human, and subsequent exposure to new environmental pathogens.

In terms of designing a cluster analysis study, important dates should be captured for reassignment of "attacks." Ideally, the exact locations of habitation from birth until the first attack would be captured for each patient. This would give invaluable information for the purposes of comparisons between regions. For the purpose of incidences, date and place of residence of first attack instead of date of diagnosis is more physiologically useful. Date and place of residence for (i) puberty, (ii) an estimated latency period before puberty, (iii) an estimated latency period before first attack, (iv) at birth, and (v) a mean time to exposure of viral illnesses after birth are all equally relevant depending on the intent of the study.

In order to search for an etiology for this complex and enigmatic disease, a migrationally stable and sufficiently large population with very little inter-regional travel is desired in order to strengthen conclusions about exposure/disease occurrence. A population with access to a sufficient number of neurologists and medical facilities (MRI, facilities to test for oligoclonal banding) is also required so that regional differences can be stated to be due to variations in exposure/genetics and not due to lack of health care access. Uniform diagnostic criteria are essential and since MS is purported to be a heterogeneous disease, diagnoses should also capture the specific type of MS

because relapsing-remitting MS can have different epidemiological characteristics than primary progressive MS.

Additionally, regions do not have to be geographical regions per se. Regionalization in a cluster analysis is usually geographical, but could also be done virtually with other categorizations. An example would be water supply source. Towns in the same region could have chlorinated water, be on boil order, have fluorination, etc. These categories could be the "subregions" and time and space clusters could be analyzed in the "water domain" rather than the "geographical domain."

CONCLUSIONS

The natural history of a disease guides the design of epidemiological studies of that disease. This paper has presented suggestions for study design in the context of a formally described natural history for MS. Specifically, studies should analyze disease prevalence and incidence based on the type of MS. Studies may analyze subregions based on geography, but also may subregionalize based on virtual domains not tied to geography. Ideally, one should study homogeneous populations that are migrationally stable. Reference points within an individual's lifetime (i.e. time/place of first attack, time/place of birth, time/place of puberty) may be equally important in determining clusters. Studies should look for "critical ages" based on these reference points. Most importantly, one should account for any of these possibilities early in the design of the study so one may maximize the information that can be analyzed from the data. If the design of the cluster study is flexible enough, the search for an ecological etiology can be sought from any clusters that are found.

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REFERENCES

1. Paty DW, Ebers GC. Multiple Sclerosis. Contemporary Neurology Series 1998; 50:Chapter 4 - Diagnosis.
2. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med 2000; 343(13):938-952.
3. Wolfson MC. Health-adjusted life expectancy. Health Rep 1996; 8(1):41-46.
4. Lauer K. Ecologic studies of multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S18-S26.
5. Hodge MJ, Wolfson C. Canine distemper virus and multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S62-S69.
6. Hernan MA, Zhang SM, Lipworth L, Olek MJ, Ascherio A. Multiple sclerosis and age at infection with common viruses. Epidemiology 2001; 12(3):301-306.
7. Landtblom AM. Exposure to organic solvents and multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S70-S74.
8. Lauer K. Diet and multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S55-S61.
9. Irvine DG, Schiefer HB, Hader WJ. Geotoxicology of multiple sclerosis: the Henribourg, Saskatchewan, cluster focus. II. The soil. Sci Total Environ 1988; 77(2-3):175-188.
10. Fukazawa T, Yamasaki K, Ito H, Kikuchi S, Minohara M, Horiuchi I et al. Both the HLA-CPB1 and -DRB1 alleles correlate with risk for multiple sclerosis in Japanese: clinical phenotypes and gender as important factors. Tissue Antigens 2000; 55(3):199-205.
11. Marrosu MG, Murru MR, Costa G, Murru R, Muntoni F, Cucca F. DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population. Hum Mol Genet 1998; 7(8):1235-1237.
12. Lucchinetti C, Bruck W, Noseworthy J. Multiple sclerosis: recent developments in neuropathology, pathogenesis, magnetic resonance imaging studies and treatment. Curr Opin Neurol 2001; 14(3):259-269.
13. Lucchinetti CF, Rodriguez M. The controversy surrounding the pathogenesis of the multiple sclerosis lesion. Mayo Clin Proc 1997; 72(7):665-678.
14. McCall MG, Brereton TL, Dawson A, Millingen K, Sutherland JM, Acheson ED. Frequency of multiple sclerosis in three Australian cities--Perth, Newcastle, and Hobart. J Neurol Neurosurg Psychiatry 1968; 31(1):1-9.
15. Hammond SR, McLeod JG, Millingen KS, Stewart-Wynne EG, English D, Holland JT et al. The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle and Hobart. Brain 1988; 111 (Pt 1):1-25.
16. Compston A. The epidemiology of multiple sclerosis: principles, achievements, and recommendations. Ann Neurol 1994; 36 Suppl 2:S211-S217.
17. Hibberd PL. Use and misuse of statistics for epidemiological studies of multiple sclerosis. Ann Neurol 1994; 36 Suppl 2:S218-S230.
18. Lauer K. Ecologic studies of multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S18-S26.
19. Poser CM, Benedikz J, Hibberd PL. The epidemiology of multiple sclerosis: the Iceland model. Onset- adjusted prevalence rate and other methodological considerations. J Neurol Sci 1992; 111(2):143-152.
20. Compston A. The epidemiology of multiple sclerosis: principles, achievements, and recommendations. Ann Neurol 1994; 36 Suppl 2:S211-S217.
21. Boiko A. Data collection guidelines for questionnaires to be used in case- control studies of multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S75-S80.
22. Kurtzke JF. Epidemiology of multiple sclerosis. Does this really point toward an etiology? Lectio Doctoralis. Neurol Sci 2000; 21(6):383-403.
23. Compston A. Genetic epidemiology of multiple sclerosis. J Neurol Neurosurg Psychiatry 1997; 62(6):553-561.
24. Cowan LD, Leviton A, Dammann O. New research directions in neuroepidemiology. Epidemiol Rev 2000; 22(1):18-23.
25. Granieri E, Casetta I, Tola MR. A multicenter study methodologic experience from a multicenter case- control study in Italy. The Italian Multiple Sclerosis Study Group. Neurology 1997; 49(2 Suppl 2):S33-S41.
26. Lauer K. Environmental associations with the risk of multiple sclerosis: the contribution of ecological studies. Acta Neurol Scand Suppl 1995; 161:77-88.
27. Weinshenker BG. Epidemiologic strategies to detect an exogenous cause of MS. Acta Neurol Scand Suppl 1995; 161:93-99.
28. Pryse-Phillips WE. The incidence and prevalence of multiple sclerosis in Newfoundland and Labrador, 1960-1984. Ann Neurol 1986; 20(3):323-328.

29. Broadley SA, Deans J, Sawcer SJ, Clayton D, Compston DA. Autoimmune disease in first-degree relatives of patients with multiple sclerosis. A UK survey. Brain 2000; 123 (Pt 6):1102-1111.
30. Hader WJ, Elliot M, Ebers GC. Epidemiology of multiple sclerosis in London and Middlesex County, Ontario, Canada. Neurology 1988; 38(4):617-621.
31. Warren S, Warren KG. Prevalence, incidence, and characteristics of multiple sclerosis in Westlock County, Alberta, Canada. Neurology 1993; 43(9):1760-1763.
32. Warren S, Warren KG. Prevalence of multiple sclerosis in Barrhead County, Alberta, Canada. Can J Neurol Sci 1992; 19(1):72-75.
33. Klein GM, Rose MS, Seland TP. A prevalence study of multiple sclerosis in the Crowsnest Pass region of southern Alberta. Can J Neurol Sci 1994; 21(3):262-265.
34. Sweeney VP, Sadovnick AD, Brandejs V. Prevalence of multiple sclerosis in British Columbia. Can J Neurol Sci 1986; 13(1):47-51.
35. Ghadirian P, Jain M, Ducic S, Shatenstein B, Morisset R. Nutritional factors in the aetiology of multiple sclerosis: a case-control study in Montreal, Canada. Int J Epidemiol 1998; 27(5):845-852.
36. Granieri E, Casetta I, Tola MR. A multicenter study methodologic experience from a multicenter case- control study in Italy. The Italian Multiple Sclerosis Study Group. Neurology 1997; 49(2 Suppl 2):S33-S41.
37. Esbjerg S, Keiding N, Koch-Henriksen N. Reporting delay and corrected incidence of multiple sclerosis. Stat Med 1999; 18(13):1691-1706.
38. Cooper GS, Miller FW, Pandey JP. The role of genetic factors in autoimmune disease: implications for environmental research. Environ Health Perspect 1999; 107 Suppl 5:693-700.
39. Weinshenker BG. Epidemiologic strategies to detect an exogenous cause of MS. Acta Neurol Scand Suppl 1995; 161:93-99.
40. Rothman KJ. Induction and latent periods. Am J Epidemiol 1981; 114(2):253-259.
41. Fischman HR. Multiple sclerosis: a two-stage process? Am J Epidemiol 1981; 114(2):244-252.
42. Ebers GC, Sadovnick AD, Risch NJ. A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. Nature 1995; 377(6545):150-151.
43. Sadovnick AD, Armstrong H, Rice GP, Bulman D, Hashimoto L, Paty DW et al. A population-based study of multiple sclerosis in twins: update. Ann Neurol 1993; 33(3):281-285.
44. Jersild C, Fog T, Hansen GS, Thomsen M, Svejgaard A, Dupont B. Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. Lancet 1973; 2(7840):1221-1225.
45. Schrijver HM, Crusius JB, Uitdehaag BM, Garcia Gonzalez MA, Kostense PJ, Polman CH et al. Association of interleukin-1beta and interleukin-1 receptor antagonist genes with disease severity in MS. Neurology 1999; 52(3):595-599.
46. Myhr KM, Raknes G, Nyland H, Vedeler C. Immunoglobulin G Fc-receptor (FcgammaR) IIA and IIIB polymorphisms related to disability in MS. Neurology 1999; 52(9):1771-1776.
47. Evangelou N, Jackson M, Beeson D, Palace J. Association of the APOE epsilon4 allele with disease activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 1999; 67(2):203-205.
48. Acheson ED. Epidemiology of multiple sclerosis. Br Med Bull 1977; 33(1):9-14.
49. Vassallo L, Elian M, Dean G. Multiple sclerosis in southern Europe. II: Prevalence in Malta in 1978. J Epidemiol Community Health 1979; 33(2):111-113.
50. Dean G, Grimaldi G, Kelly R, Karhausen L. Multiple sclerosis in southern Europe. I: Prevalence in Sicily in 1975. J Epidemiol Community Health 1979; 33(2):107-110.
51. Leibowitz U, Kahana E, Alter M. The changing frequency of multiple sclerosis in Israel. Arch Neurol 1973; 29(2):107-110.
52. Alter M, Kahana E, Loewenson R. Migration and risk of multiple sclerosis. Neurology 1978; 28(11):1089-1093.
53. Alter M, Okihiro M, Rowley W, Morris T. Multiple sclerosis among Caucasians and Orientals in Hawaii. Neurology 1970; 20(4):399.
54. Alter M, Leibowitz U, Speer J. Risk of multiple sclerosis related to age at immigration to Israel. Arch Neurol 1966; 15(3):234-237.
55. Dean G, Elian M. Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. J Neurol Neurosurg Psychiatry 1997; 63(5):565-568.
56. Detels R, Visscher BR, Haile RW, Malmgren RM, Dudley JP, Coulson AH. Multiple sclerosis and age at migration. Am J Epidemiol 1978; 108(5):386-393.
57. Hammond SR, English DR, McLeod JG. The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain 2000; 123 (Pt 5):968-974.
58. Gale CR, Martyn CN. Migrant studies in multiple sclerosis. Prog Neurobiol 1995; 47(4-5):425-448.
59. Hammond SR, English D, de Wytt C, Maxwell IC, Millingen KS, Stewart-Wynne EG et al. The clinical profile of MS in Australia: a comparison between medium- and high-frequency prevalence zones. Neurology 1988; 38(6):980-986.
60. Alter M, Halpern L, Bornstein B. Multiple Sclerosis in Israel. Arch Neurol 1962; 7:253-263.
61. Kurland L, Reed D. Geographic and climatic aspects of multiple sclerosis: a review of current hypotheses. Am J Public Health 1964; 54:588-597.
62. Larsen JP, Kvaale G, Riise T, Nyland H, Aarli JA. Multiple sclerosis--more than one disease? Acta Neurol Scand 1985; 72(2):145-150.
63. Weinshenker BG. Progressive forms of MS: classification streamlined or consensus overturned? Lancet 2000; 355(9199):162-163.
64. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology 1996; 46(4):907-911.
65. Kremenchutzky M, Cottrell D, Rice G, Hader W, Baskerville J, Koopman W et al. The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a re-evaluation. Brain 1999; 122 (Pt 10):1941-1950.
66. Confavreux C, Aimard G, Devic M. Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. Brain 1980; 103(2):281-300.
67. Bashir K, Whitaker JN. Clinical and laboratory features of primary progressive and secondary progressive MS. Neurology 1999; 53(4):765-771.
68. McDonnell GV, Hawkins SA. Primary progressive multiple sclerosis: a distinct syndrome? Mult Scler 1996; 2(3):137-141.
69. Weinshenker BG, Bass B, Rice GP, Noseworthy J, Carriere W, Baskerville J et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain 1989; 112 (Pt 1):133-146.
70. Thompson AJ, Polman CH, Miller DH, McDonald WI, Brochet B, Filippi MM, X et al. Primary progressive multiple sclerosis. Brain 1997; 120 (Pt 6):1085-1096.
71. Weiner HL, Mackin GA, Orav EJ, Hafler DA, Dawson DM, Lapierre Y et al. Intermittent cyclophosphamide pulse therapy in progressive multiple sclerosis: final report of the Northeast

Cooperative Multiple Sclerosis Treatment Group. Neurology 1993; 43(5):910-918.
72. Goodkin DE, Rudick RA, VanderBrug MS, Daughtry MM, Schwetz KM, Fischer J et al. Low-dose (7.5 mg) oral methotrexate reduces the rate of progression in chronic progressive multiple sclerosis. Ann Neurol 1995; 37(1):30-40.
73. Prat A, Pelletier D, Duquette P, Arnold DL, Antel JP. Heterogeneity of T-lymphocyte function in primary progressive multiple sclerosis: relation to magnetic resonance imaging lesion volume. Ann Neurol 2000; 47(2):234-237.
74. Olerup O, Hillert J, Fredrikson S, Olsson T, Kam-Hansen S, Moller E et al. Primarily chronic progressive and relapsing/remitting multiple sclerosis: two immunogenetically distinct disease entities. Proc Natl Acad Sci U S A 1989; 86(18):7113-7117.
75. Weinshenker BG, Santrach P, Bissonet AS, McDonnell SK, Schaid D, Moore SB et al. Major histocompatibility complex class II alleles and the course and outcome of MS: a population-based study. Neurology 1998; 51(3):742-747.
76. Hillert J, Gronning M, Nyland H, Link H, Olerup O. An immunogenetic heterogeneity in multiple sclerosis. J Neurol Neurosurg Psychiatry 1992; 55(10):887-890.
77. Rovaris M, Bozzali M, Santuccio G, Iannucci G, Sormani MP, Colombo B et al. Relative contributions of brain and cervical cord pathology to multiple sclerosis disability: a study with magnetisation transfer ratio histogram analysis. J Neurol Neurosurg Psychiatry 2000; 69(6):723-727.
78. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. A study of 113 cases. Brain 1999; 122 (Pt 12):2279-2295.
79. Riise T, Gronning M, Klauber MR, Barrett-Connor E, Nyland H, Albrektsen G. Clustering of residence of multiple sclerosis patients at age 13 to 20 years in Hordaland, Norway. Am J Epidemiol 1991; 133(9):932-939.
80. Eastman R, Sheridan J, Poskanzer DC. Multiple sclerosis clustering in a small Massachusetts community, with possible common exposure 23 years before onset. N Engl J Med 1973; 289(15):793-794.
81. Wikstrom J. Studies on the clustering of multiple sclerosis in Finland. Riv Patol Nerv Ment 1976; 97(4):199-204.
82. Hoffman RE, Zack MM, Davis LE, Burchfiel CM. Increased incidence and prevalence of multiple sclerosis in Los Alamos County, New Mexico. Neurology 1981; 31(11):1489-1492.
83. MacGregor HS. Multiple sclerosis clusters in Florida. J Epidemiol Community Health 1991; 45(1):88.
84. Hader WJ, Irvine DG, Schiefer HB. A cluster-focus of multiple sclerosis at Henribourg, Saskatchewan. Can J Neurol Sci 1990; 17(4):391-394.
85. Armon C, Daube JR, O'Brien PC, Kurland LT, Mulder DW. When is an apparent excess of neurologic cases epidemiologically significant? Neurology 1991; 41(11):1713-1718.
86. Riise T. Cluster studies in multiple sclerosis. Neurology 1997; 49(2 Suppl 2):S27-S32.
87. Granieri E, Casetta I, Tola MR, Govoni V, Paolino E, Malagu S et al. Multiple sclerosis: does epidemiology contribute to providing etiological clues? J Neurol Sci 1993; 115 Suppl:S16-S23.

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ORIGINAL ARTICLE

Efficacy of Leukotriene Modifiers for the Treatment of Persistent Asthma in Children

Duarte G. Machado*

ABSTRACT The purpose of this study was to evaluate the use of the leukotriene modifiers (LTMs), zafirlukast and montelukast, in children with asthma managed by an inner city pediatric pulmonary practice. A retrospective chart review was done of children 6 years of age with persistent asthma seen at Connecticut Children's Medical Center and prescribed LTM drugs. Eighty-three children whose asthma control was adequately assessed both before and after addition of a LTM to his/her treatment regimen was included in the study. There were statistically significant improvements in several parameters of asthma control following initiation of LTM use, including provider assessment score ($p = 0.0005$), number of hospitalizations and unscheduled visits (clinic or emergency department; $p < 0.0001$), use of oral corticosteroids ($p = 0.0015$), spirometry severity score ($p = 0.0015$), and spirometry test results (FEV1, FEV1/FVC, FEF, FEF25-75%; $p < 0.005$ for all). These results suggest that montelukast and zafirlukast help to improve asthma control in young patients with persistent asthma.

INTRODUCTION

Asthma is the most common chronic illness of childhood in the United States, affecting an estimated 4.8 million children, or 10% of the pediatric population (1). In 1997, the National Heart, Lung and Blood Institute (NHLBI) issued a report of recommended guidelines for the diagnosis and management of asthma, which included the use of inhaled corticosteroids (ICS) as the primary controller for persistent disease (2). Asthma is classified as persistent when symptoms occur >2 times a week and nighttime symptoms occur >2 times a month (2). According to the above guidelines, the standard therapeutic regimes for children with mildly persistent asthma include a daily anti-inflammatory (either ICS, cromolyn or nedocromil) or sustained-release theophylline, while a short-acting bronchodilator, such as an inhaled beta2-agonist, is used for quick relief of acute attacks.

However, the use of ICS in pediatric asthma raises several concerns. ICS can adversely affect several systems of the body and lead to adrenal axis suppression and growth inhibition (3). Prepubertal children are at greatest risk for experiencing growth suppression induced by ICS (4). In addition, ICS may be complicated to administer because proper metered-dose inhaler technique, spacers, and multiple doses during the day may be required (5). Other controller medications for persistent asthma also have undesirable characteristics. Patients who use beta2-agonists may experience anxiety, tremor, restlessness, irritability, insomnia, tachycardia, elevated blood pressure, paradoxical bronchospasm, and hypokalemia (6). Moreover, theophylline has a narrow therapeutic range and its use in children has been associated with restlessness, agitation, diuresis, and fever (7). Since rates of theophylline absorption and metabolism vary widely among patients and in the same patient at different times, regular monitoring of plasma drug concentration is necessary for optimal control (7).

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Table 1. Comparison of Leukotriene Modifiers (11,13,16,17,18)

Name	zafirlukast (Accolate)	zileuton (Zyflo)	montelukast (Singulair)
Mechanism of action	selective and competitive cys-LT1 receptor antagonist	selectively and reversibly inhibits the 5-lipoxygenase pathway	selectively blocks the cys-LT1 receptor
Age Indication	>/= 5 years	>/= 12 year	>/= 2 years
Usual dose	20 mg tablet bid (12 yrs of age); 10 mg tablet bid non-flavored mini tablet (5-11 yr old children),	600 mg tablet qid (12 yrs of age)	10 mg tablet Qhs (15 yrs of age); 5 mg chewable tablets (6-14 yr children); 4 mg cherry-flavored chewable tablets (2-5 yr old children)
Warnings	? Churg-Strauss syndrome?	elevated LFTs, monitor LFTs at initiation and during therapy	? Churg-Strauss syndrome?
Dosing considerations	empty stomach (1 hr before or 2 hrs after); food decreases absorption by 40%	none	none
Drug interactions	warfarin (increase PT), phenytoin, carbamazepine	warfarin (increase PT) theophylline (increase) propranolol (increase)	none

Abbreviations: cys-LT1, cysteinyl leukotriene; LFT, liver function test; PT, prothrombin time

The newest class of drugs, leukotriene modifiers, may be an effective alternative, especially for children. Produced and released from eosinophils, mast cells, and alveolar macrophages, cysteinyl leukotrienes are thought to play a direct role in the pathogenesis of asthma. Through activation of at least two seven transmembrane-spanning receptors, CysLT1 and CysLT2, leukotrienes can stimulate mucus formation and secretion, edema, and contraction and proliferation of smooth muscle cells (8). These effects are associated with cellular infiltration of the airways and decreased mucociliary transport. Cysteinyl leukotrienes are also classified as strong bronchoconstrictors as they are up to 1,000 times more potent than histamine (9). There is now sufficient evidence to suggest that drugs that target and modify the leukotriene pathway have the potential to alter the pathogenesis of asthma (10).

To date, three chemically distinct LTMs are available by prescription. Zafirlukast (Accolate ; Astra Zeneca Pharmaceuticals, Wilmington, DE) was approved in September 1996, followed by zileuton (Zyflo ; Abbott Laboratories, Chicago, IL) in January 1997, and montelukast (Singulair ; Merck & Co., Inc. Whitehouse Station, NJ) in February 1998 (11-13). LTMs comprise two pharmacologic classes of compounds. Zileuton inhibits the enzyme 5-lipoxygenase, thereby preventing the biosynthesis of leukotrienes. In contrast, zafirlukast and montelukast are CysLT1 selective receptor antagonists that block the binding of leukotrienes to this receptor type (14). Importantly, these antagonists selectively block binding to only the CysLT1 receptor, which is highly expressed in lung smooth muscle cells and interstitial lung macrophages (as well as the spleen and peripheral blood leukocytes including eosinophils) (15). In contrast, the CysLT2 receptor is not expressed in the lung at all, but instead in the heart, adrenal medulla, placenta, and peripheral blood leukocytes (15). Important features of the three drugs are given in Table 1.

The clinical trials that led to the FDA approvals of the different LTMs had all demonstrated improvements in several parameters of asthma control to some degree. In a study of asthmatic adults, it was found that patients treated with zafirlukast experienced a significant decrease in nocturnal symptoms, a significant improvement in forced expiratory volume in one second (FEV1), and a 30% or greater improvement of asthma symptoms compared to those given placebo (19). Improvement in asthma symptoms was found to occur within hours after administration of zafirlukast (11). In these studies, zafirlukast was used alone or in combination with rescue medication as needed. Approval for use in patients 12 years of age was granted

two years later based on these studies. Studies of zafirlukast use in children aged five to 11 years were subsequently performed to assess whether or not similar results as found in adult patients could be achieved. A four-week, double blind trial and 52 week open-label extension of children with mild to moderate asthma concluded that zafirlukast was generally well-tolerated and effective (20). A 10 mg pediatric formulation of zafirlukast was subsequently granted approval for use in patients 5 years of age.

Use of montelukast in 6 to 14-year-old children was first addressed in an eight-week, double blind trial completed in April 1996. It was found that montelukast significantly decreased daily as-needed use of agonists, the mean percentage of days with an asthma exacerbation, and the percentage of patients who experienced at least one asthma exacerbation (5). Montelukast also allowed for significant tapering of inhaled corticosteroids. This study provided sufficient evidence to allow for the approval of montelukast two years later for use in children 6 years of age. A 4 mg tablet was approved for patients 2 years of age in March 2000 following extrapolation of the efficacy of montelukast (13). Clinical studies have now been extended to patients <6 years of age, for whom montelukast was found to significantly protect against bronchoconstriction following a cold, dry air challenge (21). Moreover, both zafirlukast and montelukast have been demonstrated to provide modest but statistically significant improvement in lung function when used as monotherapy in children as young as 6 years of age (5,22,23).

Although LTMs have been shown to be safe and effective in clinical trials, the optimum use of LTMs in asthma management is still evolving (24). Since LTMs had only become commercially available shortly before the 1997 NHLBI guidelines were drafted, the guidelines were based on limited published data examining their use in asthma treatment (25). Moreover, there are still inadequate data regarding the efficacy of regular use of LTMs in children with chronic asthma (5). The current study was undertaken to evaluate the use of LTMs in the treatment of children with asthma managed by a pediatric pulmonary practice in Connecticut. Our hypothesis was that addition of LTM therapy would result in improved control of symptoms and lung function in children with chronic persistent asthma.

MATERIALS AND METHODS

Study Design

All patients greater than or equal to 6 years of age with persistent asthma who had at least two asthma-related clinical encounters with the pediatric pulmonary practice at Connecticut Children's Medical Center (CCMC) from September 1996 to March 2000 were eligible for inclusion in this study and were identified using a computerized database. The cutoff date was selected as the date when zafirlukast gained FDA approval for use in children. Since zileuton was not used in this practice, all patients were prescribed either montelukast or zafirlukast.

The medical records of 279 children with asthma at CCMC were specifically reviewed for LTM use. Pre and post clinical information was then compiled from their charts. Pre was defined as the first encounter in which a clinical assessment, which included exercise tolerance, spirometry and other pulmonary function tests performed by a pediatric pulmonology technician, concluded with a prescription for a LTM. Since the effects of LTMs were previously noted to occur as early as within one day after the first dose (21), post was defined as the first clinical encounter since initiation of LTM therapy.

Subjects

Of the 279 children, 113 met the initial inclusion criterion of having used a LTM. Of these, 30 children were excluded from the study for one or more of the following reasons: (a) the majority of the pre and post-treatment information was not available, (b) they were already taking LTMs at the first clinical encounter via prescription from another provider, or (c) they had failed to keep one or more follow-up appointments at the clinic such that post-treatment asthma control could not be ascertained. The final sample size was therefore 83 children.

The 83 patients with complete data for review had a mean age of 10.6 ± 0.3 years (ranging from 5 to 16 years). More subjects were in the montelukast group (72, or 86.7%) compared to the zafirlukast group (11, or 13.3%). The fewer subjects in the latter group reflected an early concern in using zafirlukast because it is also an inhibitor of the CYP450 isoenzyme CYP3A4, such that zafirlukast can increase concentrations of certain concomitant medications, including theophylline (26). In addition, the greater number of children in the montelukast group was not biased by the 30 children who were excluded since most of these were also prescribed montelukast (26, or 86.7%) rather than zafirlukast (4, or 13.3%).

Asthma severity was classified as severe persistent for 42 patients (50.6%), moderate persistent for 32 (38.6%), and mild persistent for 9 (10.8%), respectively. Sixty (87%) subjects reported allergies or were skin-tested positive for one or more allergens. Moreover, sixty (74%) subjects, not necessarily the same ones, also had a diagnosis of allergic rhinitis. At

Table 2. Pre and Post Assessment Score Ratings

Name	0	1	2	3
Frequency of Nocturnal awakenings	none	<1/week	>=1/week	
Exercise Tolerance	good	limited	severly limited	
School absenteeism	none	<=10/year	>10/year	
Provider's assessment of asthma control	satisfactory	marginal	unsatisfactory	
Rescue use of short acting bronchodilators	none	<=1/week	> 1/week	
Number of hospitalizations or sick visits	none	1/year	2/year	>=3/year
Use of oral steroids in past six months	no	yes		
Spirometry Severity	normal	mild	moderate	severe

Table 3. Patient Demographics

	Overall	Male	Female	Level of Significance
Number	83	40	43	
Age (yrs)	10.6 ± 0.3	10.3 ± 0.3	10.9 ± 0.3	
Allergies (n=69)*	60 (87%)	33 (94%)	27 (79%)	
Allergic rhinitis (n=81)*	60 (74%)	27 (69%)	33 (79%)	
Spirometry severity score		1.4 ± 0.12	0.9 ± 0.11	0.0014
FEV1/FVC ratio		76.6 ± 1.4	82.7 ± 1.0	0.0005
FEF (25-75%)		62.2 ± 2.9	80.8 ± 3.3	<0.0001

* Only those with a $p < 0.05$ are reported here
* This information was not available for all patients in study

baseline, there were no significant differences in the numbers of male and female subjects or in their mean ages (Table 3). However, there were significant differences in spirometry severity score, FEV1/FVC ratio, and FEF 25-75% between the males and females in this study.

Outcome Measures

The demographic information collected for each subject included age, sex, asthma severity as defined by NHLBI criteria (27), allergy skin test results, the diagnosis of allergic rhinitis, and LTM use. Assessment of asthma control was done pre and post introduction of LTM therapy. The measures recorded for each patient included frequency of nocturnal awakenings, exercise tolerance, school absenteeism, provider's assessment of asthma control, rescue use of short acting bronchodilators, number of hospitalizations or sick visits, and spirometry severity. A score was assigned for each of these parameters as shown in Table 2. Additional spirometry data obtained for each subject included FEV1, FEV1 to forced vital capacity (FEV1/FVC) ratio, peak expiratory flow (PEF), and forced expiratory flow measured between 25% and 75% of the vital capacity (FEF 25-75%). Documented reference standards (28) were used to determine baseline lung functions. Pulmonary function tests were standardized and performed by the same technician for all subjects, and results were recorded as percentages (actual/ reference x 100).

The dose and type of ICS used by each subject were also noted before treatment with a LTM and at the follow-up appointment after initiating treatment. Each dose was standardized by conversion to beclomethasone dipropionate (BDP) equivalence units (mcg) as described (29) Finally, it was also noted if and why LTM therapy was discontinued.

Statistical Analysis

All statistical analyses were performed using a computer program (StatView). The efficacy of LTMs was assessed by comparing changes in pre and post outcome measures in the form of scores, percentages, and BDP dose. For the analyses of spirometry data, each percent change was tabulated as the change in percent predicted values. Statistical comparisons were performed using paired t-tests for the pre and post comparisons, and unpaired t-tests with Bonferonni corrections for the specific LTM comparisons (given as $p < 0.05/ n$, where n is the number of comparisons).

Table 4. Before and After Assessment Scores and Pulmonary Function Test Results* with Standard Deviations for Each

Variable	Pre	Post	Change	Level of Significance
Frequency of nocturnal awakenings	1.0 ± 0.11	0.7 ± 0.11	-0.3	0.07
Exercise tolerance	0.5 ± 0.07	0.4 ± 0.06	-0.1	0.26
School absenteeism	0.9 ± 0.10	0.7 ± 0.10	-0.2	0.45
Provider's assessment of asthma control	1.0 ± 0.10	0.6 ± 0.09	-0.4	0.0005
Rescue use of short acting bronchodilators	1.0 ± 0.12	0.7 ± 0.11	-0.3	0.07
Number of hospitalizations or sick visits	0.9 ± 0.14	0.2 ± 0.06	-0.7	<0.0001
Inhaled corticosteroid dose+	1683.5 ± 136.5	1636.2 ± 130.0	-47.3	0.72
Use of oral steroids in past six months	0.5 ± 0.05	0.3 ± 0.05	-0.2	0.0015
Spirometry severity	1.3 ± 0.12	1.0 ± 0.12	-0.3	0.0015
FEV1	82.0 ± 2.05	87.8 ± 2.01	+5.7	0.0002
FEV1/FVC	78.0 ± 1.19	81.5 ± 1.28	+3.5	0.0033
PEF	91.3 ± 2.39	98.6 ± 2.61	+7.3	0.0039
FEF (25-75%)	67.8 ± 3.36	76.0 ± 3.20	+8.2	0.0042

*Based on the % reference according to each individual patient's age, sex, and height.
+This parameter is not a score but the actual dose converted into beclomethasone units (mcg)
d Statistically significant p-values accounting for non-independence

Measurements are expressed as means, standard error of the mean, and a p value of < 0.05 was considered significant.

RESULTS

Overall treatment comparison. Statistically significant treatment effects of montelukast and zafirlukast were noted in 8 of the 13 parameters studied. Notably, there were significant improvements in providers' assessment of asthma control and in the number of hospitalizations or sick visits (Table 4). There were also significant improvements in all of the spirometry parameters measured, including the spirometry severity score (Table 4).

Furthermore, LTM therapy resulted in a statistically significant decline in the use of oral steroids during the past six months. About half (50.6%) of the patients in this study had severe persistent asthma, and naturally more of these patients (66.6%) utilized oral steroids than patients with moderate (37.5%) or mild (22.2%) persistent asthma. After LTM treatment, only 35.7% of patients with severe persistent asthma, for example, were on oral steroids, indicating that almost half of this group of patients was tapered off of oral steroids within six months. In contrast, there appeared to be no effect of LTM treatment on ICS dosage overall, such that twenty patients had their dosage increased, 21 patients had their dosage decreased, and 42 patients remained on the same dosage.

Subgroup analysis I: Comparison of continued and discontinued group. Fifteen patients (18.1%) discontinued their LTM treatment but were included in all of the analyses as an intention-to-treat. The reasons for discontinuing LTMs were varied, with the two most important being lack of effectiveness (n = 7) and side effects such as headaches (n = 3). Other reported reasons for discontinuing were difficulty of administration (n = 2; disliked the taste and disliked swallowing the pills), switching from one LTM to the other (n = 2; switched from montelukast to zafirlukast and vice-versa), or problems with compliance (n = 1). There were no statistically significant differences between the continued (n = 68; 81.9%) and discontinued groups in age, asthma severity, presence of skin allergies

Table 5. Patient Comparison in Relation to Specific LTM Used (Pre-treatment)

	Montelukast	Zafirlukast
Age (yrs) (range 5-16 yrs) (p = 0.0235)	10.4 ± 0.24	11.9 ± 0.51
Beclomethasone dose of ICS (p = 0.0001)	1479 ± 91.9	2841 ± 266.6
Number of patients	72 (86.7%)	11 (13.3%)
Mild asthma (n = 9)	9	0
Moderate asthma (n=32)	26	6
Severe asthma (n=42)	37	5
Number of points that discontinued prescribed LTM	8	7

and/ or allergic rhinitis, or any pre and post outcome parameter (data not shown).

Subgroup analysis II: Comparing montelukast and zafirlukast. The characteristics of subjects prescribed montelukast versus zafirlukast differed in only a few parameters as shown in Table 5. Patients who used montelukast used statistically significant lower dosages of ICS at the start of treatment compared with zafirlukast users, although there was no overall change in ICS dose after initiation of LTMs in either group. However, there were no statistically significant differences between the two groups in terms of night symptoms, exercise tolerance, school absenteeism, providers' assessment, use of short acting bronchodilators, number of hospitalizations or sick visits, spirometry severity score, or any spirometry measurements (data not shown). It is unknown why zafirlukast was not prescribed for any patient with mild persistent asthma.

DISCUSSION

Leukotriene antagonists represent a new class of asthma therapy, and it is yet unclear how best to utilize LTMs. This study evaluated outcomes that might be expected with the integration of LTMs into the management of children with persistent asthma.

Main Findings

The results from the present study indicate that the greatest benefit in terms of asthma improvement to be gained from LTM use is in pulmonary function. All spirometry parameters increased following the use of montelukast or zafirlukast. Moreover, significant treatment effects were noted in four other parameters: physician assessment, number of hospitalizations and sick visits (both emergency department and clinic visits), use of oral steroids, and spirometry severity score. LTMs may thus help to reduce the asthma hospitalization rate among children 14 years of age living in Hartford, which is currently much higher (at 57.1 per 10,000) than the rates in the entire state and in the US-37 (30). Likewise, utilization of LTMs has the potential to help reduce the rate of annual asthma emergency room visits among children 14 years of age, which are currently higher in Hartford (at 256 per 10,000) than anywhere else in the state (30).

The frequency of nocturnal awakenings did not decline significantly, a result consistent with previous studies using both zafirlukast19 and montelukast.5 However, other symptom scores, namely 2 agonist requirement, days missed from school and ICS dose, were not affected by LTM treatment, contradicting previous studies (31,32). For example, one study found that LTM use allowed for an existing ICS dose to be halved without deterioration in symptoms or lung function (32). This result had provided the impetus for initiating LTM treatment in some of the children in this study who had experienced growth and/or height retardation due to ICS use. The present study, however, found no overall change in ICS dose most likely because an equal number of patients had increases or decreases in their dosage during addition of LTMs to their existing treatment.

The 1997 NHLBI asthma treatment guidelines specify the use of LTMs only as alternatives to low-dose inhaled corticosteroids, disodium cromoglycate, nedocromil, or sustained-release theophylline in patients with mild persistent asthma (2). However, since then, studies have shown that LTMs are efficacious over a wider range of asthma severity. One study found that zafirlukast given to patients > 12 years with continuing asthma symptoms despite a high dose of ICS resulted in a significant reduction in the risk of worsening asthma symptoms as compared to placebo (33). Similarly, it has been reported that montelukast may confer additional improvement of asthma control when used concurrently with ICS in moderate to severe asthma (31). The present study supports these findings since addition of LTMs to the treatment regimen of patients with moderate to severe asthma and already on ICS (with the exception of two subjects) led to a significant improvement in a majority of assessment parameters. This finding points to the importance of LTMs in helping to control mediators of asthma inflammation (i.e. leukotrienes) that cannot be controlled with corticosteroids alone.

Additional Findings

The data for the continued and discontinued groups are similar to those found in other studies. The Mediplus database, which includes the prescription records of 123 patients on montelukast, indicate that less than 25% of these patients discontinued the drug (34). Unfortunately, the exact reasons for discontinuing in that study are not available, although it is speculated that the reasons may include lack of effectiveness and/or adverse effects. The same reasons for cessation were found in the present study, with lack of effectiveness as the reason for 7 of 15 patients.

A comparison of montelukast and zafirlukast use in this study implies that these two LTMs have similar efficacy and tolerability. Since previous studies of LTMs have focused on only a single LTM, such comparisons are lacking in the literature. It has been proposed that montelukast, with its lack of dosing considerations and drug interactions (35) may be better tolerated than zafirlukast.

Characteristics and Limitations

Baseline measures indicate that the patient population examined in this study is not characteristic of all asthma patients. First, the number of children with asthma in Hartford is significantly higher than the national average. Second, as reported in other studies (36), there is a referral bias in this population such that the children seen by the pediatric pulmonary practice are more severely asthmatic. In this study, 51% of the patients had severe asthma while only 10% had mild asthma. This trend is nearly opposite of estimates of national asthma rates in which 60% of children have mild asthma, 30% have moderate asthma, and 10% have severe asthma (37). A population of children more equal with regards to the type of persistent asthma would perhaps be a more ideal population to have studied. However, it is already recommended that LTMs could or should be considered as alternative long term controllers in patients with mild persistent asthma (2). Moreover, it has been suggested that patients at all levels of asthma severity and requiring high doses of inhaled corticosteroids should be given a trial of LTMs (38). Thus, this study sample is valuable given that the full therapeutic potential range of LTMs is as yet unconfirmed.

Another baseline characteristic worth mentioning is the statistically different spirometry results for males and females. The reason why males performed worse on lung function tests overall is unknown but may be related to the higher degree of bronchial lability (39) and higher occurrence of respiratory infections among males compared to females (37). Another reason may be that asthma is generally worse in boys than in girls. It has also been suggested that pulmonary function differs between male and female children due to gender differences in both mechanical properties of the lung and the inflammatory process (40).

Improvements seen in this cohort of patients may not be solely due to addition of LTMs to their treatment regimens. Confounding variables include concomitant variations in all other medications taken by a patient during therapy with a LTM. For example, twenty of the patients in this study had their ICS dose increased at the same time as a LTM was added to their list of daily medications. It is thus not possible to accurately know how much the observed improvements were due to the increased ICS doses. Nonetheless, these results suggest that montelukast and zafirlukast are effective in treating persistent asthma when used concomitantly with an ICS. Another limitation is selection bias since the characteristics of the 30 patients excluded from the study are unknown and may have influenced the overall results of this study.

Future Studies

In summary, the results from this study thus indicate that CysLT1 selective receptor antagonists are clinically effective in the treatment of children with persistent asthma. This conclusion is most applicable to montelukast given the relatively small number of patients in the zafirlukast group. Additional efficacy data from long-term studies are required to establish the position of montelukast and zafirlukast in asthma treatment guidelines. More research will also be needed to assess the long-term efficacy and safety of LTMs.

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REFERENCES

1. Adams PF, Marano MA. Current estimates from the National Health Interview Survey, 1994. Vital Health Stat 10:94; 1995.
2. National Asthma Education and Prevention Program. Highlights of the Expert Panel Report II: Guidelines for the Diagnosis and Management of Asthma. Bethesda, MD: National Institutes of Health. Publication No. 97-4051A; 1997.
3. Allen DB. Do inhaled steroids impair growth? It all depends. J Respir Dis 19:1023-1036; 1998.
4. Lemanske RF, Allen DB. Choosing a long-term controller

medication in childhood asthma. Am J Respir Crit Care Med 156:685-687; 1997.
5. Knorr B, Matz J, Bernstein JA, et al. Montelukast for chronic asthma in 6- to 14- year-old children: a randomized, double-blind trial. Pediatric Montelukast Study Group. JAMA 279:1181-1186; 1998.
6. Fraser CM, Nelson HS, Middleton E Jr. Adrenergic agents. In: Middleton E Jr, Reed CE, Ellis EF, et al, editors. Allergy: Principles and Practice. St Louis, MO: Mosby-Year Book, 1993.
7. Powell EC, Reynolds SL, Rubenstein JS. Theophylline toxicity in children: a retrospective review. Pediatr Emerg Care 9(3):129-33; 1993.
8. Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. N Engl J Med 340:197-206; 1999.
9. Korenblat PE. The role of antileukotrienes in the treatment of asthma. Ann Allergy Asthma Immunol 86(Suppl):31-39; 2001.
10. Weisberg SC. Pharmacology of asthma in children, with special reference to leukotriene receptor antagonists. Pediatr Pulmonol 29:46-61; 2000.
11. Accolate. Manufacturer's prescription information. Zeneca Pharmaceuticals: Carolina, Puerto Rico; 2001.
12. Zyflo Filmtab. Manufacturer's prescription information. Abbott Laboratories: Chicago, IL, USA; 2000.
13. Singulair. Manufacturer's prescription information. Merck & Co, Inc: Whitehouse Station, NJ, USA; 2001.
14. Rachelefsky G, Bruton S, Barnes PJ, et al. Airway remodeling: The inflammatory response in asthma therapy. Dialogues in Asthma Management 1:1-9; 1998.
15. Evans JF. Cysteinyl leukotriene receptors. Prostaglandins Other Lipid Mediat 68-69:587- 597; 2002.
16. Adkins JC, Brogden RN. Zafirlukast. A review of its pharmacology and therapeutic potential in the management of asthma. Drugs 55:121-144; 1998.
17. McGill KA, Busse WW. Zileuton. Lancet 348:519-524; 1996.
18. Lazarus SC, Lee T, Kemp JP, et al. Safety and clinical efficacy of zileuton in patients with chronic asthma. Am J Manag Care 4:841-848; 1998.
19. Spector SL, Smith LJ, Glass M. Effects of 6 weeks of therapy with oral doses of ICI 204,219, a leukotriene D4 receptor antagonist, in subjects with bronchial asthma. ACCOLATE Trialists Group. Am J Respir Crit Care Med 150:618-623; 1994.
20. Lampl KL, Dixon SJ, Bonuccelli CM. Long-term safety and efficacy of zafirlukast (Accolate) in pediatric patients with mild-to-moderate asthma. Am J Respir Crit Care Med 159(3):S138; 1999.
21. Bisgaard H, Nielsen KG. Bronchoprotection with a leukotriene receptor antagonist in asthmatic preschool children. Am J Respir Crit Care Med 162:187-190; 2000.
22. Kemp JP, Dockhorn RJ, Shapiro GG, et al. Montelukast once daily inhibits exercise- induced bronchoconstriction in 6-14-year old children with asthma. J Pediatr 133(3):424-8; 1998.
23. Pearlman DS, Lampl KL, Dowling PJ Jr., Miller CJ, Bonuccelli CM. Effectiveness and tolerability of zafirlukast for the treatment of asthma in children. Clin Ther 22(6):732-47; 2000.
24. Kemp JP. Guidelines update: where do the new therapies fit in the management of asthma? Drugs 59 Suppl 1:23-28; discussion 43-5; 2000.
25. Strek ME. Consensus guidelines for asthma therapy. Ann Allergy Asthma Immunol 86(Suppl):40-44; 2001.
26. Katial RK, Stelzle RC, Bonner MW, Marino M, Cantilena LR, Smith LJ. A drug interaction between zafirlukast and theophylline. Arch Intern Med 158(15):1713-5; 1998.
27. National Asthma Education and Prevention Program. Highlights of the Expert Panel Report II: Guidelines for the Diagnosis and Management of Asthma. Bethesda, MD: National Institutes of Health. Publication No. 97-4051A; 1997. Figure 1-3.
28. Taussig LM, Chernick V, Wood R, Farrell P, Mellins RB. Standardization of lung function in testing in children. Proceedings and recommendations of the GAP conference committee, Cystic Fibrosis Foundation. J Pediatrics 97:668-676; 1980.
29. National Asthma Education and Prevention Program. Highlights of the Expert Panel Report II: Guidelines for the Diagnosis and Management of Asthma. Bethesda, MD: National Institutes of Health. Publication No. 97-4051A; 1997. Tables 3-5b and 3-5c.
30. Connecticut Department of Public Health: Asthma in Connecticut. Hartford, CT; 2001.
31. Lipworth BJ: Systemic adverse effects of inhaled corticosteroid therapy. A systemic review and meta-analysis. Arch Intern Med 159:941-955; 1999.
32. Tamioki J, Kondo M, Sakai N, et al. Leukotriene antagonist prevents exacerbation of asthma during reduction of high-dose inhaled corticosteroids. Am J Respir Crit Care Med 155:1235-1240; 1997.
33. Tashkin DP, Nathan RA, Howland WC, et al. An evaluation of zafirlukast in the treatment of asthma with exploratory subset analyses. J Allergy Clin Immunol 103 (2 Pt 1):246-254; 1998.
34. Price D. Tolerability of montelukast. Drugs 59 Suppl 1:35-42; 2000.
35. Storms W, Michele TM, Knorr B, et al. Clinical safety and tolerability of montelukast, a leukotriene receptor antagonist, in controlled clinical trials in patients aged > or = 6 years. Clin Exp Allergy 31(1):77-87; 2001.
36. Ruurd JR, Gerritsen J, Van Aalderen WMC, et al. Risk factors for the persistence of respiratory symptoms in childhood asthma. Am Rev Respir Dis 148:1490-1495; 1993.
37. Canny GJ, Levison H. Asthma. In: Loughlin G, Levison H, editors. Respiratory Diseases in Children: Diagnosis and Management. Baltimore, MD: Williams and Wilkins,1994.
38. Wenzel SE. Antileukotriene drugs in the management of asthma. JAMA 280:2068-69; 1998.
39. Verity CM, Ven Heule B, Carlswell F, Hughes AO. Bronchial lability and skin reactivity in siblings of asthmatic children. Arch Dis Child 42:542-548; 1984.
40. Gold DR, Wypij D, Wang X, et al. Gender- and race-specific effects of asthma and wheeze on level and growth of lung function in children in six U.S. cities. Am J Respir Crit Care Med 149:1198-1208; 1994.

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ORIGINAL ARTICLE

Evaluation of tumor viability in Post radiation therapy pediatric brain tumors with ^{99m}Tc-glucoheptonate single photon emission computed tomography (SPECT)

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ABSTRACT Brain single photon emission computed tomography (SPECT) with ^{99m}Tc-glucoheptonate, a blood brain barrier imaging agent, is rapidly regaining interest after it has been shown that the uptake of tumor seeking agents like thallium, tetrofosmin, sestamibi and pertechnate by brain tumors is solely dependent on disruption of the blood brain barrier. Therefore, the use of ^{99m}Tc-glucoheptonate may yield the same diagnostic information as other agents such as the much more expensive ^{99m}Tc-sestamibi. The purpose of the study was to evaluate ^{99m}Tc-glucoheptonate as an imaging agent for recurrent primary brain tumors in children. Methods: Fifty-one patients aged 5-18 years were evaluated for tumor recurrence following radiotherapy for primary malignant brain tumors, using brain single photon emission computed tomographies (SPECT) with ^{99m}Tc-Glucoheptonate. Contrast enhanced computerized tomography (CT) of brain was performed in all patients within ± 1 week of brain SPECT as a diagnostic standard and compared. Results: Recurrent tumors showed avid ^{99m}Tc-glucoheptonate concentration and a high ^{99m}Tc-glucoheptonate retention index (6.06 ± 1.41) compared with post radiation gliosis, which showed no ^{99m}Tc-glucoheptonate concentration over the affected site and had a ^{99m}Tc-glucoheptonate retention index of 1.10 ± 0.18 (p=0.001). ^{99m}Tc-glucoheptonate SPECT had a sensitivity of 79.48% and a specificity of 91.66% when compared with contrast-enhanced computed tomography as a gold standard. However, this technique did not show good performance in the differential diagnosis of lesions in posterior fossa. Conclusion: This study suggests that ^{99m}Tc-glucoheptonate brain SPECT can be used as a sensitive and specific diagnostic test to differentiate recurrent tumor from post radiation gliosis, with the exception of tumors located in posterior fossa. Further studies should address this limitation before definite protocols are established. Key words: Glucoheptonate, Fanbeam collimator, Glucoheptonate retention index, post radiation gliosis

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Survivors of brain tumors in childhood are at substantial risk of increased morbidity and late mortality. Five-year survivors of brain tumors are 13 times more likely to die than healthy age and sex matched controls. Tumor recurrence remains the single most common cause of late death, accounting for about 70% of the cases (1,2). Unfortunately, its clinical presentation can resemble that of post radiation necrosis (3). If

specific radiological features are not prominent, an elapse of time often occurs before investigations based on anatomical imaging allow to reach a conclusive diagnosis. This can be avoided by utilizing a functional imaging study. Functional imaging demonstrating blood flow and tissue metabolism can greatly help in differentiating a tumor, with increased blood flow and metabolism, from a post-radiation necrotic mass. The most often performed studies of this type for brain imaging are single photon emission tomography (SPECT) using thallium, sestamibi or tetrofosmin, and positron emission tomography using flurodeoxyglucose as a radiotracer. Tumoral lesions and normal brain tissue have different uptake properties for these tracers (4,5,6,7). The use of ^{99m}Tc-glucoheptonate (^{99m}Tc-GHA), an early brain SPECT tracer, has been disregarded over the years due to concerns that its accumulation in the tumors could be attributed to disruption of the blood-brain barrier caused by the tumor, rather than active extraction of the tracer in relation to tumor metabolism. Newly introduced tracers, such as 201Thallium in the late seventies and technetium-based thallium analogs in the mid eighties, were shown to accumulate in viable myocardium and became increasingly used for brain tumor imaging under the assumption that their uptake was independent of blood-brain barrier disruption. However, it has been suggested that disruption of the blood-brain barrier is a necessary condition for the uptake of any tumor seeking agent. Plain pertechnate, whose uptake solely dependes on disruption of the blood-brain barrier by the tumor, has been suggested to yield the same clinical information as the much more costly ^{99m}Tc-sestamibi (8,9). In this regard, there is a lack of studies testing the performance of ^{99m}Tc-GHA in pediatric patients with brain tumors. ^{99m}Tc-GHA is more economic than tetrofosmin, sestamibi or thallium, and is easily radiolabeled with technetium (^{99m}Tc) in a standard nuclear medicine pharmacy. The aim of the study was to assess if ^{99m}Tc-GHA can be used as a tumor-seeking substance for the diagnosis of recurrent brain tumors in childhood by functional imaging. We examined the performance of ^{99m}Tc-GHA in 51 patients with a previous diagnosis of pediatric brain tumors who were referred for evaluation of disease status by brain SPECT.

METHODS

Subjects

We recruited the patients for this study at the Nuclear Medicine Department, All India Institute of Medical Sciences, New Delhi, between 1998 and 2001. Eligible patients were up to 18 years old, had anatomopathological diagnosis of primary brain tumor and received surgical treatment and postoperative radiotherapy. The patients were followed up at the Radiotherapy Cancer Clinic and referred to the Nuclear Medicine Department for brain imaging studies. Patients with undetermined tumor histology or who did not complete radiotherapy treatment were not included in the study. Out of 51 patients (33 male and 18 female) falling into the inclusion criteria and evaluated with ^{99m}Tc-GHA brain SPECT, 10 had medulloblastoma, 4 had ependymoma, one had dysgerminoma, one had atypical meningioma, one had invasive pituitary adenoma, 24 had low-grade glioma, 4 had glioblastoma multiforme (GBM) and 6 had anaplastic astrocytoma. For the purpose of this study, the 4 cases of GBM and 6 cases of anaplastic astrocytoma were grouped as high grade tumors, and tumor of all other histology were considered as low grade tumors. All studied subjects received postoperative radiotherapy course 4-38 months prior to brain imaging study with a mean elapsed time of 12.6 months from the end of the treatment to the brain imaging study, and were clinically followed for a 10 - 35 month period (mean 18.2 months) after the brain SPECT. Brain contrast-enhanced computerized tomography (CT) was used as a gold standard for the diagnosis of tumor recurrence, and was performed in all subjects within +1week of the brain SPECT study. A repeated Brain SPECT using Tc-^{99m}-Tetrofosmin as tumor seeking agent was performed in patients with positive CT but negative ^{99m}Tc-GHA brain SPECT. Informed consent was taken from all patients' guardian.

^{99m}Tc-GHA study

To perform brain ^{99m}Tc-GHA SPECT, the patients were administered 370 - 740 MBq (10-20mCi) of in house prepared ^{99m}Tc-GHA i.v. For each individual, the dose was calculated as the body surface area divided by 1.73 and multiplied by the adult dose of 1,000 MBq. Brain SPECT images were acquired one hour post injection using a dual head single photon emission computed tomography system (Varicam from Elscint) fitted with fan beam collimator. Energy settings were 140 KeV with 20% energy window. A 128x128 matrix with 90 views every 4° for 25 seconds per view was obtained. Planar data were prefiltered prior to back projection and reconstruction with a two-dimensional Metz filter (cutoff=0.43 cm, P=30, Value of max=124, position of max=23, FWHM=100). Attenuation correction was done by Chang's method (10). Reconstructed images were displayed and analyzed using transverse, sagittal and coronal views.

Brain contrast-enhanced computerized tomography

Contrast-enhanced CT was performed 15 minutes after i.v. injection of a 2ml/kg-body weight contrast

Table 1. Tumor histology, location and approximate tumor size on CT of patients with false negative SPECT

Histology	Location	Size (in cm)
Atypical Meningioma	Brain stem	1.9x1.3x2.0
Low Grade Glioma	Pons	0.9x1.2x1.6
Low Grade Glioma	Pons	1.5x2.2x1.0
Glioblastoma Multiforme	Medulla	1.1x2.3x2.1
Ependymoma	4th Ventricle	1.3x0.8x1.2
Astrocytoma Grade 2	Cerebellum	2.3x2.9x3.1
Low Grade Glioma	Medulla	2.3x1.4x1.6
Dysgerminoma	Cerebellum	1.4x0.8x1.3

dose. Sensitivity to contrast was tested prior to injection. The region of interest was scanned with 3x3 mm axial cuts and 10x10 mm cuts were taken through the rest of the brain.

In-house Preparation of ^{99m}Tc-GHA

Glucoheptonate was prepared and labeled with ^{99m}Tc (Amersham Health Care Ltd., UK) by the following method. Five mg of glucoheptonate powder (Sigma Aldrich Corporation, Bangalore, India) were dissolved in 1 ml sterile water and 0.1 to 0.2 ml of stannous chloride (5mg stannous chloride in 1 ml 1N HCl) were added. The pH was adjusted to 6.5-7 by adding 1N NaOH. This solution was then passed through a filter (Millipore) and technetium pertechnetate was added. Instant thin layer chromatography (ITLC) was performed after every preparation of ^{99m}Tc-GHA to check the percentage of glucoheptonate molecules labeled with ^{99m}Tc (11). Any preparation with less than 98% labeling was discarded.

Data analysis

Two experienced nuclear medicine physicians blinded to the CT scan results evaluated the SPECT images independently. The images were interpreted as either showing or not showing evidence of tumor. Abnormally increased radiotracer uptake over the affected region was considered indicative of viable tumor. Absence of any abnormally increased tracer uptake over the site of the tumor was considered indicative of post radiotherapy gliosis. Preferential accumulation of the tumor seeking tracer in the tumor was defined as lesion-to-background ratio (glucoheptonate retention index). Two radiologists experienced in neuroradiology interpreted the CT findings independently and were blinded to the SPECT findings. Lesions were interpreted as post radiation gliosis if their Hunsfield unit values were close to cerebrospinal fluid density with no evidence of any mass effect, whereas lesions showing effacement of adjacent sulcal spaces (mass effect), with or without contrast enhancement, were reported as recurrent tumor.

Table 2. Validation parameters of ^{99m}-Tc-GHA SPECT derived from this study

Parameter	^{99m} TC-GHA Brain SPECT
Sensitivity	79.48%
Specificity	91.66%
Positive Predictive Value	96.87%
Negative Predictive Value	57.89%
Percentage of false neg. results	20.51%
Percentage of false pos. results	8.33%

^{99m}Tc-GHA index analysis

A region of interest (ROI) was drawn on the transverse slice showing the greatest tumor activity and an averaged pixel count was obtained. To obtain the background activity, a similar ROI was drawn on the opposite lobe or site. The ratio of the two values was obtained.

The ^{99m}Tc-GHA index was calculated as:

$$^{99m}\text{Tc - GHA Index} = \frac{\text{Average pixel count on Tumor ROI}}{\text{Average pixel count on contralateral ROI}}$$

Statistical analysis

^{99m}Tc-GHA index distributions in the high versus low tumor grade groups were compared using Mann-Whitney test. CT versus SPECT tumor diagnosis association was contrasted with Chi-square test. SPECT sensitivity and specificity were referenced to CT diagnosis as gold standard. A p value less than 0.05 was considered statistically significant.

RESULTS

Clinical follow-up and SPECT/CT evaluation after treatment of primary brain tumor

Brain SPECT revealed abnormally increased ^{99m}Tc-GHA uptake over the affected site in 32 of 51 patients, a scan feature consistent with viable tumor. The images were interpreted as either showing or not showing evidence of a tumor and there was no significant interobserver variability. Brain CT revealed tumor mass in 39 patients, including 31 patient who had positive SPECT (Figure 1 a,b). One patient with SPECT positive for recurrent tumor had a normal CT brain and was clinically asymptomatic, and was interpreted as a false positive SPECT study. Eight patients had mass lesions with features consistent with recurrent tumor in contrast-enhanced CT, and clinical course suggestive of recurrent tumor, but did not show any ^{99m}Tc-GHA concentration. These cases were interpreted as false negative SPECT studies (Figure 2 a,b). Repeated brain SPECT using Tc-^{99m}-Tetrofosmin, a better established brain tumor imaging agent, was performed in the eight patients where SPECT

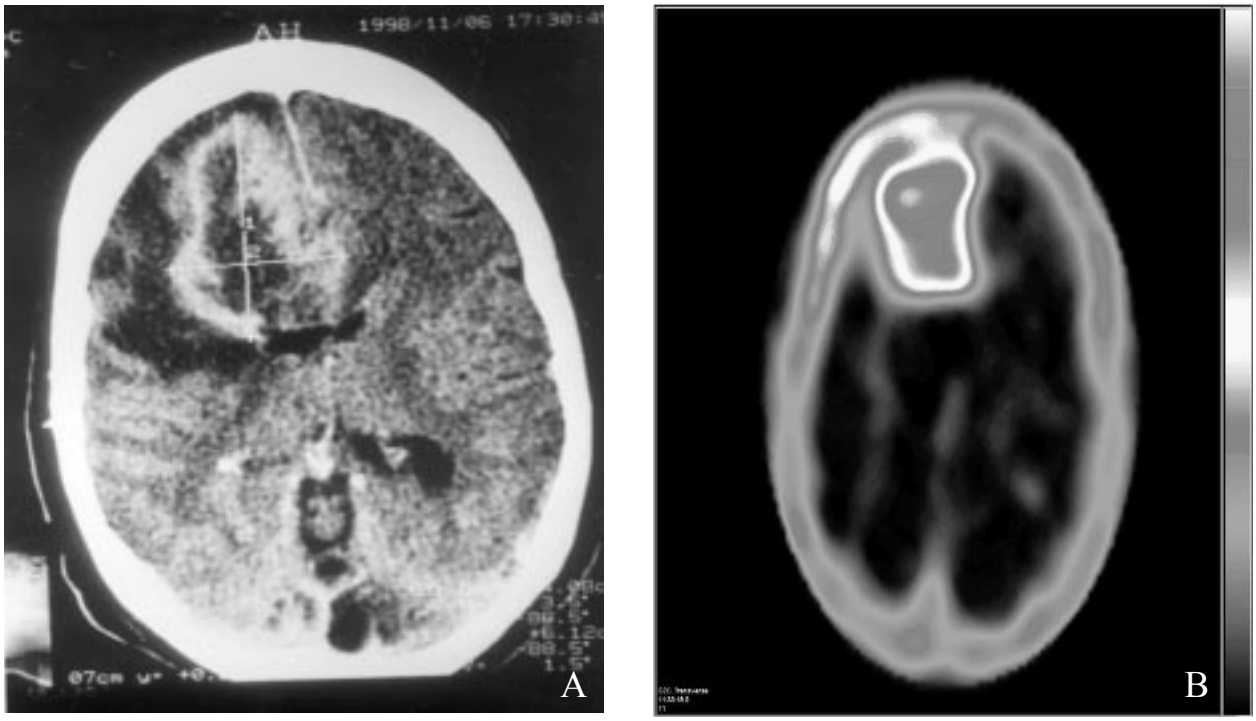

Figure 1: A. Coronal section of frontal glioblastoma multiforme showing contrast enhancement in CT. B. Corresponding SPECT slice of the same patient showing avid ^{99m}TC-GHA concentration.

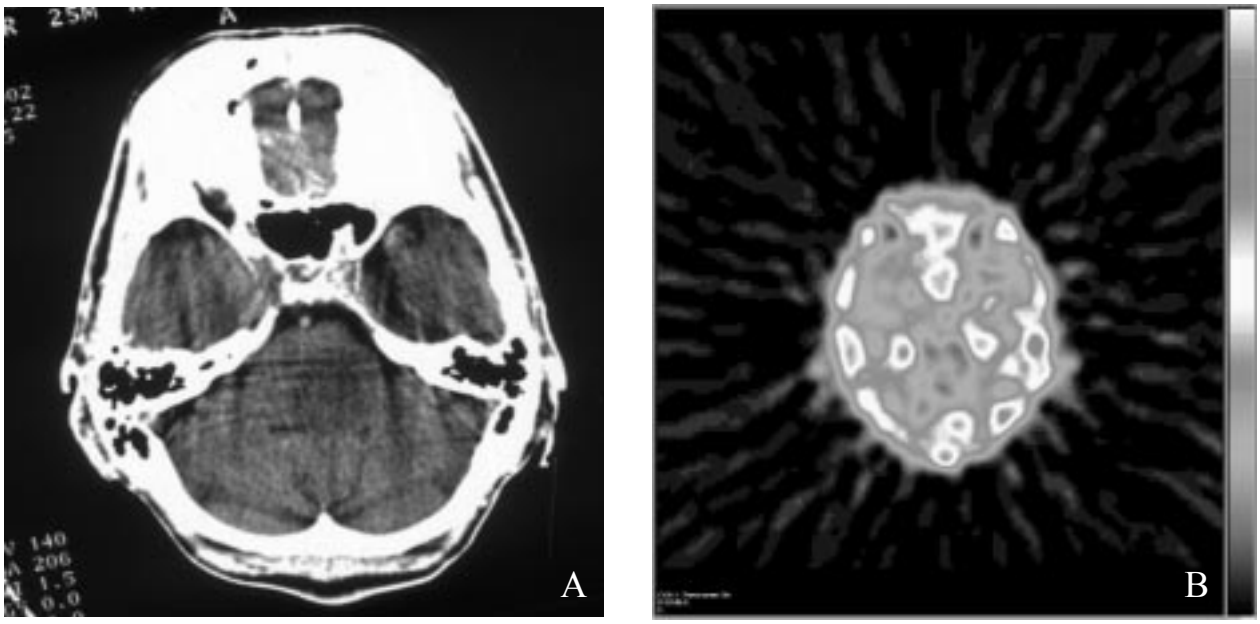

Figure 2. A. Coronal section of a low-grade glioma of medulla as seen on CT scan. B. A corresponding section from the SPECT study on the same patient shows no tracer concentration.

was normal but CT revealed a tumor mass (7). All of them had a normal Tc-^{99m}-Tetrofosmin SPECT study. Table 1 shows the distribution, histology and approximate tumor size in the 8 patients who had positive brain CT and negative SPECT. Table 2 summarizes the validation parameters of ^{99m}Tc-GHA SPECT referenced to contrast-enhanced CT.

^{99m}Tc-GHA uptake in recurrent tumor versus gliosis

A higher ^{99m}Tc-GHA index was found in recurrent tumors (6.06 ± 1.41), as compared to 1.10 ± 0.18 in post radiation gliosis (Fig. 3). The difference was statistically significant ($p = 0.001$). All of the subjects who developed post radiation gliosis had low grade

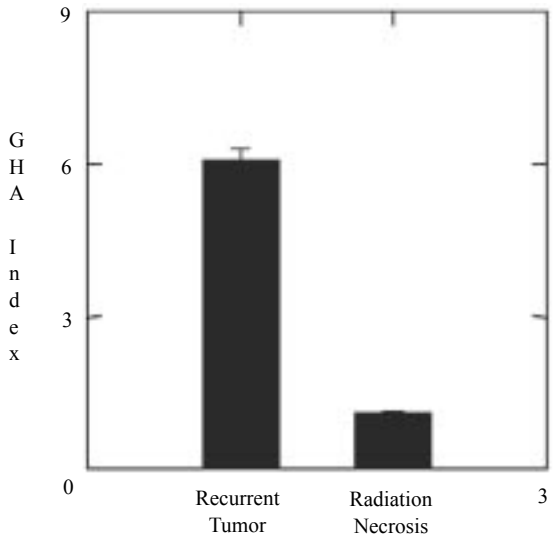

Figure 3. ^{99m}Tc-GHA uptake in recurrent and post radiation gliosis

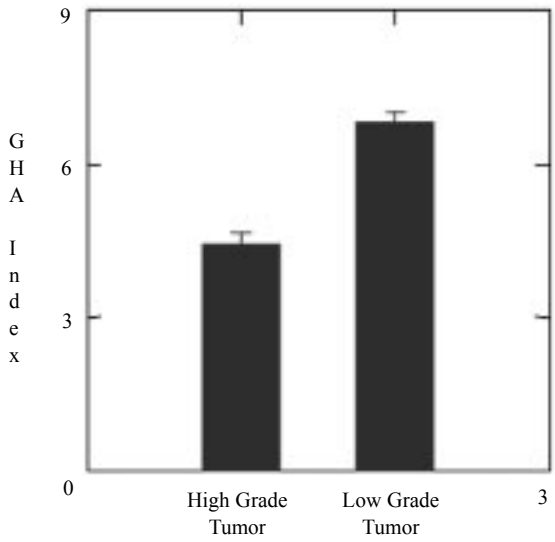

Figure 4. ^{99m}Tc-GHA uptake in high and low grade tumors

tumors, and none of the cases with high grade tumors developed post radiation gliosis.

Relationship between tumor grade and ^{99m}Tc-GHA uptake

The mean ^{99m}Tc-GHA index was lower in high-grade tumors (glioblastoma multiforme and anaplastic astrocytoma; ^{99m}Tc-GHA index = 4.40 ± 0.79) compared with low-grade tumors (remaining tumor of all other histology; ^{99m}Tc-GHA index = 6.42 ± 0.83) (Fig. 4). The difference was statistically borderline ($p = 0.05$).

DISCUSSION

Functional imaging provides physiological information about body function. The main role of functional imaging in oncological practice is to determine whether a lesion observed in an anatomical study such as CT scan, ultrasound or MRI consists of tumor cells or is formed by fibrotic tissue only. The demonstration of increased tracer extraction and subsequent accumulation in the lesion indicates viability of the suspected tumor mass. Single photon emission computed tomography using suitable radiotracers is exquisitely sensitive in demonstrating viable tumor tissue at any anatomical location in the body.

In contrast-enhanced CT studies, tumors are considered viable if they show a focal mass-effect in the form of effacement of adjacent sulcal spaces with Hunsfield unit values equal to or higher than brain parenchyma, with or without enhancement using contrast. Conversely, lesions are interpreted as post-radiation gliosis if there is evidence of focal volume loss with no enhancement with contrast. An increase in the size of the lesion after a temporal gap indicates a residual/recurrent tumor rather than focal gliosis. Compared with CT scans, ^{99m}Tc-GHA brain SPECT can identify viable tumoral tissue in a single study, tumor tracer retention being dependent upon an active uptake mechanism, thus eliminating the requirement of a temporal gap and a second study to establish a definite diagnosis (12). Pediatric brain tumors bear a high incidence of recurrence. Therefore, SPECT and positron emission tomography (PET) are routine investigations in oncological practice including pediatric brain tumor patients (1,4). In our unit we perform a SPECT study before the initiation of radiotherapy, after twelve weeks of completion of radiotherapy and then at six-month intervals. A final diagnosis of recurrent tumor or post radiation gliosis is established by combining the SPECT studies with the findings in CT scans and the clinical response to chemotherapy during the follow-up period.

The main observation in this study is the suitability of ^{99m}Tc-glucoheptonate as a potential radiotracer for the imaging of pediatric brain tumors. ^{99m}Tc-GHA shows intense physiological uptake in nasal mucosa, and large intracranial venous sinuses also retain a significant amount of radioactivity. Therefore, ^{99m}Tc-GHA brain SPECT may not be very suitable for the evaluation of tumors close to the nasal mucosa, like those situated in basifrontal lobe region, but it otherwise allows a good visualization of tumor margins (13). We observed a lower mean value for ^{99m}Tc-GHA index in high-grade tumors versus low-grade tumors. This finding may suggest a greater response to radiotherapy by more anaplastic tumors compared with low-grade tumors, which would result in higher levels of tumor cell death

or damage and, subsequently, in lower tracer uptake and retention.

Mechanism of Technetium-^{99m}-Glucoheptonate Uptake

Glucoheptonate is a seven-carbon sugar. Tc-^{99m}-glucoheptonate is a 1:2 Tc(v) complex with two glucoheptonate molecules combined with the metal (Technetium) through carboxyl and alphahydroxyl groups (14). The mechanism of ^{99m}Tc-Glucoheptonate accumulation in brain tumors is not completely understood. The mechanism of uptake has been studied in proximal tubular cells in the kidney, and it seems to be dependent on cellular metabolism (15). In the present study, no ^{99m}Tc-GHA uptake was detected in normal brain tissue, suggesting that a breakdown or increased permeability at the blood-brain barrier (BBB) seems to be a condition necessary for ^{99m}Tc-GHA tumor uptake, similarly to any other brain tumor imaging agent (9). Leveille et al. suggested that glucoheptonate also acts as a substrate for the malignant tissue thus enhancing its uptake (16). The possibility of intracellular binding was also suggested by Tanasescu et al. (17).

The mechanism of ^{99m}Tc-Tetrofosmin accumulation in tumor

The mechanism of ^{99m}Tc-Tetrofosmin accumulation has been studied in myocardial cells, and it seems to be dependent on cellular metabolism because mitochondria take up the tracer through a process that is dependent on their membrane potential and their coupling state (i.e. their ability to couple oxidative phosphorylation) (18,19). In the present study no tetrofosmin uptake was observed in normal brain tissue, suggesting that the breakdown or an increased permeability of the blood brain barrier (BBB) seems to be a condition necessary for tetrofosmin uptake by the tumor. Nevertheless, studies using a tumor cell line showed that the uptake mechanism, intracellular distribution and washout kinetics of tetrofosmin are influenced by compounds that interfere with metabolic processes and that the mechanism by which the tracer enters the cells depends upon both cell membrane (Na+/K+ pump) and mitochondrial potential (20,21).

Recurrent tumor versus post-radiation gliosis

Establishing the cause of clinical deterioration in malignant glioma patients treated with high dose radiation therapy is critical because recurrent tumor may require repeated surgery or adjuvant therapy in order to improve the quality of life and survival rate, while radiation necrosis can be managed conservatively (22,23). Active ^{99m}Tc-GHA uptake by brain lessions can allow to differentiate between tumor recurrence and post radiation changes. However, SPECT may fail to detect some tumoral lesions. In this study, all the tumors that escaped detection by SPECT were located in the posterior fossa compartment (Table 1). The posterior fossa is a compact anatomical space that allows relatively less expansion for the tumor to grow without compressing the neuronal structures. Thus, a tumor smaller than one cm can produce considerable clinical symptoms without being detected on SPECT, as the resolution of brain SPECT is around one cm. There are relatively more venous sinuses packed into a smaller space in the posterior fossa, and these sinuses frequently retain relevant amounts of the tracer, which sometimes may mask an adjacent tumor with less or equal intensity of tracer uptake. Another possibility is the existence of tumors that constitutively do not concentrate glucoheptonate or tetrofosmin. Blood-brain barrier endothelial cells may also be implicated in preventing tumors from concentrating a tracer because they express the multidrug resistance 1 gene, whose product is an adenosine triphosphatase membrane pump that extrudes a variety of toxins from the cells. ^{99m}Tc-tetrofosmin is one of these substrates. The inhibition of this multidrug resistance feature has been shown to delay the excretion of ^{99m}Tc-Tetrofosmin (24). Without inhibition, the pump prevents the tracer from reaching the interstitial space. It is possible that glucoheptonate is also a substrate for adenosine triphosphatase membrane pump.

CONCLUSIONS

In summary, this study shows that ^{99m}Tc-GHA brain SPECT can be used to differentiate recurrent primary tumors from post radiation gliosis in a pediatric population, with a sensitivity of 79.48% and a specificity of 91.66% in reference to contrast-enhanced CT. The low negative predictive value obtained in the tested population suggests that ^{99m}Tc-GHA SPECT would not be appropriate as a screening test on asymptomatic subjects, to discard tumor recurrence during follow-up. Tumors located in the posterior fossa encephalic compartment seem to be particularly conflictive for SPECT discrimination. In this case, ^{99m}Tc-GHA SPECT may not be an appropriate diagnostic test, and oncologists should interpret with caution a negative ^{99m}Tc-GHA brain SPECT in subjects with tumors located in posterior fossa.

Limitations of this study

Histopathological analysis of the recurrent lesions was not feasible because repeated surgery of primary brain tumors is rarely indicated in pediatric patients. Therefore we have adopted contrast-enhanced CT as gold standard for this study.

Future Directions

A study with a larger sample size may help to evaluate the implications of the observed lower ^{99m}Tc-GHA uptake by more malignant tumors, and to better assess the application of brain SPECT to tumors in posterior fossa.

REFERENCES

1. Sklar CA. Childhood brain tumors. J Pediatr Endocrinol Metab 15 Suppl 2:669-73; 2002.
2. Chan JL, Lee SW, Fraass BA, Normolle DP, Greenberg HS, Junck LR, Gebarski SS, Sandler HM. Survival and failure patterns of high grade gliomas after three-dimensional conformal radiotherapy. J Clin Oncol 20:1635-42; 2002.
3. Shaw E, Arusell R, Scheithauer B, et al. Prospective randomized trial of low- versus high-dose radiation therapy in adults with supratentorial low-grade glioma: initial report of a North Central Cancer Treatment Group/Radiation Therapy Oncology Group/Eastern Cooperative Oncology Group study. J Clin Oncol 20: 2267-76; 2002.
4. Utriainen M, Metsahonkala L, Salmi TT, et al. Metabolic characterization of childhood brain tumors: comparison of 18F-fluorodeoxyglucose and 11C-methionine positron emission tomography . Cancer.95:1376-86; 2002.
5. Kaplan WD, Takvorian T, Morris JH, Rumbaugh CL, Conndly BT, Atkins HL. Thallium-201 brain tumor imaging. A comparative study with pathological correlation. Journal of Nuclear Medicine 28: 47 - 52; 1987.
6. Nishiyama Y, Yamamoto Y, Fukunaga K, Satoh K, Kunishio K, Ohkawa M. Comparison of 99Tcm-MIBI with 201Tl chloride SPET in patients with malignant brain tumours. Nucl Med Commun 22: 631-9; 2001.
7. Choi JY, Kim SE, Shin HJ, Kim BT, Kim JH. Brain tumor imaging with ^{99m}Tc-tetrofosmin: comparison with 201Tl, ^{99m}Tc-MIBI, and18F-fluorodeoxyglucose. J Neurooncol; 46: 63-70; 2000.
8. Soricelli A, Cuocolo A, Varrone A, et al. Technetium-^{99m}-Tetrofosmin uptake in brain tumors by SPECT: Comparison with Thallium-201 imaging. Journal of Nuclear Medicine 39: 802-806; 1998.
9. Staudenherz A, Fazeny B, Marosi C, et al. Does (^{99m}) Tc-sestamibi in high-grade malignant brain tumors reflect blood-brain barrier damage only? Neuroimage 12: 109-11; 2000.
10. Chang LT. A method for attenuation correction in radionuclide computed tomography. IEEE Trans Nucl Sci NS 25: 638-643.
11. Pauwels EKJ, Feitsma RIJ. Radiochemical quality control of ^{99m}Tc-labeled radiopharmaceuticals. Eur J Nucl Med 2: 97; 1977.
12. Dooms GC, Hecht S, Brant-Zawadzki M, et al. Brain radiation lesion: MR imaging. Radiology 158: 149-155; 1986.
13. Waxman AD, Tanasescu D, Siemsen JK et al. Techenetium-^{99m}- glucoheptonate as a brain screening agent. Journal of Nuclear Medicine 17; 345-348;1977.
14. Roland Muller-surr. Radiopharmaceuticals: their intrarenal handling and localization. In: Nuclear medicine in clinical diagnosis and treatment Vol. 1. New York, US Churchill Livingstone, 1994.
15. Lee HB, Blaufox MD. Mechanism of renal concentration of technetium-^{99m}-glucoheptonate. Journal of Nuclear Medicine 26: 1308-1313; 1985.
16. Levielle J, Pision C, Karakand Y et al. Technetium-^{99m} glucoheptonate in brain tumor detection: an important advance in radiotracer technique. Journal of Nuclear Medicine 18: 957-961;1977.
17. Tanasescu D, Wolfstein R, Waxman AD. Technetium-^{99m}-glucoheptonate as a brain scanning agent. Editorial Journal of Nuclear Medicine18: 1037-1038; 1977.
18. Platts EA, North TL, Pickett RD, Kelly JD. Mechanism of uptake of technetium tetrofosmin 1: uptake into isolated adult rat ventricular myocyte and subcellular localization. Journal of Nuclear Cardiology 2: 317-316; 1995.
19. Younes A, Singled JA, Maublant J, Platts E, Pickett R, Veyre A. Mechanism of uptake of technetium-tetrofosmin, II: uptake into isolated adult rat heart mitochondria. Journal of Nuclear Cardiology 2: 317-316; 1995.
20. Perek N, Prevot N, Koumanov F, Frere D, Sabido O, Beauchesne P, Dubois F. Involvement of the glutathione S-conjugate compounds and the MRP protein in Tc-^{99m}-tetrofosmin and Tc-^{99m}-sestamibi uptake in glioma cell lines. Nucl Med Biol 27: 299-307; 2000.
21. Arbab AS, Koizumi K, Toyama K, Arai T. Uptake of ^{99m}Tc-tetrofosmin, technetium-^{99m}-MIBI and thallium-201 in a tumor cell line. Journal of Nuclear Medicine 37: 1551-1556; 1996.
22. Kristiansen K, Hagen S, Kollevolt T, et al. Combined modality therapy of operated astrocytoma grade 3 and 4: Confirmation of the value of post operative irradiation and lack of potentiation of Bleomycin on survival time: a prospective multicenter trial of the Scandinavian Glioblastoma study group. Cancer 47: 649; 1981.
23. Yamamoto M, Oshiro S, Tsugu H, et al. Treatment of recurrent malignant supratentorial astrocytomas with carboplatin and etoposide combined with recombinant mutant human tumor necrosis factor-alpha. Anticancer Res 22: 2447-53; 2002.
24. Bae KT, Piwnica-Worms D. Pharmacokinetic modeling of multidrug resistance P-glycoprotein transport of gamma-emitting substrates. Q J Nucl Med Jun 41:101-10; 1997.

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CASE REPORT

Postpartum Depression: Making the Case for Routine Screening

Aaron Keshen* B.Sc, and Joanne L. MacDonald M.D.

CASE REPORT

Mrs. R is a 29-year-old woman primipara who presents for a routine check-up 6 weeks postpartum. Her history and physical are unremarkable except that during the past month she has found it harder to concentrate on simple tasks, such as making the baby's formula. Also, despite always feeling exhausted, she has been having trouble falling asleep after the baby has been put to sleep. When questioned about her mood, she replies, "I feel fine", but offers little else. You know that these complaints may be symptoms of postpartum depression (PPD), but because they are non-specific, often non-pathologic (especially postpartum) and she denies feeling depressed, you are not sure whether the clinical presentation warrants a more thorough investigation of Mrs. R's mental state. Should you investigate more thoroughly? If so, instead of taking a lengthy psychiatric history, what fast and effective methods could be used to explore the possibility that this patient has, or is at risk of developing PPD? Also, what are the current guidelines for diagnosing and treating PPD?

DISCUSSION

At least 10% of mothers will suffer from postpartum depression (PPD) (1,2,3) a debilitating condition that is defined as a Major Depressive Episode, which has an onset within 4 weeks postpartum (See Table 1 for the DSM-IV4 diagnostic criteria and differential diagnosis for PPD). Although clinicians have come to accept the view that PPD is a common and serious medical condition, they are still debating the ideas concerning the etiology of the illness. Biological theorists have suggested that postpartum fluctuations in hormones and/or other biological factors are responsible for causing PPD (1,2,3) Psychological models have also attempted to explain the etiology of PPD. For instance, the cognitive model suggests that factors such as low self-esteem and dysfunctional relationships predispose the mother to depression, which is then precipitated by postpartum stress (1). Although evidence exists for both biological and psychological theories, conflicting data has made it impossible to definitively claim that any one of these theories is alone capable of explaining the etiology of PPD (1). Instead, research suggests that a multifactorial etiology, which includes both biological and psychological factors, is probably responsible for the illness (8).

While the etiology of PPD is still debated, the implications of accurately diagnosing the condition remains important for the immediate and long-term mental health of the mother (1), her spousal relationship (1) and even the cognitive and behavioral development of her child (1,2). Since PPD detrimentally affects so many lives, it is unfortunate that many cases go undiagnosed (1,2). One major reason for under diagnosis is that many physicians only inquire casually about a new mother's mental state (1,2) In addition, there are several reasons why some mothers with PPD will not disclose their symptoms. One reason is that there is still a stigma surrounding mental illness. Another reason is that some women harbor guilt about feeling depressed during a period when they are expected to be blissful (1). Yet another reason is that some women assume that they should experience some physiological changes (e.g. insomnia) during the first few postpartum months, and are therefore embarrassed to "complain" about certain symptoms to their family doctors (16).

Since it is beneficial for physicians to explore the issue of PPD more thoroughly, what fast and effective

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Table 1. Diagnosing Postpartum Depression

Definition of PPD:
The DSM-IV4 defines PPD as a Major Depressive Episode*, which has an onset within 4 weeks postpartum. In order for the diagnosis to be made at least five of the symptoms (see bottom of Table 1) must be present, one of which must be depressed mood or diminished pleasure or interest in activities. The symptoms must be present most of the day nearly every day for two weeks. Also, there must be an associated decline in social and /or occupational functioning.

Differential Diagnosis of PPD:
Baby Blues:
In order for the diagnosis of PPD to apply, the DSM-IV criteria must be met for 2 consecutive weeks.4 If the symptoms are observed for less than 2 weeks, then a diagnosis of "baby blues" is more appropriate.16 "Baby blues", which is experienced by approximately 80% of new mothers, usually lasts for hours to days, and in most cases resolves spontaneously by the 10th postnatal day.

Postpartum Psychosis:
Postpartum psychosis is a medical emergency that affects about 0.2 percent of new mothers.16 Symptoms usually begin by the first postpartum month and resemble those seen during a manic episode (i.e. insomnia, agitation, irritable mood, hallucinations and delusions).4 If hallucinations and/or delusions are present they often involve the infant, and can lead to harm directed towards self or the infant.

General Medical Conditions that Present like Depression:
In particular, thyroid disorder and anemia can be exacerbated postpartum, and should therefore be ruled out with a careful history, physical, and appropriate laboratory tests.

Suicide Risk Assessment:
As with other forms of depression, the risk of suicide is increased for women with PPD.16 For this reason, it is essential to determine whether a patient with PPD is considering suicide. The first step in a suicide risk assessment should be inquiring about passive ideation . One way to do this is by asking, "Have you felt so low that you've thought life is not worth living?" If an affirmative answer is given, the active intent/plan should be determined. This can be done by asking, "Have you ever thought of how you might end your life?" or "what types of things have you considered doing?" If the patient does have active intent, you should ask whether they have the means to carry out the plans they have devised. If you are concerned that a patient is a suicide risk, you should insist that they go to the hospital for psychiatric evaluation. If they refuse, and you suspect they are truly a threat to themselves, you must notify the authorities.

Symptoms(4) of PPD*

- **Depressed mood**
- **Diminished pleasure or interest in activities**
- **Sleep disturbance (insomnia or hypersomnia)**
- **Weight loss or weight gain**
- **Psychomotor agitation or retardation**
- **Loss of energy**
- **Feelings of worthlessness or inappropriate guilt**
- **Diminished concentration, or indecisiveness**
- **Frequent thoughts of death or suicide**

*See the DSM-IV(4) for a more detailed description of the diagnostic criteria and symptoms of depression.

methods can be used to accomplish this goal? Two accepted approaches that can be used concomitantly are: a) routine screening of all postpartum mothers and b) heightened attention to the risk factors for PPD. The next section of this paper describes how these two methods can be used effectively to increase the detection of this devastating disease.

Routine Screening and Risk Factors for PPD

One way for physicians to explore the issue of PPD is to routinely screen all postpartum mothers, a procedure that is very easy and effective, but for unknown reasons is almost never used (1). Among various screening tests for PPD, experts consider the Edinburgh Postnatal Depression Scale (EPDS) (1) to be the best choice in terms of its ease of administration, validity, specificity and sensitivity (16,19,1,2,38) (See Table 2 for the EPDS scale and guidelines for raters.) This scale has been validated using standardized psychiatric interviews and translated into fourteen languages (19,1,2,3). Furthermore, evidence from four studies has confirmed that the tool is approximately 86% sensitive and 90% specific for PPD. These are impressive statistics when one considers that the test consists of only 10 multiple choice questions and takes mothers less than 5 minutes to complete (16,19). As further evidence of the scale's effectiveness, one study showed that routine screening with the EPDS in family practices increased the rate of diagnosis of PPD from 3.7% to 10.7% (18)

In addition to routinely screening postpartum mothers,

Table 2. Edinburgh Postnatal Depression Scale (EPDS)19 and Guidelines for Raters

Guidelines for Raters (19)
1. The mother is asked to underline the response which comes closest to how she has been feeling in the previous 7 days.
2. All ten items must be completed.
3. Care should be taken to avoid the possibility of the mother discussing her answers with others.
4. The mother should complete the scale herself, unless she has limited English or has difficulty with reading.
The child health clinic, postnatal check-up or a home visit may provide suitable opportunities for its completion.

Scoring the EPDS
Response categories are scored 0, 1, 2, and 3 according to increased severity of the symptoms. Items marked with an asterisk are reverse scored (i.e. 3, 2, 1, and 0). The total score is calculated by adding together the scores for each of the ten items. Individual items are totalled to give an overall score. A score of 12+ indicates the likelihood of depression (with about 90% specificity and 86% sensitivity), but not its severity (19,22,23,24). If a women scores 12+, this warrants a full psychiatric history during which a DSM-IV diagnosis of PPD is considered. A woman who scores 5-11 should be evaluated again in 2-4 weeks in order to assess whether there has been a worsening of symptoms. A patient who scores less than 12 on the EPDS, but scores 3 or 2 on Question 10 warrants a full psychiatric evaluation. The EPDS Score is designed to assist, not replace clinical judgement.

Edinburgh Postnatal Depression Scale19

As you have recently had a baby, we would like to know how you are feeling. Please UNDERLINE the answer which comes closest to how you have felt IN THE PAST 7 DAYS, not just how you feel today.

1. I have been able to laugh and see the funny side of things.
As much as I always could
Not quite so much now
Definitely not so much now
Not at all

2. I have looked forward with enjoyment to things.
As much as I ever did
Rather less than I used to
Definitely less than I used to
Hardly at all

3. * I have blamed myself unnecessarily when things went wrong.
Yes, most of the time
Yes, some of the time
Not very often
No, never

4. I have been anxious or worried for no good reason.
No, not at all
Hardly ever
Yes, sometimes
Yes, very often

5. * I have felt scared or panicky for not very good reason.
Yes, quite a lot
Yes, sometimes
No, not much
No, not at all

6. * Things have been getting on top of me.
Yes, most of the time I haven't been able to cope at all
Yes, sometimes I haven't been coping as well as usual
No, most of the time I have coped quite well
No, I have been coping as well as ever

7. * I have been so unhappy that I have had difficulty sleeping.
Yes, most of the time
Yes, sometimes
Not very often
No, not at all

8. * I have felt sad or miserable.
Yes, most of the time
Yes, quite often
Not very often
No, not at all

9. * I have been so unhappy that I have been crying.
Yes, most of the time
Yes, quite often
Only occasionally
No, never

10. * The thought of harming myself has occurred to me.
Yes, quite often
Sometimes
Hardly ever
Never

(Taken from the British Journal of Psychiatry, June, 1987, Vol. 150 by J.L. Cox, J.M. Holden, R. Sagovsky)

physicians can further explore the possibility of PPD by asking patients about the risk factors for the illness. This is an effective strategy because certain risk factors are very strong predictors of whether a patient is susceptible to PPD (33-37). Some important risk factors for physicians to keep in mind are included in Table 3.

Table 3. Risk Factors for PPD

- History of depression prior to conceiving.(32)
- History of PPD in previous pregnancies.(33)
- Family history of depression.(32)
- Few supportive family members or friends.(34)
- Financial or housing difficulties.(35)
- Severe premenstrual syndrome.(35)
- Dysfunctional spousal relationship.(34,36)
- Other stressful life events during the pregnancy or after the childbirth.(37)

Management of PPD

The management of PPD parallels that of Major Depressive Disorder; however, there are some special management considerations for patients with PPD (1,2,3,4,5). Some of these considerations are described in Table 4.

Case Revisited

Since casual inquiry may not reveal PPD symptoms that Mrs. R could be experiencing, you decide to investigate her complaints more thoroughly. You screen Mrs. R using the EPDS and she has a score of 14, which suggests that there is a 90% probability that she suffers from PPD. She also has several of the risk factors for the condition (i.e. marital discord and financial difficulties), which corroborates the positive EPDS score. Because of the high probability that Mrs. R has PPD you take a detailed psychiatric history during which you apply the DSM-IV criteria of PPD, and also rule out disorders that present like PPD.

You determine that she does in fact have moderate PPD, so you recommend SSRI antidepressant administration, with follow-up within 2 weeks to assess side effects and response. As well, you provide her and her husband with patient education materials. You also initiate or make a

Table 4. Management of PPD

Mild PPD Symptoms that barely fulfill the DSM-IV diagnostic criteria for PPD and cause a minor impairment of social/occupational functioning.	**Moderate/Severe PPD** Symptoms in excess of the bare requirements to fulfill the DSM-IV diagnostic criteria for PPD and cause a major impairment of social/occupational functioning.
• Individual interpersonal therapy or group counselling, and couples therapy if marital discord is a factor. Also, patient education materials should be provided for the mother and spouse (16) • If there is no response to talk therapy, consider adding a SSRI or tricyclic antidepressant*. • If the depression worsens, or there are suicidal/infanticidal thoughts, or symptoms of psychosis, or inadequate response to an antidepressant, then refer to a psychiatrist (16).	• Consider a SSRI or tricyclic antidepressant* +/- individual interpersonal therapy or group counselling, and couples therapy if marital discord is a factor. Also, patient education materials should be provided for the mother and spouse (16) • If the depression worsens, or there are suicidal/infanticidal thoughts, or symptoms of psychosis, or inadequate response to an antidepressant, then refer to a psychiatrist (16)

Notes on antidepressant administration:

• Selective serotonin-reuptake inhibitors (SSRIs) (25,27), venlafaxine (28) and tricyclic antidepressants (TCAs) (29) have been shown to be more effective than placebo for treating PPD, and are therefore considered to be appropriate therapy for PPD. Fluoxetine was shown to be as effective as psychotherapy for treating PPD (27).

• SSRIs or venlafaxine should be considered as first-line drug therapy rather than TCAs because they are associated with a lower risk of toxic effects in patients who have taken an overdose (3). Also, women with PPD are more likely to have a response to SSRIs or venlafaxine than to TCAs (25,28,29) However, TCAs should be considered for women who have responded to them in the past (38).

• Administration of TCAs or SSRIs is not contraindicated during breastfeeding. However, small amounts of the drugs do reach the infant via breast milk. Since the long-term effects of this exposure are not known, parents should make informed decisions that are documented in their medical records. It should be emphasised to women with moderate/severe depression, women who have not responded to psychotherapy, and women who are suicidal, infanticidal or psychotic that the benefits of taking antidepressants are considered to outweigh the risks of exposure to the infant.

• If an exposed infant seems irritable, plasma concentration of the drug should be determined and appropriate dosage adjustments made (38).

• Antidepressant therapy should be started at the starting doses used for nonpuerperal depression. Once a full remission is achieved, therapy should continue for a minimum of six months, in order to prevent relapse (38).

referral for individual and relationship therapy if functioning or insight remains seriously impaired.

REFERENCES

1. O'Hara MW. Postpartum Depression. In: Alloy LB, editors. Series in Psychopathology. New York: Springer-Verlag; 1995.
2. Kumar R, Robson KM. A prospective study of emotional disorders in childbearing women. Br J Psychiatry. 144:35-47; 1984.
3. O'Hara MW, Neunaber DJ, Zekoski EM. A prospective study of postpartum depression: prevalence, course, and predictive factors. J Abnormal Psychology. 158-171;1984.
4. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, D.C.: American Psychiatric Association, 1994.
5. Harris B, Lovett L, Smith J, et al. Cardiff puerperal mood and hormone study. III. Postnatal depression at 5 to 6 weeks postpartum, and its hormonal correlates across the peripartum period. Br J Psychiatry. 168(6):739-744; 1996.
6. Stowe ZN, Nemeroff CB. Women at risk for postpartum-onset major depression. Am J Obstet Gynecol. 173(2):639-645;1995.
7. McEwen BS. Ovarian steroids have diverse effects on brain structure and function. In: Hammar GBaM, editors. The Modern Management of Menopause. New York: Parthenon Publishing; 1993.
8. Hendrick, V, Altshuler, L, Suri, R. Hormonal changes in the postpartum and implications for postpartum depression. Psychosomatics: Journal of Consultation Liasion Psychiatry. 39(2): 93-101;1998.
9. Cooper PJ, Murray L. The course and recurrence of postnatal depression. Br J Psychiatry 166:191-95;1995.
10. Boyce P. Personality dysfunction, marital problems and postnatal depression. In: Cox J, Holden J, editors. Perinatal psychiatry: use and misuse of the Edinburgh Postnatal Depression Scale. London, England: Gaskell, 1994.
11. Cogill SR, Caplan HL, Alexandra H, Robson KM, Kumar R. Impact of maternal postnatal depression on cognitive development of young children. BMJ 292:1165-67;1986.
12. Whiffen VE, Gotlib IH. Infants of postpartum depressed mothers: temperament and cognitive status. J Abnorm Psychol 98:274-97;1989.
13. Whitton A, Warner R, Appleby L. The pathway to care in post-natal depression: women's attitudes to post-natal depression and its treatment. Br J Gen Pract 46:427-28;1996.
14. Hirschfield RMA, Keller MB, Panico S, et al. The national depressive and manic-depressive association consensus statement on the undertreatment of depression. JAMA 277:333-40;1997.
15. Yawn, B. Recent tragedies focus attention on postpartum depression. http://www.ama-assn.org/sci-pubs/amnews/pick_01/hlsb0730.htm
16. Epperson, C. N. Postpartum Major Depression: Detection and Treatment. American Family Physician April 15:2247-2259;1999.
17. Cox, JL. Postnatal Depression. Churchill Livingston; 1986
18. Georgiopoulos, AM, Bryan, TL, Woolan, P.,and Yawn, BP. Routine Screening for Postpartum Depression. The Journal of Family Practice 50, No. 2;2001.
19. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression: development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry 150:782-86;1987.
20. O'Hara MW. Postpartum depression: identification and measurement in a cross-cultural context. In: Cox J, Holden J, editors. Perinatal psychiatry: use and misuse of the Edinburgh Postnatal Depression Scale. London, England: Gaskell; 1994.
21. Schaper AM, Rooney BL, Kay NR, Silva PD. Use of the Edinburgh Postnatal Depression Scale to identify postpartum depression in a clinical setting. J Reprod Med 39:620-24;1994.
22. Boyce P, Stubbs J, Todd A. The Edinburgh Postnatal Depression Scale: validation for an Australian sample. Aust N Z J Psychiatry 27:472-6;1993.
23. Murray L, Carothers A. The validation of the Edinburgh Postnatal Depression Scale on a community sample. Br J Psychiatry. 157:288-90;1990.
24. Harris B, Huckle P, Thomas R., John S., Fung H. The use of rating scales to identify postnatal depression. Br J Psychiatry. 154: 813-817;1989.
25. Stowe ZN, Casarella J, Landry J, Nemeroff CB. Sertraline in the treatment of women with postpartum major depression. Depression 3:49-55;1995.
26. Epperson CN, McDougle CJ, Ward-O'Brien D, Price LH. A controlled study of sertraline versus placebo in the treatment of postpartum depression: preliminary findings [Abstract 76.3]. Soc Neurosci 22:179;1996.
27. Appleby L, Warner R, Whitton A, Faragher B. A controlled study of fluoxetine and cognitive-behavioural counselling in the treatment of postnatal depression. BMJ 314:932-6;1997.
28. Cohen LS, Viguera AC, Bouffard, et al. Venlafaxine in the treatment of postpartum depression. J. Clin. Psychiatry. 62:592-596;2001.
29. Wisner KL, Peindl KS, Gigliotti TV. Tricyclics vs SSRIs for postpartum depression. Arch Womens Mental Health. 1:189-91;1999.
30. Gerstein HC. How common is postpartum thyroiditis? A methodologic overview of the literature. Arch Intern Med 150:1397-400;1990.
31. Rouse, T., Tang, G., Torgerson, C., and Van Spall, H. Essentials of Clinical Examination Handbook. 3rd Ed. The Medical Society, Faculty of Medicine, U. of Toronto, 2000.
32. Beck CT. A meta-analysis of predictors of postpartum depression. Nursing Res. 45:297-303;1996.
33. Llewellyn AM, Stowe ZN, Nemeroff CB. Depression during pregnancy and the puerperium. J Clin Psychiatry. 58(suppl 15):26-32;1997.
34. O'Hara MW. Social support, life events, and depression during pregnancy and the puerperium. Arch Gen Psychiatry 43:569-73;1986.
35. Czarkowski, K. Postpartum Depression and the "Baby Blues". American Family Physician April 15:1259-1263;1999
36. Misri, S. The impact of partner support in the treatment of postpartum depression. Can J Psychiatry. 45(6): 554-8;2000.
37. Paykel ES, Emms EM, Fletcher J, Rassaby ES. Life events and social support in puerperal depression. Br J Psychiatry 136:339-46;1980.
38. Wisner, K.L., Parry, B.L. and Piontek, C.M. Postpartum Depression. NEJM. 347, No. 3:194-199;2002.

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REVIEW ARTICLE

Midwifery in Canada: Development and Integration

Karen Born*

More drugs and technologies are now used in 'normal births' in North American than anywhere else in the world. This reflects in art the desire to master, conquer and control nature that was present among the colonist from the beginning (1)

Apparently the post-'60s, earth-mother garbage didn't go out with the love beads and bell bottoms. . . . Things can go sour at any step in the birth process. That women continue to risk their babies' lives by buying into a self-gratifying scenario which lets them spin pretty fantasies about home births with incense burning is pretty scary (2).

Since childbirth is a core aspect of the human experience and a dramatic life cycle event, the practices associated with it can become quite contentious. The protest and celebration that accompanied the emergence and increasingly visible role of midwives in the Canadian health care system is understandable, considering the issues and context. The contradictory reactions of Canadians towards midwifery correlated with the many fears and misconceptions about the nature of birth, as well as the scope and role of midwives in the entire experience, from pregnancy to delivery and beyond. Many of these misconceptions influenced politics and policy makers, and entrenched oppositional view points of midwifery through powerful lobbies and interest groups. The historical forces and movements of the twentieth century, however, empowered the midwife lobby to propagate midwifery as a viable alternative to childbirth, stirred women to demand access to midwifery and enabled a greater acceptance of midwifery as a practice and profession within the health care system. Extensive studies and inquiries in the past thirty years suggest that midwifery may be a cost-effective, efficient alternative to obstetric, physician services.

Midwifery as Integrated into the Health Care System There are growing concerns that Canada is facing a crisis in maternity care because of looming shortages in professionals available to provide newborn and maternity care. The proportion of family physicians providing obstetric services has decreased from 36% in 1982 to 18% in 2000 (3). Moreover, due to increased lengths in postgraduate training and medical school enrollment limits, there has been a shortage of younger physicians, upon whom obstetrics traditionally relies for staffing (4). Midwives, as the only health professionals educated specifically to care for normal childbearing and newborns, are prepared to alleviate the burden of physician shortages in the future. Canada's current health care reforms and visions of complete community health would be greatly enhanced by further integration of midwifery into family medicine and women's health. Canada is the last industrialized country to formally legalize midwives as health care practitioners. In all other industrialized countries, with the exception of the United States (U.S.), most babies are delivered by professional midwives who are integrated into the health care system (5). The World Health Organization defines a midwife as a person who is qualified to practice midwifery:

She is trained to give the necessary care and advice to women during pregnancy, labour and the post natal period, to conduct normal deliveries on her own

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responsibility, and to care for the newly born infant. At all times she must be able to recognize the signs of abnormal or potentially abnormal conditions which necessitate referral to a doctor, and to carry out emergency measures in the absence of medical help. She may practice in hospitals, health units or domiciliary services. In any one of these situations she has an important task in health education within the family and community. In some countries, her work extends into the fields of gynecology, family planning or child care (6).

Midwives, as the deliverers of primary care in low risk pregnancies, can alleviate some of the current pressures on obstetricians, hospitals, physicians and nurses through taking on more cases, and developing collaborative relationships with other health care practitioners. In order for midwifery to become a legal, cost effective, and medically-sound practice uniformly across Canada, legal recognition, standardization of education, and funding would be required.

A History of Midwifery in Canada: Practice on the Periphery

The trajectory of midwifery in Canada reveals how the practice was marginalized and regenerated due to various influences, including the medical profession, state-imposed legal regulations and changes in cultural perspectives of childbirth. Midwifery was formally legislated out of medical practice in 1895 when the parliament, under pressure from the physician lobby, passed a law placing childbirth under the sole jurisdiction of physicians (7). Midwifery had been particularly prevalent in smaller, remote and indigenous communities, however, it became increasingly difficult for midwives to maintain the profession's viability due to this legislation. Midwives left the profession in fear of legal prosecution, and hence the technical skills and culture of midwifery was not transmitted to the next generation, though in some districts of Canada, the profession of the nurse-midwife began to form to counter the decline of traditional midwifery (8). Furthermore, the advent of insured medicine in Canada reinforced the abandonment of the midwife profession, due to a lack of clients and social support, as most Canadian women knew of no alternatives to birth attended by a physician. The absence of formalized midwifery-training programs, coupled with the dwindling number of practicing midwives, forced this once-mainstream practice to the periphery of health care.

Midwifery persisted during these difficult years in a few, isolated communities because of socio-cultural, geographical and historical factors. Many of these communities where midwifery was practiced lacked access to physicians and hospital facilities. This occurred mostly in isolated Northern and rural regions, such as Rankin Inlet in Northwest Territories. Unlike the rest of Canada, where maternal and infant care was and still is under provincial legislation, midwifery was self-regulated in these indigenous communities (7).

The marginalization of midwifery by the Canadian political and medical mainstream was a manifestation of the aversion towards midwifery by the Canadian health care establishment. The Canadian Medical Association, as well as Provincial Colleges of Physicians and Surgeons, opposed midwifery, citing studies deeming it inferior and unsafe in comparison to medicine's approach to childbirth. They strongly opposed homebirth, which they asserted constituted an inherent, unavoidable risk, and punitive measures were imposed on physicians in Alberta and Ontario to deter them from attending home births or providing backup support for planned home births (9,11). The Canadian Nurses Association, though not as oppositional, was quite cautious since it perceived midwifery as encroaching on nursing interests and jurisdiction (7). Some Canadian nurses advocated an increased role for nursing professionals in maternal care, citing the U.S. model of the nurse-midwife as being amenable to their potential role in the Canadian system (7).

Historical currents during the World Wars slowly fostered resurgence in interest and support for midwifery in urban areas and in the political arena, as groups of women organized for increased access to midwifery, since many male physicians were on the front (8). This small lobby persisted and was strengthened exponentially with the advent of feminism and advances in perceptions of women's health. Despite the wider acceptance of midwifery, there was no standardization of midwife training and education in that era. Midwifery was a private practice, remunerated directly by the patient. Standards governing midwifery were not clearly defined and the practice was still considered outside of the usual legal modalities of health care.

Acquiescing to this lobby, government commissions to investigate midwifery were initiated in the late 1960s and 1970s in order to determine a possible role for nurse-midwives in both urban and rural settings. These committees were often politically motivated, and an opportunistic method for governments to consolidate votes and support from women and feminists. The Committee on Healing Arts, set up in 1966 at the advent of universal medical care insurance in Ontario, recommended the integration of the nurse-midwife into the health care system (8). However, there was significant pressure by nursing associations, which did not wish to incorporate midwifery into their practice. These nursing professional groups suggested that

Canada adopt the British model of midwifery as an independent and separate profession (8).

Societal demands for homebirth and natural births flowed from the counter-cultural center of Canada-British Columbia-in the 1970s. Midwifery became increasingly prevalent but was strictly relegated to the home setting. Midwives practicing at that time faced potential liability as they were providing medical care and services without formal recognition or uniform standards, and were thus vulnerable to the legal charge of practicing medicine without a license (8). Subsequent high profile trials led to increased attention to this issue, both by medical practitioners and the public, and fostered a number of government inquiries into the practice. This attention aided in the genesis of provincial midwifery coalitions and organizations to lobby the government and represent the position of midwives in several provinces.

A high profile death in 1985 of an Ontario baby, delivered at home by a midwife, brought the issue to public debate, media attention and scrutiny (8). The Crown was supported by medical professionals, who blamed the midwife for the death, whereas the midwife's defense was that the death was unavoidable. Both sides, nonetheless, concluded that regulation of midwifery was needed in Ontario. The trial and subsequent inquests evolved into a public inquiry into the state of midwifery in Ontario, and was crucial to the genesis of the Midwifery Task Force of Ontario (MTFO). The MFTO advocated the implementation of midwifery as a self-regulating profession with its own college, independent of both medicine and nursing. The MTFO also recommended that midwives have the option to practice in the home, or in an institutional setting, and that a nursing background should not be a prerequisite to midwifery education (8). When this legislation passed in Ontario in the early 1990s, and midwifery became a service reimbursed by the government, Canada joined the ranks of other countries that had already accepted midwifery and integrated the practice into their health care system (10). Government support for the midwifery initiatives were forthcoming for two main reasons: i) midwifery was seen as a cost effective form of care, and ii) midwifery support positioned governments as publicly supporting women's issues and promoting women's rights (8). Governments in British Columbia, Manitoba, Ontario and Quebec followed quickly and implemented midwifery-oriented health policy including legalization of the profession, standardization of training and fees remunerated through the public, provincial insurance plan. However, Saskatchewan and the Atlantic provinces have not legalized nor formalized midwifery practice at this time.

Policy and Practice of Midwifery in Canada

Despite the legalization of midwifery in some provinces, controversy persists in the medical community about the medical efficacy of midwife care, and there are disagreements about who provides the least expensive care to women with low risk pregnancy. In terms of optimal care for low risk pregnancy and normal vaginal delivery, studies have shown nearly congruent mortality and morbidity rates whether attended by midwives or physicians (12). In a study by Janssen et al. labour interventions in comparable midwife-attended homebirth births, midwife-attended hospital births and physician-attended hospital births were analyzed. The study showed that the home birth group displayed less frequent use of analgesia, electronic fetal monitoring, augmentation or induction of labour, and episiotomy, as well as fewer caesarian sections among women in the home birth group (6.4%) compared with the midwife hospital group (11.9%) and the physician hospital group (18.2%) (12). Obstetricians are trained with a surgical orientation and some have a tendency to utilize interventional techniques to speed up labour (12).

The majority of births are low risk and can proceed without interventional, surgical techniques (12i). Therefore, midwives are critical of this surgical medical training and practice, though necessary in complicated births, for its application in low-risk births (1). The methodologies of teaching birth in medical school, and the charge that students and residents rarely witness a normal, spontaneous, unanaesthetized birth are some of the more extreme criticisms by midwives of the medical establishment (1).

The midwifery lobby advocates an increased role for themselves in natural, uncomplicated, spontaneous birth. As defined by the Ontario Midwifery Act in 1991, "the practice of midwifery is the assessment and monitoring of women during pregnancy, labour and the postpartum period and of their newborn babies, the provision of care during normal pregnancy, labour and postpartum period and the conducting of spontaneous normal vaginal deliveries."(13) Midwife advocates assert that midwifery training includes the ability to foresee complications and appropriately call for physician assistance. These precautions reduce potential problems and encourage referral for complicated births to obstetric specialists. The differing courses of treatment of medical and midwife professionals for uncomplicated birth explain the different approaches to care and methodologies employed. The obstetrician tends to see the patient for short checkups preceding birth, and then will attend to the woman while she is giving birth for short periods of time in a hospital,

assisted by nurses and working in shifts. The midwife, however, develops a more consultative, collaborative relationship with the woman in the weeks leading up to birth, coaches the woman through the duration of delivery, and gives continued, postpartum maternal and newborn consultation.

Policy perspectives regarding cost efficiency often cite midwifery as a less costly alternative to physician care (11). There are a number of important variables that must be applied to fully balance the cost comparisons of midwife and physician deliveries. These include the reduced complications in midwife-attended births, the absence of technical equipment and drugs, and less support staff. Moreover, the midwifery option is often advantageous from a subjective client perspective, as their birth experiences are generally quite positive (11). A study by Harvey et al. confirmed this by indicating "women experiencing low risk pregnancies were more satisfied with care by midwives that with care provided by doctors." (14) Physician shortages, particularly of obstetricians and family physicians trained to provide obstetrical care, has been a major concern to decision makers since the mid-1990's. Many family physicians (who traditionally delivered babies in non-urban areas) decline or withdraw from obstetric practice due to fears of litigation, insufficient training or lifestyle concerns (3). The inclusion of midwives as the providers of primary maternal and newborn care has compensated for obstetrician and physician shortages in rural regions.

However, midwives are not universally welcomed by obstetricians, physicians and hospitals since midwives deal primarily with uncomplicated cases, which siphon billings of easier, quicker deliveries from physicians, relegating more difficult, intense, and problematic patients to physicians. This is known as 'cream-skimming' or 'cherry picking.' Home birth implies less income to hospitals, and since they require a certain level of funds to properly care for complicated births that generate massive expenses of personnel time, and resources, this is seen as a problem. The issue of 'cream skimming' has not yet become critical in the midwifery debate, however, with the anticipated growth of midwifery, it will become an increasingly contentious issue in terms of allocation of government funds. Physicians who deal predominantly with complex cases confront premature personal burn-out and also reduced financial income and billings. These are among the main factors cited by physicians and hospital lobby groups to oppose greater roles for midwifery. Nevertheless, as policies evolve, there has been more impetus to include midwives in group practices to compensate for physician shortages and hospital overcrowding.

Trained, regulated and integrated midwives can potentially decrease stress on family physicians and obstetricians by attending the large number of uncomplicated births and counseling patients on mothering and lifestyle. Midwifery similarly addresses issues of determinants of health and focuses on health promotion by discussing nutrition, early breastfeeding and child care as part of the midwife repertoire of a dynamic patient-based approach focusing on interpersonal relationships and continuity of care (3).

Provincial Perspectives: Midwifery Policy in Ontario and Alberta

Much debate, particularly between different interest groups of health care practitioners and policymakers, has accompanied the implementation of midwifery into the health care system. Since each province has autonomy in health care, midwifery has been legalized and promoted to different degrees, reflecting the unique social, political and economic needs and citizen demands of each province. A comparison of the course of legalization of midwifery in Ontario and Alberta illustrates how these differences are accommodated and reflected in law. Policies of legalization and recognition tend to address midwife-training, ranges of responsibility, methods of payment, degree of autonomy and relations to other health care providers and institutions (16).

Ontario

The Ontario Midwifery Act of 1993 regulated midwifery as an autonomous health profession, established university programs for midwifery training, created the regulating body of the College of Midwives of Ontario, and additionally organized a system of provincial financial billing for midwifery services. This legislation established that midwives could be the primary practitioners of care, with the responsibilities of admission, direction of care, and discharge, and that midwives can practice at homes, hospitals, or birthing centers. This option of delivery of care validated midwifery and allowed midwives in Ontario to become increasingly familiar with other health professionals and participate more actively in research, education and policymaking regarding maternal and newborn care (17).

The practice of midwifery is growing in Ontario. There are three provincial universities (McMaster, Ryerson Polytechnic and Laurentian), which offer the Ontario Midwifery Training Programme, a four-year degree including intensive clinical exposure and training. There is also the College of Midwives, which offers the Prior Learning and Assessment Program. These programmes register approximately thirty-five

new midwives each year (17). Arguably, this progressive policy and strong government support has facilitated the remarkable success and growth of midwifery in Ontario, with nearly 3,800 births in 1999 attended by midwives, and an anticipated 12,000 births in 2004 (10). The Ontario legislation is the foundation upon which the four provinces that subsequently legalized midwifery look to for precedent and policy directives. Patients increasingly demand midwifery, and midwives are anticipated to deliver 30% of all babies in Ontario and British Columbia by 2020 (10). Physicians in some regions have maintained strong efforts to retain their labour and delivery caseloads, and often the admission privileges of midwives are not respected (9). These physician interests are driven by various factors including a desire to preserve billing privileges and delivery priority. Midwives, however, are entrenched as part of the health care system spectrum, as their presence was implemented in an incremental and organized manner and the future evolution of the profession is assured through policy mechanisms facilitating greater patient choice and services.

Alberta

The legalization and inclusion of midwifery in Alberta differs dramatically from Ontario. The legislation granting legal recognition and professional status to midwifery does not allocate funds, educational initiatives or institutions for the growth of midwifery. The failure to pay midwives from the provincial budget, and trivial government support of midwifery led to a stagnation of development, and precipitated an exodus of trained midwives from Alberta despite recognition. The conservative fiscal stance of the Albertan government illustrates the power of government policy to facilitate or hinder shifts in health care. The government justified its actions by citing unnecessary competition between practitioners and "overlapping, nonexclusive scopes of practice" as specific criticisms of midwifery (9). Consequently, with no provisions to ensure that the supply of midwives would increase through education and training, Alberta experienced a crisis in midwifery. Widespread shortages of physicians delivering acute primary care, combined with the strong midwife and feminist lobby, enabled the politicization of midwifery, with the midwife lobby utilizing the argument of consumer choice to galvanize the public and induce government action in order to sustain the profession in the province (9). Midwifery remains politicized in Alberta, as it is currently part of the provincial government's initiative to reduce government health care costs through privatization of services of choice. That policy allows Albertans to have access to regulated midwifery and trained professionals, however, they must remunerate midwives directly for their services. Although women in Alberta can no choose to have a physician or midwife for their birth experience, only the physician fees are fully covered by Medicare. With midwife-attended births comprising 6.6% of all births in British Columbia, and 4.5% in Ontario, Alberta remains at the low rate of 1%. In Alberta, the midwife-attended births occur mainly in the home and are only available to women who can afford this service. This discrepancy is directly attributed to a lack of government funds (18). This illustrates the decisiveness and impact of government support and policy, and how inclusion of midwifery services in the health care system facilitates greater access for women who choose such services.

Midwifery and the Future of Health Care in Canada

Midwifery is becoming increasingly important to future visions of health care in Canada, and is a strong political tool and issue to garner public interest and support. Despite its political salience, midwifery as a health care service and alternative to physician-assisted birth has garnered support for practical purposes so that the challenges and shortages of maternal care can be remedied by further integrating midwifery into the changing health care system as a cost effective, medically sound alternative to physician delivery. According to Monique Begin, the former Minister of Health and Welfare, the health care system must evolve to create health care providers who will be able to operate in the dynamic system of the future (19). This system must be increasingly responsive to patient needs, and linkages need to be forged between the various participants in the health care world, between traditional medicine, health promotion advocates, as well as the social and environmental determinants of health (19).

The model of care offered by midwifery is compatible with these principles, as midwifery is based upon standards such as continuity of care, informed choice and consent, and choice of birthplace. Midwives are not just trained to deliver babies, but rather to offer a range of care such as maternity care, breastfeeding instruction and support (19). The midwife-patient relationship is based upon trust, longevity and encouragement as midwifery mandates personalized, intimate care and relationships. Furthermore, within maternal care, midwifery acts as an interface between family physicians, specialists and other traditional modalities of health care.

The emphasis on group-based care within the midwifery community and the larger medical community is congruent with the current policy

direction to group-based practices. Group-based care emphasizes comprehensiveness and cooperation, and seeks to alleviate pressures of physician shortages and waiting by offering around the clock care, referrals and attention (10). This projection of group practice is particularly significant in maternal and neonatal care within the medically underserviced urban, rural and remote populations and regions of Canada, since there has been a marked decrease in trained physicians able to provide maternal and neonatal care in these communities. Government policy in the last decade restricting medical school enrollments and residency training positions for obstetricians coupled with the decreasing numbers of trained family physicians are some factors that have created this shortage. In Ontario and British Columbia, where midwifery care has had adequate public funding, midwifery has been filling this widening gap for low risk obstetric services (10).

The loss of the basic maternal and neonatal care services has the potential to undermine the overall health of a community. Maternity and neonatal services are necessary for a community that wants to grow, and the development of a flexible, well-integrated community health care system with a wide variety of basic services is critical to serve the health care needs of the increasingly diverse population in Canada. For example, the presence of regulated midwifery in Rankin Inlet has resolved some social problems associated with moving an expectant mother away from her family and community in the weeks preceding and following birth (7).

The group-based practice answers the critical aspects of Begin's vision of the future of health care, as it is a clear and concerted effort towards professional interaction, linkages and collaboration. Furthermore, access is always a primary issue for midwives and the group-based practices provide access to a wider group of women, as their family physician will be able to refer low risk pregnancies to their midwife colleagues. The health care system, operating in the diverse urban, rural and remote regions of Canada, and accommodating the varied and complicated demands of the populace must be flexible, future-oriented and creative in order to accommodate the demands of the populace. The changing role of midwifery within Canadian health care, its ongoing, incremental acceptance into the mainstream and increasingly visible role as a policy driver illustrates how the system evolves to meet patient needs. Midwifery is poised to answer the challenges facing newborn and maternal care in Canada. However, Canadian citizens, health care professionals and decision makers must recognize this and nurture the development of this dynamic profession.

REFERENCES

1. The Boston Women's Health Book Collective. Our Bodies, Ourselves. New York: Simon and Schuster, 1998.
2. An Article in Calgary Herald, May 19, 1998 as quoted by Gunhild Hoogensen "The Politics of Birth: Midwifery in Alberta" 2000. www.birthpartnershipmidwives.com
3. Buske, Lynda. " A Crisis Aborning in Newborn and Maternity Care?" Canadian Medical Association Journal, March 6, 2001; 164 (5).
4. Chan, Benjamin TB "From Percieved Surplus to Percieved Shortage: What Happenned to Canada's Physician Workforce in the 1990's?" Canadian Institute for Health Information, June 2002.
5. Blais, Regis "Commentary/Commentaire: Are Home Births Safe?" Canadian Medical Association Journal, February 5, 2002; 166 (3).
6. Legislation Concerning Nursing/Midwifery Services and Education (EURO Reports and Studies, 1981) as quoted in Blais, Maheux, Lamber, Loiselle, Gauthier, Framarin "Midwifery defined by physicians, nurses and midwives: The birth of consensus?" Canadian Medical Association Journal 1994; 150 (5): 691-697.
7. Bailey, Lehr, Nicholas, Picco "Midwifery: Promotion and integration into Canada's healthcare system" Leadership in Health Services July/August 1993: 11-13.7.
8. Bourgeault, Ivy Lynn and Mary Fynes "Integrating Lay and Nurse Midwifery into the U.S. and Canadian Health Care Systems" Social Sciences & Medicine"1997; 44 (7): 1051-1063.
9. McKendry, Rachael and Tom Langford "Legalized, regulated, but unfounded: midwifery's laborious professionalization in Alberta, Canada, 1975-99" Social Science & Medicine, 2001;53: 531-542.
10 . Canadian Midwifery Regulators Consortium, Submission to the Commission on the Future of Health Care in Canada "Regulated Midwifery and The Future of Health Care in Canada" 2001.
11. Reinharz D, Blais R, Fraser W, Constandriopolous AP "Cost-effectiveness of midwifery services vs. medical services in Quebec, L'Equipe d'Evaluation des Projets-Pilotes Sages-Femmes" Canadian Journal of Public Health 2000; 91(1): 112-115.
12. Janssen, P, Lee, SK, Ryan, E, Etches, D, Farquarson, D, Peacock,D and Klein, M. "Outcomes of planned home births versus planned hospital births after regulation of midwifery in British Columbia" Canadian Medical Association Journal 2002;166 (3):315-323.

12i. Low risk births can be identified at any stage of gestation, however in the Janssen et al. study, 3.6% of homebirths had to call for emergency transports in spite of the application of the following exclusion criteria including multiple births, heart disease, hypertensive chronic renal disease, pregnancy induced hypertension with proteinuria, insulin dependant diabetes, antepartum hemorrhage after 20 weeks gestation, active genital herpes, breech or abnormal presentation, gestational age of less than 30 weeks or greater than 41 weeks at the onset of labour, more than one previous caesarian section, and mother transferred to hospital from another health care facility. (Janssen et al.)

13. www.aom.on.ca
14. Harvey,S, Rach, D, Stainton, MC, Jarrell, J, Brant, R. "Evaluation of Satisfaction of midwifery care" Midwifery 2002; 18: 260-267.
15. Ontario Association of Midwives (AOM) as quoted by Scott Piatkowski "Midwifery Remains a Safe Alternative", June 10, 2002. www.straightgoods.ca
16. Blais, Maheux, Lamber, Loiselle, Gauthier, Framarin

"Midwifery defined by physicians, nurses and midwives: The birth of consensus?" Canadian Medical Association Journal 1994; 150 (5): 691-697.
17. www.aom.on.ca/midwifery/NowandThen/html
18. www.asac.ab.ca
19. Bennett, Carolyn. Kill or Cure? Toronto: HarperCollins Publishers Ltd.,2000.

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REVIEW ARTICLE

Gene Therapy For Adenosine Deaminase Deficiency: Successes and Limitations

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INTRODUCTION

Severe combined immunodeficiency disease (SCID), often dubbed the "baby in a bubble" syndrome, represents the most severe type of primary immunodeficiencies (1). It is a heterogeneous group of congenital disorders caused by a number of different defects of the lymphoid lineages (2) and the estimated incidence is 1 in 100,000 live births (3). Several natural mutants have been characterized in humans, all of which involve complete block of T-cell development, and will directly or indirectly impair B-cell immunity (1, 4). This leads to devastating clinical symptoms as a result of predisposition to infections from many opportunistic pathogens (4). In many cases, severe infections starting at 1 to 3 months of age will lead to death if untreated (5, 6, 7). This disease received worldwide recognition in the 1970s when the story of David Vetter, who lived all of his 12 years of life inside a sealed plastic bubble designed to protect him from infections was brought to light. Subsequently, his life story and the unique strategies used to treat David, received widespread media attention, being the subject of movies and television shows .

The adenosine deaminase (ADA) deficient variant of SCID is an autosomal recessive disorder and accounts for approximately 20% of all SCIDs (8). This inherited deficiency results in decreased enzymatic activity or the lack of production of adenosine deaminase a housekeeping enzyme of the purine salvage pathway (9, 10). ADA was the first gene associated with a SCID condition to be identified (11), and was the focus of the first gene therapy trial in 1990 (12). ADA-SCID is a lethal disorder that is now treated with either allogeneic bone marrow transplantation or enzyme replacement therapy (2, 13). Gene therapy of this disease is in clinical trials and has produced the most promising clinical experience thus far of all the genetic diseases. ADA-SCID patients have been transplanted with transduced autologous T lymphocytes and hematopoietic stem cells (HSC) (4, 7). The progress made in the attempt to treat this disease reflects both the successes of gene therapy and the limitations of it that have to be overcome before it can be a reliable and realistic treatment for ADA deficiency and other genetic diseases. The past, present and future therapies of ADA-SCID will be examined to demonstrate this progress.

WHAT IS ADENOSINE DEAMINASE?

Adenosine deaminase (ADA) is an important deaminating enzyme of the purine salvage pathway that converts adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine respectively (2, 11, 12, 14). ADA allows for the conversion of adenosine into other purines to be recycled and removed by formation of uric acid, which is the end product of purine metabolism in humans (14). ADA is especially critical for cells such as

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lymphocytes and erythrocytes that lack or have very low activity of the de novo purine synthetic pathway (2).

In humans, there are two isoforms of the ADA enzyme, adenosine deaminase1 (ADA1) and adenosine deaminase 2 (ADA2). Intracellular ADA activity is mainly mediated by ADA1, while ADA2 is the predominant isoform in human plasma and serum (14). The cellular source of the latter has been linked to the monocyte-macrophage cell system, although it is widely accepted that ADA2 activity represents T-cell function, and measurements of ADA2 activity has been used to evaluate the disease severity of patients with acquired immunodeficiency syndrome (14).

The importance of maintaining normal levels of ADA activity can be seen in patients with a genetic deficiency of this enzyme. Lack of ADA activity, which is important in T-cell development, is associated with a form of severe combined immunodeficiency disease referred to as ADA-SCID (11, 14). In terms of biochemistry, the lymphospecific toxicity associated with this disease is thought to be the result of the accumulation of 2'-deoxyadenosine (a substrate of ADA) and its conversion to the phosphorylated form (deoxyadenosine triphosphate, dATP), which is an inhibitor of ribonucleotide reductase, a key enzyme in DNA synthesis and DNA repair in dividing T cells (15). This leads to cell death in non-dividing T cells (1, 14). A second mechanism contributing to the pathology of ADA-SCID involves the methylation reactions of S-adenosylmethionine to S-adenosylhomocysteine (AdoHcy) (14). AdoHcy is hydrolyzed to adenosine and homocysteine by AdoHcy hydrolase. In patients with ADA-SCID, accumulating 2'-deoxyadenosine inhibits AdoHcy hydrolase resulting in the accumulation of AdoHcy. AdoHcy then functions as a competitive inhibitor of many transmethylation reactions critical to cellular functions (14).

CLINICAL AND PATHOLOGIC FEATURES OF ADA-SCID

Classically, SCID is defined as a fatal infantile syndrome with symptoms resulting from the absence of cellular and humoral immunity. Most infants suffering from ADA-SCID have shown the same clinical and immunological manifestations as patients with non-ADA-SCID (16, 17). Although these infants suffer from lymphopenia and absence of non-maternally derived immunoglobulin, symptoms may not appear until several weeks to several months of life (2). The full-blown syndrome includes overwhelming fungal, viral and bacterial infections and failure to thrive (10).

While the underlying immunodeficiency in ADA-SCID appears early in life, there is a progressive worsening of the condition as toxic metabolites accumulate (due to the absence of ADA) and continue to interfere with normal T-cell and B-cell function. Although 15% of ADA-SCID patients show a slightly later onset and a slower progression of symptoms, it is still fatal. The variability of disease progression may be partly a result of environmental factors and undoubtedly of genetic origin. If left untreated, ADA-SCID is fatal by 1-2 years of age; however, it is more common for death to occur during the first few months of life (2).

In addition to infantile-onset ADA-SCID, cases of later onset immunodeficiency have also been reported (2). In one of the earliest cases of ADA deficiency, clinical symptoms did not appear until two years of age and the only detectable abnormality suggestive of an immunodeficient phenotype, prior to disease onset, was a phasic appearance of lymphopenia and eosinophilia (18). Since then, other cases have been reported and reviews of patient medical history and laboratory findings reveal common features. At the time of diagnosis, all had diminished T cell counts with low mitogen responses; however, several showed normal total Ig and antibody responses to some antigens (19, 20, 21). They had substantially high IgE and/or eosinophilia, a history of recurring sinopulmonary bacterial infections including pneumococcal pneumonia and septicemia, and inability to produce antibody to some antigens such as pneumococcus (19, 20, 21). An interesting feature seen in two cases was the diagnosis of "autoimmune" hypothyroidism (20, 21), which could directly reflect toxicity to the thyroid or autoimmune disease due to abnormal regulation of the immune response. These later onset ADA-SCID patients would show more residual ADA activity than those with the infantile-onset disease (2).

CURRENT TREATMENTS FOR ADA-SCID

Bone Marrow Transplantation

The current curative treatment of choice for all SCID patients including ADA-SCID is bone marrow transplantation (BMT) from an HLA-identical sibling (11, 12, 13). HLA, an abbreviation of human leukocyte antigen, is the major histocompatibility antigen occurring on human nucleated cells, including lymphocytes. This form of treatment results in a long-term cure rate of 95-100% (22), however, less than one third of patients have access to an HLA-identical donor (11, 12). In the absence of an HLA-identical sibling donor, T-cell depleted parental bone marrow (haploidentical donor) is preferred over an unrelated donor. This alternative has provided less encouraging results and reports have shown that treatment success rates of BMT for ADA-SCID patients lacking an HLA-genotypically identical donor have not improved over the last 20 years (12). Significant side effects can result due to the need for conditioning cytoreduction and

immunosuppression with systemic chemotherapy and total body irradiation, which increases the risk of both short and long term complications. Complications include life-threatening infections, acute cardiomyopathy, progressive pulmonary fibrosis, irreversible sterility and secondary malignancies (10). For all these reasons, BMT is not a useful treatment for all SCID patients, especially those who are too sick to tolerate cytoreductive therapy or where the risks associated with this treatment is felt to be too high (10).

Polyethylene Glycol-modified Bovine ADA (PEG-ADA)

For patients with ADA-SCID who are lacking an HLA-identical bone marrow donor, an alternative treatment would be enzyme replacement therapy using polyethylene glycol-modified bovine ADA (PEG-ADA) (23). Because the toxic substrates of ADA (adenosine and deoxyadenosine) diffuses freely throughout the body, injecting this enzyme into ADA-deficient patients can replace the function of the missing enzyme (24). Infusions of purified bovine ADA linked to polyethylene glycol have been successful in decreasing the number of infections by increasing lymphocyte count and by restoring partial T-cell function. However, the formation of inactivating antibodies against the bovine ADA has been observed, and full immune reconstitution is less regularly achieved with this therapy (4, 8, 25). This treatment is also very expensive, costing an estimated US $250,000/yr./patient (11, 26).

In a study by Bordignon et al. (1993), two children suffering form ADA-SCID were treated with PEG enzyme replacement therapy and showed very promising initial results (10). Weekly doses of 20 U/kg body weight resulted in a therapeutically constant plasma level abolishing all the tested biochemical abnormalities associated with ADA deficiency. Improvements included the absence of infections and restored weight and height gain, and after three months of therapy, the patients did not require isolation or hospitalization. During these three months, their absolute lymphocyte counts normalized, as well as percentages of CD3+, CD4+, CD8+ T cell populations, and lymphocyte proliferative responses to PHA and IL-2, indicating an improved immune system. One patient, upon receiving vaccination from tetanus toxoid and FSME virus one year after initial PEG-ADA treatment, developed specific antibodies and produced specific T-cells to both antigens. Unfortunately, these improvements did not persist after discontinuation of i.v. immunoglobulin prophylaxis, with the appearance of decreased total lymphocyte counts, reduction of TCR repertoire and antigen specific responses. In addition, results indicated that intracellular production of ADA activity would be more efficient in promoting lymphocyte survival and immune functions rather than extracellular detoxification as in the case with PEG-ADA replacement therapy.

RATIONALE FOR THE GENE THERAPY OF SEVERE COMBINED IMMUNODEFICIENCIES (SCID)

Over the past 3 decades, advances in molecular biology have demonstrated the usefulness of gene therapy as a new tool to correct patient cell function and to alleviate disease. The first successful gene transfer with a retroviral vector to murine hematopoietic cells was reported in 1983 (27), and since then, numerous studies using murine hematopoietic stem cells have been done to assess the potential use for human stem cell gene therapy (12). Encouraging murine in vivo studies in which recombinant murine retroviruses infect murine hematopoietic stem cells, have demonstrated high efficiency (28). More importantly, these cells had the ability to maintain long-term expression of the transduced gene. These successes led to the belief that human stem cell gene therapy could soon be a reliable treatment for various congenital or acquired human diseases.

There are a number of reasons that have made this disease a primary focus for gene therapy. First, ADA deficiency is the most extensively studied congenital immunodeficiency disease (12). The genomic and cDNA sequences encoding ADA were identified early on (29, 30), and the structure and function of this enzyme is well understood (12). Second, it is a disease that if left untreated, results in debilitating and lethal effects. Because an alternative effective therapy is currently not available for every patient, the potential risk of gene therapy experimentation becomes more acceptable. Third, it is known that patients receiving bone marrow transplantation can be cured of this disease (9, 31), and so the target tissue for the introduction of the ADA gene is the easily accessible hemopoietic system. Fourth, based on various studies, it is expected that the genetically corrected cells should persist and should have a selective growth advantage over the non-transduced cells in vivo after transplantation (32, 33). Finally, the regulation of the ADA gene would not have to be precisely regulated nor would the expression have to be cell-type specific for beneficial effects to occur. Patients exhibiting 10% of normal ADA levels do not show any apparent immune impairments (34) while patients with ADA activity greater than 50-fold above normal suffer only from a moderate hemolytic anemia (35). If the ADA gene can be introduced in pluripotent hemopoietic stem cells

(PHSC), reimplantation and normal growth of these transduced cells could result in a life-long production of corrected immune cells.

GENE THERAPY FOR ADENOSINE DEAMINASE DEFICIENCY

Gene therapy involves the introduction of exogenous genetic material to correct or modify the function of a cell. It is an emerging medical procedure where genetic diseases could be corrected by transfer of a normal version of a relevant gene into a patient's somatic cells. Gene transfer into a patient's hematopoietic stem cells followed by their autologous transplantation could provide the same benefits as allogenic transplantation without the immunological complications such as graft rejection, graft versus host disease, and post-transplantation immunosuppressive therapy. Although gene therapy to treat blood diseases seems logical, there are still more problems than successes, mainly from inadequate tools used for gene transfer and gene expression. However, despite bouts of successes and failures, techniques for gene transfer, gene expression and hematopoietic stem cell manipulation have steadily improved.

To date, a number of clinical gene transfer trials using human hematopoietic stem cells (HSCs) have been performed worldwide to test the potential of human stem cell gene therapy to treat ADA-SCID (7, 36, 37). However, the results from these trials are somewhat disappointing, revealing that murine studies cannot always apply to humans. The transduction frequencies in human HSCs were low and clinical benefits were not apparent in all cases. In spite of these failures, many lessons were learned that could be applied to gene therapy of other genetic diseases.

The lessons learnt from preclinical studies and subsequent early clinical trials for ADA-SCID demonstrate the advances in gene therapy made so far. Preclinical studies in the 1980's showed that murine hematopoietic stem cells (HSCs) could be transduced in vitro using the Moloney murine leukemia virus (MoMLV)-based retroviral vector, containing the human copy of the ADA gene (9, 38). These cells were subsequently transplanted into irradiated mice resulting in circulating lymphohematopoietic cells that carried and expressed the human ADA gene (9, 38). These preclinical results suggested that a similar strategy could be applied in humans to successfully treat patients with ADA deficiency. As a result, researchers proposed to harvest autologous bone marrow from ADA-SCID patients, to transduce these cells in vitro with a retroviral vector carrying the normal human ADA gene, and to infuse these cells into the respective patients without using pretransplant myeloablative chemotherapy. Although it was a good idea, researchers at the time lacked in vitro and in vivo systems to evaluate human HSC gene transduction, so preclinical research could not definitively conclude that human HSC could be transduced (9). Instead, many researchers turned their attention to the transduction of peripheral blood T lymphocytes of ADA-deficient patients who were receiving PEG-ADA enzyme replacement therapy. This different approach was a result of preclinical studies showing that in vitro transduction of peripheral blood T lymphocytes from ADA-SCID patients, using a retroviral vector, was possible (33). The genetically modified lymphocytes were transplanted intraperitoneally into immunodeficient mice, and were examined one month later. Results indicated that T lymphocytes, which had been transduced with the normal human ADA gene, persisted, while T lymphocytes transduced with a control vector did not (33). This indicated that transduction of the normal ADA gene into ADA-deficient T lymphocytes was a feasible approach to treating ADA-SCID (10, 24, 33).

These preclinical experiments were very encouraging and led investigators at the National Institutes of Health (NIH) to use a similar strategy to treat humans. In 1990, the Blaese group at the NIH performed the first clinical gene therapy trial on 2 young female patients with ADA-SCID (4, 12). They targeted peripheral blood T lymphocytes from the patients with ADA-SCID on PEG-ADA therapy with a MoMLV-based retroviral vector containing the normal human ADA gene (LASN) (4). The patient's lymphocytes were harvested, genetically modified, expanded more than 50-fold, and given back by infusion (4). Over 2 years, the patients received a total of 11-12 infusions of autologous genetically corrected lymphocytes and as a result, immune functions of both patients were better than when only on PEG-ADA treatment alone (4, 12). Unfortunately, the transduction frequency of the infused T lymphocytes differed in the two patients (30% and <1%) and they also continued to receive PEG-ADA therapy throughout the procedure (4). These experiments were able to show that transduction of human peripheral blood T lymphocytes are possible and that their progeny could persist in vivo for many years. These experiments also raised many questions such as; what was the antigenic repertoire of the transduced cells? More importantly, could the transduced T-cells persist without the exogenous source of ADA that was coming from the PEG-ADA therapy?

As a result of these questions, many groups had the ambitious goal to permanently correct ADA deficiency by genetically correcting autologous haematopoietic stem cells (HSC). If successful, they would avoid the possible problem of defects in the antigenic repertoire

of the mature T cell used in previous transplantations, and only one infusion of genetically corrected stem cells would be needed to restore the patient's immune functions (23). Three clinical trials have been conducted examining the use of transduced autologous HSC to treat ADA deficiency. One trial used only bone marrow HSC, another used bone marrow HSC in addition to peripheral blood T lymphocytes, and the last used umbilical cord blood HSC (9, 24).

The group conducting the clinical trial using CD34+ cells from bone marrow alone, reported that the transduction efficiency was disappointingly low and that transduced peripheral leukocytes could not be detected in the long-term (9, 12, 37).

On the other hand, in 1992, Bordignon et al., using transduced CD34+ bone marrow cells and PEG-ADA dependent T lymphocytes, showed rapid improvements in patient immune functions after gene therapy (7). Bordignon's group used two different retroviral vectors to transduce a normal ADA cDNA separately into peripheral T-lymphocytes or bone marrow cells to determine which cell population was the major source of the circulating peripheral blood T lymphocytes. Their results show that immediately after transplantation the circulating transduced T cells originated from the infused peripheral T lymphocytes, but as time passed they were replaced with transduced T cells derived from the transduced bone marrow cells (7, 9). This suggests that bone marrow HSC can be transduced and that genetically modified HSC can give rise to functional mature cells detectible in the peripheral circulation. Unfortunately, the patients in this trial continued to receive PEG-ADA enzyme replacement therapy, so it remains unclear if transduction of T lymphocytes of HSC origin can provide clinical benefits to ADA-SCID (9).

The third study used CD34+ cells from umbilical cord blood (9, 12, 36). Three neonates were diagnosed in utero with ADA deficiency and cells were transduced with the same LASN vector used in Bordignon's study (9). Transduction of progenitor cells was efficient and longitudinal evaluation of the patients for transduced leukocytes occurred. A year after transplantation, the group reported low but sustained levels of transduced cells in mononuclear cells and granulocytes and increased ADA activity in HSC (36). These patients started PEG-ADA therapy during their first week of life and showed characteristics of a normal functioning immune system. At age 2, PEG-ADA treatment doses were decreased, resulting in a significant decrease in circulating T lymphocytes and a 100-fold increase in the frequency of T lymphocyte transduction without change in myeloid cells or B cells (36). As the dose of PEG-ADA decreased, a selective advantage in survival of the transduced T cells originating from the transduced HSC occurred, but was nonexistent in B cells or other hematopoietic cells (24, 36). At age 5, PEG-ADA therapy was discontinued in one patient, and over a two-month period, plasma ADA levels of this patient became undetectable, levels of ADA substrates increased, and there was substantial decrease in percentage and absolute levels of natural killer (NK) cells and B lymphocytes although no changes in levels of T lymphocytes occurred (36). Again, results showed a selective advantage in survival of the transduced T cell progeny of the transduced HSC. In addition, analysis of T cell function revealed a loss of antigen-specific blastogenesis to tetanus toxoid and candida (9). Interestingly, results also showed that expression of the MoMLV-based retroviral vectors were low in resting human T cells but were relatively high in dividing T lymphocytes, indicating that LASN vector is expressed during thymopoiesis and not in resting peripheral blood T lymphocytes (9). Results suggest that cessation of PEG-ADA therapy resulted in the loss of both transduced and nontransduced antigen-specific peripheral blood T lymphocytes. Since the patient started to show clinical symptoms of immune deficiency such as weight loss, oral thrush and upper respiratory infection, the patient resumed PEG-ADA therapy, resulting in restoration of good health (9, 36). The results obtained from this trial suggest that cord blood provides a stem cell population more suitable for efficient retroviral-mediated gene transfer than does bone marrow, however, significant advances are still needed in this transfer technique before human HSCs can be used to restore effective immunity and to achieve clinical benefits (11).

CONCLUSION

The current clinical results of gene therapy for ADA-SCID are encouraging but also reveal current limitations of gene therapy for immunodeficiency disorders. Results have shown that transduction and transplantation of HSC from both bone marrow and cord blood is possible, and that transduction frequency in T lymphocytes can reach as high as 10-30% if a selection advantage existed in vivo. Unfortunately, when an exogenous source of ADA was removed, results showed a lack of gene expression in nondividing T lymphocytes, indicating the loss of both transduced and nontransduced antigen-specific peripheral blood T lymphocytes. Successful treatment of SCID or diseases involving lymphoid differentiation will ultimately require expression of vectors in mature, nondividing lymphoid cells. For this reason, many investigators are focusing on improving gene transfer technologies. Recent improvements have resulted in the development of better vectors, packaging cell lines and culture conditions for human HSC transduction. For example, use of certain cytokines and recombinant fibronectin

has improved transduction efficiency of HSCs in baboon and rhesus monkeys (39, 40). These cytokines can induce cycling of immature CD34+ cells making them more receptive to transgene integration. Also, new packaging cell lines have been designed which enhances binding of retrovirus to hematopoietic cells by pseudotyping with the gibbon ape leukemia virus envelope resulting in increased rate of CD34+ cell transduction (41). To reduce risk of in vivo transgene silencing, deletion of silencing sequences from viral LTR has been examined. Design of lentiviral vectors have also provided encouraging results, being able to infect non-cycling cells. Although attempts to cure ADA-SCID with gene therapy have yet to prove successful, study of this disease has resulted, by far, in the most promising clinical experience by identifying current limitations and providing enough successes to encourage the pursuit of the solutions.

REFERENCES

1. Cavazzana-Calvo M, Hacein-Bey S, Yates F, de Villartay JP, Le Deist F, Fischer A. Gene therapy of severe combined immunodeficiencies. The Journal of Gene Medicine 3(3):201-6; 2001.
2. Hirschhorn R. Adenosine deaminase deficiency. Immunodeficiency Reviews 2(3):175-98; 1990.
3. Kalman L, Lindegren ML, Kobrynski L, et al. IL2RG and Severe Combined Immunodeficiency (SCID), a Primary Immunodeficiency Disease (PID): fact sheet. Human Genome Epidemiology Network, Centers for Disease Control; July 2002 http://www.cdc.gov/genomics/hugenet/factsheets/FS_IL2RG_ScidPid.htm.
4. Blaese RM, Culver KW, Miller AD, et al. T lymphocyte-directed gene therapy for ADA-SCID: initial trial results after 4 years. Science 270(5235):475-80; 1995.
5. Rosen FS. Successful Gene Therapy for Severe Combined Immunodeficiency. The New England journal of medicine 346(16):1241-43; 2002.
6. Hershfield MS. Adenosine deaminase deficiency: clinical expression, molecular basis, and therapy. Seminars in hematology 35(4):291-8; 1998.
7. Bordignon C, Notarangelo LD, Nobili N, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science 270(5235):470-5; 1995.
8. Aiuti A. Advances in gene therapy for ADA-deficient SCID. Current Opinion in Molecular Therapeutics 4(5):515-22; 200.2
9. Parkman R, Weinberg K, Crooks G, Nolta J, Kapoor N, Kohn D. Gene therapy for adenosine deaminase deficiency. Annual Review of Medicine 51:33-47; 2000.
10. Bordignon C, Mavilio F, Ferrari G, et al. Transfer of the ADA gene into bone marrow cells and peripheral blood lymphocytes for the treatment of patients affected by ADA-deficient SCID. Human Gene Therapy 4(4):513-20; 1993.
11. Onodera M, Ariga T, Kawamura N, et al. Successful peripheral T-lymphocyte-directed gene transfer for a patient with severe combined immune deficiency caused by adenosine deaminase deficiency. Blood 91(1):30-6; 1998.
12. Onodera M, Melson DM, Sakiyama Y, Candotti F, Blaese RM. Gene therapy for severe combined immunodeficiency caused by adenosine deaminase deficiency: improved retroviral vectors for clinical trials. Acta Haematol. 101(2):89-96; 1999.
13. Chen SH, Ochs HD, Scott CR, et al. Adenosine deaminase deficiency: disappearance of adenine deoxynucleotides from a patient's erythrocytes after successful marrow transplantation. The Journal of clinical investigation 62(6):1386-9; 1978.
14. Cristalli G, Costanzi S, Lambertucci C, et al. Adenosine deaminase: Functional implications and different classes of inhibitors. Medicinal Research Reviews 21(2):105-28; 2001.
15. Chabes AL, Pfleger CM, Kirschner MW, et al. Mouse ribonucleotide reductase R2 protein: A new target for anaphase-promoting complex-Cdh1-mediated proteolysis. Proceedings of the National Academy of Sciences of the United States of America 100(7):3925-9; 2003.
16. Hirschhorn R. Inherited enzyme deficiencies and immunodeficiency: adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiencies. Clinical immunology and immunopathology 40(1):157-65; 1986.
17. Meuwissen HJ, Pollara B, Pickering RG, et al. Combined immunodeficiency disease associated with adenosine deaminase deficiency. The Journal of pediatrics 86:169-81; 1975.
18. Giblett ER, Anderson JE, Cohen F, et al. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 2(7786):1067-9; 1972.
19. Levy Y, Hershfield MS, Fernandez-Mejia C, et al. Adenosine deaminase deficiency with late onset of recurrent infections: response to treatment with polyethylene glycol-modified adenosine deaminase. The Journal of pediatrics 113(2):312-7; 1988.
20. Cowan MJ, Martin DW Jr, Warra DW, et al. Intravenous deoxycytidine therapy in a patient with adenosine deaminase deficiency. Advances in experimental medicine and biology 165 Pt A:39-45; 1984.
21. Geffner ME, Stiehm ER, Stephure D, et al. Probable autoimmune thyroid disease and combined immunodeficiency disease. American journal of diseases of children (1960) 140(11):1194-6; 1986.
22. Hirschhorn R. Adenosine deaminase deficiency. In Rosen FS, Seligmann M, editor. Immunodeficiency Reviews. London, Harwood Academic Publications, 1990.
23. Hershfield MS, Chaffee S, Sorensen RU: Enzyme replacement therapy with polyethylene glycol-adenosine deaminase in adenosine deaminase deficiency: Overview and case reports of three patients, including two now receiving gene therapy. Pediatric Research 33(suppl 1):S42-S47; 1993.
24. Fischer A, Hacein-Bey S, Cavazzana-Calvo M. Gene therapy of severe combined immunodeficiencies. Nature Reviews Immunology 2(8):615-21; 2002.
25. Chaffee S, Mary A, Stiehm ER, et al. IgG antibody response to polyethylene glycol-modified adenosine deaminase in patients with adenosine deaminase deficiency. The Journal of clinical investigation 89(5):1643-51; 1992.
26. Blaese RM, Culver KW, Chang L, et al. Treatment of severe combined immunodeficiency disease (SCID) due to adenosine deaminase deficiency with CD34+ selected autologous peripheral blood cells transduced with a human ADA gene. Amendment to clinical research project. Project 90-C-195, January 10, 1992. Human Gene Therapy 4(4):521-7; 1993.
27. Joyner A, Keller G, Phillips RA, et al. Retrovirus transfer of a bacterial gene into mouse haematopoietic progenitor cells. Nature 305(5934):556-8; 1983.
28. Bodine DM, McDonagh KT, Seidel NE, et al. Survival and retrovirus infection of murine hematopoietic stem cells in vitro: effects of 5-FU and method of infection. Experimental hematology 19(3):206-12; 1991.
29. Wiginton DA, Adrian GS, Hutton JJ. Sequence of human adenosine deaminase cDNA including the coding region and a small intron. Nucleic acids research 12(5):2439-46; 1984.

30. Wiginton DA, Kaplan DJ, States JC, et al. Complete sequence and structure of the gene for human adenosine deaminase. Biochemistry 25(25):8234-44; 1986.
31. Parkman R, Gelfand EW, Rosen FS, et al. Severe combined immunodeficiency and adenosine deaminase deficiency. The New England journal of medicine 292(14):714-9; 1975.
32. Tjonnfjord GE, Steen R, Veiby OP, et al. Evidence for engraftment of donor-type multipotent CD34+ cells in a patient with selective T-lymphocyte reconstitution after bone marrow transplantation for B-SCID. Blood 84(10):3584-9; 1994.
33. Ferrari G, Rossini S, Giavazzi R, et al. An in vivo model of somatic cell gene therapy for human severe combined immunodeficiency. Science 251(4999):1363-66; 1991.
34. Daddona PE, Mitchell BS, Meuwissen HJ, et al. Adenosine deaminase deficiency with normal immune function. An acidic enzyme mutation. The Journal of clinical investigation 72(2):483-92; 1983.
35. Valentine WN, Paglia DE, Tartaglia AP, et al. Hereditary hemolytic anemia with increased red cell adenosine deaminase (45- to 70-fold) and decreased adenosine triphosphate. Science 195(4280):783-5; 1977.
36. Kohn DB, Weinberg KI, Nolta JA, et al. Engraftment of gene-modified cells from umbilical cord blood in Neonates with adenosine deaminase deficiency. Nature Medicine 1(10):1017-23; 1995.
37. Hoogerbrugge PM, Beusechem VW, Fisher A, et al. Bone marrow gene transfer in three patients with adenosine deaminase deficiency. Gene Therapy 3:179-183; 1996.
38. Lim B, Williams DA, Orkin SH. Retrovirus-mediated gene transfer of human adenosine deaminase: expression of functional enzyme in murine hematopoietic stem cells in vivo. Molecular and cellular biology 7(10):3459-65; 1987.
39. Kiem HP, Andrews RG, Morris J, et al. Improved gene transfer into baboon marrow repopulation cells using recombinant human fibronectin fragment CH-296 in combination with interleukin-6, stem cell factor, FLT-3 ligand, and megakaryocyte growth and development factor. Blood 92:1878-1886; 1998.
40. Huhn RD, Tisdale JF, Agricola B, Metzger ME, Donahue RE, Dunbar CE. Retrovral marking and transplantation of rhesus hematopoietic cells by nonmyeloablative conditioning. Human Gene Therapy 10(11):1783-90; 1999.
41. Fischer A, Hacein-Bey S, Le Deist F, et al. Gene therapy of severe combined immunodeficiencies. Immunological Reviews 178:13-20; 2000.

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REVIEW ARTICLE

Comparison of Christensen Prosthesis System with Autogenous Costochondral Graft for Arthroplasty of Traumatic Temporomandibular Joint Dysfunction

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INTRODUCTION

The temporomandibular joint (TMJ) is the only joint in the body that is both a hinge and a sliding joint. The TMJ is the most active joint of the body, moving up to 2000 times each day during talking, chewing, swallowing and snoring. Disorders of the TMJ can be referred biomechanically and neurologically to the upper cervical spine, due to the structural approximation and neuromuscular relationship of the TMJ area and occipitoatlantal area. When TMJ dysfunction occurs in children, it impairs mandibular growth and results in mandibular asymmetry or retrognathism. Temperomandibular joint meniscus malposition frequently produces neck pain, headaches and suboccipital muscle spasms.(1) In many cases TMJ dysfunction has a profoundly negative influence on the psychosocial development of the patient, because of the obvious facial deformity, which worsens with growth. Arthroplasty of the TMJ is an effective treatment for structural disorders. Various alloplastic materials, as well as autogenous grafts, have been used in arthroplasty of the TMJ.

Because of the growing use of both autogenous Costochondral graft (CCG) and alloplastic Christensen prosthesis system, it is important that the potential benefits of both procedures be carefully weighed against their disadvantages in different circumstances. After outlining the anatomy of the normal TMJ and the causes and effects of TMJ dysfunction, this article compares CCG and alloplastic Christensen prostheses in terms of advantages, disadvantages and patient groups in which their use is most appropriate.

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TMJ DYSFUNCTION

Anatomy of the TMJ

The TMJ hinges within the glenoid fossa of the mandible and glides anteriorly to the eminentia during normal motion. The head of the condyle and the glenoid fossa are covered with fibroid cartilage which serves as a shock absorber (1). The meniscus of the TMJ divides the joint cavity into two parts. The lower part is used during gliding motion and the upper part is used for hinge movements. The two heads of the pterygoid muscle act asynchronously to open the joint. One head of the external pterygoid muscle pulls the meniscus forward while the second head opens the joint. Secondary assistance is provided by the mylohyoid, geniohyoid and digastric muscles. In closing the jaw, the temporal, masseter and internal pterygoid muscles are activated.

Causes of TMJ dysfunction

Temporomandibular joint dysfunction results from various agents including internal derangement, congenital malformation, arthrotic changes, avascular necrosis, rheumatoid arthritis and trauma (2,3,4,5,6,7). Local and systemic infections systemic diseases like rheumatoid arthritis, ankylosing spondylitis and psoriasis are factors which have been implicated in the etiopathogenesis of TMJ ankylosis (8,9,10,11). Trauma conditions have also often been implicated in the etiology of TMJ ankylosis (12,13,14), the presence of intra-articular hematoma with intra-articular damage leads to scarring and bone formation with resultant hypomobility and ankylosis (11,15). The reported proportion of cases of TMJ dysfunction due to traumas ranges from 26% to 100% (8,9,12,16).

Physiology of traumatic TMJ dysfunction

Trauma to the TMJ can be caused by a single, acute injury (such as blow to the jaw or car accident) or more prolonged, minor, stress due to, for example, clenching or grinding of the teeth. Temperomandibular trauma results in displacement of the disk of cartilage that cushions the ball-and-socket of the joint with possible resultant entrapment of the disc (17).

In partial displacement of the TMJ, condyle translation is not blocked and when the patient moves the closed jaw forward or toward the contralateral side, the condyle will snap forward into its normal position. In complete displacement, the disc is usually dislodged anteriorly toward the front of the condyle so its translation is restricted when the mouth is opened. Persistent condyle motion on a dislocated disc encourages irregular adaptive remodeling and osteoarthritis to develop within the joint because the dislocated disc can no longer cushion the articular surfaces (1).

Symptoms and signs of traumatic TMJ dysfunction

Muscle fatigue and a severe dull facial ache that is often localized to an anterior area to the tragus of the ear are the major symptoms of TMJ dysfunction. Muscle spasm in pterygoid, masseter and temporalis; bruxism; tenderness at the proximal mandible; and typical facial neuralgia are some of local effects of TMJ dysfunction.

TMJ RECONSTRUCTION:

In recent decades, TMJ reconstruction using an autogenous costochondral graft (CCG) has gained popularity, mainly because this graft provides a functional implant with growth potential and restores the joint as closely as possible to its normal anatomy. However, there is a significant proportion of patients, including patients who have had multiple surgery, in whom success rates with autogenous grafts are low. The Christensen TMJ prosthesis system offers a significant improvement in function and reduction in pain in most of these patients. This system involves covering the articulating surface of the temporal bone and replacing the meniscal disc with synthetic prostheses. Careful selection of the treatment modality employed in surgical reconstruction of the TMJ plays a significant role in increasing the success rate of TMJ reconstruction.

AUTOGENOUS COSTOCHONDRAL GRAFT

The most widely accepted autogenous reconstruction of the TMJ involves a costochondral graft. Ease in obtaining and adapting the graft, biological similarity to the mandibular condyle and regenerative potential are some of advantages of CCG (18,19,20,21,22,23). A CCG can also keep pace with the growth of the unaffected side to maintain mandibular symmetry during the growth period (20).

Kaban et al. (1990) achieved a mean maximum postoperative interincisor opening at one year of 37.5mm using CCGs to reconstruct the mandibular ramus in treatment of TMJ ankylosis in their seven-step surgical plan. This treatment included aggressive resection of the ankylotic segment, ipsilateral and contralateral coronoidectomy, lining of the joint with temporalis fascia or cartilage reconstruction of the ramus with a CCG and rigid fixation of the graft.(18,24)

However, current evidence suggests that CCGs tend to have more vertically directed condylar growth pattern and more laterally positioned condyles than native bone tissue leading to possible mandibular prognathism (18,25). In addition, in patients with arthropathy, long-term steroids can weaken a CCG which may cause ankylosis disease in the reconstructed joint (27,28). Clark and Britton (2001) reported a case of patient who had been operated on three times after a car motor vehicle accident. During the third operation, surgeons attempted to establish TMJ function with a bilateral CCG. This ultimately fused with heterotopic bone, causing diminishing ability to chew and function and progression from fibrous to complete and total bony ankylosis (29).

CHRISTENSEN PROSTHESIS SYSTEM

The option to use an alloplastic system, instead of an autogenous one, is determined on the basis of severity of disease. The Christensen TMJ fossa-eminence prosthesis system offers a treatment modality for severe TMJ dysfunction especially in patients who have had multiple surgery in whom autografts appear to have a very low success rate (17,33,34,35).

Temporomandibular joint reconstruction with the use of the Christensen alloplastic joint system allows a close reproduction of the natural anatomy (19). The Christensen TMJ fossa-eminence prosthesis systems provide a smooth surface for articulation with the natural condyle or with a Christensen TMJ condylar prosthesis in the case of total joint replacement. The prosthesis is attached to underlying bone structure with Co-Cr bone screws. Christensen TMJ condylar protheses are designed to sit against the Christensen TMJ fossa-eminence prosthesis and are secured to the ramus of the mandible. In the case of significant bone loss or trauma, the surgeon may request that the prostheses are cast to fit the specific patient's anatomical structure. In the case of Christensen TMJ condylar prosthesis, the flange portion is always adapted to the patient's anatomy.

A study by Chase et al.(1995) indicated that total joint

reconstruction combining placement of a Co-Cr fossa-eminence with a polymethylmethacrylate (PMMA) coated condylar prosthesis led to improved function in 85% to 90% of patients (17). In a study by Mcleod et al. (2001), who undertaked hemi-arthroplasty of the TMJ with a fossa-eminence prosthesis, 73% of patients had considerable improvement in their symptoms post operatively and a further 24% had some improvement (35).

Hemi-arthroplasty with Christensen fossa-eminence involves the same procedure as placing the prosthesis during total arthroplasty of the TMJ. Total arthroplasty with Christensen prosthesis is only indicated in patients with considerable condylar disease (35). Total replacement of TMJ may be considered for disorders include rheumatoid arthritis, osteoarthritis, psoriaratic arthritis and ankylosis after trauma.

The Christensen alloplastic joint system decreases the chance of recurrent ankylosis (30). However, particles of alloplastic prostheses at articular surfaces can generate a giant cell foreign body reaction which may cause loosening of the implant, with resultant fracture or displacement (19,27,28 ,31,32). Lack of growth and complications related to dystrophic bone formation in children and implant fracture caused by the use of inappropriate alloplastic materials are some factors that precludes the use of alloplastic TMJ prostheses (20,28,30). One of the most important aspects of preoperative assessment is condylar disease. Christensen fossa-eminence prosthesis is not used alone where there is a condylar disease such as avascular necrosis, because the condyle will be less adaptable to the new articular surface opposing it.

Speculand et al. (2000) studied outcomes in 62 patients who received total prosthetic replacement of the TMJ between 1988 and 1997 (26). The proportion of patients who could eat all food increased from 23% of the total group preoperatively to 77% postoperatively. According to this study, preoperatively, 63% reported severe pain but this number reduced to 5% postoperatively. Another study by Chase et al. (1995) indicated that 82% of 22 patients with severe TMJ disorders who underwent implant of a Christensen fossa-eminence prosthesis with retention of disc, showed significant improvement in the ability to eat. In addition, incisor opening improved in 77% of these patients. The rate of significant improvement in their ability to eat in 26 patients who underwent placement of Christensen fossa-eminence without retention of disc was reported to be 96%. In this group, incisor opening improved in 86% of patients. In both groups, all patients showed a significant decrease in pain post operatively. A further 21 patients underwent, surgical placement of Christensen fossa-eminence prosthesis along with a condylar prosthesis as part of this study. Eighty six percent of patients in this group showed a significant improvement in ability to eat, 96% showed a significant decrease in pain and 91% had significantly improved in incisor opening post operatively (17).

Recurrence and relapse are the most common complications associated with release of TMJ ankylosis. Studies have reported that the incidence of re-ankylosis is between 4% and 31% (9,16,36). Recurrence is frequently associated with extent of lesion, the release of TMJ ankylosis and surgical technique employed (16,38,39).

CONCLUSION

Temporomandibular joint dysfunction creates not only functional and aesthetic problems but also interferes with adequate nutrition and oral hygiene measures. The Christensen prosthesis system and the CCG are both accepted arthroplastic methods of TMJ reconstruction in traumatic TMJ dysfunction. Although CCG has been the most popular treatment modality to date, mainly because of accessibility and its adaptability to the TMJ area, recent studies indicate that as surgery frequency goes up, the rate of success of autografts decreases.

Technical workability, functional adaptability and regenerative potential are some of the advantages of autogenous CCG. The growth potential of CCG makes it a suitable implant in children whereas the lack of growth precludes the use of Christensen alloplastic joint system in this population. Long-term treatment with steroids for an arthroplasty may reduce the physical strength of a CCG and may cause further ankylosis decreasing the utility of CCGs in such patients. The use of Christensen alloplastic joint system is determined on the basis of severity of disease and is most helpful in patients with the most severe symptoms before surgery. This system also decreases the chance of recurrent ankylosis.

REFERENCES

1. Schafer RC, TMJ Trauma and Its Rehabilitation , Oklahoma City, Associated Chiropractic Academic Press, 1998.
2. Merrill RG. Historical perspectives and comparisons of TMJ surgery for internal disk derangement and arthropathy. J Craniomandib Pract:474-83;1986.
3. Muir CB, Goss AN. The radiologic morphology of painful temporomandibular joints. ORAL SURG ORAL MED ORAL PATHOL 70:335-59;1990.
4. Schellhas KP. Internal derangement of the temporomandibularjoint: radiologic staging with clinical, surgical, and pathologic correlation. Magn Reson Imaging 7:495-515;1989.
5. Schellhas KP, Piper MA, Ornlie MR. Facial skeleton remodeling due to temporomandibular joint degeneration: an imaging study

of 100 patients. AJR 155:373-83;1990.
6. Reiskin AB. Aseptic necrosis of the mandibular condyle: a common problem? Quintessence Int 2:85-9;1979.
7. Ogus H. Rhematoid arthritis of the temporomandibular joint. Br J Oral Surg 12;275-84;1975.
8. Adekeye EO. Ankylosis of the mandible: analysis of 76 cases. J Oral Maxillofac Surg41:442-449; 1983
9. El-Sheikh MM. Temporomandibular joint ankylosis: the Egyptian experience. Ann R Coll Surg Engl 81:12-18; 1999.
10. Guven O. A clinical study on temporomandibular joint ankylosis. Auris Nasus Larynx 27:27-33; 2000.
11. Ugboko VI, Amole AO, Olasoji HO, Ndukwe KC, Temporomandibular joint ankylosis: A multicenter nigerian study, The OnLine Journal of Dentistry and Oral Medicine Vol4:No 3:2002 .
12. Obiechina AE, Arotiba JT, Fasola AO. Temporomandibular joint ankylosis in South Western Nigeria. East Afr Med J 76:683-686; 1999.
13. Roychoudhury A, Parkash H, Trikha A. Functional restoration by gap arthroplasty in temporomandibular joint ankylosis: a report of 50 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 87:166-169; 1999.
14. Nitzan DW, Bar-Ziv J, Shteyer A. Surgical management of temporomandibular joint ankylosis type 111 by retaining the displaced condyle and disc. J Oral Maxillofac Surg 56:1133-1138; 1998.
15. Izumi Y, Kino K, Ohmura Y, Waka H, Shubuya T, Amagasa T. Clinico- statistical study of temporomandibular joint ankylosis: etiology and onset age. J Jpn Soc TMJ 6:100-113. ;1994
16. Miyamoto H, Kurita K, Ishimaru J-I, Goss AN. A sheep model for temporomandibular joint ankylosis. J Oral Maxillofac Surg 57:812-817;1999.
17. Chase DC, Hudson JW, Gerard DA, et al. The Christense prosthesis ; A retrospective clinical study, Oral Surgery Oral Medicine Oral Pathology 80:273-8:1995 .
18. Rishiraj B, McFadden LR, Treatment of Temporomandibular Joint Ankylosis: A Case Report, J Can Dent Assoc 67(11):659-63;2001.
19. MacIntosh RB,The case for autogenous reconstruction of the adult temporomandibular joint. In: Worthington P, Evans JR, eds. Controversies in Oral and Maxillofacial Surgery. Philadelphia: WB Saunders 356-380;1994.
20. Fontenot MG. Temporomandibular joint devices: past present and future. In: Sessle Bl, Bryant PS, Dionne RA, eds. Temporomandibular Disorders and Related Pain Conditions. Seattle: IASP Press;1995.
21. Mercuri LG: Considering total temporomandibular joint replacement. Journal of Craniomandibular Practice 17:44-18,1999.
22. Figueroa AA, Gans BJ, Pruzansky S. Long term follow up of a mandibular costochondral graft. Oral Surg Oral Med Oral Pathol 58: 257-268;1984.
23. Wen-Ching Ko E, Huang CS, Chen YR. Temporomandibular joint reconstruction in children using costochondral grafts. J Oral Maxillofac Surg 57: 789-798;1999.
24. Kaban LB, Perrott DH, Fisher K. A protocol for the management of temporomandibular joint ankylosis. J Oral Maxilofac Surg 48:1145-1191;1990.
25. Ohara K, Nakumara K, Ohta E. Chest wall deformities and thoracic scoliosis after costal cartilage graft harvesting. Plast Reconstr Surg 99: 1030-1036;1993.
26. Speculand B, Hensher R, Powell D, Total prosthetic replacement of the TMJ: experience with two systems 1988-1997,The British Journal of Oral & Maxillofacial Surgery 38, 360-369;2000.
27. Kent JN, Misiek DJ. Controversies in disc and condyle replacement for partial and total temporomandibular joint reconstruction.In:Worthington P, Evans JR, eds. Controversies in Oral and Maxillofacial Surgery. Philadelphia: WB Saunders 397-435;1994.
28. Saeed NR, McLeod NM, Hensher R, Temporomandibular joint replacement in rheumatoid-induced disease, British Journal of Oral and Maxillofacial Surgery 39, 71-75 ;2001.
29. Clark MS, Bilateral Temporomandibular Joint Bony Ankylosis Without Mandibular Function: A Case Report, TMJournal:Vol1,No 1: 2001.
30. McBride KL. Total temporomandibular joint reconstruction. In: Worthington P, Evans JR, eds. Controversies in Oral and Maxillofacial Surgery. Philadelphia: WB Saunders 381-395;1994.
31. Henry CH, Wolford LM. Treatment outcomes for temporomandibular joint reconstruction after proplast-teflon implant failure. J Oral Maxillofac Surg 51:352-8;1993.
32. Hensher R. Treatment of TMJ ankylosis. In: Langdon JD, Patel MF, eds. Operative Maxillofacial Surgery. London: Chapman and Hall 175-185;1998.
33. McConnell TP, McCoy JM, Simpson R, Gerard DA, Chase DC. Correlation of histopathological staging of TMJ patients with longitudinal outcome. J Dent Res 70:466:1991.
34. McCoy JM, Gotcher JE, Chase DC. Histologic grading of TMJ tissues in internal derangement. J Craniomand Prac 4:213-8; 1986.
35. McLeod NM,. Saeed NR, Hensher R, Internal derangement of the temporomandibular joint treated by discectomy and hemi-arthroplasty with a Christensen fossa-eminence prosthesis, British Journal of Oral and Maxillofacial Surgery 39, 63-66;2001.
36. Lindqvist C, Pihakari A, Tasanen A, Hampf G, Autogenous costochondral grafts in temporomandibular joint arthroplasty. A survey of 66 arthroplasties in 60 patients. J Maxillofac Surg: 14 143-149;1986.
37. Popescu V, Vasiliu D. Treatment of temporomandibular joint ankylosis with particular reference to the interposition of full-thickness skin auto transplants. J Oral Maxillofac Surg 5:3-14;1977.
38. Rowe NL. Ankylosis of the temporomandibular joint.J R Coll Surg Edinb 27:67-79;1982.
39. Kim SG. Treatment of temporomandibular joint ankylosis with temporalis muscle and fascia flap. Int J Oral Maxillofac Surg 30:189-193;2001.

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CROSSROADS: WHERE MEDICINE AND THE HUMANITIES MEET

Embryonic Stem Cell Research: Is it merely the means to an end?

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I. INTRODUCTION:

To some people, the promise of embryonic stem cell (ESC) research may seem a scientific messiah, one in its infant stage and currently under the threat of being killed by Herod-a political decree of its death before its potential may never be known. A religious metaphor is used here precisely because the arguments revolving around ESC research are so powerful as to evoke an opinion from all: religious leaders, politicians, the legal community, scientists, and ethicists. While the various views are intimately related, we will attempt to categorize them for the sake of simplicity.

The goal of this paper is not to convince the reader of the opinion of its authors. Our purpose is to present an objective overview of all the relevant issues surrounding embryonic stem cell (ESC) research, including the anticipated benefits, religious, legal and ethical arguments. Although we recognize that other issues concerning reproduction technology, including human cloning as an alternative means of having a child, pre-implantation diagnosis, and abortion are all intimately linked to the question of ESC research, we have chosen to concentrate on the issues surrounding therapeutic cloning, and ESC research.

II. WHAT IS CLONING?

Reproductive cloning is the cloning of an entire organism (whether sheep, dog or human) to yield a fully developed fetus (24, 28). Reproductive cloning is a subset of cloning research that can be used to aid infertile couples as well as other applications discussed later. Another subset of cloning research is therapeutic cloning (17). This latter area studies how cloning of specific cells, such as embryonic stem (ES) cells, could be used to treat diseases (17). This type of cloning research does not involve the production of offspring, and is therefore considered ethically different from reproductive cloning (17). It is important to understand the difference between these two areas of scientific investigation, since they have altogether separate aims and therefore different results or consequences.

III. TECHNIQUES CURRENTLY USED FOR CLONING:

A) Splitting of the Morula

During the cleavage period, each individual blastomere is totipotent, and can therefore generate an entire organism on its own. The fetuses arising from each blastomere would be clones of each other, but not clones of their parents, since each arose from one fertilized ovum (24, 5, 11).

B) Somatic Cell Nuclear Transfer (SCNT)

SCNT is a technique used to produce a clonal child that is effectively a delayed identical twin of its parent. The donor is first given oral contraceptives for approximately two weeks, providing a clean slate to begin from. The donor's pituitary gland is then pharmacologically prevented from secreting gonadotropins. Finally, the donor is injected with FSH and LH to promote follicular maturation. Once follicles have matured, a mature oocyte is aspirated from the donor's ovary using a needle. The genetic material of the oocyte is then removed via the insertion of a small needle through the zona pellucida, into the cell, removing all of the chromosomes and some of the surrounding cytoplasm (ovum enucleation) (5).

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In addition, a somatic cell from the "parent" is isolated via skin biopsy, which involves the removal of fibroblasts from the skin of the donor. A fibroblast nucleus is isolated and immediately injected into the enucleated ovum. The "fertilized cell" is then stimulated to begin mitosis. After several days, the cleaving embryo is a human clone of its donor parent. However, this embryo cannot be allowed to develop beyond 14 days according to ethics guidelines (5).

The embryo is allowed to develop only up to day 14 because during the first two weeks of development, the embryo has not yet begun to construct its nervous system, and therefore cannot have a consciousness (11,28). Furthermore, in utero, the embryo would not normally implant before the second week and is therefore still independent of the mother. Spontaneous abortions are common in nature prior to implantation and thus some ethicists do not see the killing of an embryo up to the implantation stage as an immoral act (5).

C) Cloning existing stem cell lines

These stem cell lines have been previously isolated from embryos which were discarded during fertility procedures, such as IVF (in vitro fertilization), IUF (intra-uterine fertilization) etc (6). These cells are induced to replicate via morula separation, but it needs to be mentioned separately since, in some countries (e.g. the United States (U.S.) and Germany) it is the only research technique approved for public research (6, 27, 16).

III. ANTICIPATED BENEFITS OF HUMAN EMBRYONIC CLONING

Human cloning in general, and the use of ES cells specifically, have been the sources of much recent public debate. If allowed to become widely used, these procedures would have serious ethical implications, precisely because they can have a significant impact on the development of the entire human race. Decreasing the genetic variation of future human populations can reduce our capability to adapt to environmental changes and thus severely endanger the future of our race (15). So why even consider such potentially hazardous research? Because all of the aspects of human cloning research which make it potentially dangerous also give it beneficial potential. Both therapeutic and reproductive human cloning can be tools for improving the lives of many sick or infertile people.

A) Therapeutic cloning: Using ES cells to treat disease

Researchers are desperately trying to discover methods of differentiating stem cells into specific types of cells, which can then be used to replace dysfunctional and/or damaged cells in diseased patients. If scientists manage to develop ways to produce mature nerve cells from ES cells, these healthy cells can be implanted into Alzheimer's, Parkinson's, Multiple Sclerosis or nerve-injured patients (31). The implanted cells would then take on the jobs of damaged neurons and restore partial or complete functioning to the individual.

Furthermore, ES cells could replace heart muscle cells that are damaged or destroyed after a myocardial infarction (17). Damaged cartilage (e.g. osteoarthritis) or bone (e.g. trauma or surgery) could also be replaced by implanted ES cells (17). These stem cells could also be used as sources of pancreatic cells and replace dysfunctional pancreatic cells in Diabetes Mellitus patients (17). They may no longer require chronic insulin medication and may be at reduced risk for heart disease, pregnancy complications, and many other long-term effects of diabetes mellitus. Stem cells may even be capable of generating entire organs, which could be used for transplants in patients in dire need for these structures (17).

ES cells could improve the conditions of cancer patients. Cancer treatments such as chemotherapy and radiation often cause damage to the bone marrow, resulting in an underproduction of blood and immune cells. This effect could be counteracted by injections of hematopoietic stem cells (pluripotent cells capable of giving rise to red blood cells as well as cells of the immune system) (17). Replacement of immune cells would also treat many diseases of the immune system, such as AIDS (17).

If embryonic cells could be collected and frozen from each newborn, they could provide a non-depletable source of cells and/or organs for that individual should the need arise (17). Something similar has already been done in the U.S., where a Colorado couple screened 15 embryos for one whose tissue type matched those of their 6 year-old daughter suffering from Fanconi's Anemia (17). The resulting fetus would not only be the little girl's sibling, but her blood donor. Cloning one's own ES cells would provide a faster, easier and less ethically questionable way to derive the same benefit (17).

B) Reproductive cloning: another tool to increase fertility

The fertility of first world populations has been rapidly declining recently (1). More and more couples are looking to science to help them conceive and scientific research has made many births feasible where they would not have been otherwise. Human embryonic cloning can serve as an additional method of conception for homosexual couples as well as couples with one

severely infertile person (15, 28). Furthermore, an increasing percentage of people in developed countries remain single but still desire to conceive a child on their own. This cohort of the population would also benefit from reproductive cloning procedures.

PART II: THE RELIGIOUS, LEGAL, AND ETHICAL ARGUMENTS SURROUNDING CLONING AND EMBRYONIC RESEARCH

Although beneficial, the cloning techniques mentioned above must be placed under ethical scrutiny before being clinically implemented. Even though they may increase the lifespan of many and the fertility of some, these outcomes do not come without a price. The consequences must therefore be considered in their entirety and have in fact been the source of much public debate. Some of the ethical concerns around human cloning are considered below.

The arguments for and against stem cell (SC) research are numerous and diverse. Often it depends on the standpoint of the individual making them. A catholic scientist, for example, may have a great deal of internal conflict depending on which aspect of him- or herself is presenting the argument. However, no matter how we define ourselves and our views, whether by profession, religious background, or politically, eventually there must be some element of consensus to determine if, and how far we as individuals and as a human society are willing to permit this type of research to proceed. The following presents an overview of the current religious and legal arguments, followed by a longer discussion of the ethical debate on embryonic research.

IV. AN OVERVIEW OF THE RELIGIOUS ARGUMENTS

The religious argument about ESC research tends to circle around a central question: when does life begin (4, 9)? For those who believe that life begins at the moment of conception there is little need for debate: any research involving the destruction of the embryo is tantamount to murder. For others the answer is not so uni-dimensional. In this section, we present the current thinking on ESC research of three major religious traditions: Christianity, Judaism, and Islam. Although none of these religions have uniform thinking across all their denominations, there are certain commonalities to their thinking that will be presented here. For a more thorough discussion of this subject, please refer to the reference list.

In the Instruction Donum Vitae, issued in 1987 by the Vatican's Congregation for the Doctrine of the Faith, "The human being is to be respected and treated as a person from the moment of conception; and therefore from the same moment his rights as a person must be recognized, among which in the first place is the inviolable right of every innocent being in life...No objective can in any way justify experimentation on living human embryos or fetuses, whether viable or not, either inside or outside the mother's womb." (25)

A) The Roman Catholic Church

The Roman Catholic perspective may be considered the most dogmatic of religious positions. Its contention is that no form of contraception, reproductive technology or ESC manipulation, whether for research, therapeutic, or cloning purposes is permissible (25). Thus, from the Roman Catholic viewpoint that there is no debate: From the moment of conception, there is life; if there is life then destruction or manipulation of said life is murder-end of story. However, if one delves further into Catholic doctrine, one discovers that the current and popular view is not the only one available. Until Pope Pius IX's declaration in 1869, the belief remained, in accordance with Aristotle, that the conceptus was not considered to be animatus, or with a soul, until it had acquired formatus, an obvious human form. For boys this occurred at 40 days gestation; for girls at 80 days (25). Although the reason for this strange discrepancy between male and female fetuses is beyond the scope of this paper, the relevance is in the discussion as to when life begins. For those Catholics who maintain this belief that a soul must be conferred upon a fetus before it is to be considered as a person, the question of ESC research is more open to debate. No longer dealing with the heinous crime of murder, the debate delves further into the realm of ethics, which will be dealt with in a subsequent section.

B) Protestant Denominations

Protestant beliefs are very diverse. Some traditions hold a similar view to that of the Roman Catholic Church: life begins at conception. Other views may hold that an embryo gradually acquires full human status, therefore research on ESC may be considered permissible. "It is, in fact, part of the Protestant ethos that moral questions are determined by the individual conscience, and there is therefore room for a variety of stances on this point. Protestant thought, therefore, may accept that this is an issue on which Christians may have very differing views, with these differing views being compatible with Christian beliefs." (22)

C) Judaism

Jewish law is set by the Torah. According to Jewish tradition, "... a person becomes a person, only upon birth." (10, 21) Other sources suggested that an embryo only acquires the status of personhood after forty days of gestation (10). It may at least be concluded,

therefore, that Judaism does not grant full moral status to an embryo from the moment of conception (10).

From our research, Judaism comes out as the religion strongly in favour of ESC research (10, 4) In the Torah, there are 613 commandments or good deeds that a person should follow. Among these "mitzvahs" is the command: "Thou shalt be fruitful and multiply..." This has been cited in favour of in vitro fertilization techniques (21). More important to the ESC debate is the clause indicating that almost any Jewish law may be broken in order to save a life. Those who are sick, or too young, or pregnant do not participate in fasts because it could bring them harm; and those stem cells from frozen embryos should be researched as they may prevent the death of those suffering from illness. Furthermore, "Never having been implanted into a woman's uterus, Jewish law does not even accord these embryos the limited status of an ordinary fetus. And yet, flushing them down the sink seems to dishonour their potential for human life…Even though the destruction of the embryo may be a sin, that act is massively overridden by the drive to save another life" (21).

D) Islam

Islamic tradition holds that ensoulment of the fetus does not occur until four months of gestation, according to the Qur'an and Sunnah (4). In a news release accompanying a recent poll held by the Islamic Institute, a political body in the U.S., the following statement was made, "Under the Islamic principle of the 'purposes and higher causes of the Shari'ah (Islamic law)', we believe it is a societal obligation to perform research on these extra embryos instead of discarding them." (4) However, there is still controversy surrounding the question of whether or not embryos should be created for the sole purpose of research." (4)

V. LEGISLATION

Legislation on new technology often tends to lag behind its scientific development. However, the controversy surrounding cloning and other forms of embryo research, including therapeutic, has caused a great deal of public outcry. Politically speaking, the general view is that cloning is unacceptable. Nonetheless, there remains a much greater divide amongst both law makers and the public as to whether ESC research for therapeutic purposes should be legal.

Canada

On Dec 3, 2001, following the publication of a research paper reporting the first cloning of human embryos in the E-journal of Regenerative Medicine, the Canadian Medical Association (CMA) issued a statement suggesting the need for an independent regulatory agency. This statement suggests, on behalf of the Assisted Human Reproduction Health Care Providers Coalition, that there is concern that the proposed draft legislation as written would inadvertently prohibit some potentially beneficial research which Minister Rock is in favour of: "Research using human reproductive materials has the potential of bringing significant benefits to Canadians and, therefore, this research should be encouraged." (6) An independent coalition would have the ability to procure input from both the public and experts (6). Its proposed responsibilities include accrediting facilities, issuing licenses, and monitoring the physicians and scientists according to national standards of research on human subjects.

At this time, there is no comprehensive legislation on ESC research. In May 2001, Health Minister Allan Rock presented draft legislation to the Standing Committee on Health. If accepted as a law, the cloning of human beings, the sale and purchase of human embryos, and paying women to act as surrogate mothers would be considered illegal. It would further act to regulate reproductive technologies and permit limited ESC research to those who obtain a license. The minister has requested a report on the legislation by the Committee in January 2002. Currently no research is permitted on any embryo beyond the age of 14 days (23).

United States of America

In August 2001, President George Bush approved federal funding of research conducted on pre-existing ES cells. Much controversy has stemmed from the number of reported existing lines. No federal funding would be given for research on embryos whether created for research purposes, or left-over from in vitro fertilization techniques.

Aside from bans of federal funding, it would appear that all types of research are permissible within the United States as long as the finances are available from sources outside of the government.

VI. THE ETHICS OF EMBRYONIC RESEARCH: IS IT MERELY THE MEANS TO AN END?

It is incredible the number and nature of scientific discoveries and advancements the world has seen in the last quarter of a century. What was once merely the stuff of science fiction novels, movies, or cartoons is now a part of daily discoveries. A sheep was cloned, a test tube baby was born, the mysteries of the human genome are well on their way to being catalogued and characterized, and now to the question of what to do with the very beginning of human life itself. How do

we, the human race, proceed? The following is a discussion of the ethical issues and concerns surrounding embryo research: is ESC research merely a means to an end?

Is it ethical to do research on embryos with the intent of finding the potential cures to human illnesses? First, let us define ethics. Ethics is the secular or human moral contemplation of good conduct. Thus, the question becomes, "Is it right, according to human morals and values, to conduct research on embryos?" The next obvious question follows, "What are embryos?" From a scientific standpoint, embryos are the result of the union of an ovum and sperm thus creating a zygote, which then divides to become an embryo. Arbitrarily, past three months of gestation, an embryo is termed a fetus. However, it is not the scientific definition we are interested in here. What we really should be asking ourselves is, "What is the moral status of an embryo?" Or, "Is an embryo a person?" There are those who feel that an embryo is no more than a ball of cells. As such, it has no moral value at all. If a scientist is just mixing up gametes in a petri dish, and he or she is lucky or skillful enough to create an embryo, and even to encourage it to divide outside of a uterus, then what is the harm? The answer to that question is in another, "Would you care for some human caviar?" If this question does not give you any feeling of disgust, then clearly there is no problem with the harvesting of human eggs for any purpose. However, it is likely that there is a feeling a revulsion finishing the reading of that question for most readers. If that is the case, then surely there is at least some moral status to be given to human embryos.

How is moral status defined? Mary Ann Warren has described moral status as having seven criteria: 1) Respect for life; one should not kill or harm another living creature without a just cause, 2) Anti-cruelty; harm or pain should only occur to another sentient being when there is no other way of furthering the goals of one with higher moral status, 3) Agent's rights, 4) Human rights, 5) Ecological importance, 6) Interspecific communities, and 7) Transitivity of respect; moral agents should respect another's moral attribution of moral status (1). According to these criteria, the embryo has a weak moral status for two reasons: It is alive and because of the respect, we must accord to others attribution of moral status on the embryo. It is not a sentient being. It cannot live without the support of a woman's uterus, but it does have the potential to become a human being and therefore it must be respected (1).

Still, is there not an inherent conflict between the concepts of respect and destruction? Certainly, there is for those who believe that life begins at conception and that there is never any reason great enough to offset the devaluation of destroying life. Yet, this treats the concept of respect as a black and white issue. Preserving life equals respect. Destroying life equals murder. Black. White. No gray. Yet, human life itself presents us with its own shades of gray. A female fetus develops approximately 6 to 7 million oocytes, but by the time she is born, only a million or so remains. By the time she reaches puberty and begins menstruating, a mere 40% of what she had at birth remains. Nature has destroyed approximately 95% of the oocytes that the fetus once had. Furthermore, when a woman's ovaries are being prepared for ovulation, anywhere between 5 and 15 follicles begin to develop. Only one, or on a rare occasion two, is ovulated and available for fertilization. Then, should fertilization occur, there is a large possibility that the woman will never even know she was pregnant, since the majority of embryos never even implant. Thus, one could argue that nature itself does not revere life such that it cannot be destroyed. Ah, yes, but that is nature, and who are we, mere mortals, to interfere with nature then? We all have our choice as to how to live. There are certain cultures that attempt to respect our place in nature ignoring the advancement of science. But that is not the question here. The question still before us is, "Can we respect life and still destroy it?"

We can respect life by how we choose to conduct research on it. The training of many health care professions requires the study of anatomy. This study is enhanced by the use of cadavers and prosections; those who have died and given their body for teaching. Students show their respect in the way the bodies are treated, and in many institutions, such as McGill University, there are ceremonies to commemorate the lives of those who have generously contributed to students' learning. If this is an acceptable practice, then why would we not be able to afford such respect to embryos that are being experimented upon?

The decision each person must make for him or herself is whether experimentation on embryos that would lead to their destruction is inherently wrong? If it is then we need not go further. No benefit of this research could possibly outweigh the detriment that would be done to human society by the undertaking of this research. But if we maintain that we can respect human life at the same time as conducting this research, then we must move onto what are the reasons for undertaking this research in the first place? In other words, "What are the anticipated benefits of ESC research?" This topic was covered in scientific detail in a previous section. What we are concerned with here is whether the ends justify the means. Surely, one of the greatest human virtues is compassion. To want to help

another who is suffering is often one of the most compelling reasons one has for going into health sciences or into a health care profession. That being said, how do we propose to alleviate suffering by ESC research?

There is obvious potential to find cures for diseases such as Alzheimer's and Parkinson's from ESC research. However, that is all it is: potential. There is also potential to do the same type of research with adult stem cells. Here, there is much less cause for ethical alarms to sound. There is little or no harm to the donor and it may be done with the donor's fully informed consent. So why is this avenue of research not being pursued as fiercely as that of ESC? Because, scientists claim, it is more difficult to isolate the cells and it will take too long. Why is it that humans have come to demand that everything be available to them today, and if not today then tomorrow?

Allowing that we accept there is better potential from ESC to alleviate suffering, and that it is not considered inherently wrong to do research on them providing they are allocated a minimum amount of respect, the question then becomes, "Where do we get the ES cells from?" There are several sources that could potentially serve this purpose: cloning existing stem cell lines, from "left-over" embryos no longer being used for in vitro fertilization, from the ova of a woman who chooses to donate her ova for profit, from the ova of aborted fetuses, from the umbilical cords of aborted fetuses, or by creating embryos from gametes in the lab with no intention of use for implantation. This is simply a list, but it raises a lot of questions and ties in some other ethically problematic subjects, namely reproductive technology, cloning, and abortion.

The purpose of most reproductive technologies is to enable an individual or couple who cannot achieve pregnancy by natural means to become pregnant. For example, a single mother or lesbian couple who wish to have a baby may choose to be artificially inseminated. A gay couple may choose to have a child by a surrogate mother. A heterosexual couple that has fertility problems may choose to attempt in vitro fertilization. The same couple may also wish to have pre-implantation diagnosis if either or both partners or their first child has a genetic disease. There are ethical questions inherent to all of these examples that are beyond the scope of this paper. What is relevant is when a couple chooses to undergo in vitro fertilization. In order to increase the chance of having a successful pregnancy, several ova are collected from the woman and fertilized. Some of the resulting embryos are implanted, and others are maintained in stasis by freezing them with liquid nitrogen. Should the woman become pregnant, she, or the couple, may choose to keep them for the chance to have another child later on, or they may be discarded. What if, instead of discarding them, the woman, or couple, consented to having them used for research? Is there a problem with this? It becomes a problem when one realizes that often the clinics that perform in vitro fertilization are also involved in research. Thus, there is a question as to whether there would be pressure to create a greater number of embryos than needed in order to ensure there would be some left over to do research on. Another possibility is that this could become a method of coercion for infertile individuals or couples who do not have the financial means to have a child by in vitro fertilization. The clinic will provide the treatment free in exchange for a couple of extra embryos.

So, if there is a possibility that donating embryos for research could become a corrupt business, then why not just clone them? Of course, we are not suggesting that we permit reproductive cloning, as to most people, this notion is reprehensible. If the goal of therapeutic cloning is to alleviate human suffering, then what better way to accomplish that than by cloning a dead child? Here comes the slippery slope argument. There can be no doubt as to the emotional anguish that comes from losing a loved one, especially a child. If we permit the cloning of embryos as a source of material to create a new heart for someone who will otherwise die, then how can we justify not removing the suffering of a mother whose child has died? If we permit one type of cloning, aren't we just opening the door to all others? If so, have we succeeded in completely devaluing all forms of human life?

And what, then, about the young girl who finds herself in trouble? She cannot have this baby, so she seeks out an abortion. Although her world has been turned upside down, can't at least some good come out of this by allowing the stem cells of her baby, or better yet, if a female, its ova? What would be the harm in that? Perhaps it may even become a way for those in unfortunate financial circumstances to earn a little extra money: get pregnant and have an abortion.

So many questions remain unanswered in the minds of so many individuals. At heart in all of these is what the value of human life is in all its forms, from its earliest days to the time we die. More pertinent here is if we can find an acceptable balance in which human life maintains its value and allow scientific progress to continue. Dr. Margaret Somerville suggests there is such a thing as "ethics time" that is needed to determine how to proceed when science, law, and ethics do not keep pace with each other:

"A minimum amount of time is also needed for the public to become familiar with the benefits, potential benefits, risks, and harms of a new scientific

development, not only at the physical level, but also at the level of its potential impact on values, norms, traditions, customs, culture, beliefs, and attitudes."

What seems clear is that we, both as individuals and as a society, have not had adequate ethics time to determine what role embryonic research should play in a human society.

REFERENCES

1. Ahmad, ID. Federal Funding for Stem Cell Research? Minaret of Freedom Institute 2001. www.islam-online.net/english/Views/2001/08/article6.shtml
2. Anderson, DJ, Gage, FH, & Weissman, IL. Can stem cells cross linear boundaries? Nature Medicine 7(4): 393-395; 2001.
3. Biological uncertainties about reproductive cloning. The Lancet 358(9281): 519; 2001.
4. Block, BH. Stem Cells, Cloning, and Judaism. 2001. www.beth-elsa.org/be_s0914a.htm
5. Cibelli, JB, Kiesseling, AA, Cunniff, K, Richards, C, Lanza, RP, & West, MD. Somatic cell nuclear transfer in humans: Pronuclear and early embryonic development. E-biomed: The journal of regenerative medicine 2: 25-31; 2001.
6. Craft, I. Sources of research embryos for cloning. The Lancet 357: 1368; 2001.
7. Gibbs, N & Duffy, M. In a 21st century speech on stem-cell funding, Bush budges and finds compromise. Will it work? Time 158(7): 20-22; 2001.
8. Hanna, KE. Stem cell politics: Difficult choices for the White House and Congress. Hasting Center Report July-August: 9; 2001.
9. Islamic Institute supports embryonic stem-cell research and releases poll showing Muslim American support. Islamic Institute News 2001. www.islamicinstitute.org/news-stem-cell.htm.
10. Kass, L. et al. Human Cloning and Human Dignity: An Ethical Inquiry. The President's Council on Bioethics. 2002. http://bioethics.gov/pubs/cloning1/executive.htm
11. Kerschen, A. How to Clone a Human (Version 1.1) 1998. www.biofact.com/cloning/human/html
12. Kluger, J & Lemonick, MD. And what about the science? Time 158(7): 26-27; 2001.
13. Lacayo, R. How Bush got there. Time 158(7): 23-28; 2001.
14. Lachmann, P. Stem cell research --- why is it regarded as a threat? EMBO Reports 23(31): 165-168; 2001.
15. Lauritzen, P. Neither Person Nor Property: Embryo research and the status of the early embryo, America Press; 2001.
16. Lenoir, N. Europe confronts the embryonic stem cell research challenge. Science 287: 1425; 2000.
17. McCall Smith, A & Revel, M. The use of embryonic stem cells in therapeutic research. Report of the IBC on the ethical aspects of human embryonic stem cell research: 1-20; 2001.
18. McLellan, F. Bush supports limited funding for stem-cell research. The Lancet 358: 568; 2001.
19. Meilaender, G. The point of a ban: Or, how to think about stem cell research. Hasting Center Report January-February: 9-16; 2001.
20. Meyer, MJ & Nelson, LJ. Respecting what we destroy: Reflections on human embryo research. Hasting Center report January-February: 16-23; 2001.
21. Not now, Dr. Miracle, New Scientist 2001. www.newscientist.com/hottopics/clonin/cloning.jsp?id=22980400
22. www.parkridgecenter.org/cgibin/ShowPage.dll?MODE=2&ID=39
23. Proposals for legislations governing assisted human reproduction: An overview. 2001. www.hc-sc.gc.ca/english/media/releases/2001/2001_443.htm
24. Sadler, TW. Langmans's Medical Embryology, 8th Ed. New York: Lippincott, Williams, & Wilkins, 2000.
25. Somerville, M. The Ethical Canary: Science, Society and the Human Spirit, Toronto: Viking, 2000.
26. Stem-cell research: drawing the line. The Lancet 358(9277): 163; 2001.
27. Tuffs, A.Germany debates embryonic stem cell research. BMJ 323: 8; 2001.
28. Winston, R. Embryonic stem cell research. Nature Medicine 7(4): 396-399; 2001.

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CROSSROADS: WHERE MEDICINE AND THE HUMANITIES MEET

Wilder Penfield and the Montreal Neurological Institute: Heralding the Modern Age of Neurology and Neurosurgery

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The Montreal Neurological Institute (MNI) was founded in 1934 as an institute "dedicated to relief of sickness and pain and to the study of Neurology", as is inscribed on a plaque adorning its exterior (1). To its founder, Dr. Wilder Penfield, "The problem of neurology is to understand man himself" (2). Thus this institute was not only founded to treat and study human sickness through medicine, but to understand humankind and the human mind in health, through science. How did it come to be that an institute with such a seemingly lofty purpose came to be founded in Montreal? What was its place in the larger picture of medicine at the time? The MNI is now a well-known institute, but what were its initial contributions to science, medicine, and the general public?

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To understand what shaped the founding of the MNI, one first must understand what shaped its founder, Wilder Graves Penfield (1891-1976). He is described by Dr. William Feindel, his friend and later the third director of the MNI, as "having a great vision in life" (3). He acquired this vision foremost from his mother, who was convinced that her son was destined for greatness. As a student, Wilder shared his mother's belief. While studying at Princeton University, he sat down one day to make a list of possible careers in the hopes of choosing one that would fit the following criterion: "Objective: to support myself and family and somehow make the world a better place in which to live" (4). Lofty as the latter objective might seem, the young Penfield was determined to fulfill it. As a student at Oxford University during World War I, he was to travel to the continent to work in a field hospital. His ship was torpedoed during the crossing, and he feared that he might drown. Yet, he refused to die because he simply believed that, "This cannot be the end. My work in the world has only just begun. This cannot be the end" (4).

At this age, Wilder Penfield was not only obviously driven but also impressionable. Years later, his beliefs about students in general would reveal his own feelings about himself as a student; "[Students] are lonely and highly impressionable and they have almost always some hidden strength" (5). The young Penfield took his own first impressions from Edward Conklin, his biology professor during his undergraduate years at Princeton. Conklin's passionate teaching of the subject led to Penfield's initial interest in medicine. Next, he would be inspired and impressed by two very renowned professors he met at Oxford, Sir Charles Sherrington and Sir William Osler. Feindel states that "Charles Sherrington became his scientific hero and William Osler his life-long inspirational tool" (6). Penfield was immediately impressed with Sherrington, who had contributed a vast amount of scientific knowledge in his investigation of reflexes, and it was Sherrington who inspired Penfield to study neurology. Of this, Penfield wrote that:

> I looked through his eyes and came to realize that here in the nervous system was the great unexplored field - the undiscovered country in which the mystery of the mind of man might someday be explained (5).

After his studies at Oxford, Penfield obtained his

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medical degree in 1918 at the Johns Hopkins University in Baltimore, whose medical school had been founded in part by his other Oxford hero, Sir William Osler. After this, Dr. Penfield spent a year following his burgeoning interest in neurology, interning at the Peter Bent Brigham Hospital, Boston, where he had a chance to closely observe the operations of Dr. Harvey Cushing, arguably the best neurosurgeon in the world at the time, and one whose fame was bringing respectability to the still-young field (4). He went on to do further study at the National Hospital, Queen's Square, London, where Victor Horsley had essentially 'founded' British neurosurgery. There he studied with the neuropathologist Gordon Holmes (who would eventually be knighted for his discoveries) and it is there that Dr. Penfield realized that he was living in a time when "advance in knowledge of anatomy and physiology of the brain was rapid, and neurologists were beginning to apply the new understanding to brain disease" (5).

With this in mind, Penfield returned to America, in 1922, and began work as a neurosurgeon at the Presbyterian Hospital of Columbia University in New York. By now he was ready to tackle an important question that came to him one day from a senior colleague, Prof. Bill Clarke; "What causes epilepsy?" Ignorant of, but wanting to know the answer to that fundamental question, he came to see how his broad education had moulded him. He realized that:

> A brain surgeon was not different from those who operated on other parts of the body. He too should study the healing process of the organ he treats. But no one had done it, as far as I was aware (5).

This led him on a new quest to try to study the brain and its healing process as a whole. Along the way, he 'acquired' an assistant, Dr. William Vernon Cone who was to become his life-long partner in the effort.

Adopting a new method was difficult for Penfield because it required him to delve into all aspects of studying the brain: the basic sciences of pathology, physiology, cytology, and anatomy would be critical to his approach to clinical neurosurgery. He would have to become the type of neurosurgeon that his hero Osler had advocated when he had said that he:

> ...would prefer to see neurology a special department, so that there would not be neurological physicians and surgeons, but medical chirurgical neurologists, properly trained in the anatomical, physiological, clinical and surgical aspects of the subject (7).

This would require him to keep up to date in and integrate all of these fields. Yet this was an era before the internet and long distance phone plans. Keeping up to date with research that was taking place far away was not necessarily easy. Thus, by 1922, Dr. Penfield "was firmly of the opinion that 'the real future of neurology' called for a neurological institute in which neurology and neurosurgery were not to be divided" (5). Moreover, as William Feindel relates, "Penfield had the increasing conviction that it would be best to bring together under one roof neurology, neurosurgery, neuropathology and neurophysiology" (8).

Thus, by 1928 Dr. Penfield's vision was established: He wished to study the human nervous system in order to learn how to treat it and in order to begin to understand the human mind. This vision was the result of the unique experience of having studied with a plethora of great teachers and scientists. Later, Penfield would acknowledge this by saying:

> I who thought himself the neurosurgical pupil of no one in particular, was, in reality, the pupil of everyone. I was a jack-of-all-trades and I had plans that would make me a jack-of-further-skills that I would need in the years ahead (5).

* * * * *

Dr. Penfield, therefore, had a plan and he had ambition; but he needed a setting in which to execute this plan, and more importantly, he needed like-minded persons to share it with. One such person was Edward Archibald, the professor of surgery at McGill University in Montreal, Canada. Prof. Archibald had long had an interest in neurology and neurosurgery and he had studied at Queen's Square with Victor Horsley. By 1927, however, he had divided his research and work in other areas and he realized that Montreal needed a full-time neurosurgeon. Thus, he recruited Penfield to take over and expand his neurosurgical duties in Montreal at the Royal Victoria Hospital (RVH). He immediately recognized that Penfield "[had] very large plans, plans for much more than I had in mind" (5). Penfield had already established a Laboratory of Neurocytology in New York with Dr. Cone as his assistant, and thus, were he to move to Montreal, he would want to establish and expand a similar research laboratory to coincide with his practice there. Archibald was receptive to Penfield's plans because he was also a researcher, like Penfield. William Feindel writes that, "W.E. Gallie, Professor of Surgery at Toronto, credited Archibald with changing the character of surgical education in Canada from purely clinical to scientific" (9).

Thus, Penfield transplanted his research and practice

from New York to Montreal, where he now had some allies who began to share his idea - of working to advance medical knowledge, unhampered by any "artificial" division between basic and clinical science, or between medicine and surgery. Someone who obviously shared this vision was Dr. Cone, who came to Montreal with Penfield. Until the end of his life, Bill Cone would remain Penfield's friend and partner. Somewhat whimsically, Penfield realized, in retrospect, that Cone and he "...were the beginning of a team, something more considerable than any individual can be.... We had heard together, the everlasting whisper that experienced explorers hear... we were fellow explorers" (5). With additional partners sharing his dream in Montreal, Penfield decided to make this "everlasting whisper" into a concrete plan: a proposal to the Rockefeller Foundation, a philanthropic organization dedicated to the advancement of medical science, for the building of an institute such as the one he had first envisioned some seven years earlier. About this, William Feindel writes that:

> The excellent relationship that McGill University had already developed with the Rockefeller foundation since 1922 to modernize its medical school offered Penfield a favourable matrix in which he could fulfill his conviction to "provide a center for neurological thought that would serve the whole continent..." (6).

However, Penfield did not take full advantage of this "excellent relationship" and five months after having arrived in Montreal, he went alone to Richard Pearce, in charge of medical grants at the Rockefeller Foundation, with his idea. Although it was supported in principle by Archibald and by Charles Martin, the Dean of McGill's Faculty of Medicine, the application was denied.

The MNI was Dr. Penfield's brainchild. He sowed the "germinal idea" as he termed it. But in order for it to become a reality, he had to wait for this germinal idea to sprout and take firm roots in other like-minded thinkers. A key person who also came to adopt the idea was Alan Gregg, the man who replaced Pearce at the Rockefeller Foundation. Gregg was himself a physician by training and had grown very interested in the nervous system as a result of having read Osler's *Principles and Practice of Medicine*. By the time Penfield went to Gregg to follow up on McGill's request, Gregg had already decided that the Rockefeller Foundation should support neurological research. In fact, he was also considering other proposals for funding a neurological institute. Luckily for Penfield, he made a great impression on Gregg and the two men realized that they had similar visions. Gregg said of Penfield's plan, "I think I understand what you want to do. You have a plan that gives real promise in a field that is calling desperately for exploration" (5). In April 1932, the Rockefeller Foundation awarded McGill University a grant of $1 232 000 for the creation of a neurological institute. Two hundred and thirty-two thousand dollars was to contribute to half the cost of building the institute, and $1 000 000 was to establish a permanent endowment for the scientific research to be carried out there.

* * * * *

Why Montreal? There were many factors that made McGill University and Montreal an ideal setting in which to build the institute. First was the support that McGill had shown to Penfield. This support extended equally to the neurologists of Montreal with whom Penfield had obviously made friends, including Colin Russel, McGill's Professor of Neurology who would become the first neurologist on staff at the MNI. The close association of McGill's new Department of Neurology and Neurosurgery with the RVH would ensure that the RVH, McGill, and the yet-to-be MNI could all function in close association with each other, sharing resources, and more importantly, ideas. To illustrate this point, Gordon Holmes made the following comment in his Foundation Lecture for the MNI:

> The proximity of this Institute to the medical and surgical wards of the Royal Victoria Hospital, and the connexion of its staff with other institutions in your city, will, I have no doubt… provide the desirable opportunity for intellectual and practical intercourse with mutual benefit (1).

In spite of this, it seemed remarkable to some that an American philanthropy would award such a large grant to create a Canadian institute; But this action was defended by many valid arguments. One was that the institute was to be a scientific institute that would perform research without boundaries. A second was the fact that both the Montreal public and the Montreal medical community were very enthusiastic about the prospects of building such an institute and were prepared to support it. The Americans recognized and appreciated these facts. An editorial in the *New York Times* of April 21, 1932 on "Illocality" aptly illustrates these and other arguments supporting the grant. It reads:

> [McGill University's] reputation and administrative efficiency were also an element in the decision. Besides, ...the citizens of Montreal showed interest and enterprise in welcoming such a foundation...[The institute's] benefits will have no geographical boundaries... Fortunately for Canada, there was no tariff against such talents as DR. PENFIELD carried over the border, and fortunately, no

> duties can be laid against the results of the researches of the institute (10).

These events were taking place before the age of biotechnology companies and squabbles over intellectual property rights, and financial gain was no where mentioned as a desired outcome of the institute. Thus, the Americans believed that everyone, everywhere, could stand to benefit from the research that the institute would carry out.

Dr. Penfield also believed this and he saw Montreal as the place where medical discoveries could be most rapidly disseminated, and where one could also be receptive to the greatest amount of beneficial external influences. He expressed this by writing that:

> Tradition and awareness link Montreal with Europe, especially Great Britain and France, as well as with the United States. Our location here...might well prove to be the best place in which to be influenced by the work of other centers. It might be the ideal place in which to do constructive scientific work on the brain and the mind of man, work that might in time influence thinking in other centers (5).

In this quotation, Penfield also alluded to the fact that one of Montreal's great strengths was its mix of two different, but equally vibrant cultures. The mix of French and English was an asset because it meant that while many institutions in Montreal were set up in parallel, they did not necessarily mimic each other. This was true of Montreal medical and educational institutions. From his very first days in Montreal, Penfield had striven to initiate a dialogue with the French-Canadian neurologists and by doing so he doubled the amount of information and personnel to which the English ones had access. Montreal was unique in providing such a setting and it would be of immense benefit to the prospects of a new institute if people and knowledge from both cultures were incorporated into the institution.

There were further reasons why 1930's Montreal was an ideal city for such a venture as was being proposed. Depression or not, Montreal was a comparatively large and wealthy city. In such a city it would be possible to raise private funds for the institute. Many of the wealthy so-called ‘Scottish merchant princes’ were already benefactors of McGill University, and funds for the remainder of the building were solicited from them. As is recounted in the Foundation Volume of the Hospital, "The vestibule [of the institute] bears the plaque of acknowledgement to generous benefactors: Rockefeller Foundation, Province of Quebec, City of Montreal, Sir Herbert Holt, J.W. McConnell, Walter Stewart, Four anonymous donors" (1). The three named individual benefactors had assured sizable donations that would cover the costs of the half of the edifice not covered in the Rockefeller grant. J. W. McConnell should be singled out in, particular. He was a media baron who published the aforementioned *Montreal Star*. He was an ardent supporter of McGill University and the new MNI, and would continue to provide needed money even after his initial generous donation of $100 000.

Notable as well, was the support that the MNI received from the public sector. As the institute's research and hospital portions were to have independent budgets, both of which were not to draw on McGill's general budget, the hospital operations would also have to be funded. For this, Principal Currie and Dean Martin approached the provincial and city governments and obtained $20 000 per year from the Province of Quebec and $15 000 per year from the City of Montreal.

* * * * *

On September 9, 1934, the Montreal Neurological Institute was officially opened by Edward W. Beatty, K.C., Chancellor of McGill University. An article that appeared that day in the Montreal *Gazette* ran a headline that described the event as an "Epoch Making Function" and proceeded to state that the cornerstone was laid "in the presence of a distinguished gathering of leading figures in the educational, ecclesiastical, medical, business and civic life of Montreal" (11). Luckily for the MNI, ‘good things’ came from the beginning. Dr. Penfield, accompanied by Dr. Cone and those studying with them, achieved great success in neurosurgery. Penfield's knowledge of the anatomy, physiology, and pathology of the brain, in addition to his beginning to understand the ætiology of the diseases he was working on as a result of his research, led him to strike out in new directions, attempting procedures few others had before. In particular, he made remarkable improvements in curative surgery for epilepsy.

Dr. Penfield's patients appreciated how his research was enabling his clinical work. In the early case of one William Ottmann, a private patient, Penfield had cured him of debilitating seizures. This had been a particularly difficult case and Penfield had called in many experts from near and far to assist him with a novel procedure, at Ottmann's mother's expense. A very grateful Mrs. Ottmann was overjoyed and before her death a short time later, she donated $50 000 to Penfield's research, which eventually went into building the MNI. Penfield's success in treating William Ottmann came to be repeated many times at the MNI. It is not surprising, then, that quite quickly, "the institute had become the number one center for neurology and neurosurgery in Canada" (12).

In the new institute, Dr. Penfield's operations began to make headlines across the country. During his operations, he would map out the brain of his patients to locate the origin of their seizures. In doing so, Penfield was able to develop maps of the human brain, including his famous homunculus. Penfield compiled these early findings at the MNI into a monograph in 1941 (13) and another in 1954 (14).

* * * * *

'Good things' also came to the hospital portion of the MNI. This was, in fact where the public could feel the direct impact of the MNI's foundation. Patients of the MNI in its early days were well attended to by a caring staff. A large part of this was due to the influence of Dr. Bill Cone, who had become the institute's first Neurosurgeon-in-Chief. Of Cone, Preston Robb writes that:

> With the passage of time, he gave up neuropathology and devoted all his time to patient care. He developed new neurosurgical techniques and better ways of getting the patients to the hospital and looking after them when they were there.... The great neurosurgical reputation of the Neuro were largely due to the many innovations of Bill Cone (12).

Cone's innovation is described by Mary Fitzgerald, a nurse at the MNI during the early years; "He brought back ideas for equipment from all of his experiences elsewhere. For instance, the headrest with its multiple uses was his invention and of course the Cone-Barton tongs" (15). She also echoes Robb in saying that the high quality of patient care at the MNI was due to Cone, writing that, "Dr. Cone was the surgeon that showed his interest in nursing care and ways to improve it" (15). Elizabeth Barrowman, another early nurse goes on to further discuss Cone's many inventions and innovations, including the rocking 'Neuro' bed, drill hole techniques for the relieving of intracranial pressure, a wrinkle-free lining for the Minerva jacket (a sort of half-body cast immobilizing the head and neck), an improved neck collar, and the 'Pancake Turner' for prevention of bed sores, amongst many others, such as the helicopter ambulance (which would land on the turf of the Molson Stadium adjacent to the MNI) (15).

* * * * *

In developing for himself the model of how medicine should be, Dr. Penfield dreamed of creating the MNI. This happened not because Penfield was either a genius or a visionary, but simply because he was a product of his environment, and he was ambitious and observant enough that he 'caught on' to the fact that neurology was entering what Robert Aird calls its expansive "flowering phase," which would be heavily dependent upon the integration of the clinical and basic sciences (16).

Along the way, many others who were of similar mind began to share Penfield's dream. Thus, a favourable intellectual environment for the development of the MNI presented itself. Some of the like-minded thinkers, especially Alan Gregg, were in the possession of money, and they deemed this particular idea to be the right one in which to invest that money. Thus, a favourable financial setting for the development of the MNI presented itself. McGill University and the people of Montreal were receptive to the ideas that Penfield and his associates presented to them. Governments donated to the hospital budget of the institute. McGill had begun a period of modernizing its medical faculty, recently having built a new pathology institute and a new biology building. It was eager to adopt a modern new medical institute. Montreal also seemed to be the ideal facility for many of the reasons that were discussed earlier. Thus, the right location for the development of the MNI presented itself. The confluence of these factors was well-timed enough so that the institute became a reality. One could as easily argue that this was fate as one could argue that it was chance. These arguments are rendered moot when one looks at the 'big picture'; That is, that the MNI came into being because current progress in medicine, and specifically in neurology, had found in science a useful tool. In a sense, neurology and neurosurgery were becoming "the neurosciences" and adopting the biomedical paradigm. Moreover, science could no longer be performed by mere individuals, but the scope of neurological research and care had now to be expanded within an institute.

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The early years of the MNI definitely resulted in an improvement "to relief of sickness and pain". For example, Head Nurse Eileen Flanagan did much to ensure a high calibre of nurses right at the onset of the MNI's existence. The significance of this, in the 'big picture,' is that:

> ...those [nurses] who took the Post Basic Program have gone away enriched and enthused by the nursing techniques they have learned. They have spread their knowledge across Canada, the United States and abroad. The most significant thing they have done is to improve the nursing care of the neurologically disabled around the world (15).

Moreover, the scientific research at the hospital yielded some results that were directly applicable to patient care. For example, Herbert Jasper recounted the story of:

> Sam... a young 8 year old with seizures.... He was the first patient to be operated upon by Dr. Penfield with the aid or preoperative EEG localization and corticography during the procedure. His seizures were controlled and his behaviour problem eventually improved after his anticonvulsant medication was reduced. Forty-five years later... we were surprised to see a distinguished looking gentleman.... It was Sam (17).

As exemplified by this anecdote, the founding of the MNI saw an increase in quality of life for neurological patients. The early years justified the foundation of the MNI with regards to improvement in patient care and quality of life.

As far as "the study of neurology" is concerned, the MNI represented a microcosm of the modern paradigm of medicine, and as Aird states, "in the Penfield account, a noteworthy fact is that the studies on epilepsy, like the studies of [several others], strikingly exemplify the dependence of modern neurology on... scientific advances" (16). The significance of the early days of the MNI is that it used this dependence to its advantage and was thus able to help start an age of modern progress in neurology.

* * * * *

Preston Robb writes that "Wilder Penfield had a dream, and he saw his dream fulfilled" (18). Penfield was a driven and dedicated surgeon, scientist and philosopher. This helped him fulfill his dream of building the Montreal Neurological Institute. But this dream was shared by others. It has been argued here that the fulfillment of this dream was a sign of his times, of faith in "biomedicine" and in moving beyond the descriptive phase of neurology. It was also a confirmation of this faith that the dream bore the fruit which was anticipated – that is, that the MNI had early medical and scientific successes. Thus, Wilder Penfield and the MNI helped to herald the modern age of neurology.

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REFERENCES

1. Montreal Neurological Institute. Neurological Biographies and Addresses: Foundation Volume (Published for the Staff, to Commemorate the Opening of the Montreal Neurological Institute, of McGill University). London, UK: Oxford University Press, 1936.
2. Feindel W. To Praise an Absent Friend. CMA Journal 116:1365-1367; 1977.
3. Lower, Rob, director. Something Hidden: A Portrait of Wilder Penfield. [Video recording] Produced by Rob Lower, Michael Scott and Vincent Tovell. National Film Board of Canada, 1987.
4. Lewis J. Something Hidden: A Biography of Wilder Penfield. Toronto: Doubleday, 1981.
5. Penfield W. No Man Alone: A Neurosurgeon's Life. Toronto: Little, Brown, 1977.
6. Feindel W. The Montreal Neurological Institute. Journal of Neurosurgery 75:821-822; 1991.
7. Osler W. Discussion: British Medical Association Meeting. Lancet 2:334; 1907. Quoted in Feindel W. Neurosurgery at the Montreal Neurological Institute and McGill University Hospitals. Neurosurgery 39:831; 1996.
8. Feindel W. The Contributions of Wilder Penfield and the Montreal Neurological Institute to Canadian Neurosciences. In: Roland CG, editor. Health, Disease and Medicine: Essays in Canadian History (Proceedings of the First Hannah Conference on the History of Medicine, McMaster University June 3-5, 1992). Toronto: Clarke Irwin, 1993.
9. Feindel W. Neurosurgery at the Montreal Neurological Institute and McGill University Hospitals. Neurosurgery 39:830-839; 1996.
10. Illocality. New York Times. 21 April, 1932: 20.
11. Lord Bessborough Lays Cornerstone of Neurology Unit. The Gazette. Montreal, 7 October, 1933.
12. Robb JP. The Development of Neurology at McGill. (Gift of Donald J. Baxter.) Montreal: Osler Library, McGill University, 1989.
13. Penfield W and Erickson TC. Epilepsy and Cerebral Localization. Springfield, MA: Charles Thomas, 1941.
14. Penfield W and Jasper, HH. Epilepsy and the Functional Anatomy of the Human Brain. Boston, MA: Little, Brown, 1954.
15. Robertson CE, editor. Nursing Highlights: Montreal Neurological Institute & Hospital (1934-1990). Brockville, Ont.: Henderson Printing, 1992.
16. Aird RB. Foundations of Modern Neurology: A Century of Progress. New York, NY: Raven Press, 1994.
17. Jasper HH. The Centrencephalic System. CMA Journal 116:1371-1372; 1977.
18. Robb JP. The Institute and Hospital. CMA Journal 116:1368-1369; 1977.

GENERAL REFERENCES

Bates D. Lectures in Health and the Healer in Western History, McGill University, 1998.

Bell RE. Wilder Penfield: His Legacy to Neurosurgery, Introduction. CMA Journal 116:1365; 1977.

Conrad LI, Neve M, Nutton V, Porter R, and Wear A. The Western Medical Tradition: 800 BC to AD 1800. Cambridge, UK: Cambridge University Press, 1995.

Elliott K and Allan C. Neurochemistry. CMA Journal 116:1372-1373; 1977.

Evans JP. Excited Beginnings. CMA Journal 116:1367; 1977.

Fleming G. The Picture of Health. The Gazette. Montreal: 8 September, 1934.

Hebb D. The Frontal Lobe. CMA Journal 116:1373-1374; 1977.

Kyle RA and Shampo MA. Wilder Penfield - Contributor to the Surgical Treatment of Epilepsy. Mayo Clinic Proceedings 67:596; 1992.

McNaughton FL. Impact on Medical Neurology. CMA Journal 116:1370; 1977.

Milner B. Memory Mechanisms. CMA Journal 116:1374-1376; 1977.

Montreal Neurological Institute. Prospect and Retrospect in Neurology: Second Foundation Volume (Published for the Staff, to Commemorate the Opening of the McConnell Wing and the Second Foundation of the Neurological Institute, of McGill University). Toronto: Little, Brown, 1955.

Preul MC and Feindel W. Origins of Wilder Penfield's Surgical Technique. Journal of Neurosurgery 75:812-820; 1991.

Rasmussen, TB. Surgical Treatment of Epilepsy. CMA Journal 116:1369-1370; 1977.

Stevenson L. Novelist and Historian. CMA Journal 116:1376-1377; 1977.

Concentration of Brain Cases Urged. The Gazette. Montreal, 2 June, 1933: 4.

McGill Will Fly Viceregal Flag. The Gazette. Montreal, 6 October, 1933.

Founder's Day Program Includes Fall Convocation And Laying of Neurology Institute Cornerstone. McGill Daily. Montreal, 6 October, 1933: 1.

Founders of Greater Montreal. Supplemental to Montreal Star. 7 October, 1933: 6.

Earl of Bessborough Lays Cornerstone For Institute. McGill Daily. Montreal, 10 October, 1933: 1, 4.

Medical Building Opening Prepared. The Gazette. Montreal, 8 September, 1934.

The Associated Press. Science Has Failed to Explain Problem of Life, Says Marconi. The Gazette. Montreal, 11 September, 1934: 1.

Neurological Institute Opened Doors Thursday. McGill Daily. Montreal, 1 October, 1934: 1, 7.

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FEATURE REVIEW

Advances in Tuberculosis Research in the Past 10 Years: Solutions for a Global Problem

Dick Menzies, MD*, Marcel Behr, MD; MSc; FRCPC

INTRODUCTION

In the early 1990's the number of cases of active tuberculosis (TB) was increasing in almost every country in the world. In developing, or resource-poor countries, which will henceforth be termed "poor" countries, this trend was no different than the previous 50 years, despite the discovery of effective treatment, since it was inaccessible to most of those with disease. However in industrialized countries, meaning those with established market economies (a World Bank term), which will henceforth be termed simply "rich" countries, incidence had declined since the end of the 19th century. Therefore the resurgence represented a suabstantial change that had important consequences. Although there is no doubt that this resurgence resulted in increased human suffering, and death, it had an important benefit. The phenomenon resulted in heightened awareness, interest and funding for TB. Increased investment in TB was for research as well as for control. Both activities resulted in substantial advances in our understanding of TB - at cellular, individual and population levels.

This review will examine the new knowledge gained over the past decade (1992-2002) largely resulting from this increased funding. New knowledge in TB can be broadly grouped into five areas: a) understanding the epidemiology and transmission; b) new diagnostic tools; c) new treatment tools; d) new tools for prevention; e) new approaches to management of TB - at individual and population levels. For each category the major advances of knowledge and their implications will be reviewed below.

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EPIDEMIOLOGY AND TRANSMISSION

Because the incubation period of tuberculosis may range from weeks to decades, it is usually not possible to ascertain from a patient the source of their infection. As a result, much about the epidemiology of TB transmission has traditionally been inferred through indirect means, for instance by observing that close contacts of TB cases are more likely to have a positive tuberculin skin test than casual contacts. Even in outbreaks of active TB, the confirmation of epidemiologic links was difficult, as there were no reliable bacterial typing tools prior to the early 1990's.

The discovery in the late 1980's of the insertion element, IS6110, opened up new avenues of epidemiologic and public health investigation. This insertion element is present in virtually all isolates of *M. tuberculosis* - with a variable number of copies (ranging from 1-25) and at variable loci within the bacterial genome. This means that the DNA from a particular strain of *M. tuberculosis* will have a unique number and location of these insertion sequences. Therefore when the DNA strand is cut at these insertion elements, the resulting pattern of the fragments of DNA produced after Southern hybridization with a probe for the IS6110 element, will be unique - like a fingerprint. Hence this technique, called restriction fragment length polymorphism or RFLP, is also called "DNA finger-printing". This technique is highly reproducible and theoretically there can be billions, even trillions of different DNA fragment patterns. The underlying premise of strain typing is that in an outbreak, all strains will have identical or highly similar DNA fingerprints reflecting their bacterial genotypes. Epidemiologically unrelated isolates should have different RFLP patterns.

RFLP-based studies can be categorized as: patient-based, clinic-based, and population-based. In patient-

based studies, bacterial typing permits one to determine if a clinical relapse represents treatment failure (same bacteria) or exogenous reinfection (cure followed by new infection), or a false positive culture due to lab cross-contamination. At the patient level, this information helps the treating physician to decide whether treatment is needed at all, if supervision of treatment should be intensified, or an outbreak investigation is needed to find an infectious source.

The next plane of molecular epidemiologic study can be termed the clinic or outbreak-based study. At a clinic level, participants in clinical trials with apparent relapse could be distinguished from patients with recurrent disease due to re-infection. This is important because while relapse indicates failure of a treatment regimen, re-infection does not. Outbreaks are often useful to validate the bacterial typing systems, by demonstrating concordance between epidemiologic evidence of links between patients, and genetic homogeneity of strains isolated from those patients. In one highly-cited example from San Francisco, a TB outbreak was suspected in an AIDS hospice because of the occurrence of 14 cases in just under one year (1). Molecular typing demonstrated that 12 of the cases had the same bacterial genotype, but the first two cases had a different TB bacterial strain. Based on this, it could be calculated that the true outbreak involved 12 of the 14 cases, that the epidemic window was just over 3 months, and that the interval from exposure to active pulmonary TB (incubation period) was as little as 3 weeks. Subsequently, a large number of outbreak investigations have made use of bacterial typing, either to rule-out links early during the investigation, or to refine the definition of the outbreak. As a result, TB transmission has now been linked to a wide-variety of potential contexts, including airplanes, bars, lap-dancers, illegal gaming facilities, etc. While it has not always been possible to obtain independent epidemiologic confirmation of these outbreaks, the observation of identical bacterial genotypes in these venues provides compelling evidence that TB transmission is not restricted to traditional settings (home, workplace) that would normally serve as the focus of contact investigations.

Because of the observation that outbreak strains share identical genotypes in the face of diverse strains normally circulating in a community, the next step in epidemiologic study involved the collection of all isolates from a community for strain comparison studies. Here the goal was not to elucidate all potential transmission links, but rather to demonstrate whether 200 cases in a community represents mostly independent instances of TB reactivation (manifest as different genotypes) or a collection of repeated genotypes (suggestive of unsuspected outbreaks). In the first studies to employ this methodology, groups in San Francisco and New York found that about 30-40% of TB cases in their communities represented ongoing epidemic spread (2;3). Moreover, by identifying the epidemiologic features of TB patients with shared strains (deemed molecular clusters), they were able to determine that ongoing TB transmission in these American cities appeared to be greater in American-born, younger, males. Also, HIV co-infection appeared to be a risk factor for being a member of a molecular cluster. These data refuted the contention that TB in the US was the result of immigration policies and imported infections, but rather pointed to a local problem in TB control. A number of studies have employed a similar methodology to query the degree of ongoing transmission in a community, providing a great range in estimates. In Montreal, the vast majority of TB cases have unique RFLP patterns, indicative of reactivation disease (4). In contrast, in a study of gold miners in South Africa, the majority of TB was deemed by molecular typing to represent ongoing spread (5). A further refinement of population-based molecular typing studies was to perform a observational study looking at the impact of altered TB control activities on the degree of molecular clustering. Following the observation that TB was being preferentially spread among young, US-born males in San Francisco, the department of public health bolstered control programs in that constituency. Not surprisingly, over the next 5 years, rates of TB remained essentially stable in the foreign-born community of San Francisco, but a dramatic drop in TB rates and TB molecular clustering was observed in the US-born (6).

A second important use of information at a population level, is to decide whether vaccination is a tenable strategy. If re-infection rates are high among patients treated for TB, then vaccination may not be tenable, because vaccines are most beneficial when survivors of natural infections are immune to further infections. In early studies of reinfection, most of the hosts suffering from reinfection had advanced HIV/AIDS disease, therefore, it was perhaps not surprising that their immune system had failed to ward off a new assault by *M. tuberculosis* (7). Soon after, reinfection was also demonstrated in a relatively immune-competent patient with diabetes, however, reports remained anecdotal. In 1999, van Rie and colleagues reported that in a township of Cape Town, three-quarters of patients with a second diagnosis of TB had reinfection rather than relapse. This suggested that in a high incidence setting, persons who were unable to contain the organism on first exposure could be treated and cured, but were at significant risk of developing disease again on re-exposure (8).

Contemporaneous reports suggested that the risk of reinfection was considerably reduced where TB incidences were lower, therefore, the majority of second cases of TB in San Francisco and the Netherlands represented clinical relapse, while an intermediate result was observed in the Canary Islands (9-11).

From the numerous molecular epidemiologic studies of TB, important lessons have emerged. TB transmission often occurred where TB control efforts were inadequate or de-emphasized, such as New York in the late 1980's and in recent years in the former USSR. This highlights the limited perceived economic value of preventive health until the costs of neglect mount (12). HIV infection has been shown to be a powerful force in the spread of TB, but its effects on transmission are variable. This is because HIV co-infection accelerates the reactivation of TB in persons previously infected and accelerates the progression of new *M. tuberculosis* infection to disease. Studies of drug-resistance have been able to use molecular tracking to document risk factors for the acquisition of drug-resistance mutations within patients and the spread of resistant strains among them (13). Unfortunately, a sobering lesson has been that spread of drug-resistant strains has been greatly enhanced by the bringing patients together in hospitals, providing yet another example of where a community public health problem is unwittingly amplified within the health-care system. Fortunately, attentiveness to many of these issues has been associated with a recent decline in TB rates in the United States as a whole (14), and in certain high incidence urban settings (12). The challenge that remains is bringing these advances to other countries where TB continues unabated.

DIAGNOSIS OF TB – DISEASE AND LATENT INFECTION

Nucleic acid amplification (NAA)

This term refers to a technique in which the nucleic acid (DNA or RNA) of organisms is amplified, by as much as 40 orders of magnitude, after which a probe detects a target sequence of DNA or RNA unique to that organism. These probes are highly specific, allowing one to identify individual species of mycobacteria, and distinguish M Tuberculosis (the causative organism of active TB) from Mycobacterium avium or other "atypical" environmental mycobacteria (15). These are easily confused with M Tuberculosis , but have very different clinical and public health implications.

Nucleic acid amplification tests are highly sensitive, and can detect as few as 10 organisms in one mL of clinical sample (15). Over the past decade the technology has progressed to become more automated and more rapid. The technique used to take 4-6 hours, but now 40 cycles of amplification can be accomplished in 40 minutes (16). However this technology is still expensive, requiring complex equipment as well as highly trained technical staff.

The major advantage of this technique is that it is much more sensitive and rapid than the traditional technique of direct microscopic examination of a smear of sputum stained to detect Acid Fast Bacilli (AFB smear) to rapidly diagnose active tuberculosis (15;17-19). Compared to NAA, AFB smear is less sensitive as it detects patients only when they have more advanced disease. The disadvantage of NAA testing is that Mycobacterial culture still needs to be performed, because NAA is less sensitive than culture. However, culture requires 4-8 weeks for a positive result using solid media (which are much less expensive and so are commonly used throughout the world) or 2-4 weeks using liquid media (which are only used in rich countries). Therefore, NAA tests offer the advantage of more rapid diagnosis of the majority of cases of TB disease.

Impact: At the moment NAA is only used in rich countries where the equipment and well-trained staff are available. In these countries, the greatest benefit of NAA techniques is for patients with a positive sputum AFB smear, in whom NAA can distinguish rapidly and accurately between active TB and diseases caused by environmental or atypical mycobacteria (20). This is important for infection control, public health and treatment reasons. Unfortunately, the limited sensitivity on AFB smear negative samples has prevented widespread adoption of this technique for screening of all samples.

In the long term, if the NAA process can be more automated, this will diminish requirements for highly trained staff. If the cost for materials and equipment continues to diminish, as it has over the last decade, then this technology could be applicable, at least for middle income countries (21). Since this includes most countries in Latin America, Eastern Europe and much of Asia, where more than 2 million patients with active TB are diagnosed each year, NAA could bring benefits to a large population.

Cytokines

Cytokines are inflammatory mediators produced by cells of the immune system such as macrophages, monocytes, and lymphocytes. When immune cells have been sensitized by prior exposure to M Tuberculosis, and then are re-exposed to those same antigens, they increase production of certain cytokines (22). This cell-mediated immune response, known as a Th1 response, is typical for tuberculosis and similar organisms and will result in increase of cytokines such as IFN-γ, IL-6, IL-12, and IL-18 (22-24). In contrast patients with

asthma or other atopic diseases will characteristically have a Th2 cell-mediated response and produce different cytokines, including IL-4 and IL-5 (25).

Cytokines may be useful in two ways. The first is for the measurement of response by certain Th1 immune cells to specific M Tuberculosis antigens. In patients with prior sensitization to M Tuberculosis (i.e. patients with latent TB infection), lymphocytes or other immune cells will respond with increased production of IFN-γ when these cells are exposed to M Tuberculosis antigens (26). Patients who have been sensitized by other mycobacterial organisms such as M Avium or the BCG vaccine, should not respond with increased cytokine production if exposed to highly specific M Tuberculosis antigens (27;28). The uncovering of genes uniquely present in *M. tuberculosis* through the tools of comparative genomics has greatly facilitated the search for such *M. tuberculosis*-specific antigens (29). The adoption of such antigenic proteins in the coming years may permit the detection of a cytokine response that is more specific than the tuberculin skin test in detecting latent infection with M tuberculosis.

The second use may be through identification of a pattern of cytokine response that is typical and specific for active disease due to M Tuberculosis. If such a pattern could be identified, this might be useful to distinguish patients with active TB, from those with other active pulmonary diseases such as pneumonia, asthma, bronchitis etc. However, this idea is purely speculative, as there is very little supporting data at this time.

Impact: At the present time measurement of cytokine response is limited to rich countries because this is very technologically complex requiring expensive equipment and highly trained staff. However the potential long range impact is considerable. If a test to detect patients with latent TB infection was better than the current standard of the tuberculin skin test, this would have far ranging implications as the TST is one of the most commonly used tests in clinical medicine world-wide. Another impact would be the identification of persons with LTBI who are at increased risk to develop active disease. In a small study of household contacts in Ethiopia, individuals with heightened cytokine response to certain M Tuberculosis antigens had significantly higher incidence of active TB within 2 years than those who did not (30). If confirmed in other patient populations, then the cytokine response detected may be useful not only to detect LTBI, but also to identify those with LTBI who have the greatest risk of developing disease. This information would be useful to target interventions, such as LTBI therapy, to persons who are the most likely to benefit.

A further impact of research in cytokines has been to provide insights into the pathogenesis of reactivation of active TB disease. For example, TNF-α is an important cytokine, and inhibitors of this mediator represent a novel, and highly effective therapy for patients with two inflammatory disorders - rheumatoid arthritis and Crohn's disease (31). Shortly after TNF-α inhibitors were introduced into clinical practice, a number of patients developed disseminated tuberculosis (32). These patients presented soon after their first course of therapy, with clinical features similar to patients with advanced HIV infection and active TB (32). This suggests that when TNF-α is inhibited, a profound immune defect results, which causes a susceptibility to TB reactivation. Understanding the role of cytokines in the pathogenesis of reactivation of TB, may lead to new therapies involving very different mechanisms than the traditional antibiotics.

The greatest barrier to widespread use of cytokines is the cost and complexity of their measurement. If the cost can be reduced and the techniques simplified, then cytokine-based tests may be useful in the near future to accurately identify those with latent TB infection, particularly those at high risk of disease.

TREATMENT OF ACTIVE DISEASE

Almost all the first and second line drugs currently used for TB were discovered and introduced in the 1950's and early 1960's. Rifampin, introduced in 1970, was the last new drug for more than 20 years, as there was no interest in development of new drugs for TB. However, in the past decade a whole new class of agents - the quinolones - have been found to have significant anti-tuberculosis activity. The most recently marketed agents, such as Moxifloxacin, have very high in-vitro activity against M Tuberculosis. Randomized trials are now underway to test the efficacy of this agent in the treatment of active TB.

When Rifampin was introduced, the duration of standard therapy of tuberculosis could be reduced from 18 to 9 months. When Pyrazinamide (PZA) was introduced, total duration could be further reduced from 9 to 6 months (33). With Moxifloxacin it is hoped that the total duration of therapy can be reduced further to only 4 months. Shortening the total duration of therapy is very important, because longer therapy is associated with poor patient compliance necessitating closer supervision, including directly observed therapy, which is more costly

A second new drug is Rifapentine. This is a rifamycin with a very long half-life, allowing it to be given once a week. Once weekly therapy allows highly intermittent directly observed therapy (see Section 5 below), which results in far fewer total doses of therapy - thereby reducing drug costs and the cost of supervision of therapy.

Impact: At the moment these two drugs are still much more expensive than standard first line anti-TB drugs and so are accessible only in rich, and middle-income countries. However the cost of standard first-line anti-TB therapy has been substantially reduced over the past decade (see section 5 below). Therefore it seems likely that greater use will result in lower costs for these new agents, making them more accessible for use in poor countries.

VACCINATION AGAINST TB—BCG AND NOVEL VACCINES

BCG vaccines have been administered since 1921 and currently, over 100 million infants receive BCG at birth each year. The goal of BCG vaccination of newborns is to prevent invasive forms of infantile TB, most notably miliary TB and TB meningitis. As such, BCG vaccines are generally provided in high incidence countries, where infantile exposure to TB is most likely, and in Canada, is restricted to high risk communities, such as Aboriginal communities where there is documented high incidence of TB.

It is generally stated that BCG vaccines provide high rates of protection against infantile TB and limited protection against contagious forms of TB in adults, resulting in some benefit at the level of the individual but limited impact in stemming the epidemic (34). However, according to the principles of evidence-based medicine, this view does not stand the test of critical analysis, as there has never been a randomized trial of provision of BCG to newborns. Furthermore, in a number of clinical trials of BCG vaccination in adults, there has been significant protection (up to 80%) against pulmonary TB, and in certain studies, TB-associated mortality and all-cause mortality (35). Unfortunately, the results of BCG trials have been so variable that accurate estimates of BCG protective efficacy are hazardous. In the largest study, involving over a quarter of a million subjects, BCG vaccination was no better than injection with saline placebo (36).

Given the resurgence in TB in the last decade, along with the emergence of drug-resistant forms of *M. tuberculosis*, increasing attention is being directed to the development of an improved vaccine against TB. In early years, efforts were focused on a subunit vaccine, with the view to reducing the risks associated with live, attenuated vaccines in countries often suffering from a high burden of HIV/AIDS. However, most of these candidates have been less protective than BCG in animal models, and the best subunit vaccines have equaled BCG in laboratory studies. More recently, two studies have published for the first time evidence of a vaccine that is more protective than BCG in animal models. Curiously, both are not just live vaccines, but in fact, recombinant variants of BCG vaccines.

After the original introduction of BCG vaccine in 1921, a number of different manufacturers began their own stocks of BCG, resulting by the mid 20th century in a family of vaccines that had evolved in vitro for 50-60 years. By genomic study of these vaccines, it has been possible to demonstrate genetic decay in these vaccines, with regulatory genes and antigenic proteins over-represented in these genetic events (37). This has understandably raised concerns, as the usual role of a live attenuated vaccine is to present antigens to the host immune system, therefore, a vaccine that has shed antigens may have limited utility as an immunizing agent. Recently, two groups have tried to improve upon BCG by over-expressing antigenic proteins of *M. tuberculosis*. Horwitz et al. used the Tice strain of BCG as a means of producing high quantities of the antigenic protein 85B (38). Guinea pigs are vaccinated with regular BCG Tice or the recombinant BCG, followed by challenge with fully virulent *M. tuberculosis*. In studies of the growth of the virulent *M. tuberculosis* and in time to death, the recombinant vaccine is consistently more protective (39). Notably, the recombinant vaccine does not appear to produce more pathology, in other words, the vaccine is no more virulent than the parent BCG. In a different approach, Stewart Cole's group took the Pasteur strain of BCG and added back a region of the genome that is consistently missing from all BCG strains. This region encodes two important antigenic proteins, named ESAT-6 and CFP-10. In mouse and guinea pig studies, the addition of this region did not materially increase the virulence of the BCG Pasteur, however, subsequent challenge of animals with virulent *M. tuberculosis* resulted in less dissemination of the virulent strain and less tissue pathology (40). While the exact mechanisms for these improvements in BCG vaccines remain to be determined, the important advance is that something better than BCG has finally been created. Hopefully, human phase I/II studies will proceed in coming years so that one can eventually field test these and other promising new candidates.

THERAPY FOR LATENT TB INFECTION (LTBI)

In North America, the current approach to LTBI is to identify those at increased risk of reactivation of TB disease, screen them with Tuberculin Skin Tests (TST), and to offer therapy of 9 months of INH (9INH) to tuberculin reactors. The long duration of LTBI therapy (previously referred to as preventive therapy), reduces compliance, often to less than 50%. As a result, non compliance is the most important factor reducing the effectiveness of this therapy. In addition 9INH has

significant side effects which are uncommon but can be serious, and even fatal. For this reason, patients must be intensively educated and motivated at the start, and then followed closely throughout treatment. This adds substantially to the cost of care.

Therefore the search for a shorter, safer and equally efficacious therapy has been very active for the past decade. The shortest preventive therapy regimen investigated to date has been two months of daily, self-administered Rifampin and Pyrazinamide (2RIF-PZA). This regimen was highly efficacious in a mouse model of latent TB infection. In several randomized trials among HIV positive patients, 2RIF-PZA had similar efficacy as 6 or 12 months of INH (41). However among HIV negative patients, under programme conditions, or in randomized trials, tolerability and completion rates with 2RIF-PZA were low and major adverse events unacceptably frequent (42-46). As a result, this regimen should be used with caution in highly selected patients.

Two other options are available: three months of INH and Rifapentine (3INH-RPT), taken once a week under direct observation, and 4 months daily self-administered Rifampin (4RIF). The 3INH-RPT regimen has the advantage that, in total only 12 doses are given, reducing the cost of therapy and followup as well as burden to patients. However it must be given under direct supervision, which is cumbersome. The 4 RIF regimen appears to be well tolerated, and has good completion rates. At present there is insufficient data regarding the safety and efficacy in preventing reactivation of active TB of both regimens. Therefore neither can be recommended for routine use now.

Shorter therapy will likely result in better compliance if the therapy does not have unpleasant side effects. Costs should be lower, unless follow-up has to be more intensive. Compared to 9 months INH, if a shorter regimen has fewer adverse effects, and equal efficacy then the shorter regimen will be more cost-effective, and have a better risk-benefit profile. This would make it much more acceptable for widespread use, and so potentially have a large impact on a population level in many countries.

Impact: At the present time therapy of LTBI is only feasible in rich countries. This is primarily because of the high cost of follow-up and the relatively low benefit. In poor countries and even in middle income countries resources are barely sufficient to diagnose and treat all patients with active TB disease. Diversion of these scarce resources to provide therapy of latent TB infection would be inappropriate. However if regimens can be found that are effective, shorter, and safer, and with high completion rates then therapy of LTBI would be applicable in middle income and could be considered for very high risk patients (such as HIV infected) in poor countries. Provision of therapy for latent TB infection on a population basis may accelerate reduction of incidence of active TB in many countries.

TB CONTROL

DOTS (Directly Observed Therapy - Short course):

Many advances in diagnosis and therapy of TB disease are based on studies conducted in poor countries. The DOTS approach is a good example. This approach is based on a successful approach to TB control developed in Tanzania by Dr. Styblo and colleagues of the IUAT. This approach emphasizes smear microscopy for diagnosis, therapy for 6-8 months (short-course) with standardized, Rifampin-containing combination regimens, a secure and stable supply of the necessary drugs, and directly observed therapy - meaning that someone, often a health care worker, actually observes the patient take therapy (47;48). This approach was based on sound epidemiologic principals and years of practical experience and has been shown to be highly cost-effective (49) and results in slow but steady decline in incidence when applied on a country wide basis (50).

In 1993 the World Health Organization adopted the DOTS programme and promoted its application in all countries. Because of this, the DOTS programme is now applied in most countries, although often to only a small part of the total population. As a result, currently less than 1/3 of the total world's population has access to diagnosis and effective therapy using this approach. An important element of the DOTS approach is standardized therapy with 4 highly effective first line TB drugs - Isoniazid (INH), Rifampin, Pyrazinamide (PZA), and Ethambutol (48). As a result of the increased use of standardized regimens with these 4 drugs, their price has fallen dramatically. Ten years ago the cost of a full course of therapy was approximately $60 US, but now costs less than $10, even for high quality drugs purchased from international manufacturers. As a result TB therapy is even more cost effective and more accessible to the world's poor.

DOTS Plus:

The emergence of drug resistance in almost all countries, has been one of the most major challenges to global TB control. Drug resistance, particularly multi-drug resistence (MDR) defined as resistance to at least INH and Rifampin, is the result of inadequate treatment (47). This occurs because of selection of inadequate regimens, poor quality drugs, or interrupted therapy. The latter occurs because of interrupted drug supply or patient non-compliance. In some countries, 10-20% of

patients with a history of prior therapy, have MDR-TB (51). In these same countries as many as 5% of patients who have never been treated before have MDR-TB (51). This implies substantial transmission of MDR-TB strains in the community - amplifying the gravity and extent of this problem.

Therapy of patients with drug resistance requires use of second line drugs, for 12, 18, or even 24 months depending on the pattern of drug resistance and extent of disease. As a result therapy of patients with MDR TB has been estimated to cost $7,000 - $10,000 US per patient, for the drugs alone. This is far more expensive than the $10 required to treat patients who have drug sensitive organisms, and may not be feasible for national TB control programmes in many poor countries. Therefore the IUATLD and WHO had recommended a standard re-treatment regimen of 8 months duration which did not include any of the expensive second line drugs (48). However this standardized re-treatment regimen is only moderately effective for previously treated patients and completely ineffective for patients with MDR strains. In such patients the standardized re-treatment regimen will actually worsen their drug resistance pattern.

For a number of years the approach to patients with MDR-TB was very controversial. The WHO argued that treatment of a handful of MDR TB patients could divert scarce resources, and mean that treatment would not be available for hundreds of previously untreated patients. However others argued that it was unethical to offer treatment that was almost certain to be ineffective. These patients posed a real humanitarian crisis.

To resolve this, the "DOTS-plus" approach was developed. Although there is still no single standardized regimen with documented superiority, the DOTS-plus approach emphasized a standardized and strictly observed therapy with second line drugs for prolonged periods. As with DOTS approach, a standardized international approach has enabled bulk purchasing which has resulted in more than 90% reduction in cost of the second line drugs needed. This has made therapy of MDR TB more accessible in middle-income and poor countries. Nevertheless the costs of more than $500 US per patient, makes provision of this therapy beyond the capacity of most national programmes in poor countries.

Treatment of MDR-TB remains one of the most important challenges for the next decade. It is encouraging to note that in regions where DOTS has been implemented and strictly followed the incidence of MDR TB has slowly fallen (12;52). This implies that generation of new MDR cases can be stopped by a good DOTS programme. If this can be combined with access to effective DOTS plus regimens, then the problem of MDR-TB could be controlled.

CONCLUSIONS

Much has been achieved over the past decade to advance our knowledge of the epidemiology, transmission, diagnosis, therapy, prevention, and management of tuberculosis. The advances in knowledge have resulted in greater changes in patient management and TB control in rich countries. However, there have been substantial improvements in access to diagnosis and therapy in poor countries. The challenge for the next decade is to ensure that we continue to invest in TB research in, to advance our knowledge, while also looking to apply this new knowledge in the most cost-effective and practical manner in all countries of the world.

REFERENCES

1. Daley CL, Small PM, Schecter GF, Schoolnik GK, McAdam RA, Jacobs WR et al. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. New Engl J Med 1992; 3264:231-235.
2. Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC et al. The epidemiology of tuberculosis in San Francisco: A population-based study using conventional and molecular methods. New Engl J Med 1994; 33024:1703-1709.
3. Alland D, Kalkut GE, Moss AR, McAdam RA, Hahn JA, Bosworth W et al. Transmission of tuberculosis in New York City: An analysis by DNA fingerprinting and conventional epidemiologic methods. New Engl J Med 1994; 33024:1710-1716.
4. Kulaga S, Behr M, Musana K, Brinkman J, Menzies D, Brassard P et al. Molecular epidemiology of tuberculosis in Montreal. CMAJ 2002; 1674:353-354.
5. Godfrey-Faussett P, Sonnenberg P, Shearer SC, Bruce MC, Mee C, Morris L et al. Tuberculosis control and molecular epidemiology in a South African gold-mining community. Lancet 2000; 3569235:1066-1071.
6. Jasmer RM, Hahn JA, Small PM, Daley CL, Behr MA, Moss AR et al. A Molecular Epidemiologic Analysis of Tuberculosis Trends in San Francisco, 1991-1997. Ann Inter Med 1999; 130:971-978.
7. Small P, Shafer R, Hopewell P. Exogenous reinfection with multidrug-resistant Mycobacterium tuberculosis in patients with advanced HIV infection. N Engl J Med 1993; 328:1137-1144.
8. van Rie A, Warren R, Richardson M, Victor TC, Gie RP, Enarson DA et al. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N Engl J Med 1999; 34116:1174-1179.
9. Fine PE, Small PM. Exogenous reinfection in tuberculosis. N Engl J Med 1999; 34116:1226-1227.
10. de Boer AS, van Soolingen D. Recurrent tuberculosis due to exogenous reinfection. N Engl J Med 2000; 34214:1050-1051.
11. Caminero JA, Pena MJ, Campos-Herrero MI, Rodriguez JC, Garcia I, Cabrera P et al. Epidemiological Evidence of the Spread of a Mycobacterium tuberculosis Strain of the Beijing Genotype on Gran Canaria Island. AM J Resp Crit Care Med 2001; 164:1165-1170.
12. Frieden T, Fujiwara P, Washko R, Hamburg *M. Tuberculosis* in New York City - Turning the TIde. The New England Journal of Medicine 1995; 3334:229-233.

13. Frieden TR, Sherman LF, Maw KL, Fujiwara PI, Crawford JT, Nivin B et al. A multi-institutional outbreak of highly drug-resistant tuberculosis. Epidemiology and clinical outcomes. JAMA 1996; 27615:1229-1235.
14. U.S. Department of Health and Human Services PHS. Reported Tuberculosis in the United States, 2001: Tuberculosis Case Rates: United States, 2001. Center for Disease Control and Prevention, editor. 2001.
15. Schluger NW, Rom WN. the polymerase chain reaction in the diagnosis and evaluation of pulmonary infections. Am J Respir Crit Care Med 1995; 152:11-16.
16. Meuer S, Wittwer C, Nakagawara K. Rapid Cycle Real-Time PCR. Springer Verlag Berlin Heidelberg 2001.
17. Walker DA, Taylor IK, Mitchell DM, Shaw RJ. Comparison of polymerase chain reaction amplification of two mycobacterial DNA sequences, IS6110 and the 65kDa antigen gene, in the diagnosis of tuberculosis. Thorax 1992; 47:690-694.
18. Pfyffer GE, Kissling P, Jahn EMI, Welscher H-M, Salfinger M, Weber R. Diagnostic performance of amplified mycobacterium tuberculosis direct test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. Journal of Clinical Microbiology 1996; 344:834-841.
19. Bradley SP, Reed SL, Catanzaro A. Clinical efficacy of the amplified mycobacterium tuberculosis direct test for the diagnosis of pulmonary tuberculosis. Am J Respir Crit Care Med 1996; 153:1606-1610.
20. Cohen RA, Muzaffar S, Schwartz D, Bashir S, Luke S, McGartland LP et al. Diagnosis of pulmonary tuberculosis using PCR assays on sputum collected within 24 hours of hospital admission. Am J Respir Crit Care Med 1998; 157:156-161.
21. Roos BR, van Cleeff MRA, Githui WA, Kivihya-Ndugga L, Odhiambo JA, Kibuga DK et al. Cost-effectiveness of the polymerase chain reaction versus smear examination for the diagnosis of tuberculosis in Kenya: a theoretical model. Int J Tuber Lung Dis 1997; 23:235-241.
22. Anderson P, Munk ME, Pollock S, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet 2000; 356:1099-1104.
23. Taha RA, Minshall EM, Olivenstein R, Ihaku D, Wallaert B, Tsicopoulos A et al. Increased Expression of IL-12 Receptor mRNA in Active Pulmonary Tuberculosis and Sarcoidosis. Am J Resp Crit Care Medicine 1999; 160:1119-1123.
24. Zhang Y, Broser M, Cohen H, Bodkin M, Law K, Reibman J et al. Enhanced Interleukin-8 Release and Gene Expression in Macrophages after Exposure to Mycobacterium tuberculosis and Its Components. J Clin Invest 1995; 95:586-592.
25. Surcel HM, Troye-Blomberg M, Paulie S, Anderson G, Moreno C, Passvol G et al. TD1/TD2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. Immunology 1994; 81:171-176.
26. Mazurek G, LoBue PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR. Comparison of a whole-blood interferon gamma assay with tuberculin skin testing for detecting latent Mycobacterium tuberculosis infection. JAMA 2001; 25614:1740-1747.
27. Laurens A.H.van Pixteren, Ravn P, Agger EM, Pollock J, Andersen P. Diagnosis of Tuberculosis Based on the Two Specific Antigens ESAT-6 and CFP10. Clinical & Diagnostic Labratory Immunology 2000; 72:155-160.
28. Johnson PDR, Stuart RL, Grayson ML, Olden D, Clancy A, Ravn P et al. Tuberculin-Purified Protein Derivative-, MPT-64-, and ESAT-6-Stimulated Gamma Interferon Responses in Medical Students before and after Mycobacterium bovis BCG Vaccination and in Patients with Tuberculosis. Clinical & Diagnostic Labratory Immunology 1999; 66:934-937.
29. Sorensen AL, Nagai S, Houen G, Andersen P, Andersen AB. Purification and Characterization of a Low-Molecular-Mass T-Cell Antigen Secreted by Mycobacterium Tuberculosis. Infection and Immunity 1995; 635:1710-1717.
30. Ulrichs T, Anding P, Porcelli S, Kaufmann SHE, Munk ME. Increased Numbers of ESAT-6 and Purified Protein Derivative-Specific Gamma Interferon-Producing Cells in Subclinical and Active Tuberculosis Infection. Infection and Immunity 2000; 6810:6073-6076.
31. Lipsky PE, Heijde D, St.Clair W, Furst DE, Breedveld FC, Kalden JR et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. The New England Journal of Medicine 2000; 34322:1594-1602.
32. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD et al. Tuberculosis associated with infliximab, a tumor necrosis factor a - neutralizing agent. The New England Journal of Medicine 2001; 34515:1098-1104.
33. Fox W. The current status of short-course chemotherapy. Bull Int Union Tuberc 1978; 534:1-13.
34. ten Dam HG, Toman K, Hitze KL, Guld J. Present knowledge of immunization against tuberculosis. Bulletin of the World Health Organization 1976; 54:255-267.
35. Comstock GW. Identification of an effective vaccine against tuberculosis. Am Rev Resp Dis 1988; 138:479-480.
36. The Tuberculosis Prevention Trial M. Trial of BCG vaccines in south India for Tuberculosis prevention: first report. Bulletin of the World Health Organization 1979; 575:819-827.
37. Mostowy S, Cousins D, Brinkman J, Aranaz A, Behr MA. Genomic deletions suggest a phylogeny for the Mycobacterium tuberculosis complex. J Infect Dis 2002; 1861:74-80.
38. Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic' S. Recombinant bacillus calmette-guerin BCG vaccines expressing the Mycobacterium tuberculosis 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. Proc Natl Acad Sci 2000; 9725:13853-13858.
39. Horwitz MA, Harth G. A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the Guinea Pig Model of Pulmonary Tuberculosis. Infect Immun 2003; 714:1672-1679.
40. Pym AS, Brodin P, Majlessi L, Brosch R, Demangel C, Williams A et al. Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. Nat Med 2003; 95:533-539.
41. Gordin FM, Chiasson RE, Matts JP, et al. Rifampin and Pyrazinamide vs Isoniazid for Prevention of tuberculosis in HIV-infected Persons. JAMA 2000; 28311:1445-1450.
42. Center for Disease Control. Fatal and Severe Hepatitis Associated with Rifampin and Pyrazinamide for the Treatment of Latent Tuberculosis Infection - New York and Georgia, 2000. MMWR 2001; 5015:289-291.
43. Fatal and severe liver injuries associated with rifampin and pyrazinamide for latent tuberculosis infection, and revisions in the American Thoracic Society / CDC recommendations. MMWR 2001; 5034:733-735.
44. McNeill L, Allen M, Estrada C, Cook P. Pyrazinamide and Rifampin vs Isoniazid for the Treatment of Latent Tuberculosis. Chest 2003; 123:102-106.
45. Stout JE, Engemann JJ, Cheng AC, Fortnberry ER, Hamilton CD. Safety of 2 Months of Rifampin and Pyrazinamide for Treatment of Latent Tuberculosis. Am J Crit Care Med 2003; 167:824-827.
46. Jasmer RM, Saukkonen JJ, Blumberg HM, Daley CL, Bernardo J, Vittinghoff E et al. Short-course rifampin and pyrazinamide compared with isoniazid for latent tuberculosis infection: a multicenter clinical trial. Ann Intern Med 2002; 137:640-647.
47. Rieder HL. Interventions for Tuberculosis COntrol and Elimination. International Union Against Tuberculosis and Lung

Disease 2002.
48. Enarson DA, Rieder HL, Arnadottir T, Trebucq A. Tuberculosis guide for low income countries. Tuberculosis guide for low income countries. Paris: International Union Against Tuberculosis and Lung Disease, 2000.
49. Murray CJL, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. Bull Int Union Against Tuberculosis 1990; 651:2-20.
50. Suarez PG, Watt CJ, Alarcon E, Portocarrero J, Zavala D, Canales R et al. The Dynamics of Tuberculosis in Response to 10 Years of Intensive Control Effort in Peru. The Journal of Infectious Diseases 2001; 184:473-478.
51. Pablos-Mendez A, Raviglione MC, Laszlo A, Binkin N, Rieder HL, Bustreo F et al. Global surveillance for antituberculosis-drug resistance. 1994-1997. N Engl J Med 1998; 338:1641-1649.
52 . Weis S, Slocum PC, Blais FX, King B, Nunn M, Matney B et al. The effect of directly observed therapy on the rates of drug resistant and relapse in tuberculosis. New Engl J Med 1994; 33017:1179-1184.

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FEATURE REVIEW

Host Genetics of Tuberculosis Susceptibility[1]

Tania Di Pietrantonio[†], Caroline Gallant[†], Erwin Schurr[*], Ph.D.

INTRODUCTION

Tuberculosis, primarily caused by the human pathogen *Mycobacterium tuberculosis*, continues to be a major global health concern affecting an estimated 8 million people annually and resulting in approximately 2 million deaths. Interestingly, only about 10% of those infected with *M. tuberculosis* develop clinical disease (1, 2). The disparity in progression from infection to disease points to the possible importance of the host genetic background in susceptibility to tuberculosis. Hence, the identification of host susceptibility genes is important to aid our understanding of tuberculosis pathogenesis and to identify new therapeutic and preventive strategies. New approaches in tuberculosis control are especially relevant now due to the synergistic relationship between tuberculosis and HIV/AIDS making tuberculosis the single biggest killer of people living with HIV/AIDS (3).

Understanding the natural history of *M. tuberculosis* and distinguishing between infection and disease progression are essential to dissect the genetic basis of tuberculosis. Upon inhalation of the air-borne tubercle bacilli into the lung, two courses of progression are possible. In the majority of individuals, the bacilli are ingested by phagocytic alveolar macrophages and either killed or grow to a limited extent intracellularly. Infrequently, in children and in immuno-compromised individuals, the pathogen disseminates and forms small miliary lesions or life-threatening meningitis. More commonly, within 2 to 6 weeks after infection, a cell-mediated immune response contains the localized, granulomatous lesions, killing most, but not necessarily all of the bacilli. If the cellular immune response is not effective, which occurs in approximately 5% of cases, the primary infection will progress into active disease. In addition, approximately 5% of those 95% who contained the primary infection will develop clinical tuberculosis over the course of their lifetime. In general, *M. tuberculosis* has a strong predilection for the lungs and the majority of tuberculosis patients develop pulmonary disease. Once an infected individual converts to active pulmonary disease, cavitary lesions develop and the mycobacteria proliferate. If the cavity expands into the alveoli, the patient becomes infectious and spreads the bacilli by speaking, coughing and sneezing (4).

Population variability in susceptibility to tuberculosis

There is significant historical evidence demonstrating the importance of host genetic factors in susceptibility to tuberculosis. Present day resistance to mycobacterial infection is determined in part by a population's history of exposure. Infectious disease outbreaks with high morbidity select for genetic variants that confer resistance (5). Populations with a long history of exposure, such as Europeans, compared with populations only recently exposed, such as North American Natives and sub-Saharan Africans, show greater resistance to tuberculosis (6). Two historical events illustrate population differences in tuberculosis susceptibility and point to variable a resistance pattern in both "resistant" and "susceptible" populations.

The accidental administration to infants of the *M. bovis* Bacille Calmette-Guérin (BCG) vaccine with a virulent strain of *M. tuberculosis* in Lübeck, Germany, in 1929 provided an inadvertent experimental opportunity to verify that human individual variation

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exists in response to uniform infectious exposure. Of 251 immunologically naïve infants accidentally inoculated with virulent *M. tuberculosis*, 4 showed no signs of infection, 72 died of tuberculosis within 1 year of infection, and 175 overcame the infection (7). In contrast to the high survival rate of the immunologically naïve infants in Germany, North American Natives were devastated by tuberculosis upon initial exposure. The death rates during the late 19th century were the highest recorded world-wide and exceeded by 10 times the peak death rate observed in Europe during the 17th century (6).

MOUSE STUDIES

As in many human diseases, studies employing animal models have provided important clues for the mechanisms of susceptibility to tuberculosis and related mycobacteria that could not easily have been obtained from studies in humans alone. Specifically, studies employing mouse models have provided critical insights into the role of host genetics in susceptibility to *M. tuberculosis* infection. Although *M. tuberculosis* is not a natural mouse pathogen, inbred strains of mice vary extensively in their susceptibility to tuberculosis (8, 9, 10, 11). Preliminary work involving crosses between susceptible and resistant mice has indicated that, as in humans, susceptibility to the disease is under multigenic control (10). Consequently, mouse models have become powerful tools for the identification of candidate tuberculosis susceptibility genes. One such example is the discovery of the *Nramp1* gene, which subsequently led to the identification of *Nramp1* as a susceptibility gene in human tuberculosis, leprosy and HIV (12, 13, 14).

The *Nramp1* gene

Segregation analysis in inbred mouse strains led to the identification of a gene on chromosome 1 that controlled the early splenic replication of an attenuated vaccine strain derived from *M. bovis*, bacillus Calmette-Guérin (BCG). This gene, initially designated *Bcg* (15) and later redefined as *Nramp1* (natural resistance-associated macrophage protein 1) (16), had a dominant resistance effect on the multiplication of various mycobacterial species as well as a number of taxonomically unrelated intracellular pathogens including *Leishmania donovani* and *Salmonella typhimurium* (17). The *Nramp1* gene exists in two allelic forms in inbred mouse strains(17). Resistant *Nramp1*^{r} strains are able to control microbial proliferation at the initial phase of infection whereas *Nramp1*^{s} mice are permissive for rapid uncontrolled proliferation of the mycobacteria (15). Susceptibility to infection was determined to be the result of a single, non-conservative, glycine-to-aspartate substitution at position 169 of the *NRAMP1* protein, a 12-transmembrane divalent cation transporter (16) expressed by professional phagocytes (18). Although the *Nramp1* gene is protective against infection with attenuated BCG vaccine strains, its function in modulating infection with fully virulent *M. tuberculosis* is unclear. In a resistance ranking study, *Nramp1*^{r} mice appeared to have shorter survival times than *Nramp1*^{s} strains (9, 19). Furthermore, mice with a functionally deleted *Nramp1* gene appeared to be as resistant to virulent *M. tuberculosis* as their wild-type counterparts (20).

H-2 and non-H-2 genes

The development of cell mediated immunity by preferential induction of the Th1 proliferation pathway has been postulated to be the underlying mechanism of genetic resistance to several intracellular pathogens, including *M. tuberculosis*. The T helper1 (Th1) phenotype is defined by the profile of type specific cytokines including interferon-gamma (IFN-γ) and interleukin-12 (IL-12). To understand the significance of specific cytokines in immunity to tuberculosis, numerous gene deletion mouse strains have been generated. IFN-γ knockout (GKO) mice are the most susceptible to infection with virulent *M. tuberculosis*. Since macrophage activation is defective in GKO animals (21), they develop a fatal disseminated infection in response to a sublethal dose of *M. tuberculosis* (22). Bacterial growth in these mice is virtually unrestricted and, although granulomas develop, they become rapidly necrotic (23). The principal effector mechanism for IFN-γ is the production of reactive nitrogen intermediates (RNI) by nitric oxide synthase (*Nos2*) (24). Important evidence for the role of the *Nos2* locus in protection against tuberculosis arose from studies in mice with a targeted *Nos2* deletion (*Nos2-/-*). Infection of *Nos2-/-* mice with *M. tuberculosis* produced a severe pathological condition that closely resembled that of GKO mice (25, 26).

The only other gene disruption known to cause such a fulminant *M. tuberculosis* infection is that of tumor necrosis factor-α (IFN-α). Both TNF-deficient (*Tnf* -/-) (27, 28) and Tnf receptor-1 knockout (Tnfr1 KO) (29) mice are unable to form functional granulomas, have increased bacterial loads and, consequently, succumb quickly to infection. Interestingly, macrophages from both IFN-γ receptor and Tnfr1 deficient mice are unable to produce IL-12 in response to mycobacteria (30). IL-12 is pivotal in the eradication of *M. tuberculosis* since it serves primarily in the induction of

IFN-γ (31). Direct evidence for the involvement of IL-12 in antimycobacterial mechanisms was provided by a mouse strain with a genetic disruption in *IL-12p40* (*IL-12p40-/-*) (32). *M. tuberculosis*-infected *IL-12p40-/-* mice were shown to develop substantially higher bacterial burdens than control mice and had shorter survival times.

Although IL-18 can potentially induce both a Th1 and Th2 response (33), its significance in anti-*M. tuberculosis* immunity lies, as with IL-12, in its ability to stimulate IFN-γ production (34). Reduced IFN-γ expression in *IL-18*-gene disrupted mice resulted in a slightly enhanced susceptibility to *M. tuberculosis* (35, 36). In addition, a reduced production of IFN-γ in IL-6 KO animals resulted in an early rise in mycobacterial loads when a low dose of *M. tuberculosis* was administered (37) but caused rapid mortality with a high dose (38). Furthermore, in IL-1 type I receptor-deficient (IL1R-/-) mice, an increase in susceptibility was the result of defective IL-1 signaling which subsequently led to decreased IFN-γ production (39). Thus, gene deletion mouse strains have clearly proven that IFN-γ is the key cytokine in the defense against *M. tuberculosis*.

In contrast to IFN-γ however, the function of Th2 cytokines such as IL-4 and IL-10 in host defence against *M. tuberculosis* has not yet been defined. Targeted gene disruption of either *IL-4* or *IL-10* on a tuberculosis resistant C57BL6/J background did not appear to drastically alter susceptibility to *M. tuberculosis*-triggered disease (37, 40). In fact, a study employing IL-10 deficient animals observed enhanced antimycobacterial immunity in the absence of this cytokine (41). In yet another study however, *M. tuberculosis*-infected IL-4 KO mice had an increased pulmonary bacterial burden compared to wild-type mice (42), suggesting a subtle but protective role for this immune mediator.

A role for H-2 genes in susceptibility to tuberculosis has also been established in the mouse. Carriers of the $H\text{-}2^{k}$ haplotype appear more susceptible to *M. tuberculosis* than $H\text{-}2^{b}$ and $H\text{-}2^{d}$ haplotype carriers on the basis of response phenotypes such as the bacterial burden in the lung (43) and median survival times (9). In contrast, Apt and colleagues (44) observed that $I\text{-}A^{b}/D^{b}$ allele combinations were associated with shorter survival times compared to $I\text{-}A^{k}/D^{d}$ combinations. This discrepancy may be partially explained by the differences in the infectious doses administered. In this same study, Apt and colleagues also determined that expression of the $H\text{-}2^{f}$ haplotype did not confer protective immunity by BCG vaccination. Furthermore, although H-2 genes have been implicated in the antibody response to mycobacterial antigens (45, 46), the generation of a granulomatous inflammatory response to *M. tuberculosis* does not appear to be under H-2 control (47). Hence, although the H-2 genes exert some influence on susceptibility to tuberculosis, other more significant genes are yet to be identified.

Quantitative trait locus analysis

Due to the multigenic control of host resistance to tuberculosis, an alternative strategy to identifying susceptibility genes has been adopted. Quantitative trait locus (QTL) analysis entails performing a genome-wide scan employing mice generated by experimental crosses between inbred mouse strains that represent polar ends of a resistance/susceptibility spectrum. QTLs are then assigned to specific chromosomal regions by the use of sophisticated analytical tools (48, 49) and high-density genome-wide maps.

Using different murine models, three groups have identified various genetic loci of yet unknown molecular identities that are implicated in tuberculosis susceptibility. In the first of these studies, Lavebratt et al. (50) investigated *M. tuberculosis*-triggered body weight loss in a panel of [(A/Sn I/St)F1 (I/St)] backcross animals derived from "resistant" A/Sn mice and "susceptible" I/St mice. QTLs impacting on *M. tuberculosis*-induced weight loss were identified on distal chromosome 3 and proximal chromosome 9 in females only, and suggestive linkages were observed on chromosomes 8 and 17 in females and chromosomes 5 and 10 in males. Recently, linkage of the aforementioned chromosomal regions to loss of body weight and duration of survival was studied in *M. tuberculosis*-infected [(A/Sn (I/St)F2] mice (51). The QTLs on chromosomes 3 and 9, designated (tuberculosis severity 1) *tbs1* and *tbs2* respectively, were only suggestively linked to postinfection body weight loss in F2 mice of both sexes. In addition, the previously identified QTL on chromosome 17, located in the proximity of the H-2 complex, was also involved in the control of tuberculosis and appeared to interact with *tbs1*.

Another important tuberculosis susceptibility locus was recently mapped to a 9-cM interval on mouse chromosome 1 using an F2 informative population derived from C57BL/6J (resistant) and C3HeB/FeJ (susceptible) progenitor strains (52). This locus, termed *sst1* for susceptibility to tuberculosis, controls progression of lung disease, specifically lung-specific granuloma formation, caused by virulent *M. tuberculosis*. Although the *sst1* locus is located only 10 cM of the *Nramp1* gene, these loci appear mutually exclusive given that the C57BL/6J strain carries both the resistant allele of *sst1* (*sst1*^{r}) and the susceptible

allele of *Nramp1* (*Nramp1*^{s}). It is important to note, however, that *Nramp1*^{s} strains are known to be more resistant to *M. tuberculosis* than their *Nramp1*^{r} counterparts.

Using survival time as an expression of tuberculosis susceptibility, Mitsos and colleagues (53) performed a genome-wide QTL analysis in a panel of F2 mice derived from "susceptible" DBA/2J and "resistant" C57BL6/J parental strains. These authors identified two significant linkages on the distal portion of chromosome 1 and the proximal portion of 7, termed Tuberculosis resistance locus-1 (*Trl-1*) and *Trl-3* respectively. *Trl-2* was the designation given to the third suggestive linkage detected on the proximal portion of chromosome 3. Together, *Trl-1*, *Trl-2* and *Trl-3* accounted for approximately half of the phenotypic variance observed between the two progenitors with respect to duration of survival. Furthermore, homozygosity for the parental C57BL/6J allele at each of the three loci was associated with a significantly longer survival time.

Mouse models have helped uncover numerous genes involved in the control of host response to infection with human bacterial pathogens. In terms of tuberculosis susceptibility, the H-2 major histocompatibility genes as well as several non-H-2 genes such as *Nramp1*, *Tnfa* and *Infg* genes have been clearly implicated in susceptibility. The creation of novel and improved analytical and experimental tools will further facilitate the study of complex diseases such as tuberculosis and consequently lead to the discovery of new tuberculosis candidate genes.

HUMAN STUDIES

For human populations, Abel and Casanova (54) have described the genetic control of tuberculosis as a continuous spectrum of genetic complexity, with simple Mendelian disease at one extreme, and complex polygenic disease control at the other. Presently, mutations involved in Mendelian susceptibility to mycobacterial infections are very rare and cannot account for the global burden of disease. In contrast, numerous polymorphisms contributing moderately to susceptibility have been identified but their functional relevance and their impact at the population level remains elusive. There is evidence suggesting major gene control of susceptibility in certain populations or epidemiologic contexts where gene-environment interactions can be modeled (54, 55). It seems likely that the molecular genetic dissection of tuberculosis will depend on studying all aspects of the spectrum, on distinguishing susceptibility to infection versus susceptibility to disease progression, on distinguishing primary and reactivation infection, and on using both mouse and human models.

Several different but complementary study designs can be used to identify human host genetic factors involved in disease susceptibility. These methods include: the study of individuals displaying extreme phenotypes (or Mendelian inheritance of susceptibility); case-control, candidate gene studies; and family-based, genome-wide linkage studies.

Mendelian susceptibility to mycobacterial disease

Recently, specific mutations conferring susceptibility to mycobacteria and occasionally salmonella species have been grouped under the genetic syndrome Mendelian susceptibility to mycobacterial disease (MIM 209950). Individuals with the syndrome are unable to produce or respond to IFN-γ and are therefore highly vulnerable to weakly virulent non-tuberculous mycobacteria, such as ubiquitous environmental mycobacteria and live-attenuated *M. bovis* BCG vaccine strain. Several individuals with the syndrome have been diagnosed with clinical tuberculosis but it is unclear to what extent the mutations are important in *M. tuberculosis* infection or disease progression (56, 57, 58, 59).

The mutations resulting in Mendelian susceptibility to mycobacteria are present in genes essential in host cellular immunity, or more specifically, the type-1 cytokine cascade. The genes include those encoding interleukin 12 subunit p40 (*IL12B*), interleukin 12 receptor beta-1 subunit (*IL12RB1*), interferon gamma receptor 1 (*IFNGR1*), interferon gamma receptor 2 (*IFNGR2*) and signal transducer and activator of transcription 1 (*STAT1*) (60, 61, 62, 63, 64, 65). The mutations result in three classes of alleles and several corresponding clinical, immunological and histopathological outcomes: recessive or non-functional alleles; recessive, partially functional alleles; and dominant-negative alleles resulting in partial functionality (66, 67). The identification of individuals with infections to otherwise avirulent pathogens has helped dissect and identify essential pathways crucial for immunity to mycobacteria.

An important but unanswered question is whether more common polymorphisms of the type-1 cytokine cascade genes contribute at a population level to susceptibility to tuberculosis (66, 68). Recently, two studies showed an association between a genetic defect involved in decreased production of IFN-γ with increased risk of developing tuberculosis (69,70). In addition, specific *IL12Rβ1* polymorphisms are associated with increased tuberculosis risk in a Japanese population (71). Although the importance of IFN-γ in host response to mycobacteria is well established, more studies are needed to understand the

importance of common type-1 cytokine polymorphisms in anti-mycobacterial immunity.

CANDIDATE TUBERCULOSIS SUSCEPTIBILITY GENES

Candidate genes, identified by their known or suspected involvement in disease pathogenesis, are tested by association using population or family-based case-control designs (72). "Major" susceptibility genes that account for a significant proportion of the genetic contribution to disease at the population level have not been identified. However, numerous "moderate" effect genes are associated with tuberculosis. Several of these genes will be reviewed briefly.

Natural resistance associated macrophage protein 1 (*NRAMP1*)

The human homologue to murine *Nramp1* has been tested in numerous association studies. Most notably, *NRAMP1* variants were found to be strongly associated to tuberculosis susceptibility in a West African population (12). Individuals with tuberculosis were four-times as likely to have a disease-associated *NRAMP1* genotype compared with healthy controls. Additional associations have also been detected in smaller studies of patients from Japan, Korea, Guinea-Conakry and Cambodia (73, 74, 75, 76). The independent replication of *NRAMP1* association with tuberculosis in multiple studies across different populations provides very strong evidence for *NRAMP1* as a tuberculosis susceptibility gene. The modest genetic impact of the gene on susceptibility has been interpreted to suggest that the gene accounts for only a small proportion of the total genetic contribution to susceptibility (77). However, an alternative explanation is provided by a recent genetic study of tuberculosis susceptibility in an Aboriginal Canadian community. In this study, it was possible to detect a very strong genetic effect (relative risk = 10) of *NRAMP1* on tuberculosis. Of note, this strong genetic effect was only detected when essential gene-environment interactions were introduced into the analysis. Despite substantial genetic evidence implicating *NRAMP1* in tuberculosis susceptibility, a causal relationship between *NRAMP1* variants and increased susceptibility has not been established.

Vitamin D Receptor (*VDR*)

During the 19th century, cod-liver oil and sunlight, both important sources of vitamin D, were prescribed as treatment for tuberculosis. It has since been discovered that the biologically active metabolite form of vitamin D, 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), interacting with the vitamin D receptor (*VDR*), is an important immunomodulatory hormone (78). It plays a role in activating monocytes as well as suppressing lymphocyte proliferation, immunoglobin production and cytokine synthesis (79, 80, 81). *In vitro*, $1,25(OH)_2D_3$ has been shown to enhance the ability of human monocytes to restrict *M. tuberculosis* growth (82, 83). Alveolar macrophages from tuberculosis patients produce large quantities of the vitamin (84) suggesting a role in restricting mycobacterial growth within granulomas (77). In addition, results from epidemiologic studies point to a link between vitamin D deficiency and a higher risk of tuberculosis. This is demonstrated by seasonal variation of tuberculosis incidence, lower vitamin D serum levels in untreated tuberculosis patients, and a higher incidence of tuberculosis in individuals with relatively low serum vitamin D levels, such as the elderly, uremic patients and Asian immigrants in the United Kingdom (85).

Given that vitamin D exerts its effects via the vitamin D receptor (*VDR*), and that the receptor is present on monocytes and on T and B lymphocytes (86, 87), several studies have investigated the association between *VDR* gene variants and tuberculosis. In a Gambian population, the *VDR* genotype "tt" at codon 352, associated with increased levels of $1,25(OH)_2D_3$, was found to be over-represented in healthy controls, supporting the hypothesis that vitamin D protects against tuberculosis (88). A study investigating the interaction between serum vitamin D concentrations and *VDR* genotype in a Gujarati population living in London, England, failed to show a significant association between *VDR* genotype and increased risk of tuberculosis. However, a strong association was between undetectable vitamin D serum levels and tuberculosis was observed. Moreover, the study was able to detect evidence for gene-environment interaction between the TT/Tt genotype and vitamin D deficiency and susceptibility to tuberculosis (89). In contrast, no association was found when testing for the effect of *VDR* on tuberculosis in a Cambodian population (76).

Major histocompatibility complex (*MHC*)

Reports of association between highly polymorphic class II human leukocyte antigen (HLA) alleles and tuberculosis susceptibility are conflicting and vary among populations. Studies in different populations show an association with HLA-DR2 alleles (90, 91, 92, 93, 94) and with HLA-DQB1*0501 (94) and DQB1*0503 alleles (95). Other studies failed to detect the HLA-DR2 or DQB1/DQA1 associations (96). One of the earlier studies reported HLA-DR3 specificities enriched in healthy controls suggesting a protective

role of the antigen (97). The functional significance of these associations is not known. Given the complexity of the MHC, and the large number of immunomodulatory genes within it, a greater understanding of the role of MHC in tuberculosis pathogenesis, whether in infection, progression, or response to chemotherapy, is necessary before any real conclusions can be made.

Interleukin-1 and Interleukin-1Ra (*IL1B* and *IL1RN*)

The cytokines interleukin-1 β (IL-1 β encoded by *IL1B*) and interleukin-1 receptor antagonist (*IL-1Ra* encoded by *IL1RN*), produced by monocytes, macrophages and neutrophils, are involved in the regulation of immunological and inflammatory responses and are thought to be important regulators of tuberculosis disease progression (98, 89). Both cytokines interact with and compete for the IL-1 β receptor: IL-1 β induces a strong pro-inflammatory response whereas IL-1Ra, as a receptor antagonist, inhibits it. Although an initial pro-inflammatory response is important in host defense, sustained expression of IL-1 β can lead to tissue destruction (98). Therefore, the ratio of IL-1Ra to IL-1 β may be important in *M. tuberculosis* infection since overproduction of IL-1Ra may block the anti-microbial activity of IL-1 β during the early stages of infection (or early in the establishment of lung granulomas). Increased serum levels of IL-1Ra, and a high ratio of IL-1Ra to IL-1 β in bronchoalveolar lavage, were found in patients with active pulmonary tuberculosis (99, 100). In the same Gambian population tested for associations in *NRAMP1* and *VDR*, a weak association was found between *IL1RN* and tuberculosis susceptibility (101). However, when corrected for multiple testing these associations are no longer significant. Finally, *IL1RN* was tested in a Cambodian population for association with tuberculosis but no association was found (76). Taken together, these results suggest a modest contribution at best of *IL1* and *IL1RN* polymorphism to tuberculosis susceptibility.

Tumor Necrosis Factor α (TNF-α)

TNF-α plays an important role in host immune response to *M. tuberculosis* and the immunopathology of tuberculosis. TNF-α is pro-inflammatory cytokine and is produced mainly by monocytes and macrophages. In-vitro studies show that the cytokine increases the ability of macrophages to phagocytose and kill mycobacteria (102, 103). TNF-α is also required for the formation of granulomas which sequester and contain the mycobacteria. The importance of the pro-inflammatory cytokines TNF-α and IL-1 β in tuberculosis is demonstrated by the increased risk of reactivation in rheumatoid arthritis patients receiving anti-TNF-α and anti-IL-1 β therapy (104, 105). Despite its importance in immunity and its association to leprosy, another mycobacterial disease, few studies have evaluated TNF-α polymorphisms in tuberculosis susceptibility (106, 107). Studies in two populations showed conflicting results: there was no association between a polymorphism linked to TNF-α production and tuberculosis in Cambodian patients whereas the opposite was found in an Italian population (95, 108). Further investigation in different populations is needed to clarify the importance of TNF-α polymorphisms in modulating disease susceptibility.

Linkage studies

Complementary to candidate-gene studies are genome-wide scans, a powerful approach to identify major susceptibility loci. Genome scans, a linkage-based study method, evaluate the significance of excess-allele sharing among affected pairs of offspring. A large study was performed in 92 sib-pairs with tuberculosis from Gambia and South Africa. Weak evidence for linkage was detected on chromosome regions 15q and Xq. Given that linkage analysis are more powerful to detect disease-susceptibility loci conferring high risk, the two loci identified in this study are probably different, and might have substantially larger effects than previously identified loci (109). Unfortunately, this expectation was not borne out in a follow-up association study of the chromosome 15q region (110).

Two linkages studies have assessed the role of *NRAMP1* in tuberculosis susceptibility. An analysis of families with multiple cases of tuberculosis in Brazil did not show significant linkage to *NRAMP1*, but two markers tightly linked to the gene were weakly linked to disease susceptibility (111). A linkage study of a large Aboriginal Canadian family took into account gene-environment interactions, such as vaccination status, tuberculin skin-test result, age and previous disease, and showed significant linkage between tuberculosis susceptibility and a marker just distal to *NRAMP1* (55). In this study, *NRAMP1* appeared to modulate the progression from infection to active disease.

CONCLUSION

There is clear and unambiguous evidence that human genetic variability is an important modulator of susceptibility to tuberculosis. Several tuberculosis risk variants have already been described and it is likely that others will follow. The methodological challenge

for the future will be to properly capture, and to incorporate into the analysis, gene-gene and gene-environment interactions. However, the biggest challenge will be to advance the basic genetic findings into the arena of public health and tuberculosis control. How this will happen is difficult to predict. Given the present efforts in generating better tuberculosis vaccines, a potentially fruitful application of tuberculosis genetics is the exploitation of host genetics for vaccine development. At any rate, to what extent modern genetics will be able to facilitate disease control will be an important measure to judge the benefits of the human genome project for medicine and human health.

REFERENCES

1. WHO. Global Tuberculosis Control. WHO Report. Geneva, Switzerland. WHO/CDS/TB/2001.28.
2. Sreevatsan S, Pan X, Stockbauer KE, Connell ND et al. Restricted Structural Gene Polymorphism in the Mycobacterium tuberculosis Complex Indicates Evolutionarily Recent Global Dissemination. Proceedings of National Academy of Sciences USA 94:9869-9874; 1997.
3. Maher D, Floyd K, Raviglione M. A Strategic Framework to Decrease the Burden of TB/HIV. WHO Report. Geneva, Switzerland. WHO/CDS/TB/2002.296.
4. Kaufmann SHE. How Can Immunology Contribute to the Control of Tuberculosis? Nature Reviews Immunology 1:20-30; 2001.
5. Haldane JBS. Disease and Evolution. Ric Sci (Suppl. A) 68-76; 1949.
6. Daniel TM, Bates JH, Downes KA. History of Tuberculosis. In: Bloom BR, ed. Tuberculosis: Pathogenesis, Protection, and Control. Washington, DC: American Society for Microbiology, 1994.
7. Schürmann; Beobachtungen bei den Lübecker Säuglingstuberkulosen; Beit z Klin Tuberk 81: 294; 1932.
8. Pierce C, Dubos RJ, Middlebrook G. Infection of Mice with Mammalian Tubercle Bacilli Grown in Tween-Albumin Liquid Medium. Journal of Experimental Medicine 86:159-174; 1947.
9. Medina E, North RJ. Resistance Ranking of Some Common Inbred Mouse Strains to Mycobacterium tuberculosis and Relationship to Major Histocompatibility Haplotype and Nramp1 Genotype. Immunology 93:270-274; 1998.
10. Lynch CJ, Pierce-Chase CH, Dubos R. A Genetic Study of Susceptibility to Experimental Tuberculosis in Mice Infected with Mammalian Tubercle Bacilli. Journal of Experimental Medicine 121:1051-1070; 1965.
11. Musa SH, Kim Y, Hashim R, Wang GZ, Dimmer C, Smith D. Response of inbred mice to aerosol challenge with Mycobacterium tuberculosis. Infection and Immunity 55:1862-1866; 1987.
12. Bellamy R, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. New England Journal of Medicine. 338:640-644; 1998.
13. Abel L, Sanchez FO, Oberti J, et al. Susceptibility to leprosy is linked to the human NRAMP1 gene. Journal of Infectious Diseases 177:133-145; 1998.
14. Marquet S, Sanchez FO, Arias M et al. Variants of the human NRAMP1 gene and altered human immunodeficiency virus infection susceptibility. Journal of Infectious Diseases 180:1521-1525; 1999.
15. Gros P, Skamene E, Forget A. Genetic control of natural resistance to Mycobacterium bovis (BCG) in mice. Journal of Immunology. 127:2417-2421; 1981.
16. Vidal SM, Malo D, Vogan K, Skamene E, Gros P. Natural Resistance to infection with Intracellular Parasites: Isolation of a Candidate for Bcg. Cell 73:469-485; 1993.
17. Skamene E, Gros P, Forget A, Kongshavn PAL, St. Charles C, Taylor BA. Genetic regulation of resistance to intracellular pathogens. Nature 297:506-509; 1982.
18. Stach JL. Gros P, Forget A, Skamene E. Phenotypic expression of genetically-controlled natural resistance to Mycobacterium bovis (BCG). Journal of Immunology 132:888-892; 1984.
19. Medina E, North RJ. Evidence inconsistent with a role for the Bcg gene (Nramp1) in resistance of mice to infection with Mycobacterium tuberculosis. Journal of Experimental Medicine 183:1045-1051; 1996.
20. North RJ, LaCourse R, Ryan L, Gros P. Consequence of Nramp1 deletion to Mycobacterium tuberculosis infection in mice. Infection and Immunity 67:5811-5814; 1999.
21. Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science 259:1739-42; 1993.
22. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. Journal of Experimental Medicine 178:2243-2247; 1993.
23. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. Journal of Experimental Medicine 178:2249-2254; 1993.
24. Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages: Comparison of activating cytokines and evidence for independent production. Journal of Immunology 141:2407-2412; 1988.
25. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proceedings of National Academy of Sciences U S A 94:5243-5248; 1997.
26. Adams LB, Dinauer MC, Morgenstern DE, Krahenbuhl JL. Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to Mycobacterium tuberculosis using transgenic mice. Tubercle and Lung Disease 78:237-246; 1997.
27. Bean AG, Roach DR, Briscoe H et al. Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol Mycobacterium tuberculosis infection, which is not compensated for by lymphotoxin. Journal of Immunology 162:3504-3511; 1999.
28. Roach DR, Bean AG, Demangel C, France MP, Briscoe H, Britton WJ. TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. Journal of Immunology 168:4620-4627; 2002.
29. Jacobs M, Brown N, Allie N, Chetty K, Ryffel B. Tumor necrosis factor receptor 2 plays a minor role for mycobacterial immunity. Pathobiology 68:68-75; 2000.
30. Flesch IE, Hess JH, Huang S et al. Early interleukin 12 production by macrophages in response to mycobacterial infection depends on interferon gamma and tumor necrosis factor alpha. Journal of Experimental Medicine 181:1615-1621; 1995.

31. Murphy EE, Terres G, Macatonia SE et al. B7 and interleukin 12 cooperate for proliferation and interferon gamma production by mouse T helper clones that are unresponsive to B7 costimulation. Journal of Experimental Medicine 180:223-231; 1994.
32. Cooper AM, Magram J, Ferrante J, Orme IM. Interleukin 12 (IL-12) is crucial to the development of protective immunity in mice intravenously infected with Mycobacterium tuberculosis. Journal of Experimental Medicine 186:39-45; 1997.
33. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annual Reviews: Immunology 19:423-474; 2001.
34. Okamura H, Tsutsi H, Komatsu T et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 378:88-91; 1995.
35. Sugawara I, Yamada H, Kaneko H, Mizuno S, Takeda K, Akira S. Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. Infection and Immunity 67:2585-2589; 1999.
36. Kinjo Y, Kawakami K, Uezu K, et al. Contribution of IL-18 to Th1 response and host defense against infection by Mycobacterium tuberculosis: a comparative study with IL-12p40. Journal of Immunology 169:323-329; 2002.
37. Saunders BM, Frank AA, Orme IM, Cooper AM. Interleukin-6 induces early gamma interferon production in the infected lung but is not required for generation of specific immunity to Mycobacterium tuberculosis infection. Infection and Immunity 68:3322-3326; 2000.
38. Ladel CH, Blum C, Dreher A, Reifenberg K, Kopf M, Kaufmann SH. Lethal tuberculosis in interleukin-6-deficient mutant mice. Infection and Immunity 65:4843-4849; 1997.
39. Juffermans NP, Florquin S, Camoglio L et al. Interleukin-1 signaling is essential for host defense during murine pulmonary tuberculosis. Journal of Infectious 182:902-908; 2000.
40. North RJ. Mice incapable of making IL-4 or IL-10 display normal resistance to infection with Mycobacterium tuberculosis. Clinical and Experimental Immunology 113:55-58; 1998.
41. Murray PJ, Young RA. Increased antimycobacterial immunity in interleukin-10-deficient mice. Infection and Immunity 67:3087-3095; 1999.
42. Sugawara I, Yamada H, Mizuno S, Iwakura Y. IL-4 is required for defense against mycobacterial infection. Microbiology and Immunology 44:971-979; 2000.
43. Brett S, Orrell JM, Swanson Beck J, Ivanyi J. Influence of H-2 genes on growth of Mycobacterium tuberculosis in the lungs of chronically infected mice. Immunology 76:129-132; 1992.
44. Apt AS, Avdienko VG, Nikonenko BV, Kramink IB, Moroz AM. Distinct H-2 complex control of mortality, and immune responses to tuberculosis in virgin and BCG-vaccinated mice. Clinical and Experimental Immunology 94:322-329; 1993.
45. Brett SJ, Ivanyi J. Genetic influences on the immune repertoire following tuberculosis infection in mice. Immunology 71:113-119; 1990.
46. Ivanyi J, Sharp K. Control by H-2 genes of murine antibody responses to protein antigens of Mycobacterium tuberculosis. Immunology 59:329-332; 1986.
47. Orrell JM, Brett SJ, Ivanyi J, Coghill G, Grant A, Beck JS. Morphometric analysis of Mycobacterium tuberculosis infection in mice suggests a genetic influence on the generation of the granulomatous inflammatory response. Journal of Pathology 166:77-82; 1992.
48. Darvasi A, Weinreb A, Minke V, Weller JI, Soller M. Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134:943-951; 1993.
49. Jansen RC. Interval mapping of multiple quantitative trait loci. Genetics 135:205-211; 1993.
50. Lavebratt C, Apt AS, Nikonenko BV, Schalling M, Schurr E. Severity of tuberculosis in mice is linked to distal chromosome 3 and proximal chromosome 9. Journal of Infectious Diseases 180:150-155; 1999.
51. Sanchez F, Radaeva TV, Nikonenko BV et al. Multigenic control of disease severity after virulent Mycobacterium tuberculosis infection in mice. Infection and Immunity 71:126-131; 2003.
52. Kramnik I, Dietrich WF, Demant P, Bloom BR. Genetic control of resistance to experimental infection with virulent Mycobacterium tuberculosis. Proceedings of National Academy of Sciences U S A 97:8560-8565; 2000.
53. Mitsos M, Cardon LR, Fortin A et al. Genetic control of susceptibility to infection with Mycobacterium tuberculosis in mice. Genes and Immunity 1:467-477; 2000.
54. Abel L, Casanova J-L. Genetic Predisposition to Clinical Tuberculosis: Bridging the Gap between Simple and Complex Inheritance. American Journal of Human Genetics 67:274-277; 2000.
55. Greenwood CMT, Fujiwara TM, Boothroyd LJ et al. Linkage of Tuberculosis to Chromosome 2q35 Loci, Including NRAMP1, in a Large Aboriginal Canadian Family. American Journal of Human Genetics 67:405-416; 2000.
56. Jouanguy E, Lamhamedi-Cherradi S, Altare F et al. Partial interferon- receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guérin infection and a sibling with clinical tuberculosis. Journal of Clinical Investigation 100:2658-2664; 1997.
57. Picard C, Fieschi C, Altare F et al. Inherited Interleukin-12 Deficiency: IL-12B Genotype and Clinical Phenotype of Thirteen Patients from Six Kindreds. American Journal of Human Genetics 70:336-348; 2002.
58. Ting L-M, Kim AC, Cattamanchi A, Ernst JD. Mycobacterium tuberculosis Inhibits IFN-γ Transcriptional Responses Without Inhibiting Activation of STAT1. Journal of Immunology 163:3898-3906; 1999.
59. Elloumi-Zghal H, Barbouche MR, Chemli J et al. Clinical and Genetic Heterogeneity to Disseminated Mycobacterium bovis Bacille Calmette-Guérin Infection. Journal of Infectious Diseases 185:1468-1475; 2002.
60. Altare F, Durandy A, Lammas D et al. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science 280:1432-1435; 1998.
61. Jounaguy E, Lamhamedi -Cherradi S, Lammas D et al. A human IFNGR1 small deletion associated with dominant susceptibility to mycobacterial infection. Nature Genetics 21:370-8; 1999.
62. Jouanguy E, Altare F, Lamhamedi S et al. A human interferon- -interferon deficiency in an infant with fatal bacille Calmette-Guérin. New England Journal of Medicine 335:1956-1961; 1996.
63. Newport MJ, Huxley CM, Huston S et al. A Mutation in the Interferon- -Receptor Gene and Susceptibility to Mycobacterial Infection. New England Journal of Medicine 355:1941-1945; 1996.
64. De Jong R, Altare F, Haagen I-A et al. Severe Mycobacterial and Salmonella Infections in Interleukin-12 Receptor-Deficient Patients. Science 280:1435-1438; 1998.
65. Dupuis S, Dargemont C, Fieschi C et al. Impairment of Mycobacterial but not Viral Immunity by a Germline Human STAT1 Mutation. Science 293:300-303; 2001.
66. Ottenhoff THM, Verreck FAW, Lichtenauer-Kaligis EGR, Hoeve MA, Sanal O, van Dissel JT. Genetics, Cytokines and Human Infectious Disease: Lessons from Weakly Pathogenic Mycobacteria and Salmonella. Nature Genetics 32:97-105; 2002.

67. Casanova JL, Abel L. Genetic Dissection of Immunity to Mycobacteria: The Human Model. Annual Reviews: Immunology 20:581-620; 2002.
68. Cooke GS, Hill AVS. Genetics of Susceptibility to Human Infectious Disease. Nature Genetics 2:967-977; 2001.
69. Lio D, Marino V, Serauto A et al. Genotype Frequencies of the +874T-- A Single Nucleotide Polymorphism in the First Intron of the Interferon-Gamma Gene in a Sample of the Sicilian Patients Affected by Tuberculosis. European Journal of Immunogenetics 29:371-374; 2002.
70. López-Maderuello D, Arnalich F, Serantes R et al. Interferon- and Interleukin-10 Gene Polymorphisms in Pulmonary Tuberculosis. American Journal of Respiratory and Critical Care Medicine 167:970-975; 2003.
71. Mitsuteru A, Nakashima H, Miyake K et al. Influence of Interleukin-12 Receptor 1 Polymorphisms on Tuberculosis. Human Genetics 112:237-243; 2003.
72. Malik S, Schurr E. Genetic Susceptibility to Tuberculosis. Clinical Chemisty and Laboratory Medicine 40:863-868; 2002.
73. Gao P-S, Fujishima S, Mao X-Q et al. Genetic Variants of NRAMP1 and Active Tuberculosis in Japanese Populations. Clinical Genetics 58:74-76; 2000.
74. Ryu S, Park YK, Bai GH et al. 3'UTR Polymorphisms in the NRAMP1 Gene are Associated with Susceptibility to Tuberculosis in Koreans. International Journal of Tuberculosis and Lung Disease 4:677-580; 2000.
75. Cervino AC, Lakiss S, Sow O, Hill AV. Allelic Association between the NRAMP1 Gene and Susceptibility to Tuberculosis in Guinea-Conakry. Annals Human Genetics 64:507-512; 2000.
76. Delgado JC, Baena A, Thim S, Goldfeld AE. Ethnic-Specific Genetic Associations with Pulmonary Tuberculosis. Journal of Infectious Diseases 186:1463-1468; 2002.
77. Bellamy R. Susceptibility to Mycobacterial Infections: The Importance of Host Genetics. Genes and Immunity 4:4-11; 2003.
78. Tsoukas CD, Provvedini DM, Manolagas SC. 1,25-dihydroxyvitamin D3: A Novel Immunoregulatory Hormone. Science 224:1438-1440; 1984.
79. Lemire JM, Adams JS, Sakai R, Jordan SC. 1 ,25-dihydroxy-vitamin D3 suppresses proliferation and immunoglobin production by normal human peripheral blood mononuclear cells. Journal of Clinical Investigation 74:657-661; 1984.
80. Rook GAW, Taverne J, Leveton C, Steele J. The Role of gamma-interferon, vitamin D3 metabolites and tumor necrosis factor in the pathogenesis of tuberculosis. Immunology 62:229-234; 1987.
81. Christakos S, Dhawan P, Liu Y, Peng Xizorong, Porta A. New Insights into the Mechanism of Vitamin D Action. Journal of Cellular Biochemistry 88:695-705; 2003.
82. Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D. 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of Mycobacterium tuberculosis in a human macrophage-like cell line. Infection and Immunity 66:5314-5321; 1998.
83. Rook GAW, Steele J, Fraher L et al. Vitamin D3, gamma interferon, and the control of Mycobacterium tuberculosis by human monocytes. Immunology 57:159-163; 1986.
84. Cadrenel J, Hance AJ, Milleron B, Paillard F, Akoun GM, Garabedian M. The Production of 1,25(OH)2D3 by Cells Recovered by Bronchoalveolar Lavage and the Role of This Metabolite in Calcium Homostasis. American Review of Respiratory Diseases 138:984-989; 1988.
85. Chan TYK. Vitamin D Deficiency and Susceptibility to Tuberculosis. Calcified Tissue International 66:476-478; 2000.
86. Reichel H, Koeffler HP, Tobler A, Norman AW. 1 ,25-dihydroxy-vitamin D3 inhibits gamma-interferon synthesis by normal human peripheral blood lymphocytes. Proceedings of the National Academy of Sciences USA 84:3385-3389; 1987.
87. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1 ,25-dihydroxyvitamin D3 receptors in human leukocytes. Science 221:1181-1183; 1983.
88. Bellamy R, Ruwende C, Corrah T et al. Tuberculosis and Chronic Hepatitis B Virus Infection in Africans and Variation in the Vitamin D Receptor Gene. Journal of Infectious Diseases 179:722-724; 1999.
89. Wilkinson RJ, Llewelyn M, Toossi Z et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis amongst Gujarati Asians in west London. Lancet 355:18-621; 2000.
90. Sing SPN, Mehra NK, Dingley HB, Pande JN, Vaidya MC. Human Leukocyte Antigen (HLA)-linked Control of Susceptibility to Tuberculosis and Association with HLA-DR Types. Journal of Infectious Diseases 148:676-681; 1983.
91. Bothamley GH, Beck JS, Schreuder et al. Association of tuberculosis and tuberculosis-specific antibody levels with HLA. Journal of Infectious Diseases 159:549-555; 1989.
92. Brahmajothi V, Pitchappan RM, Kakkanaiah VN et al. Association of pulmonary tuberculosis and HLA in South India. Tubercle 72:123-132; 1991.
93. Rajalingam R, Mehra NK, Jain RC, Myneedu VP, Pande JN. Polymerase chain reaction-based sequence-specific oligonucleotide hybridization analysis of the HLA class II antigens in pulmonary tuberculosis: relevance to chemotherapy and disease severity. Journal of Infectious Diseases 173:669-676; 1996.
94. Teran-Escandon D, Teran-Ortiz L, Carnarena-Olvera A et al. Human Leukocyte Antigen-Associated Susceptibility to Pulmonary Tuberculosis: Molecular Analysis of Class II Alleles by DNA Amplification and Oligonucleotide Hybridization in Mexican Patients. Chest 115:428-433; 1999.
95. Goldfeld AE, Delgado JC, Thim S et al. Association of an HLA-DQ Allele with Clinical Tuberculosis. Journal of the American Medical Association 279:226-228; 1998.
96. Sanjeevi CB, Narayanan PR, Prabakar R et al. No Association or Linkage with the HLA-DR or -DQ Genes in South Indians with Pulmonary Tuberculosis. Tuberulosis and Lung Disease 73:280-284; 1992.
97. Cox RA, Downs M, Neimes RE, Ognibene AJ, Yamashita TS, Ellner JJ. Immunogenetic analysis of human tuberculosis. Journal of Infectious Diseases 1988; 158:1302-1308.
98. Arend, WP. The Balance Between IL-1 and IL-Ra in Disease. Cytokine and Growth Factors Review 13:323-240; 2002.
99. Tsao TC, Hong J, Huang C, Yang P, Liao SK, Chang KS. Increased TNF-alpha, IL-1 beta and IL-6 Levels in the Bronchoalveolar Lavage Fluid with the Upregulation of Their mRNA in Macrophages Lavaged from Patients with Active Pulmonary Tuberculosis. Tuberculosis and Lung Disease 1999; 79:279-285.
100. Juffermans N, Verbon A, van Deventer H et al. Tumor Necrosis Factor and Interleukin-1 Inhibitors As Markers of Disease Activity of Tuberculosis. American Journal of Respiratory and Critical Care Medicine 157:1328-1331; 1998.
101. Bellamy R, Ruwende C, Corrah T, McAdam KPWJ, Whittle HC, Hill AVS. Assessment of the Interleukin 1 Gene Cluster and other Candidate Gene Polymorphisms in the Host Susceptibility to Tuberculosis. Tubercle and Lung Disease 1998; 79:83-89.
102. Havell EA. Evidence that Tumor Necrosis Factor has an Important Role in Antibacterial Resistance. Journal of Immunology 143:2894-2901; 1989.
103. Denis M. Tumor Necrosis Factor and Granulocyte Macrophage-Colony Stimulating Factor Stimulate Human Macrophages to Restrict Growth of Virulent Mycobacterium avium and to Kill

Avirulent M. avium: Killing Effector Mechanism Depends on the Generation of Nitrogen Intermediates. Journal of Leukocyte Biology 49:380-387; 1991.
104. Mohan AK, Cote TR, Siegal JN, Braun MM. Infectious Complications of Biologic Treatments of Rheumatoid Arthritis. Current Opinions in Rheumatology 15:179-184; 2003.
105. Gardam MA, Keystone EC, Menzies R et al. Anti-Tumor Necrosis Factor Agents and Tuberculosis Risk: Mechanisms of Action and Clinical Management. Lancet Infectious Diseases 3:148-155; 2003.
106. Moraes MO, Duppre NC, Suffys PN. Tumor Necrosis Factor-Promoter Polymorphisms TNF2 is Associated with a Stronger Delayed-Type Hypersensitivity Reaction in the Skin of Borderline Tuberculoid Leprosy Patients. Immunogenetics 53:45-47;2001.
107. Roy S, McGuire W, Mascie-Taylor GN. Tumor Necrosis factor Polymorphism and Susceptibility to Lepromatous Leprosy. Journal of Infectious Diseases 176:530-532; 1997.
108. Scola L, Crivello A, Marino V, Gioia V et al. IL-10 and TNF-alpha Polymorphisms in a Sample of Sicilian Patients Affected by Tuberculosis: Implications for Ageing and Life Spam Expectancy. Mechanisms of Ageing and Development 124:569-572; 2003.
109. Bellamy R, Beyers N, McAdam KPWJ et al. Genetic Susceptibility to Tuberculosis in Africans: A Genome-Wide Scan. Proceedings of the National Academy of Science USA 97:8005-8009; 2000.
110. Cervino AC, Lakiss S, Sow O et al. Fine Mapping of a Putative Tuberculosis-Susceptibility Locus on Chromosome15q11-13 in African families. Human Molecular Genetics 11:1599-603; 2002.
111. Shaw M-A, Donaldson IJ, Collins A et al. Association and Linkage of Leprosy Phenotypes with HLA Class II and Tumor Necrosis Factor Genes. Genes and Immunity 2:196-204; 2001.

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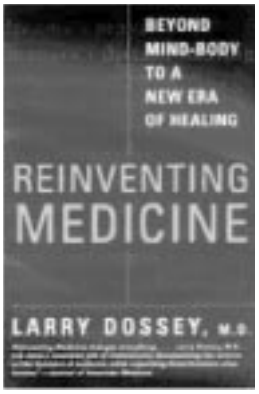
BOOK REVIEW



Reinventing Medicine
Written by Larry Dossey

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There are many aspects of medical treatment which lie outside the boundaries of the raw science. Indeed, many common observations in medicine (the placebo effect, for example) rely on psychological factors which are not readily explained by our current knowledge.

The degree to which these factors influence outcomes in medical treatment is a subject of dispute which Larry Dossey addresses in his book, "Reinventing Medicine." In order to explain this point of view the author has divided medicine into three eras. Era I started in the 1800's when medicine began to be practised with attention to evidence and science. In this era, only physical phenomena were considered and the superstition and "pseudoscience" of the ancient times were given less credibility. Eventually, near the time of the second World War, experiments showed psychological effects in medical treatment, most notably the placebo effect. This marked the second era of medicine, characterised by the consideration of both physical and psychological aspects in medical treatment. The principles of era II are those currently practised by medicine.

In era III, the author claims we will additionally make use of other special insights, which are referred to as "nonlocal." The idea of nonlocal phenomena originates from quantum physics: two particles which have a nonlocal connection are able to influence each other instantaneously, no matter how large the distance between them. Some physicists, like Albert Einstein, were so surprised by this they called them "spooky" phenomena.

The author claims that for lack of a better word, "nonlocal" best describes the new way in which medicine should be practised. For the purposes of this book, nonlocal phenomena are defined as a number of interactions (such as prayer and intuition) which have effects that cannot be accounted for by our current scientific understanding. The discussion in the book which follows is a lengthy review of experimental studies which have shown (among other things) the ability of prayer to destroy cancer cells in culture, and improving treatment outcomes in mice and even in hospital studies on humans.

These ideas are problematic for most individuals, and the author points out that many people have the reaction that "this is the sort of thing I wouldn't believe, even if it was true." This may reflect the general attitude of the scientific community, which does not place faith in nonlocal phenomena despite the large number of scientific studies which have demonstrated their effects. One explanation which is offered claims that the nature of our education and society results in a great personal reluctance by numerous individuals to even consider the concepts of era III medicine.

The last (and shortest) part of the book describes how nonlocal phenomena can be employed within the health care system. The emphasis in this section is that all nonlocal "treatment" is only to be combined in addition to (and not instead of) the best medical treatments that exist. Furthermore, any such treatment should be given only to those who request it. However, despite giving an interesting practical discussion of how these ideas might be implemented, there are areas which could have been given further consideration.

For example, the author's vision includes a network of praying volunteers, who receive photographs and descriptions of individuals recently admitted to hospital. These volunteers are then instructed to pray for the health of the patients. One important question here (among others) is whether this goes against the spirit of prayer. There is something that seems intuitively wrong about having a whole sector of health care praying for people they never meet. There is also no discussion on how medicine would be perceived within society if these changes took place; even if it is proven that nonlocal effects are true, many people might wonder why these effects are being exploited, when it is impossible to know why or how they work. I believe these are fair questions which would have been appropriate for discussion in a book regarding the reinvention of medicine. Instead, the book focuses mainly on convincing the sceptical reader about the truth of nonlocal effects.

This book is mind-opening in its discussion of the

various types of nonlocal effects, but does not go to sufficient length to explain its main objective. It is an interesting emerging area of medicine which should be considered, and this book represents the background and main practical principles of this area well.

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Journal articles

1. Bunny B, Coyote WE, Le Pew P, et al. Impact trauma Caused by Descending GPI Anchors. McGill Journal of Medicine 2003; 7: 1-2.

Books

2. Bunny B, Coyote WE, Le Pew P. Subdural Hematomas. In: Jones J, ed. Head Injuries. New York: Acme Publishers; 1994: 249-260.

Internet

3. Bunny B. Computer-induced psychosis. Society for Cartoon-Computer Interactions. http://www.SCarComI.com/psychosis.html. 1999.

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STRATEGY A CANADIAN MEDICAL ASSOCIATION PUBLICATION

MD MANAGEMENT A CMA SUBSIDIARY

Best Financial Practices for Students

Medical students and residents face a very different financial situation than their counterparts in other academic disciplines. Their tuition fees are among the highest in the country, they must live without salary income for an extended period of time, and they face repayment of a substantial debt load during residency, when their salary is at its lowest. Without a solid financial plan, this situation can not only delay their opportunity to invest in their financial future, but add undue stress to the high pressures of studying and practicing medicine.

■ MEDICAL STUDENTS ■

The good news is that when you do begin to practice medicine, you will see your earnings improve significantly. The not-so-good news is that it isn t unusual for physicians in their first year of practice to be facing a debt load of $60,000 to $100,000. But if you follow these proven best practices, you can enter your career armed with the knowledge to help you succeed financially.

❑ 1. START WITH A PLAN: BUILD A BUDGET AND FOLLOW IT

Now is the time to get organized and track where you spend your money, then create a road map for yourself and follow it. The earlier you start, the better. Make a point of understanding the challenges you face, including tuition fees, debt repayment, and earning potential. With this knowledge in hand, you can work with your MD Financial Consultant to build a budget and create a long-term plan that will evolve with your financial situation. For a detailed look at budgeting and debt management for medical students, see the article "Debt Management for Medical Students" in the October 2002 edition of *Strategy* magazine. *Strategy* articles can be accessed on the MD Management Web site at *http://mdm.ca/md/strategy*.

❑ 2. EXPLORE ALL OPTIONS TO FINANCE YOUR EDUCATION

Julie Gauthier is an MD Financial Consultant who provides financial planning service to medical students and residents. She stresses the importance of applying for student loans and bursaries, even if you think you aren t eligible. "Students should try as much as possible to obtain financing that does not require repayment while they are completing their studies," she says. "They may think they aren t eligible, when in fact they are." Any option that does not require repayment until after you have finished your education will help, even personal options like loans from family members. The goal is to finance your education so you don t have to work while in school, and to do so as cheaply as possible to minimize your burden when your studies are complete.

❑ 3. KEEP YOUR COSTS DOWN NOW SO YOU PAY LESS LATER

Committing to medicine means committing to living the student life long after it has become a nostalgic memory for your friends in other disciplines. But every penny you don t spend today will make a big difference by the end of medical school. The biggest favour you can do yourself now is to keep all of your costs down. Share rent, shop for bargains, keep transportation costs low, minimize service charges, borrow books from other students, buy second-hand. Resist the pressure of keeping up with people who have completed their studies and are working full-time. You will be glad you did.

❑ 4. SEEK OBJECTIVE ADVICE TO MANAGE YOUR DEBT

Banks may make lines of credit readily available, but keep in mind that as businesses, lenders are looking to maximize their profitability. They know that you will be a physician someday, and may offer you more financing than you re seeking. Remember that you aren t just being offered more money, you re being offered more debt. Right now, you re facing high tuition fees and no income, and the temptation to take on more debt is strong. MD Financial Consultants can help you develop a reasonable cashflow plan to minimize unpleasant surprises at the end of medical school.

❑ 5. CLARIFY YOUR INSURANCE NEEDS AND EVALUATE YOUR OPTIONS

Doctors earning potential, debt load, physically and mentally challenging career, level of responsibility and (in many cases) self-employed status, make insurance an absolute necessity. There are many options, and some kinds of insurance, such as disability, are best obtained while you are young and in good health. By getting insured early, you can increase your insurance amount when you begin practicing without requiring proof of health. This may keep your premiums down. An MD Insurance Consultant can help you evaluate your choices and determine what premium and insurance amount is right for you.

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LIPITOR
atorvastatin calcium
tablets

EFFICACY TO REACH TARGETS

PrLIPITOR*
(atorvastatin calcium)
10 mg, 20 mg, 40 mg and 80 mg tablets
THERAPEUTIC CLASSIFICATION: Lipid Metabolism Regulator

ACTIONS AND CLINICAL PHARMACOLOGY

LIPITOR (atorvastatin calcium) is a synthetic lipid-lowering agent. It is a selective, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol.

LIPITOR lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of Low Density Lipoprotein (LDL).

LIPITOR reduces LDL-Cholesterol (LDL-C) and the number of LDL particles. LIPITOR also reduces Very Low Density Lipoprotein-Cholesterol (VLDL-C), serum triglycerides (TG) and Intermediate Density Lipoproteins (IDL), as well as the number of apolipoprotein B (apo B) containing particles, but increases High Density Lipoprotein-Cholesterol (HDL-C). Elevated serum cholesterol due to elevated LDL-C is a major risk factor for the development of cardiovascular disease. Low serum concentration of HDL-C is also an independent risk factor. Elevated plasma TG is also a risk factor for cardiovascular disease, particularly if due to increased IDL, or associated with decreased HDL-C or increased LDL-C.

Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Atorvastatin tablets are 95% to 99% bioavailable compared to solutions.

Mean distribution of atorvastatin is approximately 381 litres. Atorvastatin is ≥98% bound to plasma proteins. Atorvastatin is extensively metabolized by cytochrome P-450 3A4 to ortho- and para-hydroxylated derivatives and to various beta-oxidation products. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites.

Atorvastatin and its metabolites are eliminated by biliary excretion. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration. Mean plasma elimination half-life of atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of longer-lived active metabolites.

INDICATIONS AND CLINICAL USE

LIPITOR (atorvastatin calcium) is indicated as an adjunct to lifestyle changes, including diet, [at least equivalent to the Adult Treatment Panel III (ATP III) TLC diet], for the reduction of elevated total cholesterol, (total-C), LDL-C, TG and apolipoprotein B (apo B) in hyperlipidemic and dyslipidemic conditions, when response to diet and other nonpharmacological measures alone has been inadequate, including:

- Primary hypercholesterolemia (Type IIa);
- Combined (mixed) hyperlipidemia (Type IIb), including familial combined hyperlipidemia, regardless of whether cholesterol or triglycerides are the lipid abnormality of concern;
- Dysbetalipoproteinemia (Type III);
- Hypertriglyceridemia (Type IV);
- Familial hypercholesterolemia (homozygous and heterozygous). For homozygous familial hypercholesterolemia, LIPITOR should be used as an adjunct to treatments such as LDL apheresis, or as monotherapy if such treatments are not available.

LIPITOR also raises HDL-cholesterol and therefore lowers the LDL-C/HDL-C and total-C/HDL-C ratios in patients with primary hypercholesterolemia and combined (mixed) hyperlipidemia (Fredrickson Type IIa and IIb dyslipidemia). In pooled data from 24 controlled clinical trials, LIPITOR raised HDL-C levels 5%-7% in primary hypercholesterolemic (type IIa) patients and 10%-15% in mixed (type IIb) dyslipidemic patients. These changes in HDL-C with HMG-CoA reductase inhibitors should be considered as modest when compared to those observed in LDL-C and do not play a primary role in the lowering of LDL-C/HDL-C and total-C/HDL-C ratios.

In clinical trials, LIPITOR (10 to 80 mg/day) significantly improved lipid profiles in patients with a wide variety of hyperlipidemic and dyslipidemic conditions. In 2 dose-response studies in mildly to moderately hyperlipidemic patients (Fredrickson Types IIa and IIb), LIPITOR reduced the levels of total cholesterol (29-45%), LDL-C (39-60%), apo B (32-50%), TG (19-37%), and increased high density lipoprotein cholesterol (HDL-C) levels (5-9%). Comparable responses were achieved in patients with heterozygous familial hypercholesterolemia, non-familial forms of hypercholesterolemia, combined hyperlipidemia, including familial combined hyperlipidemia and patients with non-insulin dependent diabetes mellitus. In patients with hypertriglyceridemia (Type IV), LIPITOR (10 to 80 mg daily) reduced TG (25-56%) and LDL-C levels (23-40%). LIPITOR has not been studied in conditions where the major abnormality is elevation of chylomicrons (TG levels > 11 mmol/L), i.e. types I and V.

In an open-label study in patients with dysbetalipoproteinemia (Type III), LIPITOR (10 to 80 mg daily) reduced total-C (40-57%), TG (40-56%) and IDL-C + VLDL-C levels (34-58%).

In an open label study in patients with homozygous familial hypercholesterolemia (FH) LIPITOR (10 to 80 mg daily) reduced mean LDL-C levels (22%). In a pilot study, LIPITOR 80 mg/day showed a mean LDL-C lowering of 30% for patients not on plasmapheresis and of 31% for patients who continued plasmapheresis. A mean LDL-C lowering of 35% was observed in receptor defective patients and of 19% in receptor negative patients (see PHARMACOLOGY, Clinical Studies).

For more details on efficacy results by pre-defined classification and pooled data by Fredrickson types, see PHARMACOLOGY, Clinical Studies.

Prior to initiating therapy with LIPITOR, secondary causes should be excluded for elevations in plasma lipid levels (e.g. poorly controlled diabetes mellitus, hypothyroidism, nephrotic syndrome, dysproteinemias, obstructive liver disease, and alcoholism), and a lipid profile performed to measure total cholesterol, LDL-C, HDL-C, and TG. For patients with TG <4.52 mmol/L (<400 mg/dL), LDL-C can be estimated using the following equation:

LDL-C (mmol/L) = total-C - [(0.37 x (TG) + HDL-C)]

LDL-C (mg/dL) = total-C - [(0.2 x (TG) + HDL-C)][1]

For patients with TG levels >4.52 mmol/L (>400 mg/dL), this equation is less accurate and LDL-C concentrations should be measured directly or by ultracentrifugation.

Patients with high or very high triglyceride levels, i.e. >2.2 mmol/L (200 mg/dL) or >5.6 mmol/L (500 mg/dL), respectively, may require triglyceride-lowering therapy (fenofibrate, bezafibrate or nicotinic acid) alone or in combination with LIPITOR.

In general, combination therapy with fibrates must be undertaken cautiously and only after risk-benefit analysis (see WARNINGS, Muscle Effects, PRECAUTIONS, Pharmacokinetic Interaction Studies and Potential Drug Interactions).

Elevated serum triglycerides are most often observed in patients with the metabolic syndrome (abdominal obesity, atherogenic dyslipidemia [elevated triglycerides, small dense LDL particles and low HDL-cholesterol], insulin resistance with or without glucose intolerance, raised blood pressure and prothrombic and proinflammatory states).

(For the treatment of specific dyslipidemias refer to the Report of the Canadian Working Group on Hypercholesterolemia and Other Dyslipidemias or to the US NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [Adult Treatment Panel III], under SELECTED BIBLIOGRAPHY in product monograph).

When drugs are prescribed attention to therapeutic lifestyle changes (reduced intake of saturated fats and cholesterol, weight reduction, increased physical activity, ingestion of soluble fibres) should always be maintained and reinforced.

The Atorvastatin Versus Revascularization Treatments (AVERT) study examined the effect of intensive lipid-lowering in patients with stable coronary artery disease and LDL-C at least 3.0 mmol/L in patients referred for percutaneous transluminal coronary angioplasty (PTCA). Patients were randomized for 18 months to LIPITOR 80 mg daily or to PTCA with usual medical care which could include lipid metabolism regulators. The results of the AVERT study should be considered as exploratory since several limitations may affect its design and conduct. In the medical-treated group with LIPITOR there was a trend for a reduced incidence of ischemic events and a delayed time to first ischemic event. The results also suggest that intensive treatment to target LDL-C levels with LIPITOR is **additive and complementary** to angioplasty and would benefit patients referred for this procedure (see SELECTED BIBLIOGRAPHY in product monograph).

CONTRAINDICATIONS

Hypersensitivity to any component of this medication.

Active liver disease or unexplained persistent elevations of serum transaminases exceeding 3 times the upper limit of normal (see WARNINGS).

Pregnancy and lactation (see PRECAUTIONS).

WARNINGS

Pharmacokinetic Interactions

The use of HMG-CoA reductase inhibitors has been associated with severe myopathy, including rhabdomyolysis, which may be more frequent when they are co-administered with drugs that inhibit the cytochrome P-450 enzyme system. Atorvastatin is metabolized by cytochrome P-450 isoform 3A4 and as such may interact with agents that inhibit this enzyme. (See WARNINGS, Muscle Effects and PRECAUTIONS, Drug Interactions and Cytochrome P-450-mediated Interactions).

Hepatic Effects

In clinical trials, persistent increases in serum transaminases greater than three times the upper limit of normal occurred in <1% of patients who received LIPITOR. When the dosage of LIPITOR was reduced, or when drug treatment was interrupted or discontinued, serum transaminase levels returned to pretreatment levels. The increases were generally not associated with jaundice or other clinical signs or symptoms. Most patients continued treatment with a reduced dose of LIPITOR without clinical sequelae.

Liver function tests should be performed before the initiation of treatment, and periodically thereafter. Special attention should be paid to patients who develop elevated serum transaminase levels, and in these patients measurements should be repeated promptly and then performed more frequently.

If increases in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) show evidence of progression, particularly if they rise to greater than 3 times the upper limit of normal and are persistent, the dosage should be reduced or the drug discontinued.

LIPITOR should be used with caution in patients who consume substantial quantities of alcohol and/or have a past history of liver disease. Active liver disease or unexplained transaminase elevations are contraindications to the use of LIPITOR; if such a condition should develop during therapy, the drug should be discontinued.

Muscle Effects

Myopathy, defined as muscle aching or muscle weakness in conjunction with increases in creatinine phosphokinase (CPK) values to greater than ten times the upper limit of normal, should be considered in any patient with diffuse myalgia, muscle tenderness or weakness, and/or marked elevation of CPK. Patients should be advised to report promptly unexplained muscle pain, tenderness or weakness, particularly if accompanied by malaise or fever. LIPITOR therapy should be discontinued if markedly elevated CPK levels occur or myopathy is diagnosed or suspected.

The risk of myopathy and rhabdomyolysis during treatment with HMG-CoA reductase inhibitors is increased with concurrent administration of cyclosporin, fibric acid derivatives, erythromycin, clarithromycin, niacin (nicotinic acid), azole antifungals or nefazodone. As there is no experience to date with the use of LIPITOR given concurrently with these drugs, with the exception of pharmacokinetic studies conducted in healthy subjects with erythromycin and clarithromycin, the benefits and risks of such combined therapy should be carefully considered (see PRECAUTIONS, Pharmacokinetic Interaction Studies and Potential Drug Interactions).

Rhabdomyolysis has been reported in very rare cases with LIPITOR (see PRECAUTIONS, Drug Interactions).

Rhabdomyolysis with renal dysfunction secondary to myoglobinuria has also been reported with HMG-CoA reductase inhibitors. LIPITOR therapy should be temporarily withheld or discontinued in any patient with an acute serious condition suggestive of a myopathy or having a risk factor predisposing to the development of renal failure secondary to rhabdomyolysis (such as severe acute infection, hypotension, major surgery, trauma, severe metabolic, endocrine and electrolyte disorders, and uncontrolled seizures).

PRECAUTIONS

General

The effects of atorvastatin-induced changes in lipoprotein levels, including reduction of serum cholesterol on cardiovascular morbidity or mortality or total mortality have not been established.

Before instituting therapy with LIPITOR (atorvastatin calcium), an attempt should be made to control elevated serum lipoprotein levels with appropriate diet, exercise, and weight reduction in overweight patients, and to treat other underlying medical problems (see INDICATIONS AND CLINICAL USE). Patients should be advised to inform subsequent physicians of the prior use of LIPITOR or any other lipid-lowering agents.

Effect on the Lens

Current long-term data from clinical trials do not indicate an adverse effect of atorvastatin on the human lens.

Effect on Ubiquinone (CoQ_{10}) Levels

Significant decreases in circulating ubiquinone levels in patients treated with atorvastatin and other statins have been observed. The clinical significance of a potential long-term statin-induced deficiency of ubiquinone has not been established. It has been reported that a decrease in myocardial ubiquinone levels could lead to impaired cardiac function in patients with borderline congestive heart failure (see SELECTED BIBLIOGRAPHY in product monograph).

Effect on Lipoprotein (a)

In some patients, the beneficial effect of lowered total cholesterol and LDL-C levels may be partly blunted by a concomitant increase in Lp(a) lipoprotein concentrations. Present knowledge suggests the importance of high Lp(a) levels as an emerging risk factor for coronary heart disease. It is thus desirable to maintain and reinforce lifestyle changes in high risk patients placed on atorvastatin therapy (see SELECTED BIBLIOGRAPHY in product monograph).

Hypersensitivity

An apparent hypersensitivity syndrome has been reported with other HMG-CoA reductase inhibitors which has included 1 or more of the following features: anaphylaxis, angioedema, lupus erythematous-like syndrome, polymyalgia rheumatica, vasculitis, purpura, thrombocytopenia, leukopenia, hemolytic anemia, positive ANA, ESR increase, eosinophilia, arthritis, arthralgia, urticaria, asthenia, photosensitivity, fever, chills, flushing, malaise, dyspnea, toxic epidermal necrolysis, erythema multiforme, including Stevens-Johnson syndrome. Although to date hypersensitivity syndrome has not been described as such, LIPITOR should be discontinued if hypersensitivity is suspected.

Use in Pregnancy

LIPITOR is contraindicated during pregnancy (see CONTRAINDICATIONS).

Atherosclerosis is a chronic process and discontinuation of lipid-lowering drugs during pregnancy should have little impact on the outcome of long-term therapy of primary hypercholesterolemia. Cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they may cause harm to the fetus when administered to pregnant women.

There are no data on the use of LIPITOR during pregnancy. LIPITOR should be administered to women of childbearing age only when such patients are highly unlikely to conceive and have been informed of the potential hazards. If the patient becomes pregnant while taking LIPITOR, the drug should be discontinued and the patient apprised of the potential risk to the fetus.

Nursing Mothers

In rats, milk concentrations of atorvastatin are similar to those in plasma. It is not known whether this drug is excreted in human milk. Because of the potential for adverse reactions in nursing infants, women taking LIPITOR should not breast-feed (see CONTRAINDICATIONS).

Pediatric Use

Treatment experience in a pediatric population is limited to doses of LIPITOR up to 80 mg/day for 1 year in 8 patients with homozygous familial hypercholesterolemia. No clinical or biochemical abnormalities were reported in these patients.

Geriatric Use

Treatment experience in adults 70 years or older (N=221) with doses of LIPITOR up to 80 mg/day has demonstrated that the safety and effectiveness of atorvastatin in this population was similar to that of patients <70 years of age. Pharmacokinetic evaluation of atorvastatin in subjects over the age of 65 years indicates an increased AUC. As a precautionary measure, the lowest dose should be administered initially (see PHARMACOLOGY, Human Pharmacokinetics; SELECTED BIBLIOGRAPHY in product monograph).

Renal Insufficiency

Plasma concentrations and LDL-C lowering efficacy of LIPITOR was shown to be similar in patients with moderate renal insufficiency compared with patients with normal renal function. However, since several cases of rhabdomyolysis have been reported in patients with a history of renal insufficiency of unknown severity, as a precautionary measure and pending further experience in renal disease, the lowest dose (10 mg/day) of LIPITOR should be used in these patients. Similar precautions apply in patients with severe renal insufficiency [creatinine clearance <30 mL/min (<0.5 mL/sec)]; the lowest dosage should be used and implemented cautiously (see WARNINGS, Muscle Effects; PRECAUTIONS, Drug Interactions).

Refer also to DOSAGE AND ADMINISTRATION.

Endocrine Function

HMG-CoA reductase inhibitors interfere with cholesterol synthesis and as such might theoretically blunt adrenal and/or gonadal steroid production. Clinical studies with atorvastatin and other HMG-CoA reductase inhibitors have suggested that these agents do not reduce plasma cortisol concentration or impair adrenal reserve and do not reduce basal plasma testosterone concentration. However, the effects of HMG-CoA reductase inhibitors on male fertility have not been studied in adequate numbers of patients. The effects, if any, on the pituitary-gonadal axis in premenopausal women are unknown.

Patients treated with atorvastatin who develop clinical evidence of endocrine dysfunction should be evaluated appropriately. Caution

1. Friedewald WT *et al. Clin Chem* 1972;18(6):489-502.

should be exercised if an HMG-CoA reductase inhibitor or other agent used to lower cholesterol levels is administered to patients receiving other drugs (e.g. ketoconazole, spironolactone or cimetidine) that may decrease the levels of endogenous steroid hormones.

Pharmacokinetic Interaction Studies and Potential Drug Interactions

Pharmacokinetic interaction studies conducted with drugs in healthy subjects may not detect the possibility of a potential drug interaction in some patients due to differences in underlying diseases and use of concomitant medications (see also Geriatric Use; Renal Insufficiency; Patients with Severe Hypercholesterolemia).

Concomitant Therapy with Other Lipid Metabolism Regulators: Combined drug therapy should be approached with caution as information from controlled studies is limited.

Bile Acid Sequestrants:

Patients with mild to moderate hypercholesterolemia: LDL-C reduction was greater when LIPITOR 10 mg and colestipol 20 g were coadministered (-45%) than when either drug was administered alone (-35% for LIPITOR and -22% for colestipol).

Patients with severe hypercholesterolemia: LDL-C reduction was similar (-53%) when LIPITOR 40 mg and colestipol 20 g were coadministered when compared to that with LIPITOR 80 mg alone. Plasma concentration of atorvastatin was lower (approximately 26%) when LIPITOR 40 mg plus colestipol 20 g were coadministered compared with LIPITOR 40 mg alone.

However, the combination drug therapy was less effective in lowering the triglycerides than LIPITOR monotherapy in both types of hypercholesterolemic patients (see PHARMACOLOGY, Clinical Studies).

When LIPITOR is used concurrently with colestipol or any other resin, an interval of at least 2 hours should be maintained between the two drugs, since the absorption of LIPITOR may be impaired by the resin.

Fibric Acid Derivatives (Gemfibrozil, Fenofibrate, Bezafibrate) and Niacin (Nicotinic Acid): Although there is limited experience with the use of LIPITOR given concurrently with fibric acid derivatives and niacin, the benefits and risks of such combined therapy should be carefully considered. The risk of myopathy during treatment with other drugs in this class, including atorvastatin, is increased with concurrent administration (see WARNINGS, Muscle Effects and SELECTED BIBLIOGRAPHY in product monograph).

Coumarin Anticoagulants: LIPITOR had no clinically significant effect on prothrombin time when administered to patients receiving chronic warfarin therapy (see SELECTED BIBLIOGRAPHY in product monograph).

Digoxin: In healthy subjects, digoxin pharmacokinetics at steady-state were not significantly altered by coadministration of digoxin 0.25 mg and LIPITOR 10 mg daily. However, digoxin steady-state concentrations increased approximately 20% following coadministration of digoxin 0.25 mg and LIPITOR 80 mg daily (see Human Pharmacokinetics). Patients taking digoxin should be monitored appropriately.

Antihypertensive agents (amlodipine): In clinical studies, LIPITOR was used concomitantly with antihypertensive agents without evidence to date of clinically significant adverse interactions. In healthy subjects, atorvastatin pharmacokinetics were not altered by the coadministration of LIPITOR 80 mg and amlodipine 10 mg at steady state (see Human Pharmacokinetics).

(quinapril): In a randomized, open-label study in healthy subjects, steady-state quinapril dosing (80 mg QD) did not significantly affect the pharmacokinetic profile of atorvastatin tablets (10 mg QD) (see Human Pharmacokinetics).

Oral Contraceptives and Hormone Replacement Therapy: Coadministration of LIPITOR with an oral contraceptive, containing 1mg norethindrone and 35 µg ethinyl estradiol, increased plasma concentrations (AUC levels) of norethindrone and ethinyl estradiol by approximately 30% and 20%, respectively. These increases should be considered when selecting an oral contraceptive. In clinical studies, LIPITOR was used concomitantly with estrogen replacement therapy without evidence to date of clinically significant adverse interactions.

Antacids: Administration of aluminum and magnesium based antacids, such as Maalox® TC Suspension, with LIPITOR decreased plasma concentrations of LIPITOR by approximately 35%. LDL-C reduction was not altered but the triglyceride-lowering effect of LIPITOR may be affected.

Cimetidine: Administration of cimetidine with LIPITOR did not alter plasma concentrations or LDL-C lowering efficacy of LIPITOR, however, the triglyceride-lowering effect of LIPITOR was reduced from 34% to 26%.

Cytochrome P-450-mediated Interactions: Atorvastatin is metabolized by the cytochrome P-450 isoenzyme, CYP 3A4. Erythromycin, a CYP 3A4 inhibitor, increased atorvastatin plasma levels by 40%. Coadministration of CYP 3A4 inhibitors, such as grapefruit juice, some macrolide antibiotics (i.e. erythromycin, clarithromycin), immunosuppressants (cyclosporine), azole antifungal agents (i.e. itraconazole, ketoconazole), protease inhibitors, or the antidepressant, nefazodone, may have the potential to increase plasma concentrations of HMG-CoA reductase inhibitors, including LIPITOR (see SELECTED BIBLIOGRAPHY in product monograph). Caution should thus be exercised with concomitant use of these agents (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Endocrine Function; DOSAGE AND ADMINISTRATION; SELECTED BIBLIOGRAPHY in product monograph).

In healthy subjects, coadministration of maximum doses of both atorvastatin (80 mg) and terfenadine (120 mg), a CYP 3A4 substrate, was shown to produce a modest increase in terfenadine AUC. The QTc interval remained unchanged. However, since an interaction between these two drugs cannot be excluded in patients with predisposing factors for arrhythmia, (e.g. preexisting prolonged QT interval, severe coronary artery disease, hypokalemia), caution should be exercised when these agents are coadministered (see WARNINGS, Pharmacokinetic Interactions; DOSAGE AND ADMINISTRATION).

Antipyrine: Antipyrine was used as a non-specific model for drugs metabolized by the microsomal hepatic enzyme system (cytochrome P-450 system). LIPITOR had no effect on the pharmacokinetics of antipyrine, thus interactions with other drugs metabolized via the same cytochrome isozymes are not expected.

Macrolide Antibiotics (azithromycin, clarithromycin, erythromycin): In healthy adults, coadministration of LIPITOR (10 mg QD) and azithromycin (500 mg QD) did not significantly alter the plasma concentrations of atorvastatin. However, coadministration of atorvastatin (10 mg QD) with erythromycin (500 mg QID) or clarithromycin (500 mg BID), which are both CYP 3A4 inhibitors, increased plasma concentrations of atorvastatin approximately 40% and 80%, respectively (see WARNINGS, Muscle Effects; Human Pharmacokinetics).

Protease Inhibitors (nelfinavir mesylate): In healthy adults, coadministration of nelfinavir mesylate (1250 mg BID), a known CYP 3A4 inhibitor, and atorvastatin (10 mg QD) resulted in increased plasma concentrations of atorvastatin. AUC and C_{max} of atorvastatin were increased by 74% and 122% respectively.

Patients with Severe Hypercholesterolemia: Higher drug dosages (80 mg/day) required for some patients with severe hypercholesterolemia (including familial hypercholesterolemia) are associated with increased plasma levels of atorvastatin. **Caution should be exercised in such patients who are also severely renally impaired, elderly, or are concomitantly being administered digoxin or CYP 3A4 inhibitors (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions; DOSAGE AND ADMINISTRATION).**

Drug/Laboratory Test Interactions

LIPITOR may elevate serum transaminase and creatinine phosphokinase levels (from skeletal muscle). In the differential diagnosis of chest pain in a patient on therapy with LIPITOR, cardiac and noncardiac fractions of these enzymes should be determined.

ADVERSE REACTIONS

LIPITOR is generally well-tolerated. Adverse reactions have usually been mild and transient. In controlled clinical studies (placebo-controlled and active-controlled comparative studies with other lipid lowering agents) involving 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to LIPITOR. Of these 2502 patients, 1721 were treated for at least 6 months and 1253 for 1 year or more.

Adverse experiences occurring at an incidence ≥1% in patients participating in placebo-controlled clinical studies of LIPITOR and reported to be possibly, probably or definitely drug related are shown in Table 1 below:

TABLE 1. Associated Adverse Events Reported in ≥1% of Patients in Placebo-Controlled Clinical Trials

	Placebo % (n=270)	LIPITOR % (n=1122)
GASTROINTESTINAL		
Constipation	1	1
Diarrhea	1	1
Dyspepsia	2	1
Flatulence	2	1
Nausea	0	1
NERVOUS SYSTEM		
Headache	2	1
MISCELLANEOUS		
Pain	<1	1
Myalgia	1	1
Asthenia	<1	1

The following additional adverse events were reported in clinical trials; not all events listed below have been associated with a causal relationship to LIPITOR therapy: Muscle cramps, myositis, myopathy, paresthesia, peripheral neuropathy, pancreatitis, hepatitis, cholestatic jaundice, anorexia, vomiting, alopecia, pruritus, rash, impotence, hyperglycemia, and hypoglycemia.

Post-marketing experience: Very rare reports: severe myopathy with or without rhabdomyolysis (see WARNINGS, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Drug Interactions). Isolated reports: thrombocytopenia, arthralgia and allergic reactions including urticaria, angioneurotic edema, anaphylaxis and bullous rashes (including erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis). These may have no causal relationship to atorvastatin.

Ophthalmologic observations: see PRECAUTIONS.

Laboratory Tests: Increases in serum transaminase levels have been noted in clinical trials (see WARNINGS).

SYMPTOMS AND TREATMENT OF OVERDOSAGE

There is no specific treatment for atorvastatin overdosage. Should an overdose occur, the patient should be treated symptomatically and supportive measures instituted as required. Due to extensive drug binding to plasma proteins, hemodialysis is not expected to significantly enhance atorvastatin clearance.

DOSAGE AND ADMINISTRATION

Patients should be placed on a standard cholesterol-lowering diet [at least equivalent to the Adult Treatment Panel III (ATP III) TLC diet] before receiving LIPITOR, and should continue on this diet during treatment with LIPITOR. If appropriate, a program of weight control and physical exercise should be implemented.

Primary Hypercholesterolemia and Combined (Mixed) Hyperlipidemia, Including Familial Combined Hyperlipidemia. The recommended dose of LIPITOR is 10 mg once a day. The majority of patients achieve and maintain target cholesterol levels with LIPITOR 10 mg/day. A significant therapeutic response is evident within 2 weeks, and the maximum response is usually achieved within 2-4 weeks. The response is maintained during chronic therapy. Doses can be given at any time of the day, with or without food, and should preferably be given in the evening. Doses should be individualized according to baseline LDL-C and/or TG levels, the desired LDL-C and/or TG target (see the Detection and Management of Hypercholesterolemia, Working Group on Hypercholesterolemia and other Dyslipidemias [Canada] and/or the US National Cholesterol Education Program [NCEP Adult Treatment Panel III]), the goal of therapy and the patient's response. Adjustments of dosage, if necessary, should be made at intervals of 4 weeks or more. The recommended dose range for most patients is 10 to 40 mg/day. The maximum dose is 80 mg/day, which may be required in a minority of patients (see section below).

Lipid levels should be monitored periodically and, if necessary, the dose of LIPITOR adjusted based on target lipid levels recommended by guidelines.

The following reductions in total cholesterol and LDL-C levels have been observed in 2 dose-response studies, and may serve as a guide to treatment of patients with mild to moderate hypercholesterolemia:

TABLE 2. Dose-Response in Patients With Mild to Moderate Hypercholesterolemia
(Mean Percent Change from Baseline)[a]

Lipid Parameter	LIPITOR Dose (mg/day)			
	10 (N=22)	20 (N=20)	40 (N=21)	80 (N=23)
Total-C: 7.1 mmol/L[b] (273 mg/dL)[b]	-29	-33	-37	-45
LDL-C: 4.9 mmol/L[b] (190 mg/dL)[b]	-39	-43	-50	-60

a. Results are pooled from 2 dose-response studies.
b. Mean baseline values.

Severe Dyslipidemias

In patients with severe dyslipidemias, including homozygous and heterozygous familial hypercholesterolemia and dysbetalipoproteinemia (Type III), higher dosages (up to 80 mg/day) may be required (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions).

Concomitant Therapy

See PRECAUTIONS, Drug Interactions.

Dosage in Patients With Renal Insufficiency

See PRECAUTIONS.

PHARMACEUTICAL INFORMATION

Drug Substance

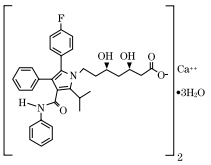
Proper Name: Atorvastatin calcium

Chemical Name: [R-(R*,R*)]-2-(4-fluorophenyl)-ß, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate

Empirical Formula: $(C_{33}H_{34}FN_2O_5)_2Ca\bullet 3H_2O$

Molecular Weight: 1209.42

Structural Formula:



Description: Atorvastatin calcium is a white to off-white crystalline powder that is practically insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

Tablet Composition:

Each tablet contains either 10 mg, 20 mg, 40 mg or 80 mg atorvastatin as the active ingredient. Each tablet also contains the following non-medicinal ingredients: calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, hydroxypropyl methylcellulose, polyethylene glycol, talc, titanium dioxide, polysorbate 80 and simethicone emulsion.

Stability and Storage Recommendations:

Store at controlled room temperature 15 to 30ºC.

AVAILABILITY OF DOSAGE FORMS

LIPITOR (atorvastatin calcium) is available in dosage strengths of 10 mg, 20 mg, 40 mg and 80 mg atorvastatin per tablet.

10 mg: White, elliptical, film-coated tablet, coded "10" on one side and "PD 155" on the other. Available in bottles of 90 tablets.

20 mg: White, elliptical, film-coated tablet, coded "20" on one side and "PD 156" on the other. Available in bottles of 90 tablets.

40 mg: White, elliptical, film-coated tablet, coded "40" on one side and "PD 157" on the other. Available in bottles of 90 tablets.

80 mg: White, elliptical, film-coated tablet, coded "80" on one side and "PD 158" on the other. Blisters of 30 tablets (3 strips x 10).

References:

1. LIPITOR (atorvastatin calcium) Product Monograph, Pfizer Canada Inc., February 2002. **2.** IMS Global Services; March 1997 – September 2002. **3.** Pitt B, *et al.* Aggressive lipid-lowering therapy compared with angioplasty in stable coronary artery disease. *N Engl J Med* 1999;341:70-76. **4.** Data on File, Pfizer Canada Inc. **5.** Simon Day. Dictionary for Clinical Trials, 1999, John Wiley & Sons Ltd. 137-38.

For a copy of the Product Monograph or full Prescribing Information, please contact:

Pfizer

Life is our life's work

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