The NEW ENGLAND JOURNAL of MEDICINE

Perspective

JANUARY 12, 2006

Research in the Hot Zone

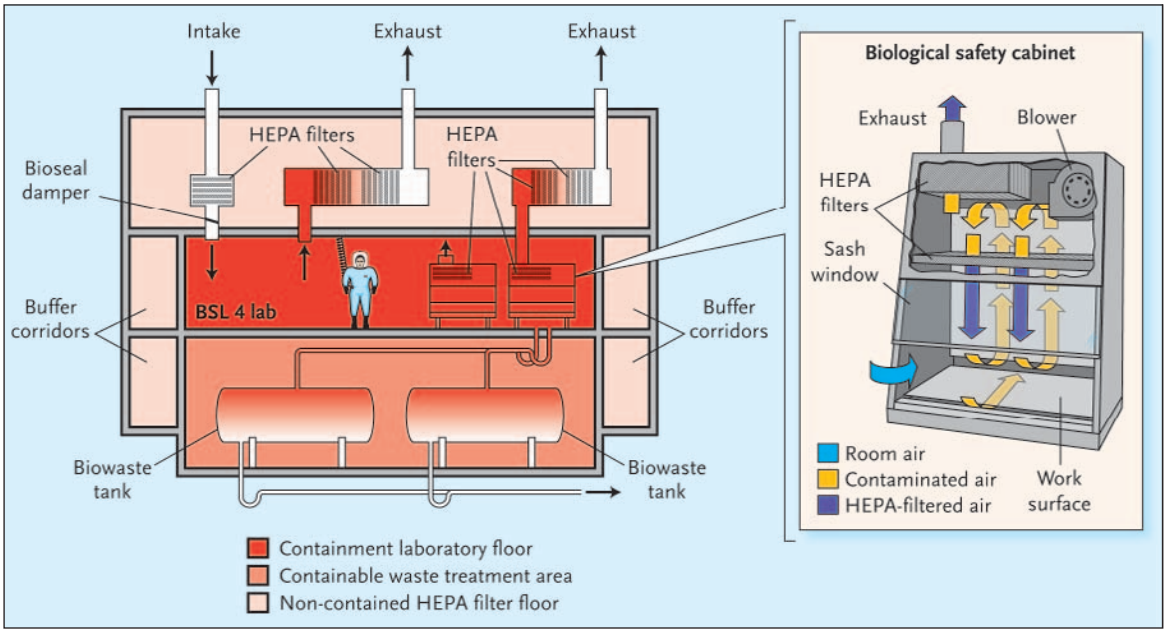
Robert Steinbrook, M.D.

Research conducted at biosafety level 4, popularly known as the hot zone, continues to be both revered and feared. Biosafety level 4 laboratories are the places to study dangerous and exotic agents that cannot safely be studied anywhere else, such as pathogens that can be transmitted within a laboratory through the air. This level of biosafety is often required for the development of diagnostic tests, therapies, and vaccines and as the starting point for work on newly recognized agents whose risks have yet to be established.[1] At the same time, these laboratories are seen as potential targets of a terrorist attack or as possible sources of the very contagions that their researchers seek to prevent. Some cities and regions want no part of such laboratories (for example, a facility was built in Toronto that never went "hot") despite their extensive and redundant safety features.

A biosafety level 4 laboratory is a box within a box — a sealed environment within a larger building (see diagram), buttressed by strict security measures. Since mid-2004, one such laboratory, 2000 ft^2 in size, has operated in the middle of a medical school campus, at the University of Texas Medical Branch (UTMB) in Galveston (see photo). Led by David Walker, chair of the pathology department, planning for the facility began at UTMB in the 1990s — years before the attacks on September 11, 2001, and before emerging infectious diseases and bioterrorism became widespread concerns. Walker explained the rationale to me when I visited the university; it is to allow deadly pathogens to be studied with the same rigor as are other organisms.

The pathogens currently under study include the viruses that cause hantavirus pulmonary syndrome, a public health problem in North and South America; viruses that cause tick-borne encephalitis; Crimean–Congo hemorrhagic fever virus, a bunyavirus found in Asia and Africa; and Rift Valley fever virus, another bunyavirus found in Africa. Work is also focusing on avian influenza A (H5N1) virus, the arenavirus that causes Lassa fever, and other arenaviruses that are found in South America.

Named after Robert Shope, the eminent virologist who worked at UTMB from 1995 until his death in 2004, the laboratory contains areas for research involving cell cultures; research in animals, such as mice, hamsters, and guinea pigs; and necropsies. For example, H5N1's ability to combine with



Cross-Sectional Diagram of a Biosafety Level 4 Laboratory (BSL 4 Lab).

Safety cabinets may recirculate HEPA-filtered air to the laboratory air space or exhaust air directly through HEPA filters. Adapted from Smith Carter.

other influenza virus strains that infect humans will be studied in cell culture. Testing of its susceptibility to antiviral medications should begin this month in ferrets, the animals thought to best reflect humans in terms of influenza infection, according to Slobodan Paessler, the project leader for these tests.

Biosafety laboratories are found at many institutions. Biosafety level 2 is for agents presenting a moderate risk; level 3 is for pathogens of greater risk that can cause lethal infections and that can be transmitted through the air. The first biosafety level 4 laboratories in the United States were at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), at Fort Detrick in Frederick, Maryland, and the Centers for Disease Control and Prevention (CDC) in Atlanta. Today, there are five such laboratories (see map). Existing facilities are being upgraded, and additional facilities are under construction. New laboratories at the CDC will more than triple their biosafety level 4 space. A new USAMRIID facility is planned. In September 2003, the National Institute of Allergy and Infectious Diseases, using money appropriated by Congress after the anthrax attacks of 2001, funded two national biocontainment laboratories: the Galveston National Laboratory at UTMB and the National Emerging Infectious Diseases Laboratories at Boston University Medical Center, in Boston.[2]

The $167 million Galveston National Laboratory is being built on the UTMB campus, immediately adjacent to the Shope Laboratory (see photo), and is expected to open in 2008. It will contain 63,000 ft² of laboratory space, including about 12,000 ft² classified as biosafety level 4, and will be surrounded by a 200-ft security perimeter from which private vehicles will be excluded. In general, the Galveston project has had the support of the community, largely because of the extensive groundwork that had been laid during the planning and construction of the current laboratory. By contrast, there has been substantial community opposition in Boston. On December 9, 2005, the final environmental impact statement for the Boston University laboratories was published in the Federal Register. A "record of decision" is expected soon. If the decision is favorable — and a building permit and state building-code variances are approved — construction could start as early as February 2006 and be completed in 2008.

The city of Galveston is a barrier island in the Gulf of Mexico with a population of nearly 60,000. The Shope Laboratory is housed in a windowless three-story pavilion that is attached to a larger research building (see photo) and is designed to withstand the battering it might receive in a hurricane-prone area. The facility's approximately 60 concrete pilings

extend 120 ft into the ground, and both building and laboratory have concrete walls 10 in. thick.

The laboratory is surrounded by a secure "buffer zone," from which windows look into the lab. The air is under negative pressure so that it will not escape. All doors and other openings in the walls are sealed so that air would be contained even in the event of a complete power failure. Both intake and exhaust air are rendered noninfectious by means of high-efficiency particulate air (HEPA) filters: intake air is filtered once to protect against back drafts, and the exhaust air is filtered twice. Material that leaves the laboratory is rendered noninfectious through processes such as autoclaving or by "cooking" liquid waste. The exception is biologic materials that need to remain viable or intact. These are transferred in sealed containers, such as plastic freezer vials or glass ampules, which are enclosed in a second unbreakable container and removed through a "dunk tank" filled with disinfectant or passed through a fumigation chamber.

Charles Fulhorst, a veterinarian and associate professor of pathology at UTMB who has extensive experience with biosafety level 4, explained, "There is nothing that can go wrong in a lab like this that will ever get outside. The exception is if somebody intentionally takes something, if they put a vial in their pocket and keep walking. So we depend on the high integrity of those selected to work in biosafety level 4 laboratories."

In the laboratory, all personnel wear full-body positive-pressure "space suits" that are connected by tether hoses to a common air supply. Research is conducted within specialized areas known as "biological safety cabinets," which contain aerosols by creating a curtain of airflow away from the open laboratory space and by filtering any aerosols generated inside the cabinet. Not infrequently, researchers who pipette a virus create an aerosol. Infected animals are also constantly shedding viruses, so their cages are opened only inside a safety cabinet. In the animal room, each cage has an air supply with HEPA filtration so that animals in different experiments are prevented from infecting one another. The laboratory is designed to remain virtually sterile at all times and to keep the air safe for breathing.

John Glowczwski/UTMB.

The Galveston National Laboratory under Construction on the Campus of the University of Texas Medical Branch, October 2005.

The arrow shows the location of the Robert E. Shope Laboratory, a biosafety level 4 facility that has been operational since mid-2004.

In 1900, Galveston was largely destroyed by one of the deadliest hurricanes in U.S. history. So in September 2005, as Hurricane Rita bore down on the Gulf Coast, the UTMB research staff followed the procedures they use whenever the Shope Laboratory is brought down for annual maintenance. They secured and fumigated the laboratory after destroying all infectious cultures and euthanizing the animals. Culture stocks were maintained in plastic vials in padlocked freezers that were sealed with duct tape and protected by dry ice or liquid nitrogen and backup generators. In the end, the power remained on, and the laboratory sustained no damage, although the work that was in progress had to start over, at additional expense.

Biosafety level 4 research is slower, more physically demanding, and more expensive than comparable research that can be conducted at lower biosafety levels. The CDC designates many of the pathogens that are worked with at level 4 as "select agents," which are subject to strict legal requirements for possession, use, and transfer — rules that complicate the research process. [1,3] For example, researchers must register with the government and be approved. Merely entering or leaving the facility is not a trivial matter. The exit procedures take about 30 minutes and include a chemical shower to decontaminate the surface of the suit, followed by a body shower after the suit is removed. In the laboratory, the full-body suits and

Biosafety Level 4 Laboratories in the United States.

The laboratories in Atlanta are at the Centers for Disease Control and Prevention and Georgia State University; the latter is a "glove-box" facility, in which the researcher's body remains outside the part of the containment area that is classified as level 4. The laboratories in Frederick, Md., are at the National Interagency Biodefense Campus at Fort Detrick.

protective gloves, which are similar to dishwashing gloves, limit manual dexterity. Researchers use cameras and video monitors when direct observation is difficult, sometimes looking at a computer screen rather than into a microscope. As they move between different areas, researchers disconnect and reconnect their space suits from the central air supply. Extensive training is required — to master the environment and to perform research procedures safely. The researchers themselves face immediate risks, such as needle sticks, cuts during necropsies, or infections from aerosols.

In the Shope Laboratory, the air supply is sufficient for only four people at a time. The Galveston National Laboratory, which is expected to have annual operating costs of $20 million or more, will support a greater volume of research, provide space for more sophisticated research tools, and have a focus on applied research and product development. Stanley Lemon, the principal investigator for the laboratory, expressed the hope that in five years "we actually are doing the kind of research with agents like Marburg and Ebola that can lead to practical vaccines." According to Scott Weaver, a virologist and director for tropical and emerging infectious diseases at UTMB's Center for Biodefense and Emerging Infectious Diseases, perhaps the best example of the anticipated tools is magnetic resonance imaging of animals, such as macaques and other primates. This imaging will enable researchers to visualize disease processes in vivo over time. Such studies will replace some experiments in which animals are sacrificed daily or every several days so that necropsies can be performed to examine their tissues.

The rapid expansion of biosafety level 4 research in the United States has left unanswered questions. There is debate about whether the current federal investment in building and upgrading laboratories is excessive, inadequate, or on target, relative to spending on other public health priorities. There is uncertainty about the level of ongoing government support for operations, for the training of researchers, and for research. Training more researchers who can safely work with deadly pathogens may also increase the likelihood that one or more will be sympathetic to a terrorist organization or misuse their expertise in other ways.[4] The risks associated with biosafety level 4 laboratories, including those to the researchers and to the surrounding communities, can be greatly minimized, but they can never be eliminated entirely. Nonetheless, a research enterprise that seeks scientific understanding of deadly pathogens, as well as effective diagnostic tests, therapies, and vaccines, needs facilities that are up to the task.

Dr. Steinbrook is a national correspondent for the *Journal*.

1. Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in microbiological and biomedical laboratories (BMBL). 4th ed. Washington, D.C.: Government Printing Office, 1999. (Accessed December 21, 2005, at http://www.cdc.gov/od/ohs/biosfty/bmbl4/ bmbl4toc.htm.)

2. Hirschberg R, La Montagne J, Fauci AS. Biomedical research — an integral component of national security. N Engl J Med 2004; 350:2119-21.

3. Committee on Research Standards to Prevent the Destructive Application of Biotechnology, National Research Council. Biotechnology research in an age of terrorism. Washington, D.C.: National Academies Press, 2004.

4. Richardson L. Buying biosafety — is the price right? N Engl J Med 2004;350:2121-3.

Bioterrorism — Preparing to Fight the Next War

David A. Relman, M.D.

The United States has become preoccupied with the threat of bioterrorism — the potential for the poisoning of the milk supply with botulinum toxin, the hypothetical dissemination of smallpox by self-infected terrorists, the possibility of a massive release of aerosolized anthrax spores in the subway, even the newly raised specter of misuse of a reconstructed 1918 influenza virus. These concerns have had important consequences for the biomedical research agenda, funding priorities, and the regulatory environment.

In fiscal year 2003, $1.5 billion was allocated for biodefense research to the National Institutes of Health (NIH). These new research dollars, which have been reallocated yearly, now account for roughly one third of the budget of the National Institute of Allergy and Infectious Diseases (NIAID) at the NIH. Although some of these funds are intended for the study of emerging infectious diseases, unprecedented attention is being paid to pathogens that currently cause rare diseases. For example, the number of NIH grants for work on *Francisella tularensis* increased from 4 in 2001 to 71 in 2003, although there are only 100 to 150 cases of tularemia in the United States each year; in October 2005, $60 million was awarded by NIAID for work on new tularemia vaccines.

Government concern about bioterrorism has also led to new federal restrictions on the handling of infectious agents; such rules have hampered both the ability of U.S. researchers to participate in international collaborations and efforts to train foreign scientists in this country. All these changes reflect a radical shift in the political and social climate — a shift highlighted by the incarceration in 2004 in federal prison, on charges of improper handling of *Yersinia pestis*, of Dr. Thomas Butler, chief of infectious diseases at Texas Tech University and an expert on plague.

How well founded is this heightened concern about bioterrorism? If it is justified, how can we best allocate our intellectual, technical, and financial resources, given the imminent dangers from avian influenza and other natural threats? On what principles should we build a biodefense strategy?

Policymakers weighing the likelihood and dangers of bioterrorism tend to seek guidance from a past era of large, state-sponsored bioweapons programs that used industrial-scale processes, emphasized quality control, and based their projections of use on traditional military doctrine. The leaders of those programs that then viewed biologic agents as credible strategic weapons believed that a few particular agents had the most potential for use and saw the technology for preparing and delivering those agents as an essential component of a weapons program.

But we cannot assume that the logic behind biowarfare programs of the past will guide future misuses of the life sciences. Indeed, the lessons of this history can be dangerously misleading. First, the notion that only a certain few agents pose a plausible threat is largely an artifact of weapons programs that predated our current knowledge of molecular biology and that selected agents on the basis of their natural properties and the limited technical expertise then available. Among the agents that remain on today's threat lists, anthrax and smallpox make particularly compelling weapons, but as science and technology advance, the number of worrisome agents is expanding greatly.

Furthermore, large-scale industrial processes are not necessary for the development of potent biologic weapons. Increasingly, the means for propagating biologic agents under controlled conditions are being made accessible to anyone. Even our traditional concept of "weaponization" is misleading: nature provides mechanisms for packaging and preserving many infectious agents that can be manipulated through biologic and genetic engineering — for example, by enhancing the virulence of naturally sporulating organisms. Materials science and nanoscale science — advances in encapsulation technology, for instance — will provide new ways to package such agents. And self-replicating agents that are highly transmissible among humans, such as variola virus and influenza virus, need little or no alteration in order to be disseminated efficiently by terrorists.

Nor should we presume, on the basis of history, that when biologic agents are used deliberately and maliciously, they are capable of causing only relatively limited harm. The large biologic-weapons programs of the late 20th century were never unleashed. And the

use of such weapons by smaller groups, such as the Aum Shinrikyo cult, has been relatively unsophisticated — far from representative of what moderately well informed groups might do today. The consequences would have been far more dire, for example, had the anthrax spores circulated in the U.S. mail in 2001 been disseminated by more effective routes. Tomorrow's science and technology will present a new landscape with features that are both worrisome and reassuring: the methods and reagents used for reverse-engineering a novel virus, for instance, can also be used to engineer a vaccine against it.

New insights into biologic systems are emerging rapidly, and new tools for manipulating these systems continue to be developed.[1,2] Information is now disseminated globally, many relevant procedures require far fewer resources than ever before, and much life-science technology has been miniaturized. Today, anyone with a high-school education can use widely available protocols and prepackaged kits to modify the sequence of a gene or replace genes within a microorganism; one can also purchase small, disposable, self-contained bioreactors for propagating viruses and microorganisms. Such advances continue to lower the barriers to biologic-weapons development.[3,4]

So far, nature has been the most effective bioterrorist. In the future, however, the ability of experimenters to create genetic or molecular diversity not found in the natural world — for example, with the use of molecular breeding technologies — and to select for virulence-associated traits may result in new biologic agents with previously unknown potency. Although such agents may not survive long in the natural world and could, from an evolutionary standpoint, be dismissed as poorly adapted competitors, they may prove extremely destructive during their lifespan.

In devising a robust biodefense strategy, a key challenge will be to define the optimal balance between fixed and flexible defenses. The Maginot Line built by the French in the 1930s serves as a symbol of static defenses designed to protect against known threats. Although these elaborate fortifications bought the French some time, the advancing German army maneuvered around them. Similarly, the creation of static defenses can be justified for clear, imminent, and potentially catastrophic biologic threats — including avian influenza virus and prominent drug-resistant bacteria, such as *Staphylococcus aureus,* as well as anthrax and smallpox.

For the vast array of other potential threats, however, we should invest even more in flexible, dynamic defenses, which will rely on integrative science, new insights into biologic systems, and advancing technology. We need methods and technologies that can generate effective diagnostics, therapeutics, and prophylactics against a new or variant infectious agent within days or weeks after its characterization.

Lists of specific agents and the scrutiny of past events can inhibit creative thinking about universal tools and generic approaches for a dynamic world. A robust biodefense plan must be anticipatory, flexible, and rapidly responsive. It should exploit crosscutting technologies and cross-disciplinary scientific insights and use broadly applicable platforms and methods that offer substantial scalability. Examples include the use of "lab-on-a-chip" technology, based on advances in microfluidics, for rapid, sensitive, point-of-care diagnostics; computational approaches for predicting drug–ligand interactions; genomic tools such as microarrays and genome-wide screening for protective antigens; and automated robotic systems for rapid, high-throughput drug screening and the scale-up of vaccine production. Efforts to understand microbial virulence should emphasize the study of mechanisms and structures that are shared by a variety of agents.

Given the importance of early intervention, a greater emphasis should be placed on approaches to diagnosing diseases early and specifically. We need such tools now for naturally occurring microbial diseases, if only to reduce the inappropriate use of antibiotics. For example, analyses of host responses to infection in which advanced mass spectroscopy or DNA microarray technology is used to assess patterns of protein abundance or genome-wide patterns of transcript abundance may lead to a new capability for diagnosing presymptomatic disease and predicting clinical outcomes or responses to therapy. The NIH, the Centers for Disease Control and Prevention, the Department of Homeland Security — in response to the federal strategic plan for defense against biologic weapons outlined in Homeland Security Presidential Directive 10 — and other agencies have discussed these needs,[5] but investments in these broad approaches have been insufficient.

Such efforts will require strengthening our public health

infrastructure, especially in terms of personnel, communications, and surge capacity. Scientists and clinicians will need to play a bigger role in biodefense planning, including the articulation of needs, policymaking, and the assessment of future threats.

It is often said that military forces are trained to fight the last war, not the next one. The same may be true of public health officials and scientists working to strengthen the public health infrastructure. But given the pace of change in the life sciences, we cannot afford to be constrained by the past, nor can we afford to make incremental, short-term fixes. Recent investments in biodefense offer immense potential benefit, if guided by a creative, future-oriented perspective. Now is the time to begin making serious, sustained investments in the science and technology on which we can build agile defenses against an ever-evolving spectrum of biologic threats.

An interview with Dr. Relman can be heard at www.nejm.org.

Dr. Relman is an associate professor in the Departments of Medicine and of Microbiology and Immunology, Stanford University, Stanford, Calif., chief of infectious diseases at the Veterans Affairs Palo Alto Health Care System, Palo Alto, Calif., and a member of the National Science Advisory Board for Biosecurity.

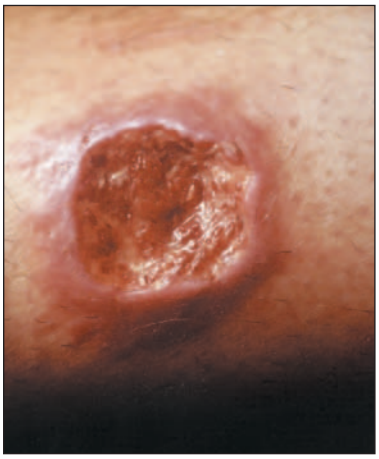
1. Segal E, Friedman N, Kaminski N, Regev A, Koller D. From signatures to models: understanding cancer using microarrays. Nat Genet 2005;37:Suppl:S38-S45.
2. Tully T, Bourtchouladze R, Scott R, Tallman J. Targeting the CREB pathway for memory enhancers. Nat Rev Drug Discov 2003; 2:267-77.
3. Petro JB, Relman DA. Understanding threats to scientific openness. Science 2003; 302:1898.
4. Petro JB, Plasse TR, McNulty JA. Biotechnology: impact on biological warfare and biodefense. Biosecur Bioterror 2003;1:161-8.
5. Hirschberg R, La Montagne J, Fauci AS. Biomedical research — an integral component of national security. N Engl J Med 2004; 350:2119-21.

The Burden of Illness in International Travelers

David R. Hill, M.D., D.T.M.&H.

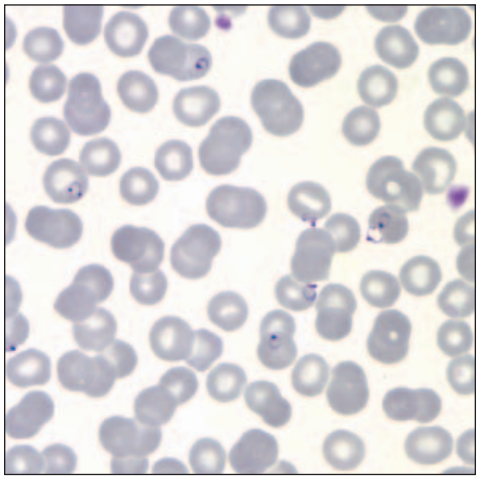
Related article, page 119

In 2004, 763 million people crossed international borders, reflecting an increase of 73 percent over the course of 15 years.[1] International travel has rebounded since the attacks of September 11, 2001, and is steadily increasing despite a variety of global health crises, the threat of terrorism, and the war in Iraq. Nearly 55 percent of travelers are vacationing, and about 15 percent are conducting business, but a growing number are visiting friends and relatives. Typically, such travelers were born in a resource-poor country, now live in a resource-rich country, and are returning to their country of birth to visit. Moreover, though most people travel voluntarily, thousands of uncounted travelers cross borders to flee war or persecution or to seek better opportunities. During the past 25 years, a new specialty of travel medicine has evolved to address the health of these international travelers — particularly those who visit resource-poor regions. Travel to these regions carries health risks and requires preventive measures that may be unfamiliar to many physicians.



Leg Ulcer from Leishmania.

In order to fully understand the risk to an individual traveler, the physician must undertake a careful pretravel assessment, determining the person's destinations, duration of travel, planned activities, and health and immunization status. Prevention and self-treatment measures can then be matched to the traveler and the planned trip. The information about imported disease that Freedman and colleagues, drawing on data from the GeoSentinel Surveillance Network, provide in this issue of the *Journal* (pages 119–130) helps to define the relative risks of various illnesses according to geographic region. For example, *Plasmodium falciparum* malaria tends to occur in travelers to sub-Saharan Africa (particularly West Africa), dengue in visitors to the Caribbean and Southeast Asia, cutaneous leishmaniasis in those who visit Central America and South America, and typhoid fever in travelers to south central Asia.



Ring Forms of *Plasmodium falciparum*.

TropNetEurop, a surveillance network of experts in infectious disease and tropical medicine throughout Europe, has reported similar trends.[2]

Travelers who return home with a tropical illness serve as windows into the diseases that are endemic in the countries they visited. By connecting tropical and travel medicine centers located around the world, surveillance networks document the spectrum of illness in returned travelers. This documentation enhances physicians' ability to recognize diseases that are rare in their own regions by alerting them and public health authorities to disease occurrence. Without information on the number of travelers to specific regions, the GeoSentinel surveillance data do not permit travel health advisers to determine the individual level of risk. However, they do help such professionals decide which diseases to emphasize. One clear message from the GeoSentinel data is the importance of measures that help travelers to avoid insect bites, given the frequency of vector-borne disease — malaria, dengue, leishmaniasis, and rickettsial infection — as well as the number of skin infections associated with these bites.

If all international travelers sought medical care before leaving home, and if all health care providers were well versed in the appropriate prophylactic regimens and in administering vaccines on the basis of risk, there would be much less travel-related illness. Unfortunately, probably less than half of travelers to regions with high risk for illness seek pretravel care, and many physicians make errors in judgment about measures such as malaria chemoprophylaxis and vaccines. Engaging travelers who are returning to their countries of origin for a visit is particularly difficult, since they often believe that they have little risk of contracting disease in a place that was once their home. This mistaken belief has translated into disproportionate burdens of such diseases as malaria and typhoid fever in this group of travelers.[3]

Even if all travelers sought health advice, however, they would still need to comply with it. For example, they would need to take their malaria chemoprophylaxis before, during, and after their trip and avoid behavior that would put them at risk for accidents, injuries, and sexually transmitted infections, including HIV infection. Data on the behavior of travelers are frequently not reassuring. Perhaps travelers would be more compliant with health recommendations if they understood the burden that diseases, such as malaria, tuberculosis, HIV infection and AIDS, and diarrheal and respiratory illnesses, have on populations in regions where the diseases are endemic.

Still, even the best-intentioned, compliant traveler cannot prevent all travel-related disease: 20 to 60 percent of visitors to resource-poor regions will have traveler's diarrhea because of widespread contamination of food and drink. Indeed, the GeoSentinel survey found a high frequency of all types of diarrhea. In addition, not all travel-related infectious disease comes from resource-poor regions: legionellosis is often associated with hotel stays in warm climates in the Mediterranean region; norovirus outbreaks, with cruise ships; and cryptosporidiosis, with exposure to water in swimming pools.

The documentation of illness in returned travelers raises several issues. First, how do we get travelers to realize that travel carries health risks and that they should seek pretravel advice from a physician or a clinic that specializes in travel medicine? As a start, we should encourage the travel industry to inform customers that health measures may need to be taken for certain destinations; we should also publicize the benefits of pretravel medical care, develop systems for providing such care specifically to travelers who visit their countries of birth, and educate physicians in the assessment of the health risks of travel.

Next, when faced with a patient, physicians need to ask, "Have you traveled abroad in the past six months?" It is important to recognize that 36 percent of travelers in the GeoSentinel survey presented with an illness a month or more after returning home. A travel history is particularly critical if the patient pre-

Courtesy of the DPDx Program, Centers for Disease Control and Prevention.

sents with a febrile syndrome, chronic diarrhea, or an unusual rash. A failure to associate a syndrome with travel may lead to delays in diagnosis and to adverse outcomes. Two common factors in death from malaria are a failure of the patient to take malaria chemoprophylaxis and a failure of the physician to consider the diagnosis early in the course of the illness.

Finally, although physicians do not need to be specialists in tropical medicine, they do need to be familiar with the current resources available for travel advice (see the Supplementary Appendix, available with the full text of this article at www.nejm.org) and with the most common travel-associated syndromes: those involving fever, acute and chronic diarrhea, skin disorders, and respiratory illness. Disease-specific testing needs to be performed in order to establish a diagnosis of an unusual parasitic, bacterial, or viral infection. And when physicians are uncomfortable making a diagnosis, they need to refer the patient to a specialist.

Given the increasing number of travelers with tropical diseases who are returning to Western regions and the heavy burden of disease in resource-poor regions, there is a clear need for continued education and training in tropical medicine, as well as for the development and supply of effective medications and vaccines. As more and more informed travelers receive pretravel health care and informed physicians increasingly consider the travel history when caring for ill returned travelers, international travel should become a healthier experience.

Dr. Hill is the director of the National Travel Health Network and Center and an honorary professor at the London School of Hygiene and Tropical Medicine — both in London.

1. WO tourism highlights, edition 2005. Madrid: World Tourism Organization, 2005: 1-12. (Accessed December 21, 2005, at http://www.world-tourism.org/facts/menu.html.)
2. Jelinek T, Mühlberger N. Surveillance of imported diseases as a window to travel health risks. Infect Dis Clin North Am 2005;19:1-13.
3. Bacaner N, Stauffer B, Boulware DR, Walker PF, Keystone JS. Travel medicine considerations for North American immigrants visiting friends and relatives. JAMA 2004;291: 2856-64.

THIS WEEK in the JOURNAL

ORIGINAL ARTICLE

Etiology of Illness in Returned Travelers

More than 17,000 ill returned travelers were evaluated within the GeoSentinel network, 30 specialized travel clinics around the globe. Some of the most common pathogens identified were those causing malaria, dengue, and rickettsial disease. The proportionate morbidity of various travel-related infectious diseases was calculated according to region of travel.

SEE P. 119; PERSPECTIVE, P. 115; CME, P. 219

ORIGINAL ARTICLE

Benazepril for Advanced Chronic Renal Insufficiency

Angiotensin-converting–enzyme (ACE) inhibitors provide renal protection in patients with mild-to-moderate renal insufficiency, slowing progression. However, the efficacy and safety of this class of medications in patients with advanced renal insufficiency are less clear. The results of this randomized, double-blind study indicate that benazepril, an ACE inhibitor, confers substantial renal benefits in patients without diabetes who have advanced renal insufficiency.

SEE P. 131; EDITORIAL, P. 189

ORIGINAL ARTICLE

Randomized Trial of Inhaled Cyclosporine in Lung-Transplant Recipients

The survival of patients receiving transplanted lungs is poorer than that of patients receiving transplants of many other organs. In this trial, inhaled cyclosporine, in addition to systemic immunosuppression, did not improve rejection rates but was associated with better overall survival and chronic rejection–free survival.

SEE P. 141; EDITORIAL, P. 191

BRIEF REPORT

Familial Sinus Bradycardia and a Mutation in the Cardiac Pacemaker Channel

Pacemaker channels in the sinoatrial node generate the sinus rhythm and regulate the heart rate. In an Italian family affected by sinus bradycardia, a mutation was identified in the α-subunit of the cardiac pacemaker channel. This mutation mimics the effect of mild vagal stimulation on heart rate.

SEE P. 151

CLINICAL PRACTICE

Chronic Daily Headache

A 36-year-old woman with a long history of catamenial migraines had had a headache almost every day for a year. The background headache was mild, but it became severe and incapacitating at least twice a week, interfering with work and sleep. She took six to eight tablets containing a combination of aspirin, acetaminophen, and caffeine per day, with minimal relief. She did not have fever, weight loss, diplopia, or tinnitus. Her headaches were not exacerbated by a Valsalva maneuver or positional change. Her physical examination was normal. How should she be evaluated and treated?

SEE P. 158; CME, P. 217

DRUG THERAPY

Treatment of Acute Lymphoblastic Leukemia

Although the overall cure rate of acute lymphoblastic leukemia (ALL) in children is about 80 percent, affected adults fare less well. This review considers recent advances in the treatment of ALL, emphasizing issues that need to be addressed if treatment outcome is to improve further.

SEE P. 166; CME, P. 218

CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL

A Male Lung-Transplant Recipient with Fever, Cough, Hemoptysis, and Abdominal Pain

A 64-year-old man who had received a double-lung allograft because of emphysema six months previously was admitted to the hospital with cough, hemoptysis, and abdominal pain of three days' duration. He had a history of smoking, chronic obstructive pulmonary disease, and lung cancer. Since the transplantation, he had had an episode of rejection and had recently had thrombophlebitis of the leg. A chest radiograph revealed a new right upper-lobe infiltrate, and a cytomegalovirus antigenemia test was positive at 185 cells per two slides. A diagnostic procedure was performed.

SEE P. 180

SOUNDING BOARD

Reform of Drug Regulation

The authors argue that extensive reform of the system of drug regulation in the United States is needed to improve drug safety. In order to develop a more comprehensive system that is not so narrowly focused on testing of drugs before marketing, the authors propose a new federal authority for drug regulation, to be made up of three independent centers: a Center for New Drug Approval, a Center for Post-marketing Studies, and a Center for Drug Information.

SEE P. 194

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 12, 2006 VOL. 354 NO. 2

Spectrum of Disease and Relation to Place of Exposure among Ill Returned Travelers

David O. Freedman, M.D., Leisa H. Weld, Ph.D., Phyllis E. Kozarsky, M.D., Tamara Fisk, M.D.,* Rachel Robins, M.D., Frank von Sonnenburg, M.D., Jay S. Keystone, M.D., Prativa Pandey, M.D., and Martin S. Cetron, M.D., for the GeoSentinel Surveillance Network†

ABSTRACT

BACKGROUND

Approximately 8 percent of travelers to the developing world require medical care during or after travel. Current understanding of morbidity profiles among ill returned travelers is based on limited data from the 1980s.

METHODS

Thirty GeoSentinel sites, which are specialized travel or tropical-medicine clinics on six continents, contributed clinician-based sentinel surveillance data for 17,353 ill returned travelers. We compared the frequency of occurrence of each diagnosis among travelers returning from six developing regions of the world.

RESULTS

Significant regional differences in proportionate morbidity were detected in 16 of 21 broad syndromic categories. Among travelers presenting to GeoSentinel sites, systemic febrile illness without localizing findings occurred disproportionately among those returning from sub-Saharan Africa or Southeast Asia, acute diarrhea among those returning from south central Asia, and dermatologic problems among those returning from the Caribbean or Central or South America. With respect to specific diagnoses, malaria was one of the three most frequent causes of systemic febrile illness among travelers from every region, although travelers from every region except sub-Saharan Africa and Central America had confirmed or probable dengue more frequently than malaria. Among travelers returning from sub-Saharan Africa, rickettsial infection, primarily tick-borne spotted fever, occurred more frequently than typhoid or dengue. Travelers from all regions except Southeast Asia presented with parasite-induced diarrhea more often than with bacterial diarrhea.

CONCLUSIONS

When patients present to specialized clinics after travel to the developing world, travel destinations are associated with the probability of the diagnosis of certain diseases. Diagnostic approaches and empiric therapies can be guided by these destination-specific differences.

From the Division of Geographic Medicine, University of Alabama at Birmingham, Birmingham (D.O.F.); the Division of Global Migration and Quarantine, Centers for Disease Control and Prevention, Atlanta (L.H.W., P.E.K., R.R., M.S.C.); the Division of Infectious Disease, Emory University, Atlanta (P.E.K., T.F.); the Department of Tropical and Infectious Diseases, University of Munich, Munich, Germany (F.S.); the Division of Infectious Diseases, University of Toronto, Toronto (J.S.K.); and CIWEC Clinic, Kathmandu, Nepal (P.P.). Address reprint requests to Dr. Freedman at the Division of Geographic Medicine, 1530 Third Ave. S., BBRB 203, Birmingham, AL 35294, or at freedman@uab.edu.

* Deceased.

† Members of the GeoSentinel Surveillance Network (GeoSentinel — The Global Surveillance Network of the International Society of Travel Medicine and the Centers for Disease Control and Prevention) are listed in the Appendix.

N Engl J Med 2006;354:119-30.

Health problems are self-reported by 22 to 64 percent of travelers to the developing world[1]; most of these problems are mild, self-limited illnesses such as diarrhea, respiratory infections, and skin disorders. More importantly, each year, up to 8 percent of the more than 50 million travelers to these regions, or 4 million persons, are ill enough to seek health care either while abroad or on returning home.[1-3]

Much of the current understanding of morbidity profiles among ill returned travelers is based on data that were collected in the 1980s.[1-4] Although some morbidity studies have been designed to examine individual diseases,[5-9] specific high-risk destinations,[10] and certain types of travelers,[11-13] a comprehensive, multicenter comparison of the spectrum of illnesses acquired by a broad range of travelers returning from developing regions on all continents has been lacking.[14]

GeoSentinel sites are specialized travel or tropical-medicine clinics on six continents that collect clinician-based surveillance data on travel-related diseases. These data include a broad sample of travel destinations and of morbidity among persons who have become ill while traveling. We used the GeoSentinel database to verify the assumption that the destination of travel is associated with the probability of each diagnosis among travelers returning from the developing world.

Methods

GeoSentinel Sites

The 30 current GeoSentinel sites are specialized travel or tropical-medicine clinics on six continents. They are recruited, on the basis of demonstrated training and experience and a record of publication in travel or tropical medicine, to contribute clinician-based sentinel surveillance data on all ill returned travelers seen (as detailed by the International Society of Travel Medicine, at www.istm.org, and by Freedman et al.[15]). The sites accounting for the majority of patients seen are within academic centers; several smaller-volume sites (almost all with a current academic affiliation) are in freestanding locations. Intake at the sites reflects a mixed population of patients requiring tertiary care and self-referred patients. Some sites are restricted to outpatient care, and at no site is practice limited to the care of ill travelers.

Data Collection

Criteria for Entry into the GeoSentinel Database

Patients must have crossed an international border within 10 years before presentation and have sought medical advice for a presumed travel-related illness. Anonymous surveillance data (including travel history) that could not be linked back to an individual patient were entered into a database at a central data center. Final diagnoses were assigned codes by the treating clinician from a standardized list of 524 possible individual diagnoses that were also categorized under 21 broad syndromes. (Diagnosis codes grouped according to syndrome are listed in the Supplementary Appendix, available with the full text of this article at www.nejm.org.) All sites used the best available reference diagnostic test in their own country. Patients were assigned as many diagnosis codes as needed. Since most infections are associated with fever, diagnoses predominantly localized to one organ system were included in that organ-system syndrome category and were not attributed to the broader category of systemic febrile disease.

The GeoSentinel data-collection protocol was reviewed by the institutional review board officer at the National Center for Infectious Diseases at the Centers for Disease Control and Prevention and classified as public health surveillance and not as human-subjects research requiring submission to institutional review boards.

Eligibility for Analysis

All travelers who presented to a GeoSentinel site after travel to the developing world were eligible for analysis. Patients with diagnoses that were either laboratory-confirmed or deemed probable were included in the analysis. Eligible patients who were ultimately found after medical assessment to have no underlying disease (1942 patients) and those found to have final diagnoses that were not confirmed or probable (1518 patients) were excluded.

Comparative Morbidity According to Travel Destination

We analyzed the six developing regions of the world where the majority of these illnesses were acquired (Fig. 1). Recent, temporally clustered travel to more than one country within a region often makes it difficult to attribute the illness to a spe-

cific country. The place of exposure during travel was therefore determined to be the single region visited or, for travelers who entered more than one region, was determined according to the data field for "most likely place of exposure" if that information could be designated as certain by the clinician by virtue of incubation period or known patterns of endemicity (Fig. 1). To calculate a cumulative morbidity profile for all ill returned travelers, data from patients for whom a single region of exposure could be determined were grouped and then combined with data for ill travelers to multiple regions in whom ascertainment of the relevant place of exposure was impossible.

STATISTICAL ANALYSIS

The data analysis was performed with the use of SAS software, version 8 (SAS Institute). The primary variable analyzed was proportionate morbidity, calculated as the number of patients with a specific diagnosis or group of diagnoses as a proportion of all ill travelers returning from a developing region. All results are given in terms of the number of patients per 1000 patients in the group. Analyses of specific etiologic organisms according to region were performed only within an individual syndrome group. This analytic approach prevented a very frequent diagnosis within a region, such as malaria among travelers to sub-Saharan Africa, from overwhelming the contributions of various causes associated with other syndrome groups, such as diarrhea, that supplied proportionately fewer cases. Chi-square tests were used to compare these proportions among regions. Because of the large numbers of statistical tests performed, a conservative two-sided significance level of $P<0.01$ was chosen.

RESULTS

OVERALL MORBIDITY

The characteristics of 17,353 patients whose data were reported to GeoSentinel from June 1996 through August 2004 and who met the inclusion criteria are shown in Table 1. These patients, with travel exposures in 230 countries, presented to GeoSentinel sites located in Europe (49 percent), the United States or Canada (33 percent), Israel (8 percent), Australia or New Zealand (8 percent), and other sites (3 percent). More than 50 percent of the ill travelers had obtained documented advice from a medical provider before traveling. Diagnoses for 67.0 percent of all the returned travelers fell into 4 of 21 major syndrome categories:

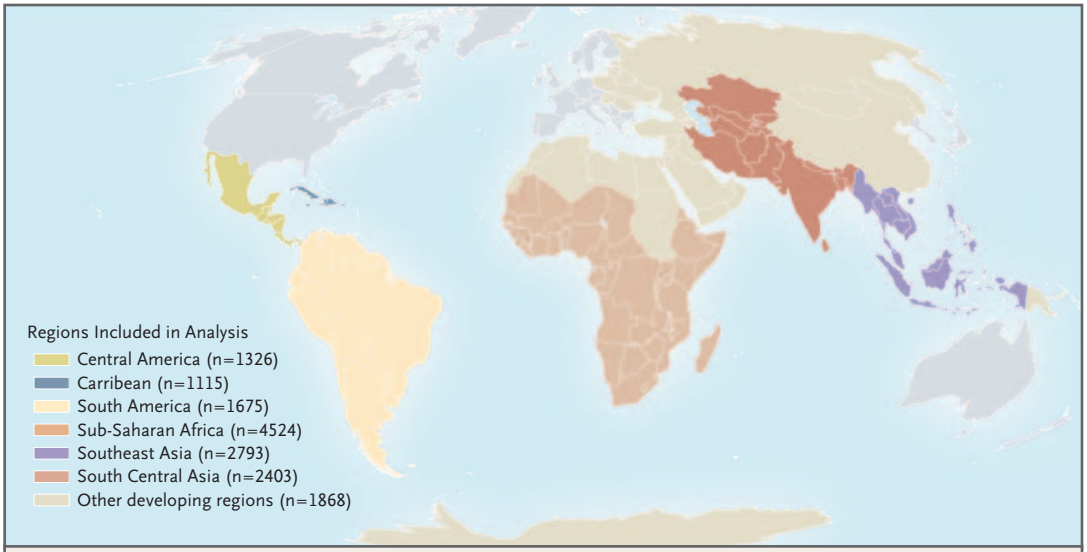


Figure 1. Regions of Travel Exposure among Ill Travelers Returning from the Developing World.

Data for 17,353 patients who presented to a GeoSentinel site were analyzed. (Ascertainment of exposure within a single developing region was not possible for 1649 travelers.) Other developing regions were West Asia, Northeast Asia, eastern Europe, Oceania, North Africa, and Antarctica.

Table 1. **Characteristics of Ill Travelers Returning from the Developing World, According to Region Visited.***

Variable	All Regions	Caribbean	Central America	South America	Sub-Saharan Africa	South Central Asia	Southeast Asia	Other or Multiple Regions†
No. of travelers	17,353	1115	1326	1675	4524	2403	2793	3517
Age (yr)								
Median	33	37	32	30	34	32	32	35
Interquartile range	26–45	27–50	24–45	23–42	27–45	25–45	25–42	27–49
Duration of most recent travel (days)								
Median	23	14	18	35	28	30	24	18
Interquartile range	14–60	7–19	9–42	20–120	14–61	17–70	15–42	10–49
Time from travel to presentation at clinic (% of travelers)‡								
≤1 mo	64	59	61	61	70	71	72	52
>1–2 mo	11	14	12	13	10	9	9	11
>2–6 mo	15	19	19	17	13	13	12	20
>6 mo	10	8	8	9	7	8	7	17
Female sex (% of travelers)‡	48	54	52	45	44	50	47	51
Reason for travel (% of travelers)‡								
Tourism	59	75	66	56	43	67	74	59
Business	14	8	8	11	17	13	11	19
Research or education	4	1	6	4	6	3	3	4
Missionary or volunteer purpose	8	4	11	15	11	3	5	7
Visit to friends or relatives	15	12	8	14	24	14	8	12
Documented pretravel health advice (% of travelers)‡	55	30	48	62	59	61	62	46
Inpatient care (% of travelers)‡	11	5	4	6	18	10	11	11

* Percentages may not total 100 because of rounding.
† This category includes travel to West Asia, Northeast Asia, eastern Europe, Oceania, North Africa, or Antarctica (1868 travelers) or to multiple developing regions, for which ascertainment of exposure was impossible (1649 travelers).
‡ P<0.01 for the comparison among regions.

systemic febrile illness, acute diarrhea, dermatologic disorders, and chronic diarrhea (Table 2). The median duration of the most recent trip ranged from 14 days for the Caribbean to 35 days for South America. Although most of the patients (64 percent) were seen within a month after travel, 10 percent had indolent diseases or diseases with a long incubation period and were not seen until more than six months after travel (Table 1). The effects of pretravel care were not measured.

In this study, diagnoses were classified as confirmed at the following rates: malaria, 98.2 percent; campylobacter infection, 98.1 percent; shigella infection, 100 percent; cyclospora infection, 100 percent; dengue, 82.6 percent; and tickborne rickettsial disease, 62.6 percent. Overall, for specific infectious causes, 90.3 percent were classified as confirmed, suggesting appropriate rigor in the use of the "probable" classification.

DESTINATION ANALYSIS

For 13,836 of the ill returned travelers (80 percent), the relevant place of exposure could be determined to be one of the six major regions that receive travelers in the developing world. Most of the patients had traveled as tourists, except for travelers to sub-Saharan Africa, which had the highest proportions of business travelers and travelers visiting friends and relatives (Table 1). Unlike those returning from all other regions, less than a third of ill persons returning from the Caribbean had received advice before traveling.

Significant differences in proportionate morbidity (P<0.01) were seen among the travel regions

Table 2. **Diagnosis According to Syndrome Group and Travel Region among Ill Travelers Returning from the Developing World.***

Diagnosis	All Regions (N=17,353)	Caribbean (N=1115)	Central America (N=1326)	South America (N=1675)	Sub-Saharan Africa (N=4524)	South Central Asia (N=2403)	Southeast Asia (N=2793)	Other or Multiple Regions (N=3517)†
	number of cases per 1000 patients							
Systemic febrile illness‡	226	166	153	143	371	171	248	145
Acute diarrhea‡	222	196	234	219	167	327	210	238
Dermatologic disorder‡	170	261	225	264	127	130	212	125
Chronic diarrhea‡	113	132	173	130	57	129	97	149
Nondiarrheal gastrointestinal disorder‡	82	87	75	82	70	74	58	121
Respiratory disorder‡	77	45	49	50	77	89	97	86
Nonspecific symptoms or signs‡	70	53	51	59	75	85	63	77
Genitourinary disorder‡	35	29	11	27	51	25	29	40
Asymptomatic parasitic infection‡	30	15	26	33	29	44	30	24
Underlying chronic disease‡	19	14	23	18	20	14	13	27
Injury‡	14	23	11	14	7	15	14	21
Neurologic disorder‡	15	23	24	16	10	15	10	16
Adverse drug or vaccine reaction‡	12	4	5	5	26	12	8	8
Psychological disorder‡	12	8	20	15	8	12	10	18
Tissue parasite‡	10	5	5	11	22	4	3	7
Cardiovascular disorder	8	12	7	5	8	7	5	10
Obstetrical or gynecologic disorder	3	3	2	2	4	3	3	3
Ophthalmologic disorder	2	2	2	2	2	1	1	2
Dental problem	1	1	1	1	1	0	2	1
Death	1	1	0	0	1	3	0	1
Loss to follow-up‡	8	9	12	9	8	5	4	13

* Diagnoses included in each syndrome category are listed in the Supplementary Appendix. Numbers may not total 1000 because patients may have had more than one diagnosis.
† This category includes travel to West Asia, Northeast Asia, eastern Europe, Oceania, North Africa, or Antarctica (1868 travelers) or to multiple developing regions, for which ascertainment of exposure was impossible (1649 travelers).
‡ P<0.01 for the comparison among regions.

for 16 of the 21 syndrome categories (Table 2). In particular, systemic febrile illness was found disproportionately among patients presenting to GeoSentinel sites after travel to sub-Saharan Africa or Southeast Asia and acute diarrhea among those presenting after travel to south central Asia. Dermatologic disorders were seen disproportionately less commonly among travelers returning from sub-Saharan Africa or south central Asia. Diagnoses contributing to death included severe and complicated malaria, pulmonary embolism, pneumonia, and pyogenic abscess.

REGIONAL MORBIDITY ACCORDING TO SPECIFIC DIAGNOSIS

Destination-specific variations in the proportionate morbidity associated with etiologic diagnoses within each of the top four syndromic categories are presented in Table 3. Etiologic diagnoses were not commonly ascertained for chronic diarrhea, so they are not listed.

Overall, malaria was the most frequent cause of systemic febrile illness without localizing organ-system findings among ill travelers returning from the developing world (Table 3). In addition, for each of the six geographic regions, malaria was one of the three most frequent specific causes of systemic febrile illness among travelers, and it was the predominant cause of systemic febrile illness among those presenting after travel to sub-Saharan Africa. Travelers with dengue presented more frequently than did those with malaria for every region except sub-Saharan Africa and Central America. Rickettsial infections, primarily tick-borne spotted fever, appeared almost exclusively among travelers returning from sub-Saharan Africa, and typhoid fever was a primary contributor to systemic febrile illness among travelers returning from south central Asia.

For all regions except Southeast Asia, parasite-induced diarrhea was more common among ill returned travelers than was bacterial diarrhea. Patients with bacterial diarrhea presented most commonly after travel in Southeast Asia; campylobacter was the predominant cause. Of the parasitic causes, giardiasis was reported disproportionately among travelers returning from south central Asia.

Overall, insect bites were the most common cause of dermatologic problems, followed by cutaneous larva migrans, allergic reactions, and skin abscesses. However, cutaneous larva migrans was the most common dermatologic disorder among patients presenting after travel to the Caribbean, among whom it was seen much more commonly than among those returning from sub-Saharan Africa, south central Asia, or Central or South America. Bacterial skin infections, including skin abscesses, were found more commonly among patients returning from sub-Saharan Africa, south central Asia, or Southeast Asia than among those returning from the other three regions. Leishmaniasis was found mostly among patients who had traveled to South America or, to a lesser extent, Central America. Myiasis was reported most frequently among patients who had traveled to South America or Central America.

Travelers returning from each of the regions presented with intestinal nematode infestations, primarily involving ascaris and intestinal strongyloides. Of all ill returned travelers who presented with nondiarrheal gastrointestinal diagnoses, 40 per 1000 seen at our sites were reported to have acute hepatitis A, 20 per 1000 hepatitis B, 6 per 1000 hepatitis C, 13 per 1000 hepatitis E, and 36 per 1000 unspecified hepatitis. The sample size was too small to allow us to assess regional differences for hepatitis. Schistosomiasis (due to *Schistosoma mansoni* or *S. haematobium*) was seen predominantly among travelers returning from sub-Saharan Africa (196 per 1000). Acute brucellosis, leptospirosis, cysticercosis, filariasis, histoplasmosis, and echinococcosis occurred too infrequently to allow comparison among regions.

RARE DIAGNOSES

Clinicians evaluating returned travelers frequently entertain rare or exotic diagnoses. Travel-related cases of Ebola virus disease, Japanese encephalitis, rabies, tetanus, diphtheria, plague, tularemia, murine typhus, Rift Valley fever, poliomyelitis, primary amebic meningoencephalitis, anthrax, or yellow fever are reported sporadically in the literature. No cases of any of these diagnoses occurred among the 17,353 travelers whose data were analyzed in this study or among any of the 25,023 patients whose records were included in any category in our database but were excluded from this study. Among the 17,353 patients in our cohort, each of the following diagnoses occurred only once: *Angiostrongylus cantonensis* infestation, *A. costaricensis* infestation, hantavirus infection, cholera, melioidosis, Ross River virus

infection, African trypanosomiasis, legionellosis, and meningococcal meningitis.

PROPORTIONATE MORBIDITY ACCORDING TO REGION

A summary profile of proportionate morbidity among ill returned travelers according to diagnosis or diagnosis group, expressed in terms of the proportion per 1000 ill returned travelers, is presented in Figure 2. Shown is the proportion, not the incidence rate, of each of the top 22 specific diagnoses among all ill returned travelers and among travelers returning from each of the regions.

DISCUSSION

Our database represents a large sample of ill travelers returning from the developing world. GeoSentinel sites are located primarily within academic centers, so some of the patients seen at each site present on the basis of consultations or outside referrals. Patients with imported illnesses that are neither self-limited nor mild are generally seen at such practices at some point during their care. At the same time, these practices are also the initial point of entry for many returned travelers who had pretravel medical preparation at the same clinic or for those who are affiliated with corporations, religious organizations, aid agencies, or governmental entities with which the clinic has an ongoing relationship or contractual arrangement. With significant growth in international travel, a travel or tropical-medicine clinic or infectious-diseases practice generally emerges in a community as the local specialized resource for providers and as the primary entry point for increasingly sophisticated travelers. Our data provide a reference for likely diagnoses, stratified according to destination, among travelers seeking care at or referred to such practices.

The data do not represent a comprehensive epidemiologic analysis of all illness in all travelers. Similarly, they do not represent a sample of illnesses in returned travelers such as those who would be seen at a nonspecialized, primary care practice, where mild or self-limited conditions would occur with higher frequency. Diagnoses with frequencies that may be underrepresented in GeoSentinel include diseases with a short incubation period, such as dengue; many cases of such diseases manifest during travel, so cases reported in returned travelers represent those acquired at the end of a trip. Finally, the small proportion of returned travelers with severe acute disease who need immediate hospitalization and are initially admitted through an emergency department may be underrepresented if the diagnosis is not considered related to travel or if care was sought outside the network. Travelers to destinations self-perceived to be particularly risky may present to a specialized unit earlier or with milder illness than travelers to more familiar destinations. Our data do not allow us to estimate incidence rates or to provide numerical risk for travel to particular destinations.

On an aggregate basis, the GeoSentinel database is a sample of illnesses acquired during exposure in 230 countries by sentinel travelers presenting in 13 countries for care. Nevertheless, biases for certain behavior-related diagnoses probably exist because of the geographic distribution and proportionate contributions of the individual sites. Patient intake at each site reflects local or national differences in the makeup of the traveling population, the distribution of travel destinations, and access to medical care. In addition, accommodations, eating habits, and other risk behaviors at a given destination may reflect the national and cultural background of the traveler. European travelers are more heavily represented in the sample than are North Americans (49 percent vs. 33 percent), and those from other regions (18 percent) are relatively underrepresented. Travel-related sexually transmitted diseases (proportionate morbidity, 8 per 1000 patients) and infections with the human immunodeficiency virus (2 per 1000 patients) were reported, but they are probably underrepresented in tropical-disease units; persons with self-recognized risk exposures or characteristic genitourinary symptoms may seek care in other settings.

At the same time, the benefit of a multicenter perspective over smaller, single-site studies, which have often reflected local patterns of travel, is demonstrated by the analysis of dermatologic disease. The most widely cited study of dermatologic disorders in travelers included 269 persons returning to France.[16] In that study, more than 60 percent of the patients had returned from travel to francophone countries of the Caribbean (Martinique and Guadeloupe), from French Guiana, and from sub-Saharan Africa. The finding that cutaneous larva migrans, pyodermas, and arthropod bites were among the top diagnoses agrees with

Table 3. **Etiologic Diagnoses within Selected Syndrome Groups, According to Travel Region.***

Syndrome and Cause	All Regions	Caribbean	Central America	South America	Sub-Saharan Africa	South Central Asia	Southeast Asia	Other or Multiple Regions†
	number of cases per 1000 patients with syndrome							
Systemic febrile illness (n=3907)								
Specific pathogen or cause reported‡	594	459	527	446	718	522	547	454
Malaria‡	352	65	133	133	622	139	130	234
Dengue‡	104	238	123	138	7	142	315	35
Mononucleosis (due to Epstein–Barr virus or cytomegalovirus)‡	32	70	69	79	10	17	32	63
Rickettsial infection‡	31	0	0	0	56	10	16	24
Salmonella typhi or *S. paratyphi* infection‡	29	22	25	17	7	141	26	24
No specific cause reported‡	406	541	473	554	282	478	453	546
Acute diarrhea (n=3859)								
Parasitic diarrhea‡	354	283	403	368	353	453	262	323
Giardiasis‡	173	132	136	158	177	286	118	132
Amebiasis‡	120	105	155	142	138	103	74	135
Presumptive parasitic cause‡	35	9	45	52	33	55	33	13
Bacterial diarrhea‡	268	260	190	253	250	294	369	227
Campylobacter infection‡	85	46	32	90	73	87	180	57
Shigella infection	41	37	26	41	46	61	26	34
Nontyphoidal salmonella infection‡	27	27	13	14	29	12	56	30
Presumptive bacterial cause	110	132	94	106	99	136	116	95
Viral diarrhea‡§	9	23	32	5	7	4	5	7
Unspecified acute diarrhea‡	385	457	377	376	397	289	393	451

Dermatologic disorder (n=2947)								
Insect bite, with or without superinfection	187	192	235	156	194	201	179	166
Cutaneous larva migrans‡	129	299	134	122	86	64	171	68
Allergic rash or reaction	113	148	128	97	105	112	93	132
Skin abscess‡	97	34	47	50	136	144	122	105
Rash of unknown cause	66	55	74	75	66	48	49	96
Mycosis, superficial	56	45	30	36	65	64	61	77
Animal bite requiring rabies postexposure prophylaxis‡	47	3	13	25	9	90	124	4
Leishmaniasis‡	38	0	64	143	14	19	0	36
Myiasis‡	35	0	101	100	40	0	0	14
Swimmer's itch‡§	28	3	0	2	117	3	9	14
Impetigo or erysipelas§	27	31	20	9	31	45	22	34
Mite infestation (e.g., scabies)§	22	21	37	39	12	29	17	14
Nondiarrheal gastrointestinal disorder (n=1421)								
Intestinal nematode infestation‡	239	278	273	256	307	202	344	141
Strongyloidiasis, simple intestinal‡	96	124	141	102	148	45	160	37
Ascaris infestation§	52	52	30	66	60	84	18	46
Gastritis or peptic ulcer disease‡	131	258	91	168	85	101	104	156
Helicobacter pylori status unknown	76	124	51	73	60	62	74	91
Positive for *H. pylori*‡§	47	103	40	80	22	28	25	60
Acute hepatitis‡	115	62	91	102	76	214	61	144
Hemorrhoids or constipation‡	89	124	192	117	54	84	74	84

* Numbers may not total 1000 because patients may have had more than one diagnosis. The most common diagnoses are listed for each category.
† This category includes travel to West Asia, Northeast Asia, eastern Europe, Oceania, North Africa, or Antarctica (1868 travelers) or ascertainment of exposure impossible subsequent to travel to multiple developing regions (1649 travelers).
‡ P<0.01 for the comparison among regions.
§ This diagnosis was listed in fewer than 100 reports.

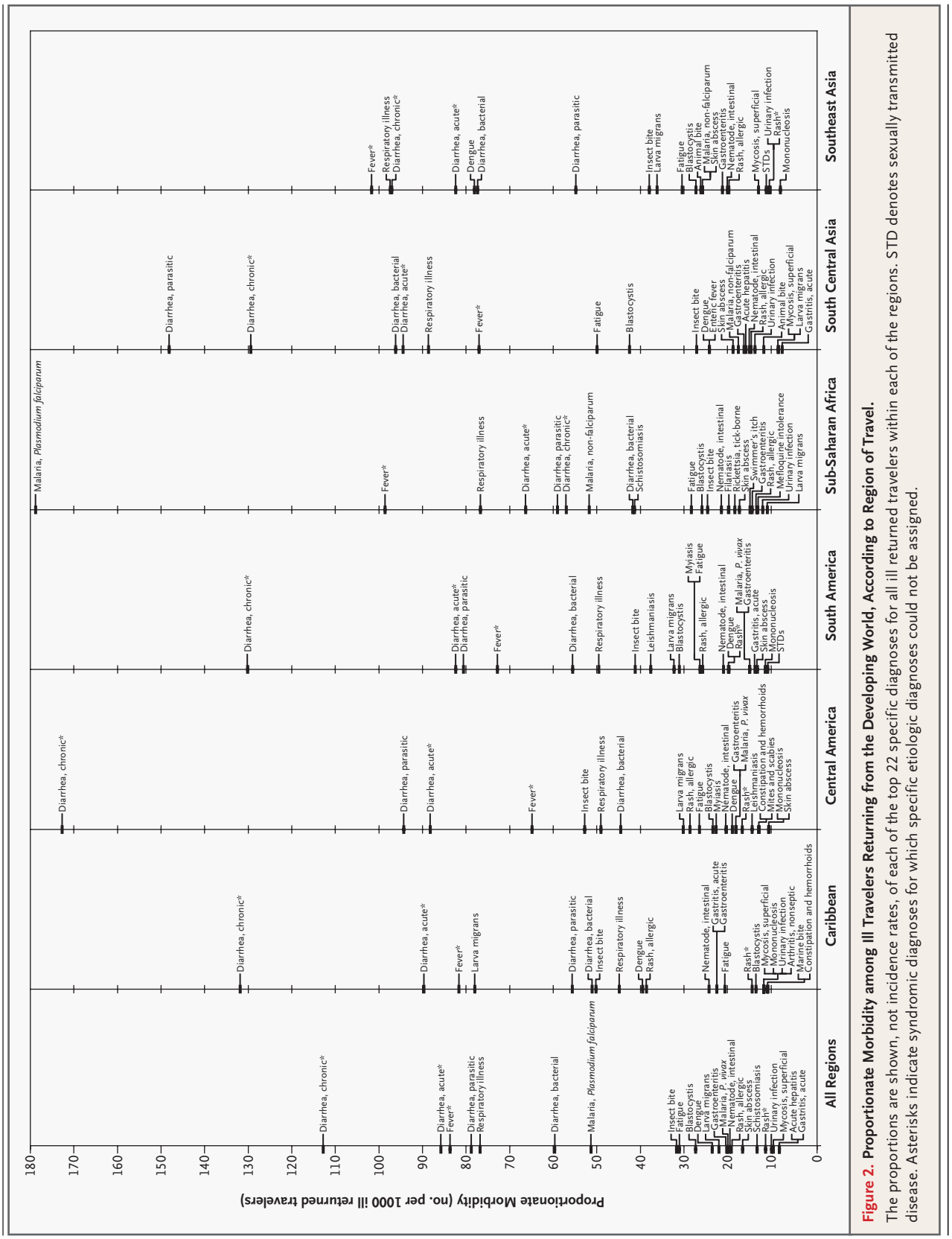


Figure 2. Proportionate Morbidity among Ill Travelers Returning from the Developing World, According to Region of Travel.

The proportions are shown, not incidence rates, of each of the top 22 specific diagnoses for all ill returned travelers within each of the regions. STD denotes sexually transmitted disease. Asterisks indicate syndromic diagnoses for which specific etiologic diagnoses could not be assigned.

our own overall findings in all ill travelers. However, the significant destination-specific differences that we are able to describe for conditions such as cutaneous larva migrans, leishmaniasis, myiasis, swimmer's itch, and animal bites were not assessed in the earlier study.

The geographic dispersion of our sites, the full range of possible diagnoses, and the range of diagnostic tests necessary to identify the causes of tropical diseases preclude centralized laboratory testing for all patients. We used the best available national reference diagnostics for confirmed diagnoses. Probable diagnoses were restricted to patients who had an indisputable physical finding (e.g., tickborne rickettsiosis, cutaneous larva migrans, myiasis, or tungiasis), a response to a highly specific therapeutic agent, or a classic clinical presentation and exposure history, with other possible causes definitively ruled out by laboratory analysis. The last situation is particularly applicable to dengue, for which results of serologic tests are often difficult to obtain immediately and are often negative during the acute illness at the time of clinical presentation; in addition, patients with dengue often do not return for follow-up when faced with the cost of expensive diagnostic evaluation of a self-limited illness, particularly when the symptoms have resolved.

Codes available for a full range of syndromic diagnoses were used to classify patients consistently when a specific etiologic diagnosis was not assignable. Reasons included the frequency of self-limited acute infections and the effectiveness of empiric treatment or self-treatment for many syndromes, as compared with the expense and difficulty of diagnostic evaluation for exotic agents. For the syndromic groups of acute diarrhea and systemic febrile illness, specific etiologic diagnoses were not made in 40 percent or more of cases. Other studies have also found similarly high rates of undiagnosed fever[17,18] and diarrhea[19] in similar patients. Nonetheless, the clinicians in our study are experienced in disease recognition and diagnosis and have access to usually available diagnostic methods; the treatable causes of illness that are routinely sought in an initial clinic visit would probably not have been missed.

Our data on diarrheal illness illustrate important differences among studies that evaluate illness occurring during travel as compared with that seen after travel. All etiologic studies of travelers' diarrhea performed in the visited country during travel have shown a predominance of bacterial causes and a relative paucity of parasitic causes.[19-21] With respect to post-travel illness seen at our sites, diarrhea caused by parasites predominated over bacterial diarrhea in the overall analysis of ill returned travelers and in the analysis of every individual region except Southeast Asia. The likely explanation is that parasite-induced diarrhea tends to have a longer incubation period than bacterial diarrhea and to be more chronic and less treatable by the empirical antibiotic agents carried by travelers or prescribed at an initial medical encounter. Similar considerations most likely apply to other syndrome groups as well.

The high frequency of rickettsial disease reflects the emergence of *Rickettsia africae* in recent years in southern Africa, as has been reported in several single-site studies.[22] Dengue in travelers is well reported in studies from the 1980s onward, but in contrast to earlier data, it now appears to occur more frequently than malaria among travelers returning from any region except Africa and Central America. This change may reflect the use of effective antimalarial chemoprophylaxis by travelers. The proportionate morbidity associated with dengue is especially high among travelers returning from Southeast Asia and the Caribbean.

The current data provide a reference for likely diagnoses in returned travelers, with stratification according to destination but not according to prophylaxis designed before travel. The profile of proportionate morbidity generated by these data is important for guiding post-travel diagnosis and empiric therapy as well as for prioritizing pretravel intervention strategies.

Supported by a cooperative agreement (U50/CCU412347) from the Centers for Disease Control and Prevention (CDC), Atlanta, and by an initial pilot grant from the International Society of Travel Medicine (both to GeoSentinel, the global surveillance network of the International Society of Travel Medicine and of the CDC).

Dr. Freedman reports having received consulting fees from Shoreland, Sanofi Pasteur, GlaxoSmithKline, and Salix Pharmaceuticals and owning equity in Shoreland. Dr. Weld reports owning equity in Amgen and Chiron. Dr. Kozarsky reports having received consulting fees from Berna Products and lecture fees from GlaxoSmithKline. Dr. Keystone reports having received consulting fees from Sanofi Pasteur and GlaxoSmithKline and speaking fees from Roche and GlaxoSmithKline. No other potential conflict of interest relevant to this article was reported.

The views expressed in this article are those of the authors and do not necessarily represent those of the CDC.

We are indebted to Elena Axelrod, Indira Srinivasan, Sue Baldwin, Christina Bernadotte, Raj Srinivas, and members of the staff at each GeoSentinel site for data management or computer programming; and to Brenda Bagwell and Adam Plier for ongoing administrative support.

APPENDIX

In addition to the authors, members of the GeoSentinel Surveillance Network include the following: *Kaiser Permanente, Honolulu* — V. Ansdell (Oct. 1997 to Jan. 2003 only); *Boston University, Boston* — E. Barnett; *Royal Melbourne Hospital, Melbourne, Australia* — G. Brown and J. Torresi; *University of Brescia, Brescia, Italy* — G. Carosi and F. Castelli; *Harvard University, Cambridge, Mass.* — L. Chen and M. Wilson; *Cornell University, New York* — B. Connor; *Hôpital Nord, Marseille, France* — J. Delmont and P. Parola; *Mount Sinai Medical Center, New York* — A. Gurtman; *University of Utah, Salt Lake City* — D. Hale and S. Gelman; *Hudson River Health Care, Peekskill, N.Y.* — N. Piper-Jenks; *University of Washington, Seattle* — E. Jong; *Travellers Medical and Vaccination Centres of Australia, Adelaide* — R. Kass (Dec. 1997 to March 2001 only); *University of Toronto, Toronto* — K. Kain; *Orlando Regional Health Center, Orlando, Fla.* — C. Licitra; *University of Geneva, Geneva* — L. Loutan and F. Chappuis; *Fresno International Travel Medical Center, Fresno, Calif.* — M. Lynch; *Tulane University, New Orleans* — S. McLellan; *Travel Clinic Services, Johannesburg, South Africa* — R. Muller; *National Institutes of Health, Bethesda, Md.* — T. Nutman and A. Klion; *Catholic University of Chile, Santiago* — C. Perret and F. Valdivieso; *Johns Hopkins University, Baltimore* — B. Sack and R. MacKenzie; *Sheba Medical Center, Tel Hashomer, Israel* — E. Schwartz; *Travellers Health and Vaccination Centre, Auckland, New Zealand* — M. Shaw; *University of Zurich, Zurich, Switzerland* — R. Steffen and P. Schlagenhauf; *Albert Einstein School of Medicine, Bronx, N.Y.* — M. Wittner; and *Royal Free Hospital, London* — J. Zuckerman (Sept. 2000 to May 2003 only).

REFERENCES

1. Steffen R, deBernardis C, Banos A. Travel epidemiology — a global perspective. Int J Antimicrob Agents 2003;21:89-95.

2. Steffen R, Rickenbach M, Wilhelm U, Helminger A, Schar M. Health problems after travel to developing countries. J Infect Dis 1987;156:84-91.

3. Hill DR. Health problems in a large cohort of Americans traveling to developing countries. J Travel Med 2000;7:259-66.

4. Reid D, Cossar JH. Epidemiology of travel. Br Med Bull 1993;49:257-68.

5. Caumes E, Ehya N, Nguyen J, Bricaire F. Typhoid and paratyphoid fever: a 10-year retrospective study of 41 cases in a Parisian hospital. J Travel Med 2001;8:293-7.

6. Shah S, Filler S, Causer LM, et al. Malaria surveillance — United States, 2002. MMWR Surveill Summ 2004;53:21-34.

7. Raoult D, Fournier PE, Fenollar F, et al. Rickettsia africae, a tick-borne pathogen in travelers to sub-Saharan Africa. N Engl J Med 2001;344:1504-10.

8. Whitty CJ, Mabey DC, Armstrong M, Wright SG, Chiodini PL. Presentation and outcome of 1107 cases of schistosomiasis from Africa diagnosed in a non-endemic country. Trans R Soc Trop Med Hyg 2000; 94:531-4.

9. Jelinek T. Dengue fever in international travelers. Clin Infect Dis 2000;31:144-7.

10. Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BL, Echeverria P. Etiology of diarrhea among travelers and foreign residents in Nepal. JAMA 1988;260: 1245-8.

11. Liese B, Mundt KA, Dell LD, Nagy L, Demure B. Medical insurance claims associated with international business travel. Occup Environ Med 1997;54:499-503.

12. Herwaldt BL, de Arroyave KR, Roberts JM, Juranek DD. A multiyear prospective study of the risk factors for and incidence of diarrheal illness in a cohort of Peace Corps volunteers in Guatemala. Ann Intern Med 2000;132:982-8.

13. Patel D, Easmon CJ, Dow C, Snashall DC, Seed PT. Medical repatriation of British diplomats resident overseas. J Travel Med 2000;7:64-9.

14. Ryan ET, Wilson ME, Kain KC. Illness after international travel. N Engl J Med 2002;347:505-16.

15. Freedman DO, Kozarsky PE, Weld LH, Cetron MS. GeoSentinel: the global emerging infections sentinel network of the International Society of Travel Medicine. J Travel Med 1999;6:94-8.

16. Caumes E, Carriere J, Guermonprez G, Bricaire F, Danis M, Gentilini M. Dermatoses associated with travel to tropical countries: a prospective study of the diagnosis and management of 269 patients presenting to a tropical disease unit. Clin Infect Dis 1995;20:542-8.

17. Doherty JF, Grant AD, Bryceson AD. Fever as the presenting complaint of travellers returning from the tropics. QJM 1995;88:277-81.

18. Humar A, Keystone J. Evaluating fever in travellers returning from tropical countries. BMJ 1996;312:953-6.

19. Steffen R, Collard F, Tornieporth N, et al. Epidemiology, etiology, and impact of traveler's diarrhea in Jamaica. JAMA 1999; 281:811-7.

20. Hoge CW, Shlim DR, Echeverria P, Rajah R, Herrmann JE, Cross JH. Epidemiology of diarrhea among expatriate residents living in a highly endemic environment. JAMA 1996;275:533-8.

21. DuPont HL, Ericsson CD. Prevention and treatment of traveler's diarrhea. N Engl J Med 1993;328:1821-7.

22. Jensenius M, Fournier PE, Vene S, et al. African tick bite fever in travelers to rural sub-Equatorial Africa. Clin Infect Dis 2003; 36:1411-7.

ORIGINAL ARTICLE

Efficacy and Safety of Benazepril for Advanced Chronic Renal Insufficiency

Fan Fan Hou, M.D., Ph.D., Xun Zhang, M.D., Guo Hua Zhang, M.D., Ph.D., Di Xie, M.D., Ping Yan Chen, M.D., Wei Ru Zhang, M.D., Ph.D., Jian Ping Jiang, M.D., Min Liang, M.D., Ph.D., Guo Bao Wang, M.D., Zheng Rong Liu, M.D., and Ren Wen Geng, M.D.

ABSTRACT

BACKGROUND

Angiotensin-converting–enzyme inhibitors provide renal protection in patients with mild-to-moderate renal insufficiency (serum creatinine level, 3.0 mg per deciliter or less). We assessed the efficacy and safety of benazepril in patients without diabetes who had advanced renal insufficiency.

METHODS

We enrolled 422 patients in a randomized, double-blind study. After an eight-week run-in period, 104 patients with serum creatinine levels of 1.5 to 3.0 mg per deciliter (group 1) received 20 mg of benazepril per day, whereas 224 patients with serum creatinine levels of 3.1 to 5.0 mg per deciliter (group 2) were randomly assigned to receive 20 mg of benazepril per day (112 patients) or placebo (112 patients) and then followed for a mean of 3.4 years. All patients received conventional antihypertensive therapy. The primary outcome was the composite of a doubling of the serum creatinine level, end-stage renal disease, or death. Secondary end points included changes in the level of proteinuria and the rate of progression of renal disease.

RESULTS

Of 102 patients in group 1, 22 (22 percent) reached the primary end point, as compared with 44 of 108 patients given benazepril in group 2 (41 percent) and 65 of 107 patients given placebo in group 2 (60 percent). As compared with placebo, benazepril was associated with a 43 percent reduction in the risk of the primary end point in group 2 (P=0.005). This benefit did not appear to be attributable to blood-pressure control. Benazepril therapy was associated with a 52 percent reduction in the level of proteinuria and a reduction of 23 percent in the rate of decline in renal function. The overall incidence of major adverse events in the benazepril and placebo subgroups of group 2 was similar.

CONCLUSIONS

Benazepril conferred substantial renal benefits in patients without diabetes who had advanced renal insufficiency. (ClinicalTrials.gov number, NCT00270426.)

From the Renal Division, Nanfang Hospital, Southern Medical University, Guangzhou, China. Address reprint requests to Dr. Hou at the Renal Division, Nanfang Hospital, 1838 N. Guangzhou Ave., Guangzhou 510515, China, or at ffhou@public.guangzhou.gd.cn.

N Engl J Med 2006;354:131-40.

Angiotensin-converting–enzyme (ACE) inhibitors slow the progression of chronic kidney disease in the presence or absence of diabetes, particularly in patients with mild-to-moderate renal insufficiency, as reflected by a serum creatinine level of 1.5 to 3.0 mg per deciliter (133 to 265 μmol per liter).[1-3] Data, however, have been limited to patients with serum creatinine levels of 3.0 mg per deciliter or less. Many physicians are reluctant to use ACE inhibitors in patients with advanced renal insufficiency because of concern that serum creatinine or potassium levels will rise.[4,5] Many infer that the unique benefits of these drugs in patients with advanced renal failure might not be superior to those afforded by blood-pressure control alone.[4] As a result, patients with advanced chronic kidney disease have not been included in prospective, large-scale clinical trials of ACE inhibitors.[1,6] Consequently, the renal outcome and the risk–benefit profile of ACE inhibitors in this population remain poorly defined.

In China, the primary cause of end-stage renal disease is nondiabetic chronic kidney disease.[7,8] In 1999, 41,000 registered patients were receiving dialysis, accounting for 5 percent of the total population requiring renal-replacement therapy.[7] Thus, therapy that could delay the progression of chronic kidney disease to end-stage renal disease is an essential management goal. Our previous open-label study involving a small number of patients with advanced chronic renal disease, some with and some without diabetes, suggested that benazepril, a nonsulfhydryl-containing ACE inhibitor, may confer renal benefits.[9] The present randomized, double-blind study was designed to determine whether benazepril could slow the progression of renal dysfunction in patients without diabetes who had advanced renal insufficiency.

METHODS

STUDY DESIGN

The study was conducted at the Nanfang Hospital Renal Division, a center of kidney disease care in southern China. The catchment area of the center includes eight cities near Guangzhou with a total population of 29.8 million as of 2000. The principal investigator and the steering committee designed the study and wrote the article. An adjudicating committee, whose members were unaware of patients' treatment assignments, reviewed the data to determine which patients had reached study end points; this committee also evaluated safety. The study protocol was approved by the Nanfang Ethics Committee, and all subjects provided written informed consent. A study period of three years was chosen on the basis of the results of previous trials involving patients with nondiabetic chronic kidney disease, which suggested that a three-year follow-up is adequate to assess efficacy.[1,2]

PATIENTS

Between May 1999 and May 2001, we screened consecutive patients with chronic kidney disease who were 18 to 70 years of age. All the patients were Chinese. Eligible patients had not received ACE inhibitors or angiotensin II–receptor antagonists for at least six weeks before screening and met the following inclusion criteria: a serum creatinine level of 1.5 to 5.0 mg per deciliter (133 to 442 μmol per liter) and a creatinine clearance[10] of 20 to 70 ml per minute per 1.73 m^2, with variations of less than 30 percent in the three months before screening; nondiabetic renal disease (as established on the basis of their history and the results of serum biochemical tests and renal biopsy); and persistent proteinuria (defined by urinary protein excretion of more than 0.3 g per day for three or more months without evidence of urinary tract infection or overt heart failure [a New York Heart Association class of III or IV]). Exclusion criteria included an immediate need for dialysis; current treatment with corticosteroids, nonsteroidal antiinflammatory drugs, or immunosuppressive drugs; renovascular disease; myocardial infarction or cerebrovascular accident in the year preceding the trial; connective-tissue disease; and obstructive uropathy.

TREATMENT

Eligible patients were divided into two groups according to their serum creatinine levels: group 1 consisted of patients with serum creatinine levels of 1.5 to 3.0 mg per deciliter, and group 2 consisted of patients with serum creatinine levels of 3.1 to 5.0 mg per deciliter (274 to 442 μmol per liter). All patients entered an eight-week run-in phase during which they received 10 mg of benazepril per day for four weeks under close observation, including weekly measurements of blood

pressure, serum creatinine, and serum potassium. The dose of benazepril was increased to 10 mg twice daily for an additional four weeks if serum creatinine levels remained unchanged or increased less than 30 percent, serum potassium levels remained below 5.6 mmol per liter, and no adverse events were reported. Open-label antihypertensive agents (diuretics, calcium-channel antagonists, alpha- or beta-blockers, or some combination of these medications, excluding ACE inhibitors and angiotensin II–receptor antagonists) were added as necessary to achieve a systolic blood pressure of less than 130 mm Hg and a diastolic blood pressure of less than 80 mm Hg.

After the run-in phase, benazepril was discontinued for three weeks and alternative antihypertensive agents (as described above) were administered as necessary to maintain blood-pressure control. After three weeks, all patients in group 1 received 10 mg of benazepril twice daily. Because of the known renal protective effects of ACE inhibitors in patients with this stage of chronic kidney disease, the ethics committee decided that these patients should not receive placebo. Patients in group 2 were randomly assigned to receive either 10 mg of benazepril twice daily or placebo, along with conventional antihypertensive therapy as required. A computer-generated list, maintained by a party not involved in the conduct of the study, was used for randomization.

A physician who was unaware of the patients' treatment assignments examined each patient every two weeks during the first month and every three months thereafter. At each examination, blood pressure was measured while the patient was seated three to four hours after the administration of the study drug and laboratory measurements were performed to assess whether adverse events had occurred or end points had been reached.

All patients were advised to reduce their salt intake to approximately 5 to 7 g of sodium chloride per day, to eat 0.5 to 0.7 g of protein per kilogram of body weight per day, and to restrict their intake of foods rich in potassium. Dietary compliance was assessed by evaluating daily urinary urea and chloride excretion.

OUTCOME MEASURES

The primary efficacy measure was the time to the first event in the composite end point of a doubling of the serum creatinine level, end-stage renal disease, or death. A doubling of the serum creatinine level was defined as two serum creatinine values obtained at least four weeks apart that were twice the baseline value. End-stage renal disease was defined by the need for long-term dialysis or renal transplantation.

Secondary end points included changes in the rate of urinary protein excretion and the progression of renal disease, as assessed by the reciprocal of the serum creatinine level,[11] creatinine clearance, and the glomerular filtration rate, as calculated with the use of the four-component Modification of Diet in Renal Disease equation incorporating age, race, sex, and serum creatinine level[12-15]: estimated glomerular filtration rate = $186 \times (\text{serum creatinine level [in milligrams per deciliter]})^{-1.154} \times (\text{age [in years]})^{-0.203}$. For women, the product of this equation was multiplied by a correction factor of 0.742.

STATISTICAL ANALYSIS

The sample size was estimated before the study with the use of nQuery Advisor software. Our preliminary study[9] of the treatment of patients with advanced renal dysfunction showed that the two-year rate of the primary end point among patients who were not taking an ACE inhibitor was 60 percent. It was estimated that ACE-inhibitor treatment would reduce this rate to 40 percent. Thus, the enrollment of 100 patients per group would provide the study with a statistical power of 80 percent at a two-sided significance level of 0.05.

The primary and secondary end points were analyzed according to the intention-to-treat principle. A Cox regression model was used to determine the hazard ratio for the primary end point. The risk reduction was calculated as 100 percent × (1 − the hazard ratio). Event curves are based on Kaplan–Meier analysis, and significance was assessed by means of the log-rank test.

Changes in the level of urinary protein excretion, creatinine clearance, and blood pressure were analyzed by repeated-measures analysis of variance. To identify interactions between the treatment groups and blood pressure, we used proportional-hazards regression and the most recent measurement of mean arterial pressure as a time-varying covariate. The relationship between the reduction in proteinuria and the rate of the decline in renal function was analyzed by Pearson

correlation. SPSS software for Windows (version 12.0) was used for analyses.

RESULTS

We screened 468 patients, and 422 entered the run-in phase: 141 had a serum creatinine level of 1.5 to 3.0 mg per deciliter and were therefore in group 1, and 281 had a serum creatinine level of 3.1 to 5.0 mg per deciliter and were thus in group 2 (Fig. 1). Before randomization, 94 patients were excluded: 72 patients had a dry cough (17 percent, 30 in group 1 and 42 in group 2), 9 had an acute increase in the serum creatinine level of more

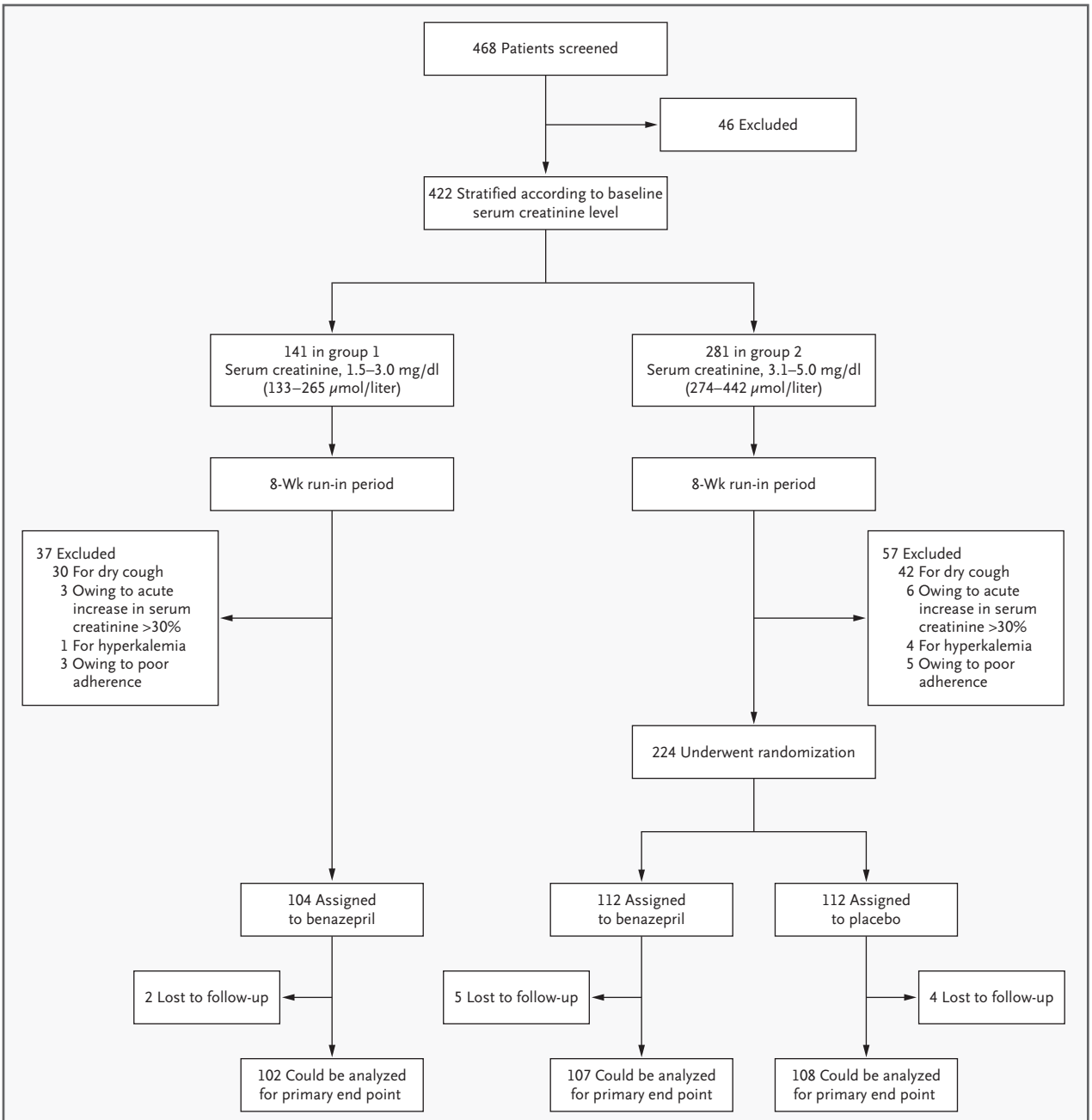


Figure 1. Enrollment and Outcomes.

Table 1. Baseline Characteristics of the Patients with Chronic Kidney Disease.*

Characteristic	Group 1 (N=104)	Group 2†	
		Benazepril (N=112)	Placebo (N=112)
Age — yr	45.1±13.0	44.4±16.8	45.0±14.1
Male sex — no. (%)	51 (49)	56 (50)	55 (49)
Body-mass index†	23.0±4.2	23.2±6.1	22.5±3.5
Body-surface area — m^2	1.7±0.1	1.8±0.2	1.7±0.1
Renal disease — no. (%)			
Glomerular disease	62 (60)	68 (61)	69 (62)
Hypertension	19 (18)	19 (17)	20 (18)
Polycystic kidney disease	13 (12)	14 (12)	11 (10)
Interstitial disease	2 (2)	3 (3)	6 (5)
Unknown	8 (8)	8 (7)	6 (5)
Hypertension — no. (%)	92 (88)	103 (92)	102 (91)
No. of antihypertensive drugs			
Median	2	2	2
Interquartile range	0–3	0–3	0–3
Blood pressure — mm Hg			
Systolic	150.6±27.6	153.2±23.8	151.6±22.5
Diastolic	85.9±11.8	87.0±10.0	84.8±10.7
Renal function‡			
Serum creatinine — mg/dl	2.3±0.5§	4.0±0.7	3.9±0.7
Calculated GFR — ml/min/1.73 m^2	37.1±6.3§	26.3±5.3	25.8±5.3
Creatinine clearance — ml/min/1.73 m^2	38.2±14.4§	27.1±13.3	26.1±8.2
Urinary protein excretion — g/day	1.6±0.8	1.6±0.7	1.7±0.8
Laboratory variables — mmol/liter¶			
Serum cholesterol	4.6±1.3	4.7±1.2	4.6±1.5
Serum triglycerides	1.9±0.8	2.0±0.9	1.9±0.8
Serum potassium	4.4±0.7	4.5±0.7	4.6±0.6
Hemoglobin — g/liter	113.2±8.2§	101.1±13.5	98.6±8.7
Urinary urea excretion — g/day	5.1±1.6	5.0±1.5	4.8±1.8
Urinary chloride excretion — mmol/day	107.2±13.8	104.5±14.2	102.6±12.8

* Group 1 had a serum creatinine level of 1.5 to 3.0 mg per deciliter, and group 2 had a serum creatinine level of 3.1 to 5.0 mg per deciliter. Plus–minus values are means ±SD. The five stages of chronic kidney disease are as follows: stage 1, kidney damage with a normal or increased glomerular filtration rate (GFR) of at least 90 ml per minute per 1.73 m^2; stage 2, kidney damage with a mild decrease in the GFR (60 to 89 ml per minute per 1.73 m^2); stage 3, moderate decrease in the GFR (30 to 59 ml per minute per 1.73 m^2); stage 4, severe decrease in the GFR (15 to 29 ml per minute per 1.73 m^2); and stage 5, kidney failure, as defined by a GFR of less than 15 ml per minute per 1.73 m^2 or the need for dialysis. The body-mass index is the weight in kilograms divided by the square of the height in meters.

† There were no significant differences between the benazepril group and the placebo group.

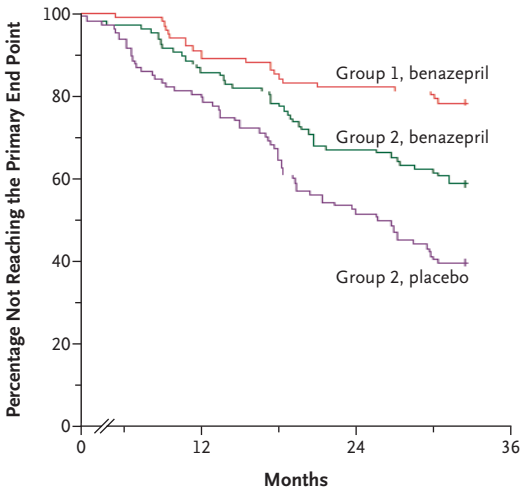
‡ To convert values for creatinine to micromoles per liter, multiply by 88.4. Serum and urinary creatinine levels were determined by an enzymatic method (sarcosine oxidase–peroxidase–antiperoxidase). The baseline serum creatinine value is the mean of all values obtained during the run-in period. The GFR was calculated with the use of the four-component Modification of Diet in Renal Disease[12] equation: $186 \times (\text{serum creatinine level [in milligrams per deciliter]})^{-1.154} \times (\text{age [in years]})^{-0.203}$. For women, this equation was multiplied by a correction factor of 0.742.

§ $P<0.05$ for the comparison with the patients in group 2.

¶ To convert values for cholesterol to milligrams per deciliter, divide by 0.02586. To convert values for triglycerides to milligrams per deciliter, divide by 0.01129.

than 30 percent (2 percent, 3 in group 1 and 6 in group 2), 5 had an increase in the serum potassium level to more than 5.6 mmol per liter (1 percent, 1 in group 1 and 4 in group 2), and 8 had a rate of adherence to medication of less than 80 percent (2 percent, 3 in group 1 and 5 in group 2). There were no significant differences in these events between the two groups.

All 104 patients in group 1 received 20 mg of benazepril per day, whereas in group 2, 112 patients were randomly assigned to receive 20 mg of benazepril per day and 112 were assigned to receive placebo. The baseline characteristics of the patients were similar in the two subgroups of group 2 (Table 1). The average age and the distribution of the primary causes of renal dysfunction in the various groups were similar to those reported in the registry of the Chinese Society of Nephrology.[7] The mean length of follow-up was 3.4 years (range, 2 to 5). Eleven patients were lost to follow-up (2 in group 1, 5 assigned to benazepril in group 2, and 4 assigned to placebo in group 2), leaving 102 patients in group 1 and 215 patients in group 2 (107 assigned to benazepril and 108 assigned to placebo) who could be included in the efficacy analysis.



No. at Risk				
Group 1, benazepril	102	96	84	40
Group 2, benazepril	107	96	73	32
Group 2, placebo	108	88	59	22

Figure 2. Kaplan–Meier Estimates of the Percentage of Patients Not Reaching the Primary Composite End Point of a Doubling of the Serum Creatinine Level, End-Stage Renal Disease, or Death.

Group 1 had a serum creatinine level of 1.5 to 3.0 mg per deciliter, and group 2 had a serum creatinine level of 3.1 to 5.0 mg per deciliter at baseline.

PRIMARY OUTCOMES

In group 2, 44 patients assigned to benazepril reached the primary end point, as compared with 65 patients assigned to placebo (41 percent vs. 60 percent, P=0.004) (Fig. 2). However, renal outcome, as measured by the number of patients who reached the primary end point, was worse among patients assigned to benazepril in group 2 than among patients in group 1, even though all patients received the same dose of benazepril (P=0.003) (Fig. 2).

In group 2, treatment with benazepril, as compared with placebo, resulted in a 43 percent overall reduction in the risk of the primary end point (P=0.005). The decrease in the risk (hazard rate, 40 percent) remained significant after adjustment for differences in the mean arterial pressure (P=0.009). The intention-to-treat analyses of the individual components of the primary end point indicated that the risk of a doubling of the serum creatinine level was 51 percent lower among patients who received benazepril than among those given placebo (P=0.02). Benazepril also reduced the risk of end-stage renal disease by 40 percent (P=0.02).

BLOOD PRESSURE

Classes of conventional antihypertensive drugs used before and during the study are listed in Table 2. Blood pressure declined progressively during the study (Fig. 3A). The decline in blood pressure was similar in the two groups (P=0.26) and in the two subgroups of group 2 (P=0.18).

SECONDARY OUTCOMES

In group 2, there was a significantly greater reduction in the level of proteinuria among patients assigned to benazepril than among those assigned to placebo (52 percent vs. 20 percent, P<0.001) (Fig. 3B). Urinary protein excretion was also decreased in group 1, with an average reduction of 49 percent (P=0.57 for the comparison with patients assigned to benazepril in group 2) (Fig. 3B). Benazepril also reduced the rate of decline in renal function by 23 percent in group 2, as assessed by the reciprocal of the serum creatinine level (median slope, −0.09 dl per milligram per year among those assigned to benazepril, as compared with −0.11 dl per milligram per year among those assigned to placebo; P=0.02).

Likewise, benazepril was associated with a 24 percent reduction in the estimated decline in the

Table 2. Use of Conventional Antihypertensive Medications at Baseline and during Study Treatment.*

Drug	Group 1 (N=104)	Group 2	
		Benazepril (N=112)	Placebo (N=112)
		number (percent)	
Calcium-channel antagonist			
At baseline	72 (69)	74 (66)	77 (69)
During treatment	79 (76)	83 (74)	88 (79)
Dihydropyridine			
At baseline	53 (51)	54 (48)	58 (52)
During treatment	62 (60)	65 (58)	69 (62)
Nondihydropyridine			
At baseline	19 (18)	20 (18)	19 (17)
During treatment	17 (16)	18 (16)	19 (17)
Diuretic			
At baseline	60 (58)	59 (53)	62 (55)
During treatment	85 (82)	90 (80)	93 (83)
Beta-blocker			
At baseline	53 (51)	57 (51)	53 (47)
During treatment	49 (47)	54 (48)	58 (52)
Centrally acting agent			
At baseline	26 (25)	36 (32)	32 (29)
During treatment	35 (34)	36 (32)	40 (36)

* Group 1 had a serum creatinine level of 1.5 to 3.0 mg per deciliter, and group 2 had a serum creatinine level of 3.1 to 5.0 mg per deciliter at baseline.

glomerular filtration rate in group 2: the median rate of decline was 6.8 ml per minute per 1.73 m² per year among the patients assigned to benazepril, as compared with 8.8 ml per minute per 1.73 m² per year among the patients assigned to placebo (P=0.006). There was no significant difference between the two benazepril groups in the rate of decline in the estimated glomerular filtration rate (P=0.23) or creatinine clearance (P=0.19) (Fig. 3C). However, there was a significant correlation between the extent of the reduction in proteinuria and the rate of decline in both the estimated glomerular filtration rate (P=0.03) and creatinine clearance (P=0.03) in patients with proteinuria of at least 1 g per day at baseline.

SAFETY

One patient assigned to benazepril in group 2 died of pneumonia. The last measurement obtained in this patient showed good blood-pressure control (120/80 mm Hg) and a normal serum potassium level. There were no significant differences in the number of nonfatal cardiovascular events between the two groups or between the two subgroups in group 2 (Table 3). The incidence of other adverse events was also similar in the two groups and in the two subgroups of group 2. Hyperkalemia (defined as a serum potassium level of at least 6.0 mmol per liter) occurred in 11 patients (5 percent) in group 2. Of these 11 patients, 8 were successfully treated with dietary modifications, concomitant diuretic therapy, and optimized acid–base balance. The remaining three patients withdrew from the study. Follow-up serum potassium levels in group 2 were higher among patients receiving benazepril than among those receiving placebo (P=0.001), but the differences never exceeded 0.5 mmol per liter. In group 2, the proportion of patients receiving recombinant human erythropoietin, the mean dose of recombinant human erythropoietin, and hemoglobin levels were similar in the two subgroups at baseline and during the study (data not shown).

DISCUSSION

Our findings indicate that benazepril, along with conventional antihypertensive treatment, as needed, confers renal protection in patients without diabetes who have advanced renal insufficiency. Benazepril therapy reduced the risk of the primary end point by 43 percent among patients quite close to end-stage renal disease during an average follow-up of 3.4 years. The difference between the slopes of the reciprocal of the serum creatinine level and the slower decline in creatinine clearance and the glomerular filtration rate with benazepril therapy provide further evidence of the renal protection afforded by this ACE inhibitor. Thus, the primary analysis of this controlled trial provides evidence that treatment with the ACE inhibitor benazepril is beneficial in patients with stage 4 chronic kidney disease (defined by a glomerular filtration rate of 15 to 29 ml per minute per 1.73 m²). Although a previous secondary analysis suggested a benefit of ACE inhibitors among patients with severe renal dysfunction,[16] evidence from a randomized, controlled trial is necessary to confirm the efficacy and tolerability of these agents in this patient population. Since current information indicates that 85 percent of patients with stage 4 chronic

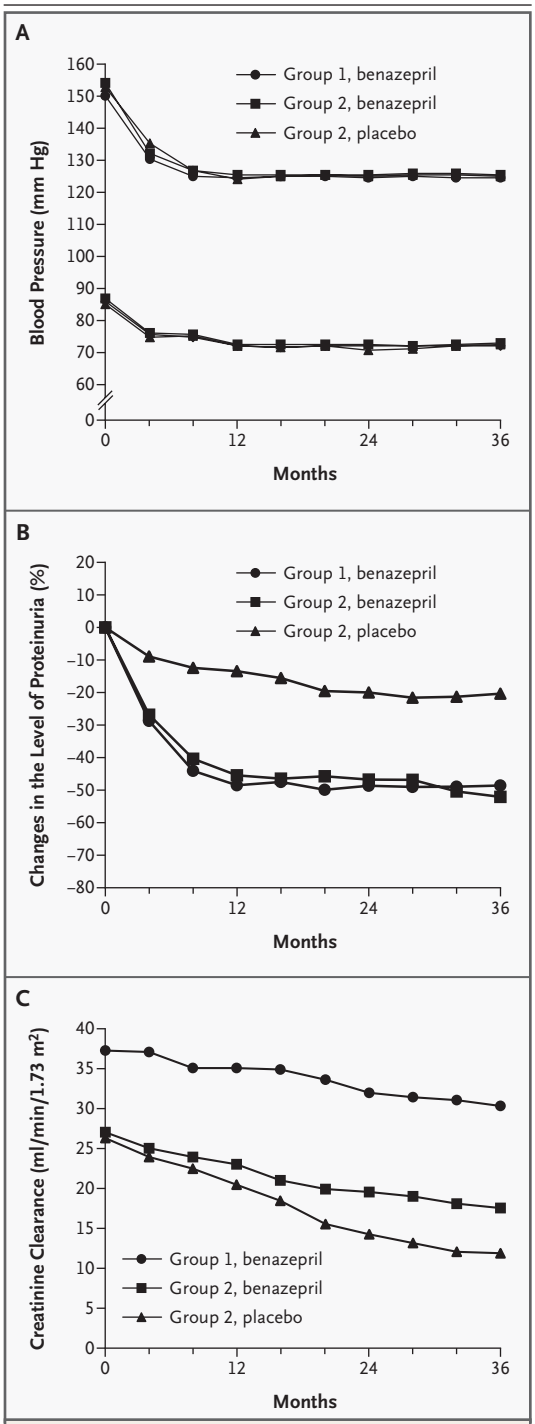


Figure 3. Changes in Blood Pressure (Panel A), the Level of Proteinuria (Panel B), and Creatinine Clearance (Panel C) during Follow-up.

Group 1 had a serum creatinine level of 1.5 to 3.0 mg per deciliter, and group 2 had a serum creatinine level of 3.1 to 5.0 mg per deciliter at baseline.

kidney disease are not offered such renal protective treatment,[16] our results may have important implications for the development of new therapeutic guidelines.

Consistent with previous findings,[16,17] our data suggest that the response to ACE inhibitors was independent of the baseline glomerular filtration rate. The rate of decrease in the glomerular filtration rate appeared to be higher among patients in group 2 than among those in group 1 and was moderately reduced by ACE inhibitors. This observation is consistent with the report that enalapril slowed the progression of advanced chronic kidney disease in patients without diabetes.[18] The higher proportion of patients reaching the primary end point in group 2 might be explained by the fact that the ACE-inhibitor–associated reduction in risk is time-dependent[18,19] and that patients with advanced chronic kidney disease had been treated for a shorter period than those with more preserved renal function. Thus, to achieve maximal renal protection, treatment with ACE inhibitors should arguably be initiated at earlier stages of chronic kidney disease.

Our study was also designed to determine whether the renal protective effect of benazepril in patients with advanced chronic kidney disease is dependent on its antihypertensive action. Blood pressure and the use of conventional antihypertensive drugs were similar at baseline and during treatment in both subgroups of group 2. Furthermore, the reduction in the risk of the primary end point changed little after correction for blood pressure, suggesting that the renal protection conferred by benazepril is not dependent on blood pressure when the drug is used as part of combination antihypertensive therapy.

The most striking difference between the patients who received benazepril and the patients who received placebo in group 2 was the change in urinary protein excretion. Benazepril greatly reduced urinary protein excretion in patients with advanced chronic kidney disease. This observation, as well as the close correlation between the extent of the reduction in proteinuria and the rate of decline in renal function, provides further support for, but does not prove, the hypothesis that proteinuria has a causal role in the progression of renal dysfunction.[17,20]

Patients with stage 4 chronic kidney disease

are particularly vulnerable to the effects of ACE inhibitors on the glomerular filtration rate and potassium excretion.[21,22] For safety's sake, as well as because of the higher incidence of dry cough among Chinese patients taking ACE inhibitors than among other ethnic groups receiving these drugs,[23] we treated all eligible patients with benazepril and observed them closely during the run-in period. Dry cough and an acute increase in the serum creatinine level occurred mostly within the first two months after the initiation of benazepril therapy, but there were no significant differences in the incidence of these adverse events between patients in group 1 and those in group 2. Benazepril-associated cough was reported in 17 percent of patients during the run-in period, though as compared with patients in other studies, fewer of those with cough reported that this was an intolerable side effect.[23] Nine patients had an increase in the serum creatinine level that exceeded 30 percent during the run-in phase, and five patients were evaluated for renal-artery stenosis (two in group 1 and three in group 2).

In the cohort with advanced renal dysfunction, the tolerability of benazepril and placebo was similar, as evidenced by the similar incidence of major adverse events in the two subgroups. As predicted, a nonsignificant trend toward a higher frequency of hyperkalemia was observed in group 2 as compared with group 1. However, the incidence of hyperkalemia was similar among patients who received benazepril and those who received placebo in group 2, and benazepril therapy resulted in an average increase in the serum potassium level that never exceeded 0.5 mmol per liter. Furthermore, benazepril had no significant effects on patients' hemoglobin levels or the dose of recombinant human erythropoietin. These data suggest that the addition of benazepril to a conventional regimen of antihypertensive therapy is acceptable, even in patients with serum creatinine levels exceeding 3.0 mg per deciliter. Nonetheless, blood pressure, renal function, and serum potassium levels should be monitored regularly in patients with advanced chronic kidney disease, especially during the first two months of ACE-inhibitor therapy and as renal function changes.

Table 3. Adverse Events after Randomization.*

Adverse Event	Group 1 (N=104)	Group 2	
		Benazepril (N=112)	Placebo (N=112)
		no. of events	
Death	0	1	0
Nonfatal cardiovascular event			
Myocardial infarction	3	5	8
Heart failure	1	3	5
Stroke	1	2	3
Other adverse events			
Hyperkalemia†	2	6	5
Acute decline in renal function	1	1	1
Dry cough	0	1	0
Hypotension‡	1	0	0
Total	9	19	22

* Group 1 had a serum creatinine level of 1.5 to 3.0 mg per deciliter, and group 2 had a serum creatinine level of 3.1 to 5.0 mg per deciliter.
† Two patients assigned to benazepril in group 2 and one patient assigned to placebo in group 2 withdrew from the study owing to persistent hyperkalemia.
‡ One patient in group 1 discontinued treatment because of hypotension (defined by a systolic blood pressure of less than 90 mm Hg).

In summary, benazepril therapy was associated with a significant improvement in renal outcome and surpassed that attributable to blood-pressure control in patients without diabetes who had advanced renal dysfunction.

Supported by a National Nature and Sciences Grant for Major Projects (30330300) and a People's Liberation Army Grant for Major Clinical Research (to Dr. Hou) and in part by Novartis.

No potential conflict of interest relevant to this article was reported.

APPENDIX

The following persons participated in the study: **Principal Investigator:** F.F. Hou; **Steering Panel:** F.F. Hou, X. Zhang, G.H. Zhang; **End-Point and Safety Monitoring Panel:** M. Liang, D. Xie, W.R. Zhang; **Primary Investigators:** Z.R. Liu, J.P. Jiang, G.B. Wang, H. Ren, H.F. Liu, X.B. Yang, Z.J. Guo, H.Y. Li, X.Y. Shi, H.X. Niu.

REFERENCES

1. Maschio G, Alberti D, Janin G, et al. Effect of the angiotensin-converting–enzyme inhibitor benazepril on the progression of chronic renal insufficiency. N Engl J Med 1996;334:939-45.
2. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. Lancet 1997;349:1857-63.
3. Ruggenenti P, Perna A, Benini R, Remuzzi G. Effects of dihydropyridine calcium channel blockers, angiotensin-convert-

ing enzyme inhibition, and blood pressure control on chronic, nondiabetic nephropathies. J Am Soc Nephrol 1998;9:2096-101.
4. Bakris GL, Weir MR. Angiotensin-converting enzyme inhibitor-associated elevations in serum creatinine: is this a cause for concern? Arch Intern Med 2000;160:685-93.
5. Moser M. Angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists and calcium channel blocking agents: a review of potential benefits and possible adverse reactions. J Am Coll Cardiol 1997;29:1414-21.
6. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting–enzyme inhibition on diabetic nephropathy. N Engl J Med 1993;329:1456-62. [Erratum, N Engl J Med 1993;330:152.]
7. Dialysis and Transplantation Registration Group, Chinese Society of Nephrology. The report about the registration of dialysis and transplantation in China 1999. Chin J Nephrol 2001;17:77-8.
8. Hou FF, Ma ZG, Mei CL, et al. Cardiovascular disease in Chinese chronic renal insufficiency patients — epidemiology survey. Zhonghua Yi Xue Za Zhi 2005; 85:458-63. (In Chinese.)
9. Zhang GH, Hou FF, Zhang X, Liu QF. Can angiotensin-converting enzyme inhibitor be used in chronic kidney disease patients with serum creatinine level greater than 266 micromol/L? Zhonghua Nei Ke Za Zhi 2005;44:592-6. (In Chinese.)
10. Levey AS. Clinical evaluation of renal function. In: Greenberg A, ed. Primer on kidney diseases. 2nd ed. San Diego, Calif.: Academic Press, 1998:20-6.
11. Mitch WE, Walser M, Buffington GA, Lemann J Jr. A simple method of estimating progression of chronic renal failure. Lancet 1976;2:1326-8.
12. Levey AS, Greene T, Kusek JW, Beck GJ. A simplified equation to predict glomerular filtration rate from serum creatinine. J Am Soc Nephrol 2000;11:155A. abstract.
13. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130: 461-70.
14. Coresh J, Astor BC, McQuillan G, et al. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. Am J Kidney Dis 2002;39: 920-9.
15. Zuo L, Ma YC, Zhou YH, Wang M, Xu GB, Wang HY. Application of GFR-estimating equations in Chinese patients with chronic kidney disease. Am J Kidney Dis 2005;45:463-72.
16. Ruggenenti P, Perna A, Remuzzi G, Gruppo Italiano di Studi Epidemiologici in Nefrologia. ACE inhibitors to prevent end-stage renal disease: when to start and why possibly never to stop: a post hoc analysis of the REIN trial results. J Am Soc Nephrol 2001;12:2832-7.
17. Ruggenenti P, Perna A, Mosconi L, Pisoni R, Remuzzi G. Urinary protein excretion rate is the best independent predictor of ESRF in non-diabetic proteinuric chronic nephropathies. Kidney Int 1998;53: 1209-16.
18. Ihle BU, Whitworth JA, Shahinfar S, Cnaan A, Kincaid-Smith PS, Becker GJ. Angiotensin-converting enzyme inhibition in nondiabetic progressive renal insufficiency: a controlled double-blind trial. Am J Kidney Dis 1996;27:489-95.
19. Ruggenenti P, Perna A, Benini R, et al. In chronic nephropathies prolonged ACE inhibition can induce remission: dynamics of time-dependent changes in GFR. J Am Soc Nephrol 1999;10:997-1006.
20. Peterson JC, Adler S, Burkart JM, et al. Blood pressure control, proteinuria, and the progression of renal disease: the Modification of Diet in Renal Disease Study. Ann Intern Med 1995;123:754-62.
21. Reams GP, Bauer JH. Effect of lisinopril monotherapy on renal hemodynamics. Am J Kidney Dis 1988;11:499-507.
22. Reardon LC, Macpherson DS. Hyperkalemia in outpatients using angiotensin-converting enzyme inhibitors: how much should we worry? Arch Intern Med 1998;158: 26-32.
23. Tomlinson B, Woo J, Thomas GN, Chau YM, Critchley JA. Randomized, controlled, parallel-group comparison of ambulatory and clinic blood pressure responses to amlodipine or enalapril during and after treatment in adult Chinese patients with hypertension. Clin Ther 2004;26:1292-304.

***JOURNAL* INDEX**

The index to volume 353 of the *Journal* will be available on February 23, 2006. At that time, it can be downloaded free in PDF format from **www.nejm.org.**

ORIGINAL ARTICLE

A Randomized Trial of Inhaled Cyclosporine in Lung-Transplant Recipients

Aldo T. Iacono, M.D., Bruce A. Johnson, M.D., Wayne F. Grgurich, B.S., J. Georges Youssef, M.D., Timothy E. Corcoran, Ph.D., Deidre A. Seiler, M.S.N., James H. Dauber, M.D., Gerald C. Smaldone, M.D., Ph.D., Adriana Zeevi, Ph.D., Samuel A. Yousem, M.D., John J. Fung, M.D., Ph.D., Gilbert J. Burckart, Pharm.D., Kenneth R. McCurry, M.D., and Bartley P. Griffith, M.D.

ABSTRACT

BACKGROUND

Conventional regimens of immunosuppressive drugs often do not prevent chronic rejection after lung transplantation. Topical delivery of cyclosporine in addition to conventional systemic immunosuppression might help prevent acute and chronic rejection events.

METHODS

We conducted a single-center, randomized, double-blind, placebo-controlled trial of inhaled cyclosporine initiated within six weeks after transplantation and given in addition to systemic immunosuppression. A total of 58 patients were randomly assigned to inhale either 300 mg of aerosol cyclosporine (28 patients) or aerosol placebo (30 patients) three days a week for the first two years after transplantation. The primary end point was the rate of histologic acute rejection.

RESULTS

The rates of acute rejection of grade 2 or higher were similar in the cyclosporine and placebo groups: 0.44 episode (95 percent confidence interval, 0.31 to 0.62) vs. 0.46 episode (95 percent confidence interval, 0.33 to 0.64) per patient per year, respectively ($P=0.87$ by Poisson regression). Survival was improved with aerosolized cyclosporine, with 3 deaths among patients receiving cyclosporine and 14 deaths among patients receiving placebo (relative risk of death, 0.20; 95 percent confidence interval, 0.06 to 0.70; $P=0.01$). Chronic rejection–free survival also improved with cyclosporine, as determined by spirometric analysis (10 events in the cyclosporine group and 20 events in the placebo group; relative risk of chronic rejection, 0.38; 95 percent confidence interval, 0.18 to 0.82; $P=0.01$) and histologic analysis (6 vs. 19 events, respectively; relative risk, 0.27; 95 percent confidence interval, 0.11 to 0.67; $P=0.005$). The risks of nephrotoxic effects and opportunistic infection were similar for patients in the cyclosporine group and the placebo group.

CONCLUSIONS

Inhaled cyclosporine did not improve the rate of acute rejection, but it did improve survival and extend periods of chronic rejection–free survival. (ClinicalTrials.gov number, NCT00268515.)

From the Division of Pulmonary, Allergy and Critical Care Medicine (A.T.I., B.A.J., W.F.G., J.G.Y., T.E.C., D.A.S., J.H.D.), the Department of Pathology (A.Z., S.A.Y.), and the Thomas E. Starzl Transplantation Institute (J.J.F.), University of Pittsburgh Medical Center; and the Division of Cardiothoracic Surgery, University of Pittsburgh (K.R.M.) — all in Pittsburgh; the Division of Pulmonary and Critical Care Medicine, State University of New York at Stony Brook, Stony Brook (G.C.S.); the Department of Pharmacy, University of Southern California, Los Angeles (G.J.B.); and the Division of Cardiothoracic Surgery, University of Maryland, Baltimore (B.P.G.). Address reprint requests to Dr. Iacono at the Division of Pulmonary Transplantation and Pulmonary and Critical Care Medicine, Medical School Teaching Facility 800, University of Maryland School of Medicine, 10 S. Pine St., Baltimore, MD 21201, or at aiacono@medicine.umaryland.edu.

N Engl J Med 2006;354:141-50.

Outcomes after lung transplantation are poor as compared with those after heart, kidney, or liver transplantation, with a three-year survival rate of only 55 percent for recipients of lung transplants. Death is commonly due to chronic rejection,[1] which presents histologically as bronchiolitis obliterans[2-7]; the latter is thought to be a complex response to immunologic, ischemic, and infectious injury.[8-11] Preventive and therapeutic strategies for this process have been largely unsuccessful.[12-14]

Since the immunosuppressive effect of cyclosporine is dose-dependent, targeted delivery of this drug might improve efficacy by increasing the concentration of cyclosporine in the allograft. In animal models of lung transplantation, inhaled cyclosporine remains in high concentrations in lung tissue and reduces rejection without toxicity.[15-18] Moreover, in human lung-transplant recipients with refractory acute and chronic rejection, open-label rescue treatment with inhaled cyclosporine improves clinical markers of rejection and improves survival.[19-25] In light of these findings, we tested whether prophylactic inhaled cyclosporine would improve outcomes after lung transplantation.

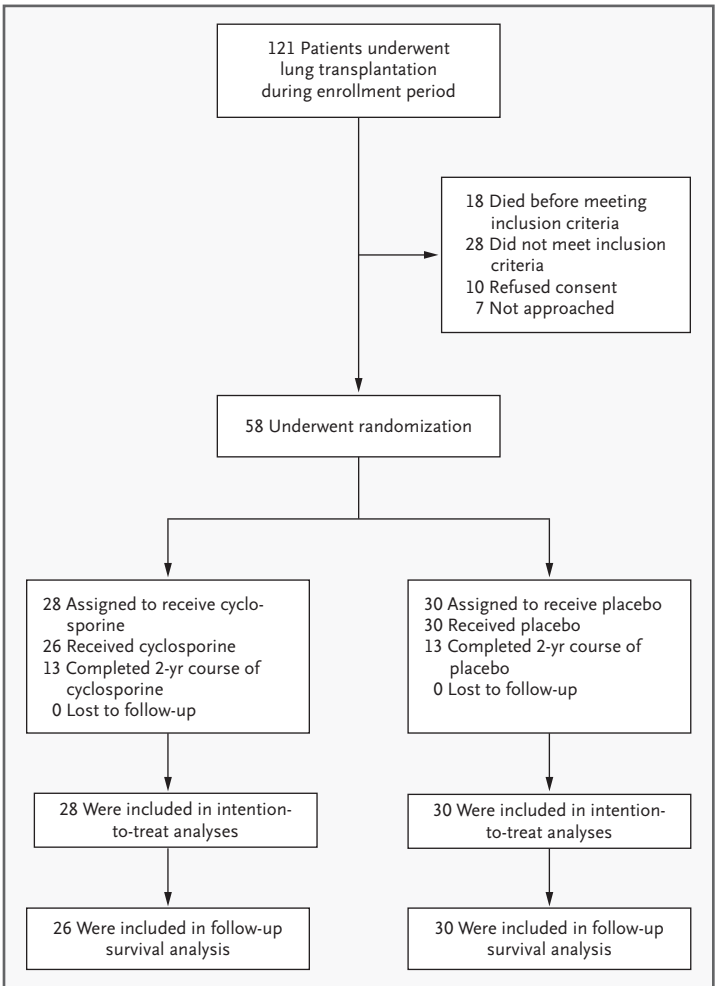


Figure 1. Study Enrollment and Inclusion in the Intention-to-Treat and Survival Analyses.

METHODS

STUDY DESIGN

A randomized, double-blind, placebo-controlled trial of aerosol cyclosporine inhalation, given in addition to conventional immunosuppression, was conducted at the University of Pittsburgh Medical Center with approval of the institutional review board. Recipients of single or bilateral lung transplants who were at least 18 years of age were eligible. Patients were excluded from the study if they had active fungal or bacterial pneumonia, unresolved diffuse alveolar damage, or untreated bronchial stenosis or if they were receiving mechanical ventilation. From November 1998 to August 2001, patients were offered enrollment if they met the study criteria before day 42 after transplantation and were randomly assigned to a treatment group immediately after the provision of written informed consent. Study treatment began as soon as was practically possible thereafter, but no more than 55 days later.

Because mismatches between donor and recipient with respect to cytomegalovirus (CMV) serologic status are known to have an adverse effect on the outcome of transplantation, the randomization was stratified according to donor–recipient CMV status. The two categories were a primary mismatch (a CMV-positive donor and a CMV-negative recipient) and all other serologic combinations. Patients were then randomly assigned to groups according to permuted blocks of four in a 1:1 ratio to receive either inhaled cyclosporine or placebo for two years. All patients were followed for clinical outcome until the last subject completed the scheduled two-year regimen (August 2003). As a result, follow-up ranged from 24 to 56 months.

Novartis Pharmaceuticals provided cyclosporine powder, which was compounded by the Uni-

versity of Pittsburgh experimental pharmacy. University investigators were solely responsible for the trial design, data accrual, and study management. After completion of the study, Chiron obtained a license agreement for inhaled cyclosporine. The analyses presented in this article are those of the university investigators, unless otherwise noted. The data and safety monitoring board took no action regarding early trial cessation.

ADMINISTRATION OF INHALED CYCLOSPORINE

Patients inhaled cyclosporine mixed in propylene glycol (62.5 mg per milliliter) or placebo (propylene glycol alone) initially for 10 consecutive days, then three times weekly with the use of a jet nebulizer (AeroTech II, CIS-US). Patients were instructed to continue treatment for two years. Patients were premedicated by inhalation of 2 percent lidocaine (3 ml) and 2.5 mg of albuterol by means of a nebulizer (Airlife, Cardinal Health). Inhaled cyclosporine was initiated at a dose of 100 mg and increased by incremental doses of 100 mg up to 300 mg or a maximally tolerated dose or an aerosol equivalent volume of placebo. Aerosols were self-administered by the patients and were temporarily discontinued if the treating physician reported an infection that persisted after antibiotic therapy. A study coordinator contacted the patients at least monthly to verify compliance.

TRANSPLANT MONITORING

Patients followed a typical care regimen for patients after lung transplantation, including surveil-

Table 1. Characteristics of the Patients.*

Characteristic	Cyclosporine (N=28)	Placebo (N=30)	P Value
Age — yr	51.3±2.3	51.7±2.0	0.89
Sex — no. (%)			0.96
Male	17 (61)	18 (60)	
Female	11 (39)	12 (40)	
Diagnosis before transplantation — no. (%)			0.18
Emphysema	10 (36)	19 (63)	
Cystic fibrosis	5 (18)	4 (13)	
Idiopathic pulmonary fibrosis or mixed connective-tissue disease	7 (25)	3 (10)	
Other condition	6 (21)	4 (13)	
Type of transplantation — no. (%)			0.11
Single lung	17 (61)	24 (80)	
Double lung	11 (39)	6 (20)	
Donor–recipient CMV status — no. (%)			0.89
+/+	9 (32)	8 (27)	
+/−	5 (18)	7 (23)	
−/+	7 (25)	9 (30)	
−/−	7 (25)	6 (20)	
No. of HLA mismatches			
HLA-A	1.43±0.12	1.33±0.12	0.58
HLA-B	1.64±0.09	1.80±0.07	0.19
HLA-DR	1.39±0.11	1.33±0.13	0.73
Ischemic time — min	254±15	245±18	0.69
Age of donor — yr	36.0±2.3	35.8±2.8	0.96

* Plus–minus values are means ±SE. Percentages may not sum to 100 because of rounding. CMV denotes cytomegalovirus.

lance bronchoscopy with transbronchial biopsy and bronchoalveolar lavage, spirometry, blood work, and a complete history and physical examination one month after transplantation and at intervals of approximately three months for the first two postoperative years, then at intervals of four to six months. Histologic rejection was defined according to established criteria.[26] Spirometry was performed according to American Thoracic Society standards[27] and the results expressed in terms of the percentage of predicted values.[28] Because bronchiolitis obliterans is not uniformly detectable by biopsy, spirometry is routinely used as a surrogate marker to diagnose chronic rejection. Airflow measurements were evaluated for criteria of the bronchiolitis obliterans syndrome, which was defined as a sustained decrease in the forced expiratory volume in one second (FEV_1) of at least 20 percent from the patient's maximum values in the absence of other causes.[29] Cytomegalovirus status was assessed by CMV pp65 antigenemia at weekly intervals for six months after transplantation.

CLINICAL MANAGEMENT

Both groups received conventional immunosuppression, including tacrolimus (0.06 mg per kilogram of body weight per day), azathioprine (2 mg per kilogram per day), and prednisone (20 mg per day). Subsequent adjustments were made at the discretion of the treating clinician on the basis of lung function and biopsy results. Enhanced immune suppression for treatment of acute rejection (grade 2 or higher), active bronchiolitis obliterans, or both consisted of pulsed corticosteroids (intravenous methylprednisolone at a dose of 1 g per day for 3 days or oral prednisone at a dose of 100 mg tapered to 10 mg over 14 days) or rabbit antithymocyte globulin (Thymoglobulin [SangStat] at a dose of 1.5 mg per kilogram per day for 5 to 7 days). Intravenous ganciclovir was

Table 2. Characteristics of the Patients after Enrollment.*

Characteristic	Cyclosporine (N=28)	Placebo (N=30)	P Value
Days from transplantation to start of treatment	26.2±3.2	23.6±2.5	0.52
Total days of aerosol administration	400±57	431±50	0.70
Percentage of eligible doses received†	57.4±7.8	65.5±6.0	0.41
Patients completing two-year study — no. (%)	13 (46)	13 (43)	
Reasons for discontinuation — no. (%)			0.85
Decision of investigator			
Infection	5 (18)	6 (20)	
Renal failure	0 (0)	1 (3)	
Smoking	0 (0)	1 (3)	
Patient entered in rescue study	1 (4)	2 (7)	
Decision of patient			
Symptoms caused by aerosol administration	2 (7)	2 (7)	
Withdrawal from study	7 (25)	5 (17)	
Duration of follow-up — yr			
Mean	3.1±0.2	2.7±0.2	0.16
Median	3.1	2.6	0.29
Interquartile range	2.4–4.1	2.1–3.9	
No. of tests of pulmonary function per patient	20.5±1.6	18.5±2.0	0.43
Time from transplantation to last test of pulmonary function — yr			
Median	2.9	2.4	0.11
Interquartile range	2.1–3.9	2.0–3.5	

used if the level of antigenemia was more than 10 CMV-positive cells per 2×10^5 leukocytes.

END POINTS

The primary end point of the study was the frequency of histologic acute rejection. Secondary end points included chronic rejection–free survival and overall survival. Chronic rejection was identified on the basis of both histologic markers (for bronchiolitis obliterans) and spirometric markers (for the bronchiolitis obliterans syndrome).[29]

Adverse events were defined as infections requiring treatment, hospitalizations, and symptoms as reported by questionnaire. All evaluations of outcomes were performed in a blinded manner.

STATISTICAL ANALYSIS

Power analysis stipulating a 33 percent difference in the frequency of acute rejection suggested the enrollment of 136 patients on the basis of a two-sided test ($\alpha=0.05$, $\beta=0.15$), an assumed acute-rejection rate of 2.8 events per year, and a dropout rate of 15 percent. The study prespecified a three-year enrollment period. During this time, all qualifying lung-transplant recipients were approached for participation. Enrollment was discontinued after three years, after the accrual of only 58 subjects. The failure to achieve enrollment goals was due to an overestimation of the number of transplantations that would be performed during the accrual period (anticipated number, 180; actual number performed, 121). The study was closed two years after the last subject had been enrolled. All outcome variables were followed until either the death of the patient or the end of the study (in August 2003), independent of the continuation or discontinuation of study medication or the conclusion of the scheduled two-year study-treatment period. Patients were analyzed according to the intention to treat, and no patients were

Table 2. (Continued.)

Characteristic	Cyclosporine (N=28)	Placebo (N=30)	P Value
No. of biopsies per patient	12.0±0.7	11.1±0.8	0.43
Time from transplantation to last biopsy — yr			
Median	2.2	2.2	0.99
Interquartile range	1.6–3.3	1.6–2.7	
Daily dose of prednisone AUC — mg‡	12.1±0.6	12.2±0.6	0.88
Calcineurin-inhibitor regimen			0.90
Tacrolimus — no. of patients (%)	23 (82)	25 (83)	
Conversion from tacrolimus to cyclosporine — no. of patients (%)	5 (18)	5 (17)	
Tacrolimus level AUC — ng/ml§	12.4±0.4	11.9±0.4	0.20
Cyclosporine level AUC — ng/ml§	190±19	179±16	0.89
Cytostatic regimen — no. of patients (%)			0.26
Azathioprine	11 (39)	16 (53)	
Mycophenolate mofetil	4 (14)	1 (3)	
Conversion from azathioprine to mycophenolate mofetil	13 (46)	13 (43)	
Daily dose of azathioprine AUC — mg‡	69.5±9.2	63.9±10	0.69
Daily dose of mycophenolate mofetil AUC — mg‡	726±194	738±175	0.96
No. of methylprednisolone pulses — 3 g/patient/yr	0.68±0.18	1.06±0.45	0.45
No. of antithymocyte globulin treatments — per patient per yr	0.17±0.06	0.60±0.45	0.37

* Plus–minus values are means ±SE unless otherwise indicated. Percentages may not sum to 100 because of rounding. AUC denotes area under the concentration–time curve.
† Compliance was determined on the basis of the number of doses patients were eligible to receive up to the time of their death or at two years as a percentage of the doses they received.
‡ Mean daily doses of prednisone, azathioprine, and mycophenolate mofetil were calculated from the reported doses at follow-up visits.
§ Mean calcineurin-inhibitor levels were calculated on the basis of values obtained for each patient.

lost to follow-up. Group means were compared with the use of unpaired, two-tailed t-tests or Mann–Whitney tests. All reported P values are two-sided and have not been adjusted for multiple testing.

The frequency of acute rejection was calculated by determining the number of rejection events of grade 2 or higher per year of study time for each subject. Differences between groups were also considered with the use of a Poisson regression model, with covariates including treatment group, CMV mismatch, and the occurrence or nonoccurrence of a rejection episode before the start of the study treatment. The Poisson model was calculated by Chiron.

Log-rank and Cox proportional-hazards analyses were used to compare survival and chronic rejection–free survival. Nonaerosol covariates that were tested in multivariate analyses included CMV-mismatch status, HLA-mismatch status, the age and sex of the recipient, the age and sex of the donor, smoking history of the recipient, diagnosis before transplantation, type of transplantation (single or double), and ischemic time. After closure of the study and unblinding, Chiron performed a follow-up analysis of survival as of June 2004. Statistical analyses were performed with Statview software (SAS).

RESULTS

CHARACTERISTICS OF THE PATIENTS

Of the 121 patients who received lung transplants during the enrollment period, 58 were randomly assigned to a study group and 56 received at least one dose of study medication (inhaled cyclosporine, 26; placebo aerosol, 30) (Fig. 1). The baseline characteristics and clinical management in the two groups were similar (Table 1). The CMV-mismatch status, the number of biopsy procedures, the number of spirometric measurements, immunosuppressive-drug regimens, and tacrolimus levels were similar in patients in the two groups (Table 1 and Table 2).

The mean duration of treatment was 400±57 days among patients receiving cyclosporine and 431±50 days among patients receiving placebo. Thirteen of the 28 patients in the cyclosporine group (46 percent) and 13 of the 30 patients in the placebo group (43 percent) completed the two-year inhalation period. Reasons for discontinuation are given in Table 2. Discontinuation that was initiated by patients (i.e., patients tolerated the aerosol administration but withdrew from the study) and concern on the part of investigators regarding infection accounted for most of the discontinuations in the cyclosporine group (43 percent) and the placebo group (37 percent), and there were no significant differences between the groups. Two patients in each group stopped treatment because of symptoms related to aerosol inhalation. Two patients in the placebo group and one in the cyclosporine group with refractory rejection were withdrawn from the study and entered into the separate open-label, "rescue" trial of inhaled cyclosporine. Two additional patients received open-label therapy after study medication was stopped. All data from these five patients after crossover were included in the statistical results in the intention-to-treat analysis.

ACUTE REJECTION

We performed a total of 335 biopsies in patients in the cyclosporine group and 333 biopsies among patients in the placebo group. The mean number of biopsies (5.5 per patient per year) exceeded the minimum protocol requirements. The mean follow-up for acute rejection (mean time from study initiation to the last biopsy) was 2.4±0.2 years for the cyclosporine group and 2.2±0.2 years for the placebo group (P=0.43). The estimated number of acute-rejection episodes of grade 2 or higher per patient per year after the start of study-drug administration was 0.44 (95 percent confidence interval, 0.31 to 0.62) for the cyclosporine group and 0.46 (95 percent confidence interval, 0.33 to 0.64) for the placebo group. In each group, 17 patients had no more than one event per year; 26 patients in the cyclosporine group and 23 patients in the placebo group had no more than two events per year; and 26 patients in each group had no more than three events per year. A Poisson regression model with control for CMV-mismatch status and the occurrence or nonoccurrence of a rejection episode of grade 2 or higher before study-drug administration demonstrated that there was no significant difference between the treatment groups (P=0.87).

CHRONIC REJECTION

Chronic rejection–free survival was improved among patients who were treated with inhaled cyclosporine as determined by spirometric and histologic evaluation. Figure 2A shows the re-

sults of a hazards analysis of survival free of the bronchiolitis obliterans syndrome, with 10 events in the cyclosporine group and 20 events in the placebo group (relative risk, 0.38; 95 percent confidence interval, 0.18 to 0.82; P=0.01). Figure 2B shows the results of a similar analysis of survival free of histologic bronchiolitis obliterans, with 6 events among patients in the cyclosporine group and 19 events among patients in the placebo group (relative risk, 0.27; 95 percent confidence interval, 0.11 to 0.67; P=0.005). None of the four patients in the cyclosporine group in whom histologic bronchiolitis obliterans was diagnosed were receiving study drug at the time of diagnosis.

There was no significant difference in the number of methylprednisolone pulses or treatments with antilymphocyte globulin between the two groups. However, none of the 28 patients in the cyclosporine group were treated with sirolimus after other therapies for chronic rejection failed (as determined by histologic or spirometric analysis), as compared with 7 of 30 patients in the placebo group (P=0.006). Overall, more patients required treatment for histologic bronchiolitis obliterans in the placebo group (8 of 30 patients) than in the cyclosporine group (2 of 28 patients) (P=0.05).

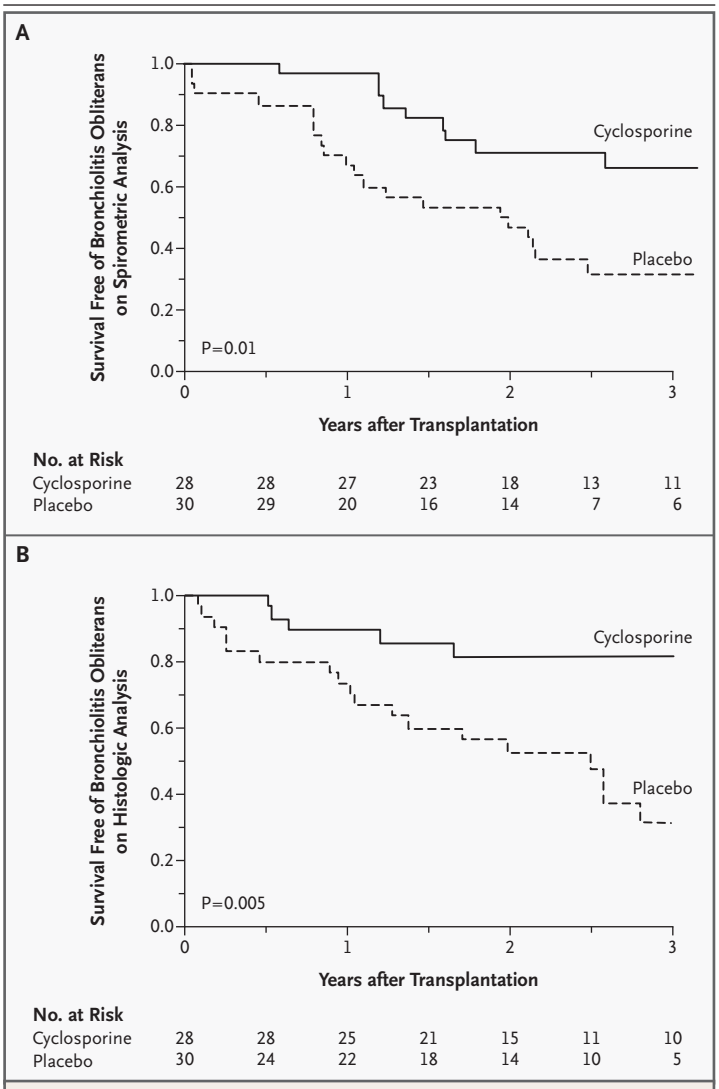


Figure 2. Kaplan–Meier Estimates of Chronic Rejection on the Basis of Bronchiolitis Obliterans Identified by Spirometric and Histologic Analyses.

Multivariate time-to-event regression on the basis of spirometric analysis (Panel A) shows a relative risk of 0.38 for inhaled cyclosporine as compared with placebo (95 percent confidence interval, 0.18 to 0.82; P=0.01 by both Cox proportional-hazards analysis and log-rank analysis). Multivariate time-to-event regression on the basis of histologic analysis (Panel B) shows a relative risk of 0.27 for cyclosporine as compared with placebo (95 percent confidence interval, 0.11 to 0.67; P=0.005 by Cox proportional-hazards analysis and P=0.004 by log-rank analysis).

SURVIVAL ANALYSIS

The use of inhaled cyclosporine was associated with a substantial survival advantage (Fig. 3). There were 14 deaths (47 percent) in the placebo group, as compared with 3 deaths (11 percent) in the cyclosporine group (P=0.005 by log-rank analysis). Multivariate survival regression confirmed that the risk of death for patients receiving placebo was higher by a factor of 5 (relative risk of death in the cyclosporine group as compared with the placebo group, 0.20; 95 percent confidence interval, 0.06 to 0.70; P=0.01) and revealed no significant effect for CMV strata, transplant type, or HLA mismatch.

Ten deaths were attributed to rejection, pneumonia, or sepsis (eight in the placebo group and two in the cyclosporine group). Three other patients with a known previous diagnosis of clinically significant rejection or pneumonia died of either pulmonary embolism (two patients in the placebo group) or congestive heart failure (one in the placebo group). Four patients with a known previous diagnosis of rejection (three with bronchiolitis obliterans) or pneumonia died outside our institution (three in the placebo group and one in the cyclosporine group) without postmortem verification of the cause of death.

A follow-up analysis of survival including all 56 patients who received study medication was conducted during the 10 months that followed the end of the study. During that period, an additional

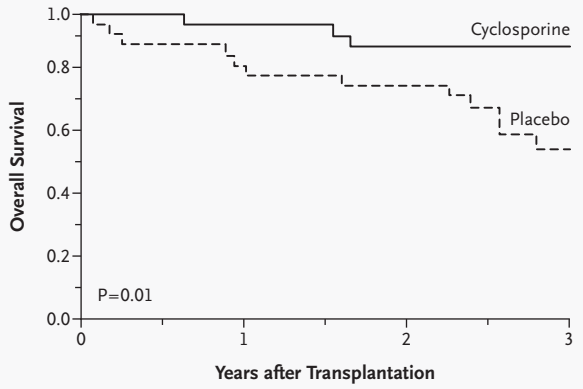


Figure 3. Kaplan–Meier Estimates of Overall Survival.

There were 14 deaths in the placebo group (47 percent) as compared with 3 deaths in the cyclosporine group (11 percent). Survival regression yielded a relative hazard of 0.20 for inhalational cyclosporine as compared with controls (95 percent confidence interval, 0.06 to 0.70; P=0.01 by Cox proportional-hazards analysis and P=0.005 by log-rank analysis).

two patients in the cyclosporine group and one patient in the placebo group died. The results of log-rank analysis of survival were similar to those reported at study closure (P=0.02).[30]

INFECTION

The pneumonia rate was not significantly different in the two groups (13 patients in the cyclosporine group and 17 patients in the placebo group, P=0.44), and patients receiving aerosol cyclosporine were no more likely to be treated for infection than were patients in the placebo group (2.3±0.4 vs. 3.1±0.6 courses of antibiotic per patient per year, respectively; P=0.29). The risk of CMV pneumonitis was less in the cyclosporine group (3 of 28 patients) than in the placebo group (10 of 30 patients; P=0.04). The difference was confirmed by Cox proportional-hazards analysis with study drug and donor–recipient CMV status as covariates, with a relative risk of assignment to cyclosporine of 0.27 (P=0.05) and of CMV mismatch of 4.33 (P=0.009) among CMV-positive donors with CMV-negative recipients, as compared with all other combinations.

ADVERSE EVENTS

Both cyclosporine and placebo aerosols were associated with local irritation, including cough, pharyngeal soreness, or dyspnea in 52 percent of study participants on the basis of responses to a questionnaire administered during clinical visits (Table 3). Such symptoms were observed in both groups, were typically transient, and were either mild or moderate in severity. When symptoms occurred, they usually resolved within 30 to 45 minutes after inhalation.

Twelve patients were given a diagnosis of cancer (6 of 28 patients in the cyclosporine group and 6 of 30 patients in the placebo group, P=0.89). Although the overall area under the concentration–time curve (AUC) for the mean serum creatinine level was not significantly different in the two groups (1.5±0.1 mg per deciliter in the cyclosporine group and 1.7±0.1 mg per deciliter in the placebo group), a higher proportion of patients in the placebo group had an AUC mean creatinine level that was more than 1 SD above the all-patient mean (0 of 28 patients in the cyclosporine group and 6 of 30 patients in the placebo group, P=0.01). There was no significant difference between the groups in the number of hospital days per patient per year (23±4 in the cyclosporine group and 48±12 in the placebo group, P=0.07) or the number of hospitalizations per patient per year (2.1± 0.5 in the cyclosporine group and 3.8± 1.0 in the placebo group, P=0.17).

DISCUSSION

Chronic rejection remains the leading cause of death after lung transplantation despite the use of systemic calcineurin inhibitors.[31-33] The immunosuppressive effects of cyclosporine have been shown to be dose-dependent. However, high systemic levels of the drug cannot be achieved without significant toxicity, especially to the kidneys. We hypothesized that the inhalation of an aerosol cyclosporine would provide high pulmonary concentrations of the drug with minimal systemic toxicity, resulting in less acute and chronic rejection. This double-blind, placebo-controlled trial of inhaled cyclosporine given in addition to conventional immunosuppression after lung transplantation was negative with respect to its primary end point, since rates of acute rejection were similar in the group receiving cyclosporine and that receiving placebo. However, survival improved significantly with aerosol cyclosporine, as did the rate of chronic rejection–free survival

(on the basis of both histologic and spirometric analysis).

In the absence of notable differences in rates of acute rejection, a positive result in terms of chronic rejection was unexpected, since previous studies have linked repeated acute rejection events with chronic rejection.[2] Histologically, chronic rejection presents in the airways as bronchiolitis obliterans, whereas acute rejection presents as vasculitis. Bronchioles would have higher local concentrations of a drug as a result of direct aerosol delivery, whereas pharmacokinetic studies suggest a much less substantial vascular concentration of the drug.[34-36] Therefore, it is possible that aerosol cyclosporine has a local airway antiinflammatory effect that decreases the likelihood of chronic rejection while having a lesser effect on vascular acute rejection.

The rates of pneumonia were similar in the two groups, as were serum creatinine levels. More than half the study patients had some level of local irritation (pharyngeal soreness, cough, or dyspnea), with no significant differences noted between patients in the two groups. Less than 10 percent of patients in the cyclosporine group withdrew because of symptoms associated with the aerosol. However, supervision during the first several treatments would probably be required, given the tenuous respiratory status of patients soon after transplantation. Many patients who had some initial minor respiratory symptoms developed a tolerance for the medication after a few treatments.

Local cyclosporine treatment had some benefit in this small, single-center trial. Further experience with inhaled cyclosporine is needed to confirm the magnitude and durability of the observed effects in recipients of single-lung and double-lung transplants.

Table 3. Adverse Events.*

Reported Event	Cyclosporine (N=28)	Placebo (N=30)	P Value
	no. of patients (%)		
Dyspnea	7 (25)	8 (27)	1.00
Wheezing	2 (7)	1 (3)	0.61
Cough	10 (36)	4 (13)	0.07
Headache	3 (11)	1 (3)	0.34
Pharyngeal soreness	12 (43)	12 (40)	1.00
Difficulty swallowing	10 (36)	8 (27)	0.57
Fatigue	0	1 (3)	1.00
Anxiety	2 (7)	0	0.23
Nausea	3 (11)	1 (3)	0.34
Dizziness	2 (7)	1 (3)	0.61
General intolerance	1 (4)	0	0.48
Tremor	1 (4)	2 (7)	1.00
Other condition	1 (4)	4 (13)	0.35

* The number of adverse events was determined on the basis of patients' answers to a questionnaire administered at regularly scheduled clinic visits. Some patients had more than one adverse event. Fisher's exact test was used to compare rates of individual events.

Supported by a grant from the National Institutes of Health (HL059490 01, to Drs. Iacono and Griffith).

A U.S. patent application (20020006901) entitled "Use of Aerosolized Cyclosporine for Prevention and Treatment of Pulmonary Disease" was submitted on February 5, 1999, and assigned to the University of Pittsburgh, with Dr. Iacono listed as inventor. The patent has been licensed by the University of Pittsburgh to Chiron and Novartis Pharmaceuticals. In the event of eventual commercialization of aerosol cyclosporine, a royalty could be paid to the following investigators at the University of Pittsburgh and the State University of New York, Stony Brook: Dr. Iacono, Dr. Dauber, Dr. Smaldone, Dr. Zeevi, and Dr. Burckart. No author reports having any equity interest in either Chiron or Novartis as a stockholder or other related ownership interests. Drs. Iacono, Johnson, and Corcoran report having received consulting fees and Dr. Zeevi lecture fees from Chiron. Dr. McCurry reports having received a grant from Pfizer. Dr. Dauber reports having received lecture fees from and serving on an advisory board for InterMune. No other potential conflict of interest relevant to this article was reported.

We are indebted to Howard E. Rockette of the Department of Biostatistics at the University of Pittsburgh for trial design and for his assistance with statistical analyses; to Laurence S. Magder of the Department of Biostatistics–Informatics at the University of Maryland for his review of statistical analyses; to William B. Capra of Chiron in Emeryville, Calif., for performing the Poisson regression analysis and reviewing statistical analyses performed at the University of Pittsburgh; to Dr. Brack G. Hattler, Carmella R. Campbell, Mary C. Williams, Jan D. Manzetti, and Judi L. Vensak of the University of Pittsburgh Medical Center for their support for this project and assistance with patient care; to Dr. N. Paul Ohori of the Department of Pathology at the University of Pittsburgh for interpretation of lung biopsies; to Frank J. Clementi of the Sunrise Medical Home Healthcare Group, Somerset, Pa., for donating compressors; to Novartis in East Hanover, N.J., for supplying the cyclosporine powder needed to conduct this study; and to Dr. Maria M. Crespo of the Pulmonary Allergy and Critical Care Division, University of Pittsburgh Medical Center, and Dr. Edward P. Ingenito of the Pulmonary and Critical Care Division, Brigham and Women's Hospital, Boston, for their thoughtful review of the manuscript.

REFERENCES

1. Lin HM, Kauffman HM, McBride MA, et al. Center-specific graft and patient survival rates: 1997 United Network for Organ Sharing (UNOS) report. JAMA 1998;280:1153-60.
2. Bando K, Paradis IL, Similo S, et al. Obliterative bronchiolitis after lung and heart-lung transplantation: an analysis of risk factors and management. J Thorac Cardiovasc Surg 1995;110:4-13.
3. Sharples LD, Tamm M, McNeil K, Higenbottam TW, Stewart S, Wallwork J. Development of bronchiolitis obliterans syndrome in recipients of heart-lung transplantation — early risk factors. Transplantation 1996;61:560-6.
4. Hosenpud JD, Bennett LE, Keck BM, Fiol B, Boucek MM, Novick RJ. The Registry of the International Society for Heart and Lung Transplantation: fifteenth official report — 1998. J Heart Lung Transplant 1998;17:656-68.
5. Trulock EP. Management of lung transplant rejection. Chest 1993;103:1566-76.
6. Yousem SA. Lymphocytic bronchitis/bronchiolitis in lung allograft recipients. Am J Surg Pathol 1993;17:491-6.
7. Yousem SA, Duncan SR, Griffith BP. Interstitial and airspace granulation tissue reactions in lung transplant recipients. Am J Surg Pathol 1992;16:877-84.
8. Date H, Lynch JP, Sundaresan S, Patterson GA, Trulock EP. The impact of cytolytic therapy on bronchiolitis obliterans syndrome. J Heart Lung Transplant 1998;17:869-75.
9. Tikkanen JM, Lemstrom KB, Koskinen PK. Blockade of CD28/B7-2 costimulation inhibits experimental obliterative bronchiolitis in rat tracheal allografts: suppression of helper T cell type 1-dominated immune response. Am J Respir Crit Care Med 2002;165:724-9.
10. Duncan SR, Leonard C, Theodore J, et al. Oligoclonal CD4(+) T cell expansions in lung transplant recipients with obliterative bronchiolitis. Am J Respir Crit Care Med 2002;165:1439-44.
11. Duncan SR, Valentine V, Roglic M, et al. T cell receptor biases and clonal proliferations among lung transplant recipients with obliterative bronchiolitis. J Clin Invest 1996;97:2642-50.
12. Kesten S, Maidenberg A, Winton T, Maurer J. Treatment of presumed and proven acute rejection following six months of lung transplant survival. Am J Respir Crit Care Med 1995;152:1321-4.
13. Trulock EP. Lung transplantation. Am J Respir Crit Care Med 1997;155:789-818.
14. Paradis I, Yousem S, Griffith B. Airway obstruction and bronchiolitis obliterans after lung transplantation. Clin Chest Med 1993;14:751-63.
15. Mitruka SN, Won A, McCurry KR, et al. In the lung aerosol cyclosporine provides a regional concentration advantage over intramuscular cyclosporine. J Heart Lung Transplant 2000;19:969-75.
16. Muggenberg BA, Hoover MD, Griffith BP. Administration of cyclosporine by inhalation: a feasibility study in beagle dogs. J Aerosol Med 1990;3:1-6.
17. Dowling RD, Zenati M, Burckart GJ, et al. Aerosolized cyclosporine as single-agent immunotherapy in canine lung allografts. Surgery 1990;108:198-204.
18. Keenan RJ, Duncan AJ, Yousem SA, et al. Improved immunosuppression with aerosolized cyclosporine in experimental pulmonary transplantation. Transplantation 1992;53:20-5.
19. Iacono AT, Corcoran TE, Griffith BP, et al. Aerosol cyclosporin therapy in lung transplant recipients with bronchiolitis obliterans. Eur Respir J 2004;23:384-90.
20. Iacono AT, McCurry KR, Corcoran TE. Inhalation cyclosporine: a new use in lung transplantation? Curr Opin Organ Transplant 2003;8:327-33.
21. Iacono AT, Keenan RJ, Duncan SR, et al. Aerosolized cyclosporine in lung recipients with refractory chronic rejection. Am J Respir Crit Care Med 1996;153:1451-5.
22. Keenan RJ, Zeevi A, Iacono AT, et al. Efficacy of inhaled cyclosporine in lung transplant recipients with refractory rejection: correlation of intragraft cytokine gene expression with pulmonary function and histologic characteristics. Surgery 1995;118:385-91.
23. Keenan RJ, Iacono A, Dauber JH, et al. Treatment of refractory acute allograft rejection with aerosolized cyclosporine in lung transplant recipients. J Thorac Cardiovasc Surg 1997;113:335-40.
24. Iacono A, Dauber J, Keenan R, et al. Interleukin 6 and interferon-gamma gene expression in lung transplant recipients with refractory acute cellular rejection: implications for monitoring and inhibition by treatment with aerosolized cyclosporine. Transplantation 1997;64:263-9.
25. Iacono AT, Smaldone GC, Keenan RJ, et al. Dose-related reversal of acute lung rejection by aerosolized cyclosporine. Am J Respir Crit Care Med 1997;155:1690-8.
26. Yousem SA, Berry GJ, Cagle PT, et al. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. J Heart Lung Transplant 1996;15:1-15.
27. Standardization of spirometry, 1994 update: American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107-36.
28. Morris JF. Spirometry in the evaluation of pulmonary function. West J Med 1976;125:110-8.
29. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. J Heart Lung Transplant 2002;21:297-310.
30. Iacono AT, Capra WB, Shrewsbury SB, et al. Long-term follow-up of a double-blind, randomized, placebo-controlled trial of Cyclosporine Inhalation Solution (CyIS) in lung transplant recipients. J Heart Lung Transplant 2005;24:S82-S83. abstract.
31. Di Padova FE. Pharmacology of cyclosporine (Sandimmune). V. Pharmacological effects on immune function: in vitro studies. Pharmacol Rev 1990;41:373-405.
32. Losa Garcia JE, Mateos Rodriguez F, Jiminez Lopez A, et al. Effect of cyclosporin A on inflammatory cytokine production by human alveolar macrophages. Respir Med 1998;92:722-8.
33. Eickelberg O, Pansky A, Koehler E, et al. Molecular mechanisms of TGF-β antagonism by interferon-γ and cyclosporine A in lung fibroblasts. FASEB J 2001;15:797-806.
34. Burckart GJ, Smaldone GC, Eldon MA, et al. Lung deposition and pharmacokinetics of cyclosporine after aerosolization in lung transplant patients. Pharm Res 2003;20:252-6.
35. Corcoran TE, Smaldone GC, Dauber JH, et al. Preservation of post-transplant lung function with aerosol cyclosporine. Eur Respir J 2004;23:378-83.
36. O'Riordan TG, Iacono A, Keenan RJ, et al. Delivery and distribution of aerosolized cyclosporine in lung allograft recipients. Am J Respir Crit Care Med 1995;151:516-21.

BRIEF REPORT

Familial Sinus Bradycardia Associated with a Mutation in the Cardiac Pacemaker Channel

Raffaella Milanesi, Ph.D., Mirko Baruscotti, Ph.D.,
Tomaso Gnecchi-Ruscone, M.D., and Dario DiFrancesco, Ph.D.

SUMMARY

We found that sinus bradycardia in members of a large family was associated with a mutation in the gene coding for the pacemaker HCN4 ion channel. Pacemaker channels of the sinoatrial node generate spontaneous activity and mediate cyclic AMP (cAMP)–dependent autonomic modulation of the heart rate. The mutation associated with bradycardia is located near the cAMP-binding site; functional analysis found that mutant channels respond normally to cAMP but are activated at more negative voltages than are wild-type channels. These changes, which mimic those of mild vagal stimulation, slow the heart rate by decreasing the inward diastolic current. Thus, diminished function of pacemaker channels is linked to familial bradycardia.

Bradycardia is conventionally defined as a heart rate lower than 60 beats per minute. Asymptomatic sinus bradycardia is usually harmless and is often a sign of good physical conditioning. On the other hand, symptomatic sinus bradycardia, such as that associated with the sick-sinus syndrome, can be a life-threatening condition and deserves prompt medical attention. The fact that sinus bradycardia can be inherited[1] indicates that it can have a genetic basis.

The pacemaker ("funny") current (I_f) of sinoatrial-node myocytes determines the slope of the diastolic depolarization of pacemaker cells and thus has a key role in the generation and autonomic regulation of sinus rhythm and rate.[2] Because of their specific involvement in pacemaking, f-channels are the target for the pharmacologic control of heart rate. Indeed, substances acting by specific f-channel blockade slow the heart rate without side effects, such as the negative inotropic effects typical of beta-blockers or calcium antagonists.[3] This property is particularly useful in the treatment of coronary heart disease.[4]

The f-channels are encoded by the hyperpolarization-activated, cyclic nucleotide–gated (HCN) channel gene family.[5,6] Of the four known HCN subunits (1 through 4), HCN4 is the most highly expressed in the mammalian sinoatrial node.[7] We therefore screened a panel of persons with asymptomatic or symptomatic bradycardia (including sick-sinus syndrome and atrioventricular block) for mutations in the *hHCN4* gene. The *hHCN4* gene is located on chromosome 15 (15q24–25)[8]; the genes coding for other channels contributing to the electrical activity of sinoatrial-node cells, including the L- and T-type calcium channels and delayed-rectifying potassium channels, are all located on different chromosomes.[9]

We report here on a family with a hereditary form of asymptomatic bradycardia associated with a mutation in the pacemaker-channel α-subunit HCN4. Functional

From the Department of Biomolecular Sciences and Biotechnology, Laboratory of Molecular Physiology and Neurobiology, University of Milan, Milan (R.M., M.B., D.D.); and the Department of Cardiology, Merate Hospital, Merate, Italy (T.G.-R.). Address reprint requests to Dr. DiFrancesco at the Department of Biomolecular Sciences and Biotechnology, Laboratory of Molecular Physiology and Neurobiology, University of Milan, Via Celoria 26, 20133 Milan, Italy, or at dario.difrancesco@unimi.it.

N Engl J Med 2006;354:151-7.

analysis revealed that the mutant channels are activated at voltages more negative than those at which wild-type channels are activated; as a result, they supply less current during diastolic depolarization, which in turn results in slowing of the heart rate. The mutation mimics the effect of moderate vagal stimulation.

METHODS

Our research protocol was reviewed and approved by the institutional review board for the Department of Biomolecular Sciences and Biotechnology, University of Milan.

SEQUENCE ANALYSIS AND MUTAGENESIS

All subjects gave written informed consent before undergoing genetic analysis. Screening for mutations was performed on genomic DNA samples extracted from whole blood, saliva, or both (Puragene, Gentra Systems). The primers were designed to amplify DNA fragments of 200 to 350 bp in order to screen all of the coding portion of *hHCN4*. The polymerase-chain-reaction (PCR) products were analyzed by single-strand conformation polymorphism (SSCP) analysis. We used the primers 5′ATGCCTCATCCTGAGTCCTG3′ (F) and 5′CTCACCAATGCGGTCCAG3′ (R) to amplify exon 7 by Pfu polymerase (Stratagene). Mutagenesis was identified by DNA sequencing (MWG Biotech). A control group of 373 healthy subjects was used to exclude DNA polymorphisms. The *hHCN4* complementary DNA was inserted in the eukaryotic expression vector pcDNA 1.1 (Clontech Laboratories), and the point mutation (2016 C→A) was generated by a PCR overlap method. The oligonucleotide used to incorporate the mutation into *hHCN4* was 5′ACAGCCAGAGTGAGGGCC3′.

ELECTROPHYSIOLOGICAL METHODS

Wild-type and mutant human (*h*) *HCN4* channel complementary DNA was transfected for transient functional expression into HEK293 cells with a plasmid containing green fluorescent protein, as described previously.[10] From one to five days after transfection, the cells were dispersed by trypsin and plated on 35-mm plastic petri dishes. A dish was placed under the stage of an inverted microscope, and GFP-expressing cells were selected for patch-clamp analysis at room temperature (25° to 26°C). The cells were initially superfused with Tyrode's solution containing 140 mM sodium chloride, 5.4 mM potassium chloride, 1.8 mM calcium chloride, 5.5 mM D-glucose, and 5 mM HEPES–sodium hydroxide buffer (pH 7.4). The pipettes used in the whole-cell patch-clamp experiment contained 10 mM sodium chloride, 130 mM potassium chloride, 1 mM egtazic acid (EGTA), 0.5 mM magnesium chloride, 2 mM ATP (sodium salt), 0.1 mM guanosine triphosphate (sodium salt), 5 mM phosphocreatine, and 5 mM HEPES–potassium hydroxide buffer (pH 7.2). The control extracellular solution in whole-cell experiments contained 110 mM sodium chloride, 30 mM potassium chloride, 1.8 mM calcium chloride, 0.5 mM magnesium chloride, and 5 mM HEPES–sodium hydroxide buffer (pH 7.4); 1 mM barium chloride, 2 mM manganese chloride, 100 μM nickel chloride, and 20 μM nifedipine were added to improve dissection of the pacemaker current. The pipettes used in inside-out macropatch[11] experiments contained 70 mM sodium chloride, 70 mM potassium chloride, 1.8 mM calcium chloride, 1 mM magnesium chloride, 1 mM barium chloride, 2 mM manganese chloride, and 5 mM HEPES–sodium hydroxide buffer (pH 7.4); a solution containing 130 mM potassium aspartate, 10 mM sodium chloride, 2 mM calcium chloride, 5 mM EGTA–potassium hydroxide, and 10 mM HEPES–potassium hydroxide buffer (pH 7.2; pCa [the negative log of the concentration of Ca^{2+} ions] 7) perfused the intracellular sides of the patches.

For the investigation of mutant channels, HEK293 cultures were split into two halves, one to express control channels and one to express mutant channels, and the two halves were treated by identical procedures; experiments comparing the properties of mutant and control channels were always performed on cells matched according to the day of culture.

The activation curves for hHCN4 currents recorded under whole-cell conditions were obtained by standard activation and deactivation protocols and analyzed by the Boltzmann equation, $y = 1 \div \{1 + \exp[(V - V_{1/2}) \div s]\}$, where y is the fractional activation, V is the voltage in millivolts, $V_{1/2}$ is the half-activation voltage in millivolts, and s is the inverse slope factor in millivolts. Mean activation curves were obtained by fitting individual curves from each cell to the Boltzmann equation and averaging half-activation voltages and inverse slope factors. The activation curves in inside-out macropatches, the time constants of current activation and deactivation, and the shifts induced

by cAMP were calculated as previously reported.[12] The dose–response curves of cAMP-induced shifts in inside-out macropatches were analyzed by the Hill equation, as follows: $S \div S_{max} = 1 \div [1 + (k_{1/2} \div \text{cAMP concentration})^h]$, where S is the shift, $k_{1/2}$ is the half-maximal concentration, and h is the Hill factor. Each patch was exposed to cAMP only once. The holding potential was −35 mV in all experiments.

MEASUREMENTS OF HEART RATE

Heart-rate measurements were performed during the daytime (between 10 a.m. and 6 p.m.) with the patient at rest. The rates for children 10 years of age or younger were normalized to adult rates by linear scaling according to published data (Table A1.46 in Macfarlane and Lawrie[13]). The mean adult rates used for normalization were calculated by averaging the rates for the age groups 18 through 29 years, 30 through 39 years, 40 through 49 years, and 50 years or more from Table A1.1 in Macfarlane and Lawrie.[13] These rates (±SE) were 71.0±0.7 beats per minute for men and 76.7±0.2 beats per minute for women.

STATISTICAL ANALYSIS

Data were compared by the independent Student's t-test or two-way analysis of variance, and P values of 0.05 or less were considered to indicate statistical significance. Genetic-linkage analysis and lod-score calculations were performed with the MLINK program of the LINKAGE software package.[14]

RESULTS

We screened 52 persons with bradycardia for mutations anywhere in the coding region of the pacemaker-channel gene *hHCN4*. This procedure led us initially to identify a missense mutation in exon 7, S672R, in one member of an Italian family (the proband) with asymptomatic sinus bradycardia (heart rate, 43 beats per minute). We then collected and examined DNA from a total of 27 members of the same family. PCR–SSCP analysis showed that the S672R mutation cosegregates with the bradycardic phenotype, indicating an autosomal dominant pattern (Fig. 1). The heart rate varied from 43 to 60 beats per minute in persons with the mutated gene (mean value for 15 persons, 52.2±1.4 beats per minute) and from 64 to 81 beats per minute in those with the wild-type gene (mean value for 12 persons, 73.2±1.6 beats per minute; P<0.001 by Student's t-test) (Fig. 1 □□□□). The presence of the S672R mutation was confirmed by electropherograms (Fig. 2A). The same mutation was absent in 746 control chromosomes from unrelated persons of Italian origin.

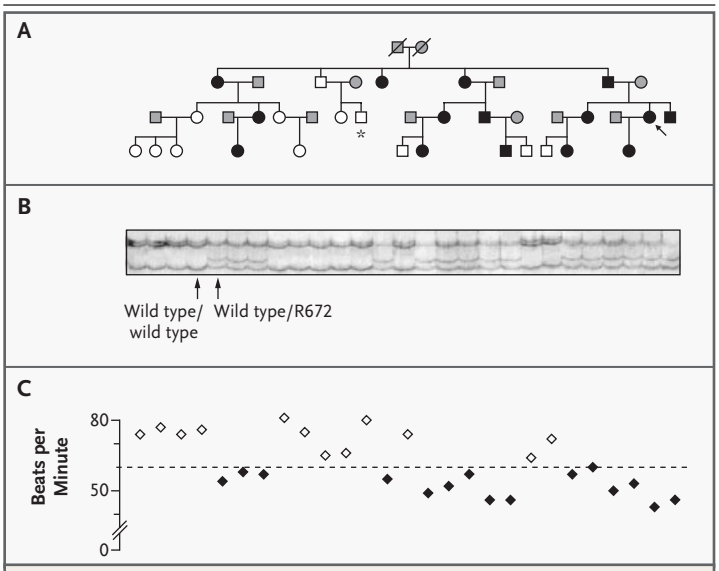


Figure 1. Mutation in *hHCN4* Resulting in Sinus Bradycardia.

Panel A shows the pedigree of a four-generation family with sinus bradycardia (the arrow indicates the proband). Solid symbols represent persons carrying the mutation, and open symbols those without the gene (termed controls). Gray symbols indicate persons who are not represented in Panels B and C. The asterisk indicates the control whose electrocardiogram is shown in Figure 2B, left-hand panel. Panel B presents an analysis of exon 7 of *hHCN4* by polymerase chain reaction–single-strand conformational polymorphism that shows control (wild type/wild type) and aberrant (wild type/R672) band patterns, aligned in correspondence with the pedigree diagram. Panel C shows a corresponding plot of the heart rates of persons carrying the mutation and controls (solid and open diamonds, respectively), measured at rest.

To quantify the cosegregation of bradycardia with the *hHCN4* gene, we calculated the two-point lod score for the family shown in Figure 1. Since no estimates of the frequency of the gene for sinus bradycardia exist, for the purposes of this study we assumed a frequency of 0.005 for this gene and a penetrance of 0.9. The maximum lod score was 5.47 (at $\theta = 0$, the range of θ is 0 to 0.5, step 0.01), indicating tight linkage. Changing the penetrance over the range from 0.7 to 1.0 generated maximum lod scores (at $\theta = 0$) in the range of 5.07 to 5.66.

The HCN channels are six transmembrane-domain channels that are dually activated by voltage hyperpolarization and cAMP,[2,5] and the

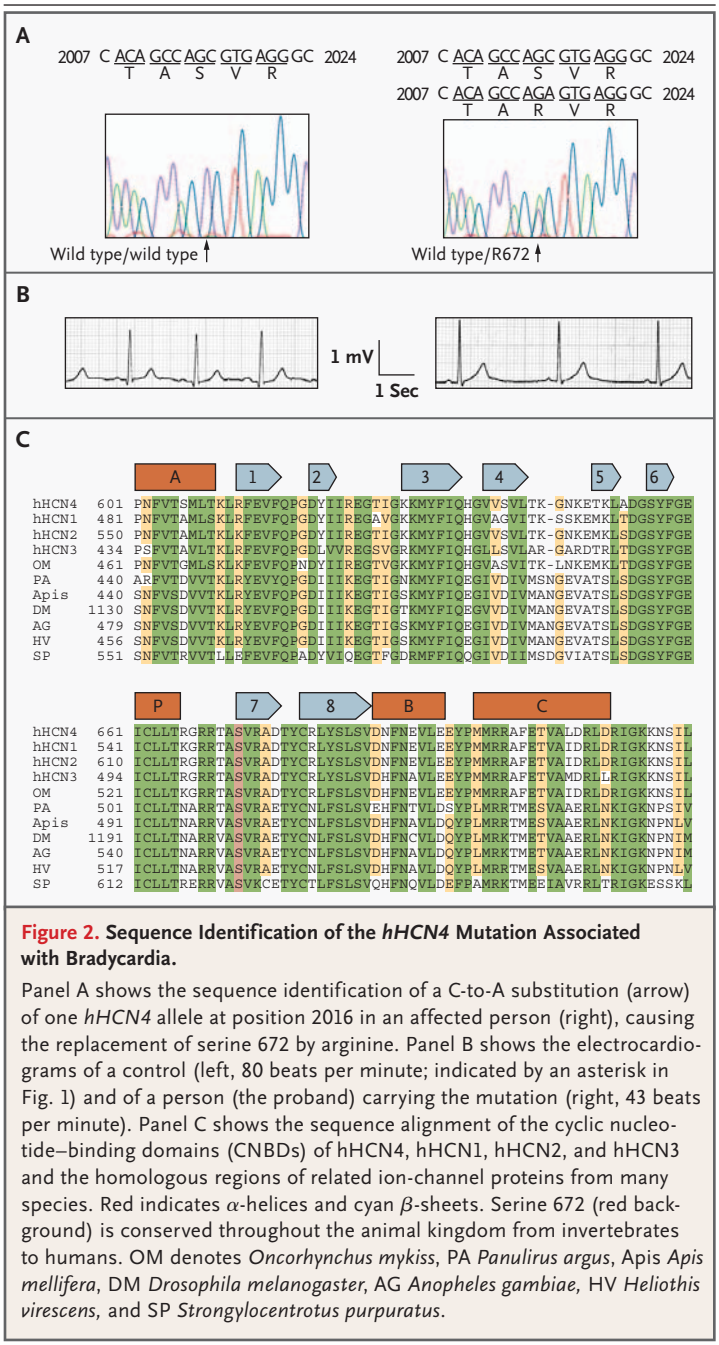


Figure 2. Sequence Identification of the *hHCN4* Mutation Associated with Bradycardia.

Panel A shows the sequence identification of a C-to-A substitution (arrow) of one *hHCN4* allele at position 2016 in an affected person (right), causing the replacement of serine 672 by arginine. Panel B shows the electrocardiograms of a control (left, 80 beats per minute; indicated by an asterisk in Fig. 1) and of a person (the proband) carrying the mutation (right, 43 beats per minute). Panel C shows the sequence alignment of the cyclic nucleotide–binding domains (CNBDs) of hHCN4, hHCN1, hHCN2, and hHCN3 and the homologous regions of related ion-channel proteins from many species. Red indicates α-helices and cyan β-sheets. Serine 672 (red background) is conserved throughout the animal kingdom from invertebrates to humans. OM denotes *Oncorhynchus mykiss*, PA *Panulirus argus*, Apis *Apis mellifera*, DM *Drosophila melanogaster*, AG *Anopheles gambiae*, HV *Heliothis virescens*, and SP *Strongylocentrotus purpuratus*.

native cardiac pacemaker f-channels are composed mainly of hHCN4 subunits.[7] The cAMP activates the channels by direct binding[11] and partial removal of an intrinsic inhibitory mechanism,[15,16] resulting in a depolarizing shift of the open probability curve[12]; this shift underlies the modulation of the heart rate by autonomic neurotransmitters.[17-19]

Since the S672R mutation is located within the cyclic nucleotide–binding domain (CNBD) of hHCN4 (Fig. 2C), it might affect cAMP binding and thus interfere with the autonomic modulation of heart rate. The fact that S672 is highly conserved throughout the animal kingdom (Fig. 2C) supports the view that it has an essential functional role.

To evaluate the functional effect of the S672R mutation, we transfected wild-type and mutated *hHCN4* complementary DNA into HEK293 cells (Fig. 3A). The mutant S672R channels were expressed in HEK293 cells as efficiently as were wild-type channels. However, the S672R mutant channels were activated at more negative voltages than were wild-type channels. The half-activation voltages, as calculated from the Boltzmann equation, were −76.1±1.3 mV for the wild-type channels and −84.5±1.5 mV for the mutant channels ($P=0.002$ by Student's t-test), with a negative shift of 8.4 mV; inverse slope factors did not change significantly (10.2±0.8 and 11.2±0.8 mV for wild-type and mutant channels, respectively; $P=0.41$) (Fig. 3A, left-hand panel). Moreover, the mutant channels were deactivated faster than were the wild-type channels, as shown by plotting mean activation and deactivation time-constant curves for wild-type and mutant channels (Fig. 3A, right-hand panel). The deactivation time constants were significantly faster in mutant than in wild-type channels, according to two-way analysis of variance ($P<0.001$). These properties of the mutant channels are compatible with a reduced contribution of the pacemaker current to diastolic depolarization.

A negative shift of the activation range could be due to a reduced ability of the basal cAMP concentration in HEK293 cells to activate S672R channels; this possibility is suggested by the localization of the mutation within the CNBD (Fig. 2C), close to residues (such as R669) affecting cAMP binding.[20] However, cAMP-induced shifts of the current activation curve measured in inside-out patches were similar for wild-type and mutant channels (Fig. 3B, left-hand panel): fitting with the Hill equation (lines) yielded maximal shifts of 9.79 and 9.67 mV, half-maximal concentrations of 1.53 and 1.66 μM, and Hill slopes of 0.787 and 0.982 for wild-type and mutant channels, respectively.

Since the S672R mutation does not interfere with cAMP-induced channel activation, the negative shift of the activation curve in Figure 3A prob-

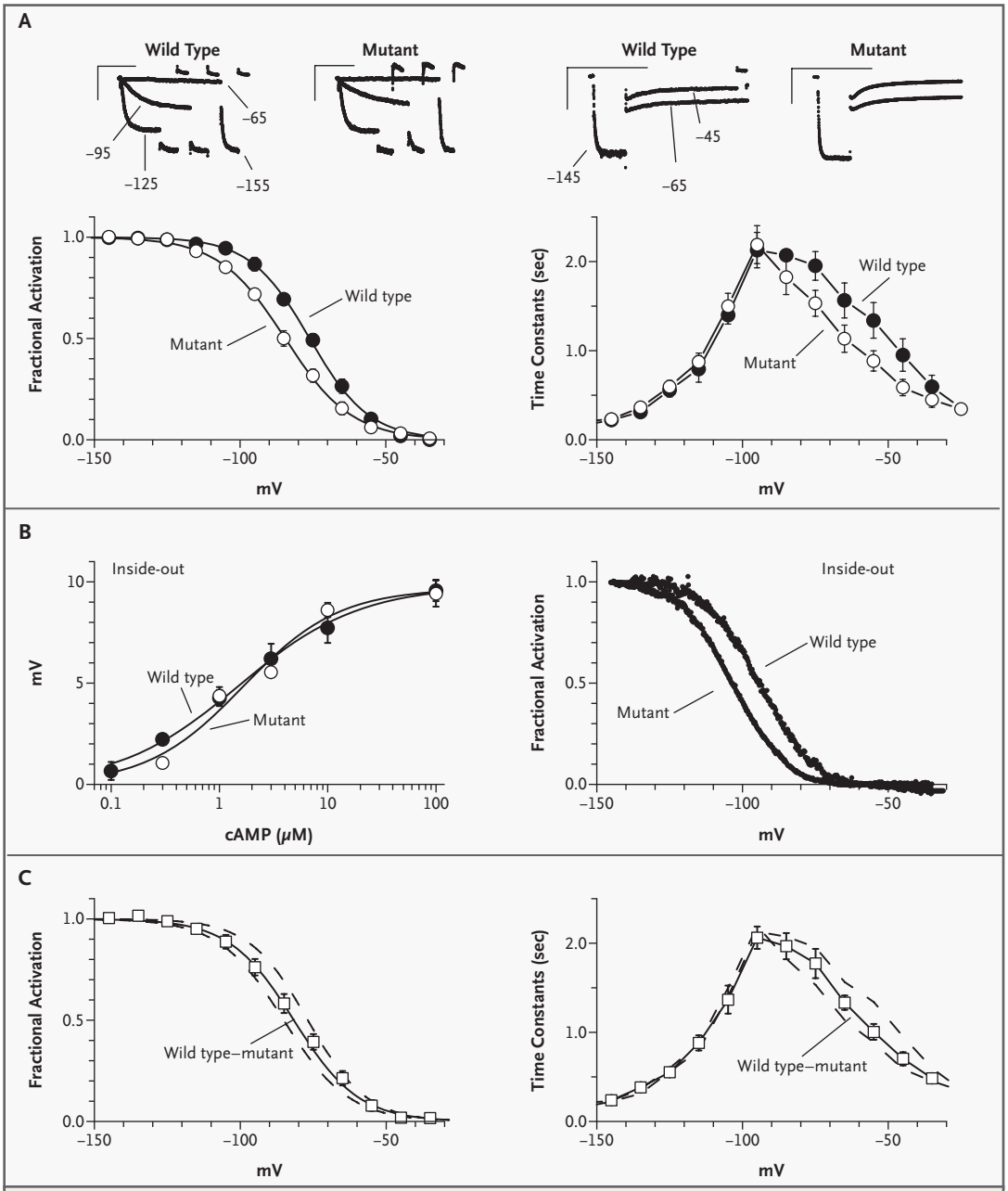


Figure 3. Kinetic Properties of Mutant hHCN4 Channels.

Panel A (left) shows the mean activation curves for wild-type channels (solid circles, six cells) and homomeric S672R mutant hHCN4 channels (open circles, eight cells) expressed in HEK293 cells. Sample traces recorded at the voltages indicated are shown at the top; the vertical scale bars represent current (50 picoamperes per picofarad) and the horizontal scale bars, time (5 seconds). Panel A (right) shows the time-constant curves of activation (less than –90 mV) and deactivation (greater than or equal to –90 mV) obtained as averages of five curves for wild-type channels and nine curves for mutant channels. Panel B (left) shows mean cAMP-dependent shifts of the activation curve measured in inside-out macropatches expressing wild-type channels (solid circles, 27 patches) and mutant channels (open circles, 25 patches). Each data point is the average of three to nine exposures. Panel B (right) shows the mean activation curves of wild-type channels (averaged from five curves) and mutant channels (averaged from nine curves) measured in inside-out macropatches by slow voltage ramps.[12] Panel C (left) shows the mean activation curve for heteromeric wild-type–mutant channels (solid line, seven cells). Panel C (right) shows the time constants of activation and deactivation, as averaged from eight curves. The curves for wild-type and homomeric mutant channels, which are also shown in Panel A, are plotted here as broken lines. Mean ±SE values are plotted in Panels A, B (left), and C.

ably reflects a constitutive new biophysical property of the channel caused by the mutation. This was confirmed by measuring activation curves in inside-out macropatches in the absence of cAMP: the $V_{1/2}$ of the mutant channels was −94.4 mV, 9.1 mV more negative than that of the wild-type channels (−103.5 mV), and s was 8.7 mV in both cases (Fig. 3B, right-hand panel). Thus, S672R channels are constitutively activated at voltages about 8 to 9 mV more negative than are wild-type channels.

Finally, in order to produce channels heteromeric for wild-type and mutant α-subunits, such as those occurring in heterozygotes, we cotransfected identical amounts of wild-type and S672R complementary DNA into HEK293 cells and measured the resulting currents (Fig. 3C). The properties of heteromeric wild-type–mutant channels were intermediate between those of wild-type and homomeric S672R channels. The mean activation curve had a $V_{1/2}$ of −81.0±1.6 mV, with a shift of −4.9 mV relative to the control ($P=0.042$ by Student's t test); s was 11.2±0.7mV ($P=0.38$) (Fig. 3C, left-hand panel); the deactivation time constants were significantly different from those of wild-type channels according to two-way analysis of variance ($P=0.008$) (Fig. 3C, right-hand panel).

Shifting of the I_f activation curve underlies physiologic frequency changes in pacemaker cells.[2] Moderate vagal activity slows the heart rate by a muscarinic-induced negative shift of the I_f activation curve,[18] a mechanism contributing to the basal slow rate associated with vagal tone[19]; this effect is accompanied by a marked acceleration of channel deactivation[18] and is fully explained by a reduction of intracellular cAMP.[11] The effects of the heterozygous S672R mutation on hHCN4 channels (a shift of −4.9 mV in the activation curve and acceleration of deactivation) are therefore analogous to those of a low dose (10 to 30 nM) of acetylcholine.[19] This led us to speculate that the bradycardic rate slowing in persons heterozygous for the S672R mutation might be similar to the slowing induced in freely beating pacemaker myocytes by 10 to 30 nM acetylcholine. Published data indicate that the slowing in pacemaker rate caused by acetylcholine at these concentrations varies between 15 and 41 percent.[19] Indeed, in the family investigated, the bradycardic mutant rates were on average 29 percent slower than nonbradycardic rates (Fig. 1), a result compatible with the above concept.

DISCUSSION

We report that familial sinus bradycardia is associated with a mutation in the HCN4 pacemaker-channel α-subunit. HCN4 is the major HCN isoform contributing to native f-channels in the sinoatrial node, the natural cardiac pacemaker region.[7] Defective HCN4 channels have been found to be associated with disorders of cardiac rhythm, but previous reports either were based on a single patient[21] or described a complex array of rhythm disturbances without clarifying which specific effect was mechanistically related to the mutation.[22]

The f-channels control pacemaker activity by generating the diastolic depolarization phase of the action potential, and they mediate the chronotropic action of autonomic neurotransmitters.[2] They are activated by cAMP[11]; by increasing cAMP levels, β-adrenergic stimulation increases diastolic I_f and consequently steepens diastolic depolarization, thus causing an acceleration of the heart rate[17]; the opposite mechanism operates when muscarinic stimulation by acetylcholine decreases cAMP levels and I_f and slows the heart rate.[18,19]

The hHCN4 mutation associated with bradycardia, S672R, is located in the CNBD (Fig. 2C), a region composed of several residues that affect cAMP binding.[23] We found, however, that the S672R mutation does not affect cAMP-induced channel activation, but it does modify channel kinetics by shifting the current activation range to hyperpolarized voltages and slowing current deactivation; these changes mimic those caused by a low concentration (10 to 30 nM) of acetylcholine[19] and lead to a reduced flow of inward current during diastolic depolarization and hence to slowing of the heart rate.

Finally, it is known that several mutations may alter the kinetics and cAMP dependence of HCN channels[24,25]; furthermore, HCN kinetics are modified by β-subunits.[6] Thus, the mutation reported here may represent a specific case of a broader mechanism for sinus bradycardia based on constitutive inhibition of pacemaker channels.

Supported by grants from the Ministero dell'Istruzione, dell'Università e della Ricerca (FISR 02.00652.ST97, Cofin 2003052919, and FIRB RBNE01LNX7) to Dr. DiFrancesco.

No potential conflict of interest relevant to this article was reported.

We are indebted to Professor D. Noble (University of Oxford) for comments; to Drs. C. Viscomi, B. Terragni, A. Moroni, G. Consalez, and L. Gianfranceschi for assistance; to D. Lopez and G. Suffia for technical help; to Dr. B.U. Kaupp (University of Jülich) for supplying *hHCN4*; and to Dr. S. Priori (University of Pavia) for giving us access to control DNA.

REFERENCES

1. Sarachek NS, Leonard JL. Familial heart block and sinus bradycardia: classification and natural history. Am J Cardiol 1972;29:451-8.

2. DiFrancesco D. Pacemaker mechanisms in cardiac tissue. Annu Rev Physiol 1993;55:455-72.

3. Singh BN. Morbidity and mortality in cardiovascular disorders: impact of reduced heart rate. J Cardiovasc Pharmacol Ther 2001;6:313-31.

4. DiFrancesco D, Camm JA. Heart rate lowering by specific and selective I_f current inhibition with ivabradine: a new therapeutic perspective in cardiovascular disease. Drugs 2004;64:1757-65.

5. Baruscotti M, Bucchi A, DiFrancesco D. Physiology and pharmacology of the cardiac pacemaker ("funny") current. Pharmacol Ther 2005;107:59-79.

6. Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 2003;65:453-80.

7. Shi W, Wymore R, Yu H, et al. Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues. Circ Res 1999; 85:e1-e6.

8. Seifert R, Scholten A, Gauss R, Mincheva A, Lichter P, Kaupp UB. Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. Proc Natl Acad Sci U S A 1999; 96:9391-6.

9. Roden DM, Balser JR, George AL Jr, Anderson ME. Cardiac ion channels. Annu Rev Physiol 2002;64:431-75.

10. Viscomi C, Altomare C, Bucchi A, et al. C terminus-mediated control of voltage and cAMP gating of hyperpolarization-activated cyclic nucleotide-gated channels. J Biol Chem 2001;276:29930-4.

11. DiFrancesco D, Tortora P. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. Nature 1991;351: 145-7.

12. DiFrancesco D, Mangoni M. Modulation of single hyperpolarization-activated channels (i_f) by cAMP in the rabbit sinoatrial node. J Physiol 1994;474:473-82.

13. Macfarlane PW, Lawrie TDV. Comprehensive electrocardiology: theory and practice in health and disease. New York: Pergamon Press, 1989.

14. Terwilliger JD, Ott J. Handbook of human genetics linkage. Baltimore: Johns Hopkins University Press, 1994.

15. Barbuti A, Baruscotti M, Altomare C, Moroni A, DiFrancesco D. Action of internal pronase on the f-channel kinetics in the rabbit SA node. J Physiol 1999;520: 737-44.

16. Wainger BJ, DeGennaro M, Santoro B, Siegelbaum SA, Tibbs GR. Molecular mechanism of cAMP modulation of HCN pacemaker channels. Nature 2001;411:805-10.

17. Brown HF, DiFrancesco D, Noble SJ. How does adrenaline accelerate the heart? Nature 1979;280:235-6.

18. DiFrancesco D, Tromba C. Inhibition of the hyperpolarization-activated current (i_f) induced by acetylcholine in rabbit sinoatrial node myocytes. J Physiol 1988;405: 477-91.

19. DiFrancesco D, Ducouret P, Robinson RB. Muscarinic modulation of cardiac rate at low acetylcholine concentrations. Science 1989;243:669-71.

20. Chen S, Wang J, Siegelbaum SA. Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. J Gen Physiol 2001;117:491-504.

21. Schulze-Bahr E, Neu A, Friederich P, et al. Pacemaker channel dysfunction in a patient with sinus node disease. J Clin Invest 2003;111:1537-45.

22. Ueda K, Nakamura K, Hayashi T, et al. Functional characterization of a trafficking-defective HCN4 mutation, D553N, associated with cardiac arrhythmia. J Biol Chem 2004;279:27194-8.

23. Zagotta WN, Olivier NB, Black KD, Young EC, Olson R, Gouaux E. Structural basis for modulation and agonist specificity of HCN pacemaker channels. Nature 2003;425:200-5.

24. Vaca L, Stieber J, Zong X, Ludwig A, Hofmann F, Biel M. Mutations in the S4 domain of a pacemaker channel alter its voltage dependence. FEBS Lett 2000;479: 35-40.

25. Chen J, Mitcheson JS, Tristani-Firouzi M, Lin M, Sanguinetti MC. The S4-S5 linker couples voltage sensing and activation of pacemaker channels. Proc Natl Acad Sci U S A 2001;98:11277-82.

CLINICAL PRACTICE

Chronic Daily Headache

David W. Dodick, M.D.

This Journal *feature begins with a case vignette highlighting a common clinical problem. Evidence supporting various strategies is then presented, followed by a review of formal guidelines, when they exist. The article ends with the author's clinical recommendations.*

A 36-year-old woman with a long history of catamenial migraines had had a headache almost every day during the previous year. The background headache was mild but became severe and incapacitating at least twice a week, interfering with work and sleep. She took six to eight tablets containing a combination of aspirin, acetaminophen, and caffeine per day, with minimal relief. She had no fever, weight loss, diplopia, or tinnitus. Her headaches were not exacerbated by a Valsalva maneuver or positional change. Her physical examination was normal. How should she be evaluated and treated?

THE CLINICAL PROBLEM

From the Department of Neurology, Mayo Clinic College of Medicine, Scottsdale, Ariz. Address reprint requests to Dr. Dodick at the Department of Neurology, Mayo Clinic College of Medicine, 13400 E. Shea Blvd., Scottsdale, AZ 85259, or at dodick.david@mayo.edu.

N Engl J Med 2006;354:158-65.

Chronic daily headache refers to the presence of a headache more than 15 days per month for longer than 3 months. Chronic daily headache is not a diagnosis but a category that contains many disorders representing primary and secondary headaches.[1,2] Secondary causes must be ruled out before the diagnosis of a primary headache disorder is made. Approximately 3 to 5 percent of the population worldwide[3-5] and 70 to 80 percent of patients presenting to headache clinics in the United States[6] have daily or near-daily headaches. The disability associated with this disorder is substantial and includes a diminished quality of life related to physical and mental health, as well as impaired physical, social, and occupational functioning.[7-9]

Risk factors for chronic daily headache as identified in population-based and clinic-based studies include obesity, a history of frequent headache (more than one per week), caffeine consumption, and overuse (more than 10 days per month) of acute-headache medications, including analgesics, ergots, and triptans.[10-14] Over half of all patients with chronic daily headache have sleep disturbances and mood disorders such as depression or anxiety, and these disorders can exacerbate the underlying headache.

Transformed migraine and medication-overuse headaches are among the most common and challenging of the chronic daily headache disorders and are the focus of this review (Table 1). The clinical features of primary chronic daily headache disorders are summarized in Table 2.[15] Primary chronic daily headache disorders are classified on the basis of the usual length of each episode — that is, prolonged (four hours or longer) or brief (less than four hours).[1]

STRATEGIES AND EVIDENCE

DIAGNOSIS

Before a primary headache can be diagnosed, secondary causes must be considered. The development of progressively frequent and severe headaches within a period

Table 1. Criteria for Transformed Migraine and Medication-Overuse Headache.

Transformed migraine*
Daily or almost daily (>15 days per month) head pain for >1 mo
Average headache lasting >4 hr per day (if untreated)
At least one of the following criteria:
History of any form of episodic migraine meeting IHS criteria†
History of increasing headache frequency with decreasing severity of migrainous features over a period of at least 3 mo
Headache at some time meets IHS criteria for migraine other than duration
Does not meet criteria for new daily persistent headache or hemicrania continua
Medication-overuse headache[2]
Headache present at least 15 days per month characterized by the development or marked worsening of pain during medication overuse and resolution of pain and reversion to previous episodic pattern (<15 days per month) within 2 mo after discontinuation of medication
Definition of overuse of medication
Regular overuse of a headache medication for >3 mo
Use of ergotamine, triptans, opioids, and combination analgesics >10 days per month
Use of simple analgesics ≥15 days per month
Total use of all headache medications ≥15 days per month

* The criteria for transformed migraine are those of Silberstein et al.[1,2] These criteria have been used in clinical, population-based, and treatment studies during the past 10 years. The criteria for chronic migraine have not been field-tested or validated.

† The criteria of the International Headache Society (IHS) for migraine without aura[2] include at least five attacks that last 4 to 72 hours (untreated or unsuccessfully treated). The headaches must have at least two of the following characteristics: unilateral location, pulsating quality, moderate or severe pain intensity, and reason for avoidance of routine physical activity (e.g., walking or climbing stairs). During the headaches, at least one of the following must be present: nausea, vomiting, or both; photophobia and phonophobia; and no attribution to another disorder.

of three months, neurologic symptoms, focal or lateralizing neurologic signs, papilledema, headaches aggravated or relieved by assuming an upright or supine posture, headaches provoked by a Valsalva maneuver such as a cough or sneeze, systemic symptoms or fever, or a history of headache of sudden onset or onset after the age of 50 years should prompt a diagnostic evaluation with appropriate imaging.

Most patients with transformed migraine and medication-overuse headache are women and have a history of episodic migraine that dates back to adolescence or early adulthood.[16,17] Patients often report a period of transformation that occurs over months or years in which headaches become more frequent, until a pattern of daily or near-daily headaches develops that clinically resembles a mixture of tension-type headache and migraine. This clinical phenotype explains why labels such as "mixed headache" and "tension–vascular headache" have been informally applied to this group of patients.

The overuse of acute-headache medications by patients with frequent headache may lead to medication-overuse headache, a syndrome of daily headaches that is induced and maintained by the very medications used to relieve the pain.[18,19] The prevalence in the population of chronic daily headache associated with the overuse of acute-headache medication was recently estimated to be 1.4 percent overall and was particularly high among women (2.6 percent), especially those over the age of 50 years (5 percent).[20] Overuse of acute-headache medications is reported by approximately 80 percent of the patients with transformed migraine who are seen in headache clinics,[21] but by fewer than a third of those with transformed migraine in the general population.[3] Furthermore, in a substantial proportion of patients, daily headache may continue once they stop overusing acute-headache medication. Therefore, the overuse of acute-headache

Table 2. Other Types of Primary Chronic Daily Headache.*

Disorder	Male:Female Ratio	Prevalence	Clinical Features
		%	
Transformed migraine	1:3	2	Migraine with or without aura >15 days per month for >3 mo
Chronic tension-type headache	1:1	2	Mild-to-moderate severity; no migrainous symptoms; bilateral
New daily persistent headache	More common in women	Rare	Bilateral, persistent, moderately severe; may be preceded by viral infection; may resemble migraine or tension-type headache
Hemicrania continua	More common in women	Rare	Rare, unilateral, and constant exacerbations of severe headache; cranial autonomic symptoms; "ice-pick" pain; responsive to indomethacin by definition
Cluster headache	3:1	0.4	Cluster periods lasting 4–8 wk 1–3 times per year; daily headaches, often nocturnal, occurring 1–8 times per day, lasting about 1 hr on average, extremely severe, mostly periorbital or temporal, and associated with motor restlessness and autonomic symptoms (tearing, rhinorrhea)
Hypnic headache	1:2 among the elderly (>60 yr of age)	0.07	Occurring daily but only during sleep; moderately severe; often bilateral; lasting about 1 hr; not associated with autonomic symptoms
Paroxysmal hemicrania	1:2	Rare	Headaches identical to cluster headaches except that attacks occur more often (>5 and up to 24 times per day) and are briefer (8–25 min); responsive to indomethacin by definition
Short-lasting, unilateral, neuralgiform headache with conjunctival injection and tearing syndrome	3:1	Rare	Headaches resembling cluster and paroxysmal hemicranias except that attacks occur more often (30–100 per day) and are much briefer (20–120 sec); may be mistaken for trigeminal neuralgia, except pain is strictly periorbital (V1) with cranial autonomic symptoms

* Secondary causes require careful consideration and exclusion. These include medication-overuse headache, cervicogenic headache (pain referred from a source in the neck and perceived in one or more regions of the head, face, or both), intracranial hypertension or hypotension, intracranial infection (meningitis or sinusitis), space-occupying lesions, post-traumatic headache, arterial dissection, venous sinus thrombosis, and giant-cell arteritis. Prolonged (four hours or longer) chronic daily headache disorders include chronic migraine, chronic tension-type headache, hemicrania continua, and new daily persistent headache. Transient (less than four hours) disorders include cluster headache, hypnic headache, paroxysmal hemicrania, and the short-lasting, unilateral, neuralgiform headache with conjunctival injection and tearing syndrome.

medications is neither necessary nor sufficient to cause transformed migraine.

Patients with transformed migraine most often overuse acute-headache medications such as analgesics (especially analgesics that combine aspirin, acetaminophen, and caffeine and those that contain butalbital), opioids, ergotamine, or triptans or a combination of these medications.

The interval from the frequent intake of these medications to the development of medication-overuse headache has been reported to be shortest for triptans (1.7 years), longer for ergots (2.7 years), and longest for analgesics (4.8 years). It is unclear whether this observation relates to the pharmacologic characteristics of the medications.[22]

Although it is often difficult to be certain whether the overuse of acute-headache medication is the cause or the consequence of the daily headaches, accurate diagnosis and management require the withdrawal of such medications in all patients, especially in the light of the observation that their overuse may preclude the efficacy of preventive medications. If a pattern of episodic headaches (fewer than 15 days per month) recurs within 2 months after drug withdrawal, medication-overuse headache is diagnosed.[2] If headache continues to occur on at least 15 days per month despite the withdrawal of acute-headache medications, a diagnosis of transformed migraine is often made. Although this distinction is obviously arbitrary, the reduction in the frequency of headache after the withdrawal of medication is often dramatic in patients who have true medication-overuse headache.

TREATMENT

Nonpharmacologic Therapy

Although data are lacking from controlled trials, clinical experience suggests that lifestyle modifications such as limiting or eliminating caffeine consumption, engaging in regular exercise, and establishing regular mealtimes and sleep schedules can be beneficial for some patients. Depression, anxiety, and sleep disturbances should be addressed.[23] Training in relaxation techniques and

Table 3. Preventive Medications Used in Cases of Transformed Migraine or Medication-Overuse Headache.*

Medication Class and Drug	Target Daily Dose	Titration Period	Common Side Effects
Antidepressants			
Tricyclic antidepressants such as amitriptyline	50–100 mg	1–2 mo	Weight gain, dry mouth, constipation, palpitation, drowsiness, dizziness, fatigue
Selective serotonin-reuptake inhibitors such as fluoxetine	20–60 mg	1 mo	Anorexia, insomnia, anxiety, tremor, asthenia, dizziness, somnolence
Anticonvulsants			
Divalproex	500–2000	2–4 wk	Nausea, somnolence, dizziness, vomiting, tremor, alopecia, weight gain
Gabapentin	900–3600 mg	1–2 mo	Dizziness, somnolence, ataxia, abnormal thinking, peripheral edema, weight gain, incoordination
Topiramate	50–200 mg	1–2 mo	Paresthesia, difficulty with word-finding and concentration, weight loss
α_2-Adrenergic agonists			
Tizanidine	8–20 mg	1–2 mo	Dry mouth, somnolence, asthenia, dizziness, constipation, hypotension, bradycardia
Neurotoxin			
Botulinum toxin type A	25–260 U every 3 mo	Injection every 3 mo	Weakness of injected muscle, ptosis, neck pain

* All the listed agents (except divalproex) have been studied in at least one randomized trial involving patients with a primary chronic daily headache (more than 15 days per month). However, these were not studies specifically of patients with transformed migraine or medication-overuse headache. Most of the patients in some studies and all the patients in other studies had a history of migraine; none of the studies evaluating these therapies uniformly used the definition of the International Headache Society for chronic migraine or the criteria of Silberstein et al.[1] for transformed migraine. Overuse of acute-headache medications was present in a substantial proportion of patients in several studies. This table is not intended to be exhaustive. No medications are approved by the Food and Drug Administration for the prevention of headache in patients with transformed migraine or medication-overuse headache.

biofeedback may be beneficial, although data to support these interventions come from patients with chronic tension-type headache, rather than transformed migraine.[24] Patients should be provided with support and close follow-up, particularly during the first eight weeks after treatment is initiated.

Preventive Medications

Randomized trials of the use of preventive medications in chronic daily headache are scarce.[25-34] In a single trial involving the tricyclic amytriptyline, the reported response rates (the percentage of patients whose frequency of headache is reduced by more than 50 percent) have exceeded 50 percent. Response rates superior to those achieved with placebo have also been reported for gabapentin (36 percent, as compared with 11 percent for placebo), topiramate (71 percent, as compared with 11 percent), and botulinum toxin type A (54 percent, as compared with 38 percent).[25,30,31] However, available studies are limited by small numbers of patients, the failure to account for the overuse of acute-headache medications, the concomitant use of other preventive medications, the lack of a specific diagnosis, or a combination of these factors.

Nonetheless, on the basis of these data and clinical experience, several potential preventive therapies are being used in patients with transformed migraine (Table 3). Given the high rate of associated sleep and mood disorders in these patients, sedating antidepressants such as amitriptyline may be particularly useful, although data are lacking to compare this category of drugs with a placebo or other preventive medications.

Preventive medications are generally titrated to the minimal effective or maximal tolerated dose over a period of one or two months. This target dose is maintained for at least two months; if the patient has a response (more than a 50 percent reduction in the number of days on which headache occurs), the medication is continued for at least three to six months. At that point, clinical experience suggests that it is reasonable to attempt to taper and discontinue the medication, after consultation with the patient.

The use of preventive medications in patients with headaches thought to be due to overuse of acute-headache medications is more controversial. Some investigators believe that most patients who overuse these medications have medication-overuse headache and that withdrawal of the overused medications alone will allow the headache to revert to an episodic pattern, without the need for preventive therapy.[35] However, given the relatively poor long-term success rates after the withdrawal of medication alone, other investigators recommend preventive therapy in such patients in an attempt to reduce the frequency and severity of the withdrawal headaches, as well as the potential for relapse, which can occur during or after the withdrawal period.

The use of daily opioid therapy in patients with chronic daily headache is controversial. A recent prospective study with an initial cohort of 160 patients who were prescribed daily opioid therapy reported the outcomes among 70 patients with medically refractory chronic daily headache who continued this therapy for at least three years.[36] Only 41 of the original 160 patients (26 percent) had greater than 50 percent improvement in a headache index that took into account the frequency and severity of headaches each week. Fifty percent of the patients had "problem drug behavior" (defined as "lost" prescriptions, seeking medication from other sources, and most commonly, dose violations). Most of these patients (74 percent) either did not show marked improvement or were dropped from the program because of problem drug behavior. These data from a highly specialized center with very close follow-up underscore the low efficacy of long-term opioid therapy and the high risk of misuse in this patient population.

WITHDRAWAL FROM ACUTE-HEADACHE MEDICATION

No controlled studies have yet assessed the efficacy of withdrawal of medication alone. Treatment strategies for patients with transformed migraine associated with overuse of acute-headache medication are therefore based on case series, prospective uncontrolled studies, controlled trials involving patients with unspecified chronic daily headache, and clinical experience. Withdrawal studies are confounded by the addition of preventive medications, behavioral techniques, lifestyle modifications, and acute-headache medications, all of which may influence the frequency and severity of headaches. Nonsteroidal antiinflammatory drugs and dihydroergotamine mesylate (unlike ergotamine tartrate) are generally considered to have a low risk of medication-overuse headache and are often used to treat breakthough headaches during the withdrawal period. It is unclear whether the rarity of overuse of acute-headache

medication associated with dihydroergotamine reflects a pharmacologic mechanism or whether the rarity is explained by the more limited use of this medication, which requires parenteral administration, as compared with ergotamine tartrate, which is taken orally.

In general, most patients can be treated on an outpatient basis (Table 4). Simple analgesics, ergotamine, triptans, and most combination analgesics can be discontinued abruptly, whereas opioids and butalbital-containing analgesics should be tapered over a period of one month. To minimize the potential for troublesome or serious withdrawal symptoms from analgesics containing barbiturates and opioids, some experts have drawn on clinical experience to recommend a short (two to six weeks), tapering course of phenobarbital or clonidine for patients who have been using such agents.

A prospective study demonstrated that withdrawal symptoms and headaches generally resolved within four days after the cessation of triptan, whereas fewer than 20 percent of patients reported being free of headache within four days after the discontinuation of analgesics.[22] Withdrawal from ergotamine tartrate and combination analgesics may cause severe headache, nausea, vomiting, hypotension, and tachycardia that can last several days to weeks.

A recent double-blind, placebo-controlled study evaluated the effect of 100 mg of prednisone for five days on the duration of severe withdrawal headache in 20 patients with presumed medication-overuse headache.[37] There was a significant reduction in the number of hours of severe withdrawal headache in the active-treatment group (18.1 vs. 36.7 hours, $P=0.04$), which confirmed earlier observations from uncontrolled studies. In a 12-week open-label study, a single bedtime dose of tizanidine ranging from 2 to 16 mg (average, 3.6) in combination with a single morning dose of a nonsteroidal antiinflammatory drug resolved chronic daily headache in 34 of 55 patients (62 percent).[38]

Inpatient treatment may be necessary if the patients are not successful in decreasing their use of acute-headache medications, if the amount of barbiturate or opioid makes withdrawal on an outpatient basis unsafe, or if there are serious coexisting medical or psychiatric conditions.[39] Although there is a paucity of controlled trials to guide inpatient therapy, a meta-analysis of uncontrolled inpatient studies demonstrated that 81 percent of patients had a decreased severity or frequency of headache of at least 50 percent after two months of follow-up and that 61 percent had such an improvement after one to four years of follow-up.[39]

However, relapse is common after the withdrawal of acute-headache medications, both in patients who were no longer experiencing chronic daily headache after medication withdrawal and in those who continued to have chronic daily headache but who were initially successful in decreasing their intake of acute-headache medications. One large, prospective study indi-

Table 4. Suggested Treatment of Transformed Migraine or Medication-Overuse Headache.

Education, support, and close follow-up for 8–12 wk
Lifestyle modifications (quitting smoking, eliminating caffeine consumption, exercising, eating regular meals, and establishing regular sleep schedule)
Behavioral therapy (relaxation therapy, biofeedback)
Abrupt withdrawal of overused medications for acute headache, except barbiturates or opioids*
Prednisone (100 mg for 5 days [optional])
Acute-headache treatment (for moderate or severe headache)
Nonsteroidal antiinflammatory drugs (e.g., 500 mg of naproxen sodium)
Dihydroergotamine (1 mg) intranasally, subcutaneously, or intramuscularly
Antiemetics (10–20 mg of metoclopramide, 10 mg of prochlorperazine, or 4–8 mg of ondansetron)
Preventive therapy

* For butalbital overuse, taper the drug over a period of two to four weeks; if there is concern about the possibility of withdrawal syndrome, provide a tapering course of phenobarbital (30 mg twice daily for two weeks, followed by 15 mg twice daily for two weeks). For opioid overuse, taper the drug over a period of two to four weeks; if there is concern about the possibility of withdrawal syndrome, provide clonidine (transdermal therapeutic system patch for one to two weeks).

cated a relapse rate of 38 percent in the first year and a rate of 42 percent after four years.[40,41] In another report, 60 percent of the patients continued to have chronic daily headache and were overusing acute-headache medications four years after the initial withdrawal of the medication.[42]

AREAS OF UNCERTAINTY

The mechanisms by which headache becomes daily and sometimes continuous remain unclear. Repeated attacks of migraine in susceptible persons may lead to a state of chronic central sensitization of trigeminal-pain pathways, resulting in continuous headache. The overuse of analgesics, especially opioids, may also sensitize central-pain pathways. Randomized, controlled trials of different treatment strategies that can be used to inform therapy for patients with transformed migraine or medication-overuse headache are lacking. The role of novel treatments for certain subtypes of chronic daily headache also remains uncertain. Occipital-nerve stimulation has shown some promise in individual patients with chronic migraine, but controlled trials are needed.[43]

GUIDELINES

There are no formal recommendations from the American Academy of Neurology or the American Headache Society for the management of transformed migraine or medication-overuse headache. However, consensus guidelines of the American Academy of Neurology for the management of chronic migraine recommend "guarding against medication overuse headache by avoiding acute headache medication escalation and initiating preventive medication in patients with frequent headache or in those who overuse acute therapies."[42]

CONCLUSIONS AND RECOMMENDATIONS

Most patients with chronic daily headache have a history of episodic migraine and overuse acute-headache medications. A careful history-taking and examination should rule out features suggestive of a secondary cause. The presentation of the patient in the vignette is consistent with a diagnosis of transformed migraine resulting from overuse of acute-headache medication. Although, strictly speaking, the diagnosis of medication-overuse headache requires the withdrawal of the overused medication and an evaluation of the frequency of headache after two months of follow-up, patients tend to tolerate this approach poorly. Thus, in this case, in combination with abruptly discontinuing the patient's overused medications, I would treat her with 60 mg of prednisone for five days to minimize withdrawal headaches and other symptoms, even though only limited data are available to support the use of this approach.

Although data are lacking from randomized trials of patients with transformed migraine or medication-overuse headache to guide the use of preventive therapy, I would also recommend starting therapy with amitriptyline (10 mg) at bedtime and increasing the dose in increments of 10 mg until the frequency of the headaches begins to decline or dose-limiting side effects occur, with monthly follow-up for the first three months. On the basis of clinical experience, I would also encourage the patient to limit or eliminate caffeine consumption, exercise regularly, and maintain a regular sleep schedule. Moderate or severe headaches that occur after the withdrawal of overused acute-headache medication could be treated with a nonsteroidal antiinflammatory agent or intranasal or parenteral dihydroergotamine; antiemetics are helpful for headache-induced or drug-induced nausea. If the patient were to make good progress and have a marked reduction in the frequency of headache for three to six months, it would be reasonable to try to taper the dose of amitriptyline gradually. However, she should be reminded that the need to use acute-headache medications for more than two days per week indicates the need to resume a preventive medication.

Dr. Dodick reports receiving research funding from Allergan.

No other potential conflict of interest relevant to this article was reported.

REFERENCES

1. Silberstein SD, Lipton RB, Sliwinski M. Classification of daily and near-daily headaches: field trial of revised IHS criteria. Neurology 1996;47:871-5.
2. Headache Classification Subcommittee of the International Headache Society. The international classification of headache disorders, 2nd edition. Cephalalgia 2004;24:Suppl 1:1-160.
3. Scher AI, Stewart WF, Liberman J, Lipton RB. Prevalence of frequent headache in a population sample. Headache 1998;38:497-506.
4. Castillo J, Munoz J, Guitera V, Pascual J. Epidemiology of chronic daily headache

in the general population. Headache 1999; 39:190-6.
5. Lanteri-Minet M, Auray JP, El Hasnaoui A. Prevalence and description of chronic daily headache in the general population of France. Pain 2003;102:143-9.
6. Mathew NT, Reuveni U, Perez F. Transformed or evolutive migraine. Headache 1987;27:102-6.
7. D'Amico D, Usai S, Grazzi L, et al. Quality of life and disability in primary chronic daily headache. Neurol Sci 2003; 24:Suppl 2:S97-S100.
8. Guitera V, Munoz P, Castillo J, Pascual J. Quality of life in chronic daily headache: a study in a general population. Neurology 2002;58:1062-5.
9. Wang SJ, Fuh JL, Lu SR, Juang KD. Quality of life differs among headache diagnoses: analysis of SF-36 survey in 901 headache patients. Pain 2001;89:285-92.
10. Scher AI, Stewart WF, Ricci JA, Lipton RB. Factors associated with the onset and remission of chronic daily headache in a population-based study. Pain 2003;106:81-9.
11. Scher AI, Lipton RB, Stewart W. Risk factors for chronic daily headache. Curr Pain Headache Rep 2002;6:486-91.
12. Wang SJ, Fuh JL, Lu SR, et al. Chronic daily headache in Chinese elderly: prevalence, risk factors and biannual follow-up. Neurology 2000;54:314-9.
13. Zwart JA, Dyb G, Hagen K, et al. Analgesic use: a predictor of chronic pain and medication overuse headache: the Head-HUNT study. Neurology 2003;61:160-4.
14. Katsarava Z, Limmroth V, Finke M, Diener HC, Fritsche G. Rates and predictors for relapse in medication overuse headache: a 1-year prospective study. Neurology 2003; 60:1682-3.
15. Welch KM, Goadsby PJ. Chronic daily headache: nosology and pathophysiology. Curr Opin Neurol 2002;15:287-95.
16. Mathew NT, Stubits E, Nigam MR. Transformation of episodic migraine into daily headache: analysis of factors. Headache 1982;22:66-8.
17. Saper JR. Daily chronic headache. Neurol Clin 1990;8:891-901.
18. Mathew NT, Kurman R, Perez F. Drug induced refractory headache — clinical features and management. Headache 1990; 30:634-8.
19. Saper JR. Ergotamine dependency. Headache 1987;27:435-8.
20. Colas R, Munoz P, Temprano R, Gomez C, Pascual J. Chronic daily headache with analgesic overuse: epidemiology and impact on quality of life. Neurology 2004; 62:1338-42.
21. Bigal ME, Sheftell FD, Rapoport AM, Lipton RB, Tepper SJ. Chronic daily headache in a tertiary care population: correlation between the International Headache Society diagnostic criteria and proposed revisions of criteria for chronic daily headache. Cephalalgia 2002;22:432-8.
22. Katsarava Z, Fritsche G, Muessig M, Diener HC, Limmroth V. Clinical features of withdrawal headache following overuse of triptans and other headache drugs. Neurology 2001;57:1694-8.
23. Verri AP, Proietti Cecchini A, Galli C, Granella F, Sandrini G, Nappi G. Psychiatric comorbidity in chronic daily headache. Cephalalgia 1998;18:Suppl 21:45-9.
24. Holroyd KA, O'Donnell FJ, Stensland M, Lipchik GL, Cordingley GE, Carlson BW. Management of chronic tension-type headache with tricyclic antidepressant medication, stress management therapy, and their combination: a randomized controlled trial. JAMA 2001;285:2208-15.
25. Spira PJ, Beran RG. Gabapentin in the prophylaxis of chronic daily headache: a randomized, placebo-controlled study. Neurology 2003;61:1753-9.
26. Saper JR, Silberstein SD, Lake AE III, Winters ME. Double-blind trial of fluoxetine: chronic daily headache and migraine. Headache 1994;34:497-502.
27. Saper JR, Lake AE III, Cantrell DT, Winner PK, White JR. Chronic daily headache prophylaxis with tizanidine: a double-blind, placebo-controlled, multicenter outcome study. Headache 2002;42:470-82.
28. Freitag FG, Diamond S, Diamond ML, Urban GJ. Divalproex in the long-term treatment of chronic daily headache. Headache 2001;41:271-8.
29. Krymchantowski AV, Silva MT, Barbosa JS, Alves LA. Amitriptyline versus amitriptyline combined with fluoxetine in the preventative treatment of transformed migraine: a double-blind study. Headache 2002;42:510-4.
30. Silvestrini M, Bartolini M, Coccia M, Baruffaldi R, Taffi R, Provinciali L. Topiramate in the treatment of chronic migraine. Cephalalgia 2003;23:820-4.
31. Mathew NT, Frishberg BM, Gawel M, et al. Botulinum toxin type A (Botox) for the treatment of chronic daily headache: a randomized, double-blind, placebo-controlled trial. Headache 2005;45:293-307.
32. Silberstein SD, Neto W, Schmitt J, et al. Topiramate in migraine prevention: results of a large controlled trial. Arch Neurol 2004;61:490-5.
33. Brandes JL, Saper JR, Diamond M, et al. Topiramate for migraine prevention: a randomized controlled trial. JAMA 2004; 291:965-73.
34. Freitag FG, Collins SD, Carlson HA, et al. A randomized trial of divalproex sodium extended-release tablets in migraine prophylaxis. Neurology 2002;58: 1652-9.
35. Limmroth V, Katsarava Z. Medication overuse headache. Curr Opin Neurol 2004; 17:301-6.
36. Saper JR, Lake AE III, Hamel RL, et al. Daily scheduled opioids for intractable head pain: long-term observations of a treatment program. Neurology 2004;62: 1687-94.
37. Pageler L, Katsarava Z, Limmroth V, et al. Prednisone in the treatment of medication withdrawal headache following medication overuse headache: a placebo-controlled, double-blind, and randomized pilot study. Cephalalgia 2004;24:72. abstract.
38. Smith TR. Low-dose tizanidine with nonsteroidal anti-inflammatory drugs for detoxification from analgesic rebound headache. Headache 2002;42:175-7.
39. Freitag FG, Lake A III, Lipton R, et al. Inpatient treatment of headache: an evidence-based assessment. Headache 2004; 44:342-60.
40. Fritsche G, Eberl A, Katsarava Z, Limmroth V, Diener HC. Drug-induced headache: long-term follow-up of withdrawal therapy and persistence of drug misuse. Eur Neurol 2001;45:229-35.
41. Pini LA, Cicero AF, Sandrini M. Long-term follow-up of patients treated for chronic headache with analgesic overuse. Cephalalgia 2001;21:878-83.
42. Silberstein SD. Practice parameter: evidence-based guidelines for migraine headache (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2000;55:754-62.
43. Matharu MS, Bartsch T, Ward N, Frackowiak RS, Weiner R, Goadsby PJ. Central neuromodulation in chronic migraine patients with suboccipital stimulators: a PET study. Brain 2004;127:220-30.

CORRECTION

Chronic Daily Headache

Chronic Daily Headache . On page 160, in Table 2, the male:female ratio for cluster headache should have read ``3:1,'' rather than ``1:3,'' as printed. Also, on page 217, in the Continuing Medical Education section, choice A under Question 1 (which was listed as an incorrect choice) should have read, ``Cluster headaches are more common among women than among men,'' rather than ``more common among men than among women,'' as printed. Likewise, the explanation that followed the choices, which appeared at www.nejm.org, should have read, ``Cluster headache is more common among men,'' rather than ``among women,'' as printed. This article and its Continuing Medical Education examination have been corrected on the *Journal*'s Web site. We regret the errors.

REVIEW ARTICLE

DRUG THERAPY

Treatment of Acute Lymphoblastic Leukemia

Ching-Hon Pui, M.D., and William E. Evans, Pharm.D.

From the Departments of Hematology and Oncology (C.-H.P.), Pharmaceutical Sciences (W.E.E.), and Pathology (C.-H.P.), St. Jude Children's Research Hospital; and the Colleges of Medicine (C.-H.P., W.E.E.) and Pharmacy (W.E.E.), University of Tennessee Health Science Center — both in Memphis. Address reprint requests to Dr. Pui at St. Jude Children's Research Hospital, 332 N. Lauderdale St., Memphis, TN 38105-2794, or at ching-hon.pui@stjude.org.

N Engl J Med 2006;354:166-78.

Almost 4000 cases of acute lymphoblastic leukemia (ALL) are diagnosed annually in the United States, approximately two thirds of which are in children and adolescents, making ALL the most common cancer in these age groups.[1] Optimal use of the same antileukemic agents that were developed from the 1950s through the 1980s, together with a stringent application of prognostic factors for risk-directed therapy in clinical trials, has resulted in a steady improvement in treatment outcome.[1] In the 1990s, the five-year event-free survival rates for childhood ALL generally ranged from 70 to 83 percent in developed countries (Table 1),[2-11] with an overall cure rate of approximately 80 percent.[1] Emerging results suggest that a cure rate of nearly 90 percent will be attained in the near future[12] (Fig. 1).

Unfortunately, the experience with adult ALL has been far less rewarding: reported cure rates seldom exceed 40 percent (Table 2), despite the use of hematopoietic stem-cell transplantation in many cases.[13-20] The poor outcome in adult ALL has been variously attributed to an increased frequency of high-risk leukemia with greater drug resistance, poorer tolerance of and compliance with treatment, reluctance to accept certain temporary toxic effects, and less effective treatment regimens, as compared with childhood ALL. In this review, we consider recent advances in the treatment of ALL, emphasizing issues that must be addressed if treatment outcome is to improve further.

Risk Assessment

A stringent assessment of the risk of relapse for subgroups of patients is critical in selecting therapy that will avoid excessive toxicity but maintain a high cure rate. Risk classification has been based mainly on readily apparent clinical features of patients and characteristics of leukemia cells.[1] Certain host factors are now recognized to exert a critical influence on treatment efficacy and toxicity.[21,22] In some patients, for example, treatment failure results from inadequate drug dosing rather than from an intrinsic drug resistance of the leukemia cells.[1,23] Although most pediatric study groups classify patients into categories of standard, high (i.e., intermediate or average), or very high risk, the Children's Oncology Group has proposed a four-category system that recognizes patients with a very low probability of relapse.[24] Adults are generally divided into only standard-risk and high-risk groups.

Factors Predicting Clinical Outcome

Treatment Regimen

Improved treatment has abolished the prognostic strength of many clinical and biologic variables that previously were related to outcome. For example, T-cell ALL or mature B-cell ALL in children, once associated with a very poor prognosis, has a cure rate of 75 to 80 percent with contemporary treatments.[25,26] Similarly, although

black children still have poor outcomes in national studies, they have the same high cure rates as white children when given equal access to effective treatment in trials at single institutions.[27] The adverse prognostic effect of male sex has also been abolished in clinical trials involving children in which the overall five-year event-free survival rate was 80 percent or more.[7,10] More interesting, perhaps, is that retrospective studies have indicated that the event-free survival rates among adolescents 15 to 20 years of age who were treated according to pediatric protocols were significantly higher than those among the same age group who were treated according to adult protocols.[28,29] Whether this discrepancy in outcome reflects differences in treatment regimens, protocol compliance on the part of the patients and physicians, or other factors is unknown. Whatever the explanation, joint efforts by pediatric and adult oncologists are under way to develop common protocols for adolescents and young adults.

CLINICAL FEATURES

Age and leukocyte count at diagnosis continue to be strong prognostic indicators of outcome, mainly among patients with B-cell precursor ALL. In this subgroup, an age of one to nine years and a leukocyte count of less than 50×10^9 per liter usually define standard-risk disease.[1] Among adults, the outcome worsens with increasing age and leukocyte count, but there are no clear guidelines for assigning adults to different risk groups on the basis of increments of age or leukocyte count. With only a few exceptions,[13,15,17] patients older than 60 years are not included in adult trials because they have many coexisting health problems and are particularly susceptible to treatment-related illness and death.

GENETICS OF LEUKEMIA CELLS

Primary genetic abnormalities of leukemia cells have important prognostic significance.[30-32] In B-cell precursor ALL, hyperdiploidy (more than 50 chromosomes per leukemia cell) and t(12;21) with the *TEL-AML1* fusion gene, accounting for approximately 50 percent of childhood cases but only 10 percent of adult cases, confer a highly favorable prognosis. Childhood cases with trisomies 4, 10, and 17 may have a particularly favorable outcome.[33] Hypodiploidy (fewer than 45 chromosomes per leukemia cell), found in less than 2 percent of pediatric or adult cases, confers a poor outcome that is even worse in subgroups with rare low hypodiploidy (33 to 39 chromosomes) or near-haploidy (23 to 29 chromosomes). A poor prognosis is also associated with t(4;11) with the *MLL-AF4* fusion gene, which occurs in approximately 50 percent of cases in infants, 2 percent of cases in children, and 5 to 6 percent of cases in adults, and

Glossary

BCR-ABL: A reciprocal t(9;22) fuses the *BCR* (breakpoint cluster region) gene from chromosome 22 to the *ABL* (Abelson) gene from chromosome 9. The fusion protein is a constitutive protein kinase that alters signaling pathways that control the proliferation, survival, and self-renewal of hematopoietic stem cells.

E2A-PBX1: In the t(1;19), the N-terminal transactivation domain of E2A (a helix–loop–helix protein-coding gene on chromosome 19) fused to the C-terminal DNA-binding homeodomain of *PBX1* (pre-B-cell transforming) gene on chromosome 1. The chimeric protein interferes with hematopoietic differentiation by disrupting patterns of gene expression that are normally regulated by HOX-PBX1 complexes.

FLT3: This class 3 receptor tyrosine-kinase (fms-related tyrosine kinase 3) gene plays an important role in normal hematopoiesis. Constitutive activation of the gene contributes to the abnormal growth of leukemic cells.

HOX: Homeobox genes, master transcriptional regulators of early development, play a critical role in regulating hematopoietic stem-cell survival and proliferation.

MLL-AF4: The t(4;11) results in a chimeric protein consisting of the N-terminal portion of MLL (mixed-lineage leukemia) encoded by the gene on chromosome 11 and the C-terminal portion of AF4 (ALL1 fused gene from chromosome 4). The fusion protein disrupts the normal expression pattern of homeobox genes, causing a change in the self-renewal and growth of hematopoietic stem cells and committed progenitor cells.

MLL-ENL: The fusion of the *MLL* gene with the *ENL* (eleven nineteen leukemia) gene.

NOTCH1: This gene (Notch homologue 1, translocation-associated [Drosphila]) encodes a member of the transmembrane protein family, which plays a role in the developmental processes of a variety of tissues. Constitutive Notch signaling in hematopoietic progenitors disrupts both normal T-cell and B-cell development and leads to T-cell cancers.

NUP214-ABL1: This fusion between *NUP214* (nucleoporin of 214 kD) and *ABL1* is associated with increased *HOX* expression and contributes to the multistep pathogenesis of T-cell ALL.

TEL-AML1: The t(12;21) creates a fusion gene that includes the 5′ portion of *TEL* (translocation-ETS-leukemia) gene on chromosome 12, which encodes a nuclear phosphoprotein that is a member of the ETS family of transcription factors, and almost the entire coding region of *AML1*, another transcription-factor gene that encodes the alpha subunit of core-binding factor, a master regulator of the formation of definitive hematopoietic stem cells. The fusion protein inhibits normal *AML1*-mediated transcriptional activity, resulting in the alteration of self-renewal capacity and the differentiation capacity of hematopoietic stem cells.

TTK: The tramtrack gene encodes a protein kinase detectable in all proliferating cells and tissues; its expression is markedly reduced or absent in normal resting cells and in tissues with a low proliferative index.

Table 1. Characteristics of the Patients and Treatment Results from Selected Clinical Trials Involving Children with Acute Lymphoblastic Leukemia.*

Study Group	Years of Study	No. of Patients	Age Range (yr)	Ph+ (%)	T Cell (%)	White-Cell Count ≥100 x 10^9/Liter (%)	Remission-Induction Therapy			5-yr Event-free Survival (%)	Data Source
							Complete Remission	Rate of Death	Resistant Disease		
							percent				
AIEOP-91	1991–1995	1194	0–15	1.6	12.1	12.1	96.5	1.4	2.1	70.8±1.3	Conter et al.[2]
BFM-95	1995–1999	2012	0–18	—	11.0	13.0	99.0	0.8	0.2	79.0±1.0	Bürger et al.[3]
CCG-1800	1989–1995	5121	0–21	2.3	13.0	11.7	—	—	—	75.0±1.0	Gaynon et al.[4]
COALL-92	1992–1997	538	0–18	1.7	15.0	12.6	98.7	0.4	0.9	76.9±1.9	Harms et al.[5]
DCLSG-ALL-8	1991–1996	467	0–18	—	12.0	10.0	98.7	0.9	0.4	73±2	Kamps et al.[6]
DFCI-91-01	1991–1995	377	0–18	1.6	7.4	10.9	98.2	0.5	1.3	83±2	Silverman et al.[7]
EORTC-CLG-58881	1989–1998	2065	0–18	3.1	14.5	14.3	97.8	0.9	1.3	70.9±1.1	Vilmer et al.[8]
NOPHO ALL92	1992–1998	1143	0–15	1.0	9.3	10.4	98.4	—	—	77.6±1.4	Gustafsson et al.[9]
SJCRH 13B	1994–1998	247	0–18	2.9	17.6	15.4	98.0	1.2	0.8	80.8±2.6	Pui et al.[10]
UKALL XI	1990–1997	2090	1–15	1.5	10.7	12.1	99.3	0.3	0.4	63.1±2.2	Hill et al.[11]

* Plus–minus values are estimates ±SE. Ph+ denotes Philadelphia chromosome–positive, AIEOP Associazione Italiana di Ematologia ed Oncologia Pediatrica, BFM Berlin–Frankfurt–Münster, CCG Children's Cancer Group, COALL Cooperative ALL Study Group, DCLSG Dutch Childhood Leukemia Study Group, DFCI Dana–Farber Cancer Institute consortium, EORTC-CLCG European Organisation for Research and Treatment of Cancer–Children's Leukaemia Cooperative Study Group, NOPHO Nordic Society of Pediatric Hematology and Oncology, SJCRH St. Jude Children's Research Hospital, and UKALL United Kingdom Medical Research Council Working Party on Childhood Leukaemia. Event-free survival was measured from the first day of remission-induction therapy to the date of induction failure, relapse, death, or the detection of a second cancer.

with t(9;22) with *BCR-ABL* fusion, which increases in frequency with age, from 3 percent in children to 20 percent in adults to more than 50 percent in patients older than 50 years.[30,31,34]

Age influences the prognostic effect of these genetic lesions. Among patients with t(9;22), children one to nine years of age have a better prognosis than adolescents with the same disease,[35] who in turn fare better than adults.[31,34] Among patients with *MLL-AF4* fusion, infants fare considerably worse than older children, and adults have an especially poor outcome.[31,36] In T-cell ALL, the presence of t(11;19) with *MLL-ENL* fusion and overexpression of the *HOX11* gene confer a good prognosis.[32,36-38] More than half of the cases of T-cell ALL have activating mutations of the *NOTCH1* gene,[39] although the prognostic significance of this finding has yet to be determined.

HOST PHARMACODYNAMICS AND PHARMACOGENETICS

Host factors can influence treatment efficacy.[21,22] With the same dosages of either methotrexate or mercaptopurine, reduced accumulation of active metabolites in leukemia cells — due to fast clearance, inactivation, or other reasons — has been associated with a poor outcome.[40] The concomitant administration of certain anticonvulsants (e.g., phenytoin, phenobarbital, or carbamazepine) significantly increases the systemic clearance of antileukemic agents by inducing the production of cytochrome P-450 enzymes and can therefore adversely affect treatment outcome.[41] Thus, it is preferable to use anticonvulsants (e.g., gabapentin or valproic acid) that are less likely to induce the activity of these enzymes.

Genetic polymorphisms in genes that encode drug-metabolizing enzymes, transporters, receptors, and drug targets result in wide differences among patients in terms of drug disposition and pharmacologic effects.[21,22] Patients who inherit homozygous or heterozygous deficiency of thiopurine methyltransferase, the enzyme that catalyzes the S-methylation (inactivation) of mercaptopurine, have a significantly increased risk for hematopoietic toxic effects,[42] but they tend to have a better treatment response than do patients without this inherited deficiency, possibly because they receive a higher dose intensity of mercaptopurine.[43,44] The null genotype of glutathione S-transferases, enzymes that catalyze the inactivation of many antileukemic agents, has been

associated with a reduced risk of relapse.[45] A tandem-repeat polymorphism within the enhancer region of the thymidylate synthase gene, one of the major targets of methotrexate, has been linked to increased expression of the enzyme and an increased risk of relapse.[45,46] However, the prognostic importance of individual pharmacogenetic variables is also influenced by the treatment.[47,48] Moreover, multiple genetic polymorphisms probably interact to influence the treatment response so that polygenic pharmacogenetic models may be necessary to predict treatment outcome reliably.[45] Finally, the acquisition of additional chromosomes in leukemia cells can create discordance between germ-line genotypes and leukemia-cell phenotypes, including pharmacogenomics.[49]

GLOBAL GENE-EXPRESSION PATTERNS

Global gene-expression profiling has the potential to reveal new dimensions of the pathologic features of ALL and to identify novel therapeutic targets. Several studies have revealed distinct gene-expression patterns in specific subtypes of leukemia.[37,50-52] In *TEL-AML1*–positive ALL, the erythropoietin receptor was found to be highly expressed in leukemia cells, potentially affecting proliferation and survival signals.[51] In T-cell ALL, the levels of expression of several known T-cell oncogenes were related to the expression of many genes that define discrete stages of T-cell differentiation.[37,53] Cases in the *HOX11*+ cluster showed an increased expression of genes that promote proliferation and a lack of expression of antiapoptotic genes, which may explain the better response of these patients to therapy.[37] In T-cell ALL in adults, *TTK*, a gene with kinase activity that is overexpressed in proliferating cells, was found to have decreased expression in the blast cells of patients whose disease ultimately relapsed.[53] Consistent with the idea that impaired cell proliferation may be a mechanism for resistance to treatment, expression of this gene was also reduced among children with B-cell precursor ALL who had poorer responses to remission-induction therapy.[54] Gene-expression profiling that was performed in patients with rearrangements of the *MLL* gene identified two subgroups of patients with prognostic significance that was independent of the gene-fusion partner and of clinical factors.[55] The identification of high levels of *FLT3* expression in cases with *MLL* rearrangement or hyperdiploidy[50,56] has led to the development of FLT3 inhibitors.[57]

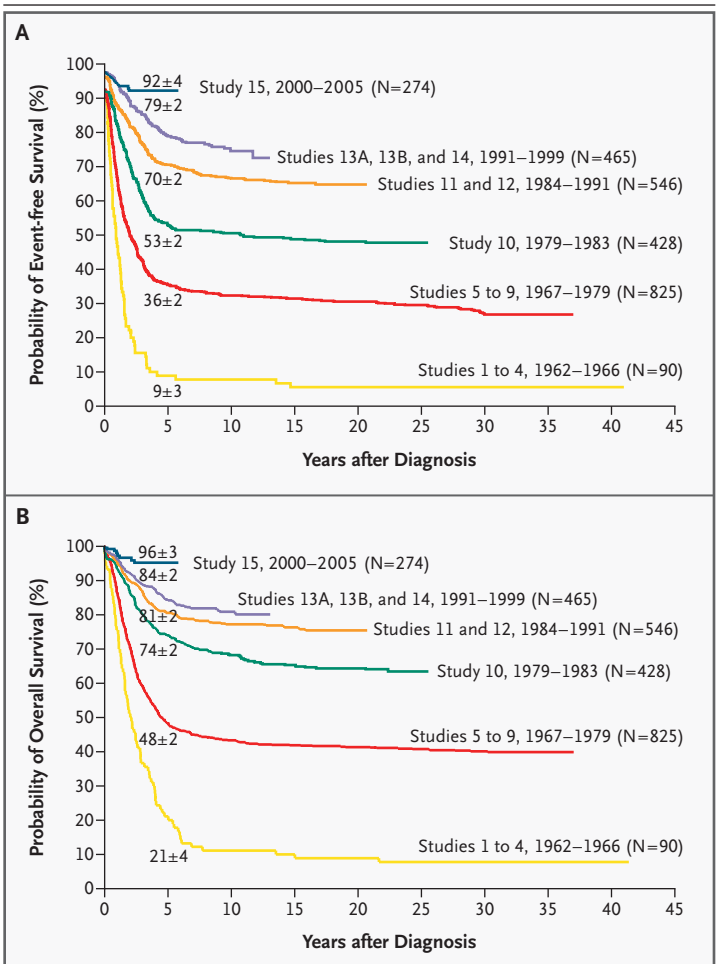


Figure 1. Kaplan–Meier Analyses of Event-free Survival (Panel A) and Overall Survival (Panel B) in 2628 Children with Newly Diagnosed ALL.

The patients participated in 15 consecutive studies conducted at St. Jude Children's Research Hospital from 1962 to 2005. The five-year event-free and overall survival estimates (±SE) are shown, except for Study 15, for which preliminary results at four years are provided. The results demonstrate steady improvement in clinical outcome over the past four decades. The difference in event-free and overall survival rates has narrowed in the recent periods, suggesting that relapses or second cancers that occur after contemporary therapy are more refractory to treatment.

The gene-expression profiles of leukemia cells have also been used to identify genes related to the intracellular disposition of antileukemia agents in vivo[58-60] and to reveal distinct sets of genes associated with resistance to each of four structurally different and widely used medications (prednisolone, vincristine, asparaginase, and daunorubicin).[61] The independent prognostic significance of the resistance patterns was validated in a separate cohort of patients treated with the same agents at a different institution.[61] A sub-

Table 2. Characteristics of the Patients and Treatment Results from Selected Clinical Trials Involving Adults with Acute Lymphoblastic Leukemia.*

Study Group	Years of Study	No. of Patients	Age (yr)		Ph+ (%)	T Cell (%)	White-Cell Count ≥30×10⁹/Liter (%)	Remission-Induction Therapy			Disease-free Survival (%)	Data Source
			Median	Range				Complete Remission	Rate of Death	Resistant Disease		
								percent				
CALGB 19802	1999–2001	163	41	16–82	18	—	—	78	11	11	~35 at 3 yr	Larson[13]
GIMEMA ALL 0288	1988–1994	778	27.5	12–60	22	22	26	82	7	11	29 at 9 yr	Annino et al.[14]
GMALL 05/93	1993–1999	1163	35	15–65	24	24	—	83	6	11	35–40 at 5 yr	Goekbuget et al.[15]
GOELAMS 02	1994–1998	198	33	15–59	22	21	42	86	3	18	41 at 6 yr	Hunault et al.[16]
Hyper-CVAD	1992–2000	288	40	15–92	17	13	25	92	5	3	38 at 5 yr	Kantarjian et al.[17]
JALSG-ALL93	1993–1997	263	31	15–59	22	21	34	78	6	16	30 at 6 yr	Takeuchi et al.[18]
LALA-94	1994–2002	922	33	15–55	23	26	38	84	5	11	36 at 5 yr	Thomas et al.[19]
UCSF 8707	1987–1998	84	27	16–59	16	21	33	93	1	6	53 at 5 yr	Linker et al.[20]

* CALGB denotes Cancer and Leukemia Group B, GIMEMA Gruppo Italiano Malattie Ematologiche dell'Adulto, GMALL German Multicenter Study Group for Adult ALL, GOELAMS Groupe Ouest-Est des Leucémies Aigués et Maladies du Sang, Hyper-CVAD hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone, JALSG Japan Adult Leukemia Study Group, LALA Leucémie Aiguës Lymphoblastique de l'Adulte, and UCSF University of California, San Francisco. Disease-free survival was measured from the date of complete remission to the date of relapse, death, or the detection of a second cancer.

sequent study revealed a robust pattern of drug cross-resistance, which identified patients at very high risk for relapse.[62]

It is not yet clear why genes associated with drug resistance are expressed at different levels in leukemia cells from different patients. Possibly, genetic polymorphisms in the germ line influence gene expression in leukemia cells.[63] Ongoing studies will determine whether germ-line polymorphisms affect the expression of genes that have been associated with resistance to antileukemic drugs and with treatment outcome. Additional studies are also needed to determine whether expression profiling of microRNAs, which reflect the lineage and state of differentiation of cancer cells, could enhance our ability to classify ALL.[64]

EARLY RESPONSE TO TREATMENT

Response to therapy, which reflects the genetics of leukemia cells and the pharmacodynamics and pharmacogenetics of the host, has greater prognostic strength than does any other biologic or clinical feature tested to date.[65] In this regard, the measurement of minimal residual disease, with the use of either flow cytometry or polymerase-chain-reaction (PCR) analysis, affords a level of sensitivity and specificity that cannot be attained through traditional morphologic assessments. A residual disease level of less than 0.01 percent during or on completion of initial remission-induction therapy reliably identifies patients with an exceptionally good treatment outcome.[65] By contrast, patients with a level of 1 percent or more at the end of remission-induction therapy or those with a level of 0.1 percent or more at later times have a very high risk of relapse. Tandem application of flow cytometry and PCR analysis has enabled the study of minimal residual disease in virtually all patients.[65] We currently treat patients who have a residual leukemia level of 0.01 percent or more after six weeks of remission-induction therapy with intensified therapy, an approach that improved outcome in patients with poor early responses according to morphologic criteria.[66]

FACTORS PREDICTING TREATMENT-RELATED TOXIC EFFECTS

With continued improvement in survival rates, the focus in clinical protocols has begun to shift to-

ward the reduction of deleterious acute and late effects of treatment. Thus, drugs with carcinogenic or major organ-damaging effects are avoided or reduced in dosage in patients who have standard-risk ALL. Attempts to reduce treatment-related toxic effects also include the preconditioning of patients with agents that counteract the toxic effects of antileukemic drugs. Use of the iron-chelating agent dexrazoxane, for example, was associated with a reduction in serum levels of cardiac troponin T, a surrogate measure of early anthracycline-induced myocardial injury,[67] but extended follow-up is needed to assess the safety and long-term cardioprotective effects of this agent.[68]

Regardless of whether patients receive dexrazoxane or some other protective agent, the toxic reactions they have to therapy are influenced by many factors, such as age, sex, race, constitutional syndrome, and pharmacogenetics.[22,44] As one example, life-threatening infections and organ failure are more likely to develop in elderly patients than in younger patients. Among children, those over 10 years of age, especially adolescents, are at increased risk for the development of osteonecrosis, hyperglycemia, mucositis, and typhlitis, and for dying from infections.[69-71] Widely adopted strategies to reduce toxic effects include the reduction of vincristine doses in infants, the omission of cranial irradiation in young children, girls, and patients with ataxia–telangiectasia, and the reduction of methotrexate doses in patients with Down's syndrome. In the estimated 10 percent of patients with thiopurine methyltransferase deficiency, we lower the starting dose of mercaptopurine to reduce the risk of acute myelotoxicity and the late development of second cancers.[22,40,44] Other pharmacogenetic factors will probably serve as the basis for treatment modifications in future clinical trials.

TREATMENT

The recognition that ALL is a heterogeneous disease has led to treatment directed according to phenotype, genotype, and risk. Thus, mature B-cell ALL is the only subtype that is treated with short-term intensive chemotherapy.[26,72] For all other patients, specific treatment approaches differ but consistently emphasize remission-induction therapy followed by intensification (or consolidation) therapy and continuation treatment to eliminate residual leukemia. Therapy directed at the central nervous system, which starts early in the clinical course, is given for varying lengths of time, depending on the patient's risk of relapse, the intensity of systemic treatment, and whether or not cranial irradiation is used. It should be stressed that the effect of individual drugs within the context of a combination regimen depends on the dosage and schedule of administration and on the types of drugs given simultaneously.

REMISSION INDUCTION

The goal of remission-induction therapy is to eradicate more than 99 percent of the initial burden of leukemia cells and to restore normal hematopoiesis and a normal performance status. This treatment phase almost always includes the administration of a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and at least one other agent (usually asparaginase, an anthracycline, or both). Children with high-risk or very-high-risk ALL and nearly all young adults with ALL receive four or more drugs during remission-induction therapy. Improvements in chemotherapy and supportive care have resulted in complete remission rates of about 98 percent for children and about 85 percent for adults (Tables 1 and 2). Attempts have been made to intensify induction therapy on the premise that more rapid and complete reduction of the leukemia-cell burden can prevent drug resistance and improve cure rates. However, intensive induction therapy may not be necessary for children with standard-risk ALL, provided that they receive adequate postinduction intensification therapy.[4,73] Overly aggressive induction therapy may, in fact, lead to increased morbidity and mortality.[74] The addition of cyclophosphamide, high-dose cytarabine, or high-dose anthracycline has yielded no clear benefit in adults, partly because such therapy is poorly tolerated by older patients.[14,17,75]

Presumably because of its increased penetration into the central nervous system and its longer half-life, the use of dexamethasone in induction and postremission therapy appears to provide better control in the central nervous system and systemically than do either prednisone or prednisolone.[76,77] However, one small study suggested that an increased dose of prednisolone in the context of other intensive treatment can yield results similar to those achieved with dexamethasone.[78]

The development of imatinib mesylate, a tyrosine kinase inhibitor, has enhanced the management of leukemia with *BCR-ABL* fusion, especially in elderly adults. Imatinib either as a single agent or as part of combination regimens has successfully induced and consolidated remissions.[79,80] Although its capacity to improve the cure rate remains uncertain, imatinib has clearly contributed to extended disease-free survival and improved quality of life among these patients.

INTENSIFICATION (CONSOLIDATION) THERAPY

When normal hematopoiesis is restored, patients in remission become candidates for intensification therapy. Commonly used regimens for childhood ALL include high-dose methotrexate with mercaptopurine, high-dose asparaginase given for an extended period, and reinduction treatment.[7,23,66,81] The use of one regimen should not preclude the use of others, and it may be desirable to use all of these treatments in patients with high-risk or very-high-risk ALL.

Very high doses of methotrexate appear to improve the outcome in patients with T-cell ALL.[24,81] This finding is consistent with a lower accumulation of methotrexate polyglutamates (i.e., active metabolites) in T-cell ALL than in B-cell precursor ALL; hence, higher serum concentrations of methotrexate are needed to produce an adequate therapeutic effect.[60] Blast cells with either *TEL-AML1* or *E2A-PBX1* fusion accumulate significantly lower levels of methotrexate polyglutamates than do those with other genetic abnormalities,[60] suggesting that patients with these genotypes may also benefit from an increased dose of methotrexate.

The intensive use of asparaginase during the postinduction period has yielded excellent treatment results with relatively low morbidity, especially in terms of thrombotic complications and hyperglycemia,[7,73] which have impeded the use of asparaginase during remission-induction therapy when a glucocorticoid is used concomitantly. There are several forms of asparaginase, each with a unique pharmacokinetic profile and hence a different dosing requirement.[82] In terms of the control of leukemia, the dose intensity and duration of asparaginase therapy are more important than the type of asparaginase used. In one study, no difference in outcome was observed between patients randomly selected to receive two different forms of asparaginase, but the clinical outcome among patients who received fewer than 25 weekly doses was significantly worse than that among patients who received the agent for 26 to 30 weeks.[7] In another study, a form of asparaginase with a shorter half-life yielded an inferior treatment outcome,[83] a finding now widely acknowledged to have resulted from the use of an inadequate dose.

Reinduction treatment — essentially a repetition of the initial induction therapy administered during the first few months of remission — has become an integral component of successful ALL treatment protocols.[4,10,81] In one study, a second, delayed intensification treatment further improved the outcome among patients with intermediate-risk ALL.[84] It is interesting to note that additional vincristine and prednisone after one reinduction treatment were not beneficial, suggesting that the observed improvement was due to the increased dose intensity of other agents, such as asparaginase.[84] Because of the frequent occurrence of osteonecrosis after reinduction treatment, glucocorticoid therapy given on alternating weeks is being investigated as a strategy to reduce this complication.

Although previous studies failed to show a benefit of intensification treatment in adults, more recent nonrandomized studies strongly suggested that intensive consolidation treatment improved outcome, especially in young adults.[17,85] In cases of T-cell ALL, the therapeutic benefit appears to have been contributed by cyclophosphamide and cytarabine, whereas in other types of ALL it reflected the use of high-dose cytarabine.[85]

ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION

Allogeneic transplantation is the ultimate form of treatment intensification. Among adults with ALL, long-term disease-free survival rates of 30 to 40 percent have been obtained with the use of chemotherapy, as compared with 45 to 75 percent with the use of allogeneic transplantation.[16,19] Interpretation of these results is complicated by the criteria used to select patients for transplantation and by the small number of patients studied. Even so, allogeneic transplantation clearly benefits certain very-high-risk pediatric and adult patients, such as those with *BCR-ABL*+ ALL or those with a poor initial response to treatment.[16,19] The procedure also appears to improve the clinical outcome among adults who have ALL with

t(4;11), but whether it is beneficial for infants with the same genotype remains controversial.[86-88] Recent studies suggest that among adults, transplantation with cells from a matched unrelated donor or from cord blood could yield results similar to those achieved with matched related-donor transplantation.[89,90] Thus, the indications for transplantation should be continuously evaluated in light of improvements in this procedure and in chemotherapy.

CONTINUATION TREATMENT

Patients with ALL generally require prolonged continuation therapy, for reasons that are still poorly understood. Attempts to shorten the duration of moderately intensive chemotherapy to 12 to 18 months or less have yielded poor results in both children and adults. Although as many as two thirds of childhood cases may be curable with only 12 months of treatment, it is not possible to reliably identify this subgroup prospectively.[91] Thus, in most contemporary clinical trials, patients are treated for two years or more.[92]

A combination of methotrexate administered weekly and mercaptopurine given daily constitutes the basis of most continuation regimens. Accumulation of increased intracellular concentrations of the active metabolites of methotrexate and mercaptopurine, and administration of this combination to the limits of tolerance, have been associated with improved clinical outcome.[40,93] Many investigators advocate the maintenance of white-cell counts below 3×10^9 per liter during continuation therapy.[92] Overzealous use of mercaptopurine is counterproductive, because it can cause severe neutropenia, which necessitates the interruption of chemotherapy and leads to a reduction in overall dose intensity.[40]

Mercaptopurine is more effective when administered in the evening than in the morning; it should not be given with milk or milk products that contain xanthine oxidase, which can degrade the drug.[92] Weekly intravenous administration at a higher dosage is ineffective[6-8,76] and may yield an inferior outcome.[93] The identification of inherited deficiency of thiopurine-*S*-methyltransferase among patients with hematopoietic toxic effects allows the clinician to lower the dose of mercaptopurine selectively without modifying the dose of methotrexate.[21,22,44] The relative merits of oral versus parenteral administration of methotrexate are uncertain, but the latter method circumvents problems of decreased bioavailability and poor compliance. Elevation of serum aminotransferase levels, a common finding during antimetabolite-based continuation treatment, appears to be caused by the methylated metabolites of mercaptopurine, resolves promptly after the completion of therapy, and correlates with a favorable outcome.[94] In the absence of other evidence of severe liver toxicity or viral hepatitis, it is generally not necessary to withhold or reduce the dose of continuation chemotherapy.

TREATMENT DIRECTED TO THE CENTRAL NERVOUS SYSTEM

Factors associated with an increased risk of relapse in the central nervous system include high-risk genetic features, T-cell immunophenotype, a large leukemia-cell burden, and the presence of leukemia cells in the cerebrospinal fluid (even from iatrogenic introduction through a traumatic lumbar puncture).[3,95] Because cranial irradiation can cause many acute and late complications, including second cancers, late neurocognitive deficits, and endocrinopathy,[96] it has largely been replaced by intrathecal and systemic chemotherapy. In most clinical trials, irradiation is still recommended for patients at very high risk for relapse, such as those with leukemia of the central nervous system or those with T-cell ALL, especially with presenting leukocyte counts of more than 100×10^9 per liter. Limited studies suggest that the radiation dose can be lowered to 12 Gy for patients with T-cell disease and to 18 Gy for those with leukemia of the central nervous system, provided that effective systemic chemotherapy is used.[81] We are testing the feasibility of omitting irradiation for all patients and reserving it for patients who have a relapse. Whether or not cranial irradiation is used, intrathecal therapy should be administered optimally, with careful attention to the prevention of traumatic lumbar punctures, especially at diagnosis, when most patients have abundant circulating leukemia cells.[95,97] To this end, patients diagnosed with overt testicular leukemia may also be spared testicular irradiation if effective systemic chemotherapy is given.[98]

FUTURE DIRECTIONS

The future of treatment for leukemia resides in defining the molecular pathways underlying the pathogenesis of this disease and in further eluci-

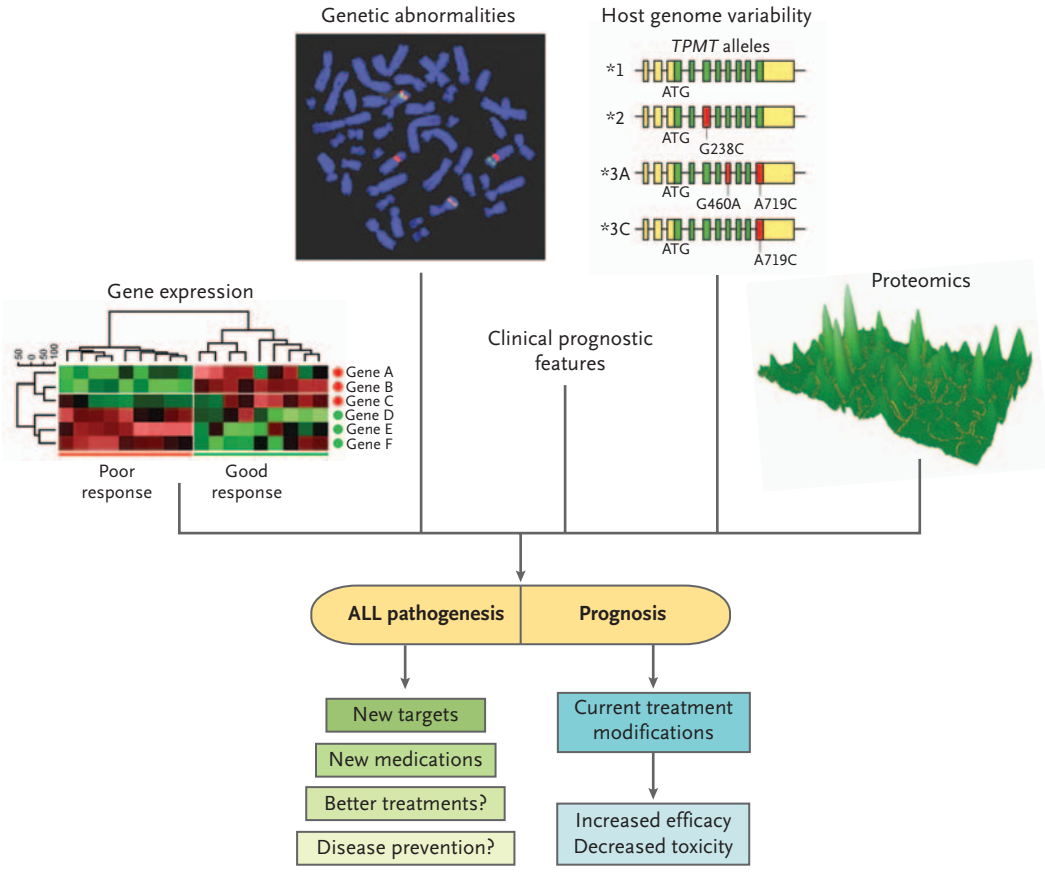


Figure 2. Factors Affecting Pathogenesis and Prognosis of ALL.

In ALL, multiple factors affect leukemogenesis and prognosis. In addition to the conventional clinical features and specific genetic abnormalities of leukemia cells, studies of global gene expression and proteomics, as well as host genome variability, should enhance our understanding of the pathogenesis of leukemia and improve risk assessment. The improved understanding of the pathologic features of the disease could in turn lead to the identification of new drugs or targets for specific treatments and may even suggest strategies for disease prevention. Precise risk assessment would ensure that patients are neither overtreated nor undertreated.

dating the pharmacogenetic factors of the host (Fig. 2). If successful, these efforts would identify new genes whose protein products are suitable for targeted therapy (Table 3).[30] The paradigm for such therapy is the use of imatinib mesylate and second-generation ABL kinase inhibitors, which were recently developed to overcome resistance to imatinib.[99] Imatinib may also benefit a subgroup of patients who have T-cell ALL with *NUP214-ABL1* fusion and extrachromosomal (episomal) amplification of the *ABL1* gene.[100] Other novel agents in the early phase of clinical testing include FLT3 inhibitors, farnesyltransferase inhibitors, γ-secretase inhibitors, and epigenetic drugs to reactivate silenced tumor-suppressor genes.[39,57,101,102] Proteasome inhibitors and short interfering RNAs are also being investigated as potential therapies.[103,104]

Although not directed at molecular targets, several new formulations of existing agents have been produced to improve the efficacy and reduce the toxic effects of the parent compounds. Asparaginase, for example, was modified by covalently conjugating it with monomethoxypolyethylene glycol to increase its half-life and reduce immunogenicity.[82] A recently developed recombinant form of asparaginase may be less immunogenic than the original product. The administration of vincristine and daunorubicin in liposomal form to decrease neurotoxicity and cardiotoxicity, respectively, is being explored. Liposomal cytara-

Table 3. Selected Recently Developed Antileukemic Agents Being Tested in Clinical Trials.

Agent	Mechanism of Action	Subtype of Leukemia Targeted
Imatinib mesylate	ABL kinase inhibition	*BCR-ABL*+
BMS-354825	ABL–SRC kinase inhibition	*BCR-ABL*+
AMN107	ABL kinase inhibition	*BCR-ABL*+
PKC412 MLN518 CEP701	FMS-like tyrosine kinase 3 inhibition	*MLL*-rearranged, hyperdiploid
Tipifarnib	Farnesyltransferase inhibition	All
MK0752	Gamma secretase inhibition (interference with NOTCH signaling)	T-cell
Decitabine	DNA demethylation	All
SAHA Valproic acid MS-275	Histone deacetylase inhibition	All
Bortezomib	Inhibition of ubiquitin proteasome pathway	All
Clofarabine	Deoxyadenosine analogue	All
Nelarabine	Deoxyguanosine analogue	T-cell
Rituximab	Anti-CD20 chimeric murine–human monoclonal antibody	CD20+
Gemtuzumab ozogamicin	Anti-CD33 monoclonal antibody conjugated with calicheamicin	CD33+
Alemtuzumab	Anti-CD52 humanized monoclonal antibody	CD52+
Epratuzumab	Anti-CD22 humanized monoclonal antibody	CD22+

bine with a prolonged half-life is also being tested as intrathecal therapy to improve the control of disease in the central nervous system.[105] Several novel nucleoside analogues, such as gemcitabine, clofarabine, and nelarabine, have shown promise in the treatment of ALL; nelarabine appears to be preferentially effective against T-cell ALL.[106,107] The monoclonal antibodies rituximab, gemtuzumab ozogamicin, alemtuzumab, and epratuzumab have been incorporated into some antileukemic regimens because of their activity against certain leukemia-associated antigens.[108] Finally, recent advances in immunology could lead to effective adoptive cellular immunotherapy.[109] Together, these advances in therapy in the future may boost the cure rate in childhood ALL to more than 90 percent and in adult ALL to more than 50 percent.

Supported in part by grants (R37 CA36401, R01 CA78224, R01 CA51001, R01 CA60419, and U01 GM61393) from the National Institutes of Health, by Cancer Center Support Grant CA21765, and by American Lebanese Syrian Associated Charities. Dr. Pui is the American Cancer Society–F.M. Kirby Clinical Research Professor.

No potential conflict of interest relevant to this article was reported.

We are indebted to Drs. Mary Relling and Hagop Kantarjian for their helpful comments on the manuscript; to Julie Groff, Betsy Williford, Deqing Pei, and Dr. Meyling Cheok for their assistance with the figures; to Kimberly Gayden for assistance in the preparation of the manuscript; and to Drs. James Downing, Mary Relling, Raul Ribeiro, Melissa Hudson, John Sandlund, Dario Campana, Jeffrey Rubnitz, Gaston Rivera, Rupert Handgretinger, Cheng Cheng, Sima Jeha, Bassem Razzouk, Fred Behm, Susana Raimondi, and Nobuko Hijiya and Ms. Vickey Simmons for their contributions to the conduct of Total Therapy Studies.

REFERENCES

1. Pui C-H, Evans WE. Acute lymphoblastic leukemia. N Engl J Med 1998;339:605-15.
2. Conter V, Arico M, Valsecchi MG, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) acute lymphoblastic leukemia studies, 1982-1995. Leukemia 2000;14:2196-204.
3. Bürger B, Zimmermann M, Mann G, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture. J Clin Oncol 2003;21:184-8.
4. Gaynon PS, Trigg ME, Heerema NA, et al. Children's Cancer Group trials in childhood acute lymphoblastic leukemia: 1983-1995. Leukemia 2000;14:2223-33.
5. Harms DO, Göbel U, Spaar HJ, et al. Thioguanine offers no advantage over mercaptopurine in maintenance treatment of childhood ALL: results of the randomized trial COALL-92. Blood 2003;102:2736-40.
6. Kamps WA, Bokkerink JP, Hakvoort-Cammel FG, et al. BFM-oriented treatment for children with acute lymphoblastic leukemia without cranial irradiation and treatment reduction for standard risk patients:

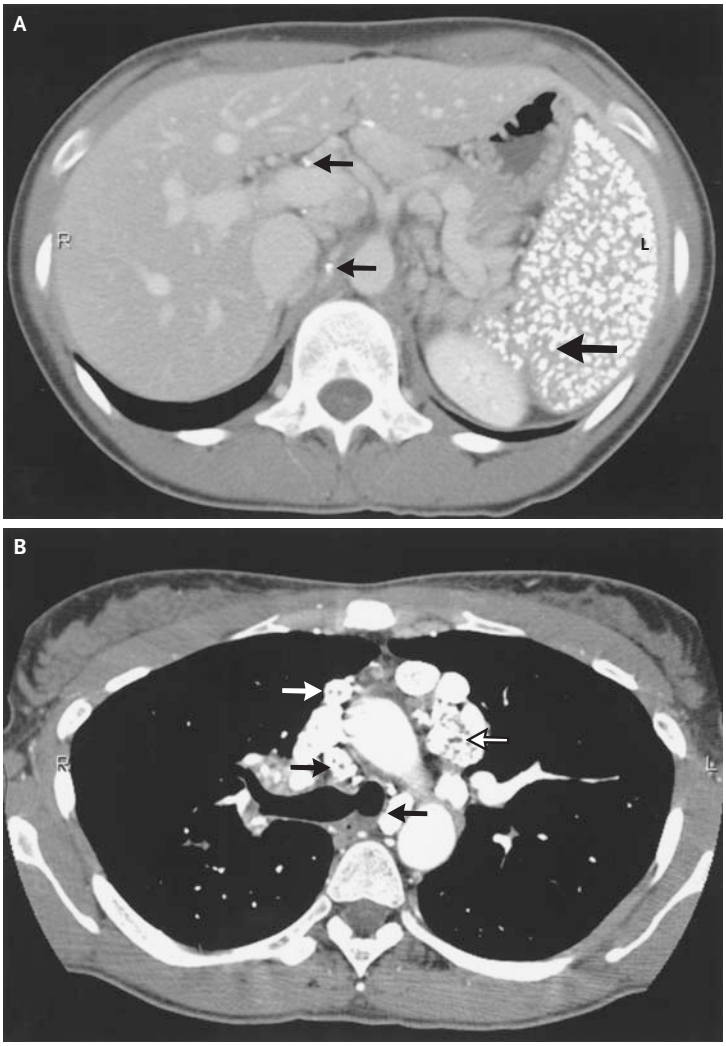
results of DCLSG protocol ALL-8 (1991-1996). Leukemia 2002;16:1099-111.
7. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-0Blood 2001;97:1211-8.
8. Vilmer E, Suciu S, Ferster A, et al. Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. Leukemia 2000;14:2257-66.
9. Gustafsson G, Schmiegelow K, Forestier E, et al. Improving outcome through two decades in childhood ALL in the Nordic countries: the impact of high-dose methotrexate in the reduction of central nervous system irradiation. Leukemia 2000; 14:2267-75.
10. Pui C-H, Sandlund JT, Pei D, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St. Jude Children's Research Hospital. Blood 2004;104:2690-6.
11. Hill FGH, Richards S, Gibson B, et al. Successful treatment without cranial radiotherapy of children receiving intensified chemotherapy for acute lymphoblastic leukaemia: results of the risk-stratified randomized central nervous system treatment trial MRC UKALL XI (ISRC TN 16757172). Br J Haematol 2004;124:33-46.
12. Pui C-H, Relling MV, Sandlund JT, Downing JR, Campana D, Evans WE. Rationale and design of Total Therapy Study XV for newly diagnosed childhood acute lymphoblastic leukemia. Ann Hematol 2004;83:Suppl 1:S124-S126.
13. Larson RA. The U.S. trials in adult acute lymphoblastic leukemia. Ann Hematol 2004;83:Suppl 1:S127-S128.
14. Annino L, Vegna ML, Camera A, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. Blood 2002;99:863-71.
15. Goekbuget N, Arnold R, Buechner T, et al. Intensification of induction and consolidation improves only subgroups of adult ALL: analysis of 1200 patients in GMALL study 05/93. Blood 2001;98:802a. abstract.
16. Hunault M, Harousseau JL, Delain M, et al. Better outcome of adult acute lymphoblastic leukemia after early genoidentical allogeneic bone marrow transplantation (BMT) than after late high-dose therapy and autologous BMT: a GOELAMS trial. Blood 2004;104:3028-37.
17. Kantarjian H, Thomas D, O'Brien S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. Cancer 2004; 101:2788-801.
18. Takeuchi J, Kyo T, Naito K, et al. Induction therapy by frequent administration of doxorubicin with four other drugs, followed by intensive consolidation and maintenance therapy for adult acute lymphoblastic leukemia: the JALSG-ALL93 study. Leukemia 2002;16:1259-66.
19. Thomas X, Boiron JM, Huguet F, et al. Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. J Clin Oncol 2004;22:4075-86.
20. Linker C, Damon L, Ries C, Navarro W. Intensified and shortened cyclical chemotherapy for adult acute lymphoblastic leukemia. J Clin Oncol 2002;20:2464-71.
21. Evans WE, McLeod HL. Pharmacogenomics — drug disposition, drug targets, and side effects. N Engl J Med 2003;348: 538-49.
22. Evans WE, Relling MV. Moving towards individualized medicine with pharmacogenomics. Nature 2004;429:464-8.
23. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui C-H. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. N Engl J Med 1998;338: 499-505.
24. Pui C-H, Sallan S, Relling MV, Masera G, Evans WE. International Childhood Acute Lymphoblastic Leukemia Workshop: Sausalito, CA, 30 November–1 December 2000. Leukemia 2001;15:707-15.
25. Goldberg JM, Silverman LB, Levy DE, et al. Childhood T-cell acute lymphoblastic leukemia: the Dana–Farber Cancer Institute acute lymphoblastic leukemia consortium experience. J Clin Oncol 2003;21: 3616-22.
26. Patte C, Auperin A, Michon J, et al. The Société Française d'Oncologie Pédiatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burden and initial response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood 2001;97:3370-9.
27. Pui C-H, Sandlund JT, Pei D, et al. Results of therapy for acute lymphoblastic leukemia in black and white children. JAMA 2003;290:2001-7.
28. Boissel N, Auclerc M-F, Lhéritier V, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. J Clin Oncol 2003;21:774-80.
29. de Bont JM, Holt B, Dekker AW, van der Does-van den Berg A, Sonneveld P, Pieters R. Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric vs adult protocols in the Netherlands. Leukemia 2004;18:2032-5.
30. Pui C-H, Relling MV, Downing JR. Acute lymphoblastic leukemia. N Engl J Med 2004;350:1535-48.
31. Mancini M, Scappaticci D, Cimino G, et al. A comprehensive genetic classification of adult acute lymphoblastic leukemia (ALL): analysis of the GIMEMA 0496 protocol. Blood 2005;105:3434-41.
32. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol 2005;23:6306-15.
33. Sutcliffe MJ, Shuster JJ, Sather HN, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI standard-risk B-precursor acute lymphoblastic leukemia: a Children's Oncology Group (COG) initiative. Leukemia 2005;19:734-40.
34. Gleissner B, Gökbuget N, Bartram CR, et al. Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. Blood 2002;99: 1536-43.
35. Aricò M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome–positive acute lymphoblastic leukemia. N Engl J Med 2000;342:998-1006.
36. Pui C-H, Gaynon PS, Boyett JM, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet 2002;359:1909-15.
37. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T-cell acute lymphoblastic leukemia. Cancer Cell 2002; 1:75-87.
38. Ferrando AA, Neuberg DS, Dodge RK, et al. Prognostic importance of *TLX1* (*HOX11*) oncogene expression in adults with T-cell acute lymphoblastic leukaemia. Lancet 2004;363:535-6.
39. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of *NOTCH1* in human T cell acute lymphoblastic leukemia. Science 2004;306:269-71.
40. Pui C-H, Relling MV, Evans WE. Role of pharmacogenomics and pharmacodynamics in the treatment of acute lymphoblastic leukaemia. Best Pract Res Clin Haematol 2002;15:741-56.
41. Relling MV, Pui C-H, Sandlund JT, et al. Adverse effect of anticonvulsants on efficacy of chemotherapy for acute lymphoblastic leukaemia. Lancet 2000;356:285-90.
42. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst 1999;91:2001-8.
43. Stanulla M, Schaeffeler E, Flohr T, et al. Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA 2005;293:1485-9.
44. Relling MV, Dervieux T. Pharmacoge-

netics and cancer therapy. Nat Rev Cancer 2001;1:99-108.
45. Rocha JC, Cheng C, Liu W, et al. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. Blood 2005; 105:4752-8.
46. Krajinovic M, Costea I, Chiasson S. Polymorphism of the thymidylate synthase gene and outcome of acute lymphoblastic leukaemia. Lancet 2002;359:1033-4.
47. Davies SM, Bhatia S, Ross JA, et al. Glutathione *S*-tranferase genotypes, genetic susceptibility, and outcome of therapy in childhood acute lymphoblastic leukemia. Blood 2002;100:67-71.
48. Lauten M, Asgedom G, Welte K, Schrappe M, Stanulla M. Thymidylate synthase gene polymorphism and its association with relapse in childhood B-cell precursor acute lymphoblastic leukemia. Haematologica 2003;88:353-4.
49. Cheng Q, Yang W, Raimondi SC, Pui C-H, Relling MV, Evans WE. Karyotypic abnormalities create discordance of germline genotype and cancer cell phenotypes. Nat Genet 2005;37:878-82.
50. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1:133-43.
51. Fine BM, Stanulla M, Schrappe M, et al. Gene expression patterns associated with recurrent chromosomal translocations in acute lymphoblastic leukemia. Blood 2004;103:1043-9.
52. Willenbrock H, Juncker AS, Schmiegelow K, Knudsen S, Ryder LP. Prediction of immunophenotype, treatment response, and relapse in childhood acute lymphoblastic leukemia using DNA microarrays. Leukemia 2004;18:1270-7.
53. Chiaretti S, Li X, Gentleman R, et al. Gene expression profile of adult T-cell acute lymphoblastic leukemia identifies distinct subsets of patients with different response to therapy and survival. Blood 2004;103:2771-8.
54. Cario G, Stanulla M, Fine BM, et al. Distinct gene expression profiles determine molecular treatment response in childhood acute lymphoblastic leukemia. Blood 2005; 105:821-6.
55. Tsutsumi S, Taketani T, Nishimura K, et al. Two distinct gene expression signatures in pediatric acute lymphoblastic leukemia with MLL rearrangements. Cancer Res 2003;63:4882-7.
56. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet 2002;30:41-7.
57. Brown P, Levis M, Shurtleff S, Campana D, Downing J, Small D. FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. Blood 2005;105:812-20.
58. Cheok MH, Yang W, Pui CH, et al. Treatment-specific changes in gene expression discriminate in vivo drug response in human leukemia cells. Nat Genet 2003; 34:85-90. [Erratum, Nat Genet 2003;34: 231.]
59. Zaza G, Cheok M, Yang M, et al. Gene expression and thioguanine nucleotide disposition in acute lymphoblastic leukemia after in vivo mercaptopurine treatment. Blood 2005;106:1778-85.
60. Kager L, Cheok M, Yang W, et al. Folate pathway gene expression differs in subtypes of acute lymphoblastic leukemia and influences methotrexate pharmacodynamics. J Clin Invest 2005;115:110-7.
61. Holleman A, Cheok MH, den Boer ML, et al. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. N Engl J Med 2004;351:533-42.
62. Lugthart S, Cheok MH, den Boer ML, et al. Identification of genes associated with chemotherapy crossresistance and treatment response in childhood acute lymphoblastic leukemia. Cancer Cell 2005;7:375-86.
63. French D, Wilkinson MR, Yang W, et al. Global gene expression as a function of germline genetic variation. Hum Mol Genet 2005;14:1621-9.
64. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-8.
65. Pui C-H, Campana D, Evans WE. Childhood acute lymphoblastic leukaemia — current status and future perspectives. Lancet Oncol 2001;2:597-607.
66. Nachman JB, Sather HN, Sensel MG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. N Engl J Med 1998;338: 1663-71.
67. Lipshultz SE, Rifai N, Dalton VM, et al. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. N Engl J Med 2004;351:145-53.
68. Lipshultz SE, Lipsitz SR, Sallan SE, et al. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. J Clin Oncol 2005;23:2629-36.
69. Mattano LA Jr, Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group. J Clin Oncol 2000; 18:3262-72.
70. McCarville MB, Adelman CS, Li C, et al. Typhlitis in childhood cancer. Cancer 2005;104:380-7.
71. Rubnitz JE, Lensing S, Zhou Y, et al. Death during induction therapy and first remission of acute leukemia in childhood: the St. Jude experience. Cancer 2004;101: 1677-84.
72. Lee EJ, Petroni GR, Schiffer CA, et al. Brief-duration high-intensity chemotherapy for patients with small noncleaved-cell lymphoma or FAB L3 acute lymphocytic leukemia: results of Cancer and Leukemia Group B study 9251. J Clin Oncol 2001;19:4014-22.
73. Pession A, Valsecchi MG, Masera G, et al. Long-term results of a randomized trial on extended use of high dose of L-asparaginase for standard risk childhood acute lymphoblastic leukemia. J Clin Oncol 2005; 23:7161-7.
74. Hurwitz CA, Silverman LB, Schorin MA, et al. Substituting dexamethasone for prednisone complicates remission induction in children with acute lymphoblastic leukemia. Cancer 2000;88:1964-9.
75. Hallböök H, Simonsson B, Ahlgren T, et al. High-dose cytarabine in upfront therapy for adult patients with acute lymphoblastic leukaemia. Br J Haematol 2002; 118:748-54.
76. Bostrom BC, Sensel MR, Sather HN, et al. Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood 2003;101:3809-17.
77. Mitchell CD, Richards SM, Kinsey SE, et al. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL 97 randomized trial. Br J Haematol 2005;129:734-45.
78. Igarashi S, Manabe A, Ohara A, et al. No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. J Clin Oncol 2005;23:6489-98.
79. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. Blood 2004;103:4396-407.
80. Lee S, Kim YJ, Min CK, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood 2005; 105:3449-57.
81. Schrappe M, Reiter A, Ludwig WD, et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. Blood 2000;95:3310-22.
82. Pinheiro JP, Boos J. The best way to use asparaginase in childhood acute lymphoblastic leukaemia — still to be defined? Br J Haematol 2004;125:117-27.
83. Duval M, Suciu S, Ferster A, et al. Comparison of *Escherichia coli*-asparaginase

with *Erwinia*-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer–Children's Leukemia Group phase 3 trial. Blood 2002;99:2734-9.
84. Lange BJ, Bostrom BC, Cherlow JM, et al. Double-delayed intensification improves event-free survival for children with intermediate-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood 2002;99:825-33.
85. Hoelzer D, Gokbuget N. New approaches to acute lymphoblastic leukemia in adults: where do we go? Semin Oncol 2000;27:540-59.
86. Pui C-H, Chessells JM, Camitta B, et al. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. Leukemia 2003;17:700-6.
87. Kosaka Y, Koh K, Kinukawa N, et al. Infant acute lymphoblastic leukemia with *MLL* gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. Blood 2004;104:3527-34.
88. Sanders JE, Im HJ, Hoffmeister PA, et al. Allogeneic hematopoietic cell transplantation for infants with acute lymphoblastic leukemia. Blood 2005;105:3749-56.
89. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med 2004;351:2276-85.
90. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 2004;351:2265-75.
91. Toyoda Y, Manabe A, Tsuchida M, et al. Six months of maintenance chemotherapy after intensified treatment for acute lymphoblastic leukemia of childhood. J Clin Oncol 2000;18:1508-16.
92. Aricó M, Baruchel A, Bertrand Y, et al. The seventh International Childhood Acute Lymphoblastic Leukemia Workshop report: Palermo, Italy, January 29–30, 2005. Leukemia 2005;19:1145-52.
93. van der Werff Ten Bosch J, Suciu S, Thyss A, et al. Value of intravenous 6-mercaptopurine during combination treatment in childhood acute lymphoblastic leukemia and non-Hodgkin's lymphoma: final results of a randomized phase III trial (58881) of the EORTC CLG. Leukemia 2005; 19:721-6.
94. Nygaard U, Toft N, Schmiegelow K. Methylated metabolites of 6-mercaptopurine are associated with hepatotoxicity. Clin Pharmacol Ther 2004;75:274-81.
95. Pui C-H. Toward optimal central nervous system-directed treatment in childhood acute lymphoblastic leukemia. J Clin Oncol 2003;21:179-81.
96. Pui C-H, Cheng C, Leung W, et al. Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. N Engl J Med 2003;349:640-9. [Erratum, N Engl J Med 2003;349:1299.]
97. Howard SC, Gajjar AJ, Cheng C, et al. Risk factors for traumatic and bloody lumbar puncture in children with acute lymphoblastic leukemia. JAMA 2002;288:2001-7.
98. Hijiya N, Liu W, Sandlund JT, et al. Overt testicular disease at diagnosis of childhood acute lymphoblastic leukemia: lack of therapeutic role of local irradiation. Leukemia 2005;19:1399-403.
99. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2004;305:399-401.
100. Graux C, Cools J, Melotte C, et al. Fusion of *NUP214* to *ABL1* on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet 2004;36:1084-9.
101. Sawyers C. Targeted cancer therapy. Nature 2004;432:294-7.
102. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature 2004; 429:457-63.
103. Mitchell BS. The proteasome — an emerging therapeutic target in cancer. N Engl J Med 2003;348:2597-8.
104. Ptasznik A, Nakata Y, Kalota A, Emerson SG, Gewirtz AM. Short interfering RNA (siRNA) targeting the Lyn kinase induces apoptosis in primary, and drug-resistant, BCR-ABL1(+) leukemia cells. Nat Med 2004;10:1187-9.
105. Bomgaars L, Geyer JR, Franklin J, et al. Phase I trial of intrathecal liposomal cytarabine in children with neoplastic meningitis. J Clin Oncol 2004;22:3916-21.
106. Pui C-H, Jeha S, Kirkpatrick P. Clofarabine. Nat Rev Drug Discov 2005;4:369-70.
107. Kurtzberg J, Ernst TJ, Keating MJ, et al. Phase I study of 506U78 administered on a consecutive 5-day schedule in children and adults with refractory hematologic malignancies. J Clin Oncol 2005;23: 3396-403.
108. Nemecek ER, Matthews DC. Antibody-based therapy of human leukemia. Curr Opin Hematol 2002;9:316-21.
109. Imai C, Iwamoto S, Campana D. Genetic modification of primary natural killer cells overcomes inhibitory signals and induces specific killing of leukemic cells. Blood 2005;106:376-83.

IMAGES IN CLINICAL MEDICINE

Splenic and Mediastinal Calcifications in Histoplasmosis



Jeremy Topin, M.D.
Gökhan M. Mutlu, M.D.

Northwestern University
Feinberg School of Medicine
Chicago, IL 60611

A 37-year-old woman from Illinois with a history of an idiopathic T-cell deficiency (CD4 cell count, 249 cells per cubic millimeter, and negative for infection with the human immunodeficiency virus), histoplasma pneumonia in childhood, and recurrent vulvovaginal candidiasis presented with nausea and odynophagia for three days. Physical examination was unremarkable except for the presence of oral candidiasis and mild tenderness in the left upper quadrant. As a part of the workup, she underwent computed tomography (CT) of the abdomen, which showed extensive, diffuse calcification of the spleen (Panel A, large arrow) with several calcified lymph nodes within the abdominal cavity (Panel A, small arrows). A chest CT also showed numerous calcified mediastinal lymph nodes (Panel B, arrows). The patient's acute symptoms were diagnosed as esophageal candidiasis. She was administered fluconazole, to which the infection responded. The calcifications visualized in the radiographic studies were diagnosed as due to a prior infection with *Histoplasma capsulatum*. The central United States is an area where histoplasmosis is endemic. Most cases of histoplasmosis are associated with enlargement of mediastinal lymph nodes, which can calcify and cause mediastinal obstructive syndromes. *H. capsulatum* infection is a common cause of diffuse splenic calcification; other considerations include brucellosis and tuberculosis.

CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL

Founded by Richard C. Cabot
Nancy Lee Harris, M.D., *Editor*
Jo-Anne O. Shepard, M.D., *Associate Editor*
Sally H. Ebeling, *Assistant Editor*
Stacey M. Ellender, *Assistant Editor*
Christine C. Peters, *Assistant Editor*

Case 1-2006: A 64-Year-Old Male Lung-Transplant Recipient with Fever, Cough, Hemoptysis, and Abdominal Pain

Robert H. Rubin, M.D., Matthew D. Gilman, M.D., and Richard L. Kradin, M.D.

From the Division of Infectious Disease, Brigham and Women's Hospital (R.H.R.); the Departments of Radiology (M.D.G.) and Pathology (R.L.K.), Massachusetts General Hospital; and the Harvard/Massachusetts Institute of Technology Division of Health Science and Technology (R.H.R), Medicine (R.H.R.), Radiology (M.D.G.), and Pathology (R.L.K.), Harvard Medical School — all in Boston.

N Engl J Med 2006;354:180-7.

PRESENTATION OF CASE

A 64-year-old man with emphysema who had received a double-lung allograft six months previously was admitted to the hospital because of cough, hemoptysis, and abdominal pain.

Three days before admission, abdominal pain that was localized to the left lower quadrant developed. Two days before admission, the patient began to have small-volume hemoptysis (approximately one teaspoon) on awakening. His appetite was poor, but he was able to drink liquids. He did not have diarrhea, melena, hematochezia, shortness of breath, or chest pain. On the evening before admission, fever to 38.3°C developed, associated with shaking chills. The patient vomited once the next morning and came to the transplantation clinic. A chest radiograph revealed a new infiltrate in the right upper lobe, and a test for cytomegalovirus (CMV) antigenemia was positive at 185 cells per two slides. He was admitted to the hospital.

The patient had smoked three packs of cigarettes per day for 25 years; he quit 9 years before admission when he started to become short of breath. Over the course of the year after he stopped smoking, his exercise tolerance declined and he was started on home oxygen therapy. Eight years before admission, a lung mass in the left lower lobe was seen on a routine chest radiograph. A thoracoscopic wedge resection and mediastinal lymph-node dissection were performed; pathological examination disclosed a moderately to poorly differentiated squamous-cell carcinoma; the surgical margins and lymph nodes were negative for carcinoma. He received no further treatment for the cancer. Five years before admission, pulmonary-function tests documented findings that were consistent with emphysema with severe obstruction, as defined by a ratio of forced expiratory volume in one second to forced vital capacity of 28 percent, a peak expiratory flow rate of 3.08 liters per second (36 percent of the predicted rate), elevated lung volumes (total lung capacity, 11.1 liters; 176 percent of predicted volume), residual volume of 7.98 liters (327 percent of the predicted volume), and decreased diffusing capacity (15 percent of the predicted capacity). The patient was placed on a waiting list for lung transplantation.

In the three years before admission, the patient had had five admissions for

acute respiratory failure. During one of those admissions, a pulmonary nodule, 2.1 cm in diameter, was revealed in the right lower lobe by computed tomographic (CT) evaluation of the chest. Wedge resection was performed, and a pathological examination revealed caseating granulomas; cultures grew *Mycobacterium avium* complex, and the condition was treated with ethambutol and clarithromycin up to the time of the patient's transplantation.

Six months before admission, bilateral pneumonectomy and double-lung transplantation were performed. The patient's initial immunosuppressive regimen consisted of antithymocyte globulin, cyclosporine, azathioprine, and prednisone. Four months before admission, he received methylprednisolone pulse treatment for a possible episode of rejection. Cyclosporine and azathioprine were replaced by tacrolimus and mycophenolate to decrease renal toxicity. The patient had been negative for CMV antibodies at the time of transplantation; the donor had been positive for CMV antibodies. Fourteen days before admission, a test for CMV antigen in the patient's blood was negative.

Two months before admission, edema of the right lower leg, dyspnea, and lightheadedness developed; the patient was admitted to the hospital and was found to have a right popliteal deep venous thrombosis. A ventilation–perfusion scan showed an intermediate probability of pulmonary embolism; the patient received five days of anticoagulant therapy and then was discharged, still taking oral warfarin.

Other medical problems included diverticulosis (with no history of diverticulitis) and chronic renal insufficiency (two weeks before admission, the blood level of urea nitrogen was 50 mg per deciliter [1.8 mmol per liter] and the creatinine level was 2.3 mg per deciliter [203.3 μmol per liter]), diabetes mellitus, benign prostatic hypertrophy, osteoporosis, and depression. The patient's medications at the time of admission included tacrolimus, mycophenolate mofetil, prednisone, acyclovir, trimethoprim–sulfamethoxazole clotrimazole troches, alendronate, erythropoietin, tamsulosin, warfarin, neutral protomine Hagedorn insulin, regular insulin, atorvastatin, furosemide, esomeprazole, docusate, senna, calcium, magnesium, folic acid, and vitamin B_6 and vitamin B_{12} supplements.

On the patient's admission to the emergency room, the blood pressure was 112/66 mm Hg, the pulse 110 beats per minute, the respiratory rate 18 breaths per minute, and the temperature 36.5°C. The oxygen saturation was 96 percent, and the patient's weight was 106.5 kg. He was comfortable and in no respiratory distress. Auscultation of the lungs revealed a few crackles at the left base, with no wheezes. The abdomen was soft and obese, with hypoactive bowel sounds and tenderness to palpation in the left lower quadrant. There were no masses, no rebound, and no guarding. A rectal examination revealed guaiac-positive brown stool. There was moderate edema of the right leg with mild erythema over the lower tibia. The remainder of the physical examination and the results of a neurologic examination were normal. Laboratory-test results are shown in Table 1.

Chest radiography showed a new infiltrate in the right upper lobe. CT scanning of the chest confirmed the presence of airway disease in the right upper lobe, consistent with pneumonia. Small bilateral pleural effusions were slightly smaller than those seen on the radiographic study obtained two months before admission. Atelectasis in the lingula and left lower lobe and air trapping in the right middle lobe were stable. CT scanning of the abdomen and pelvis showed sigmoid diverticulosis, without any signs of diverticulitis, and no other abnormalities.

Anti-CMV hyperimmune globulin was administered intravenously in the emergency room. Rigors developed after the infusion of 75 percent of the dose and resolved after treatment with acetaminophen, diphenhydramine, and meperidine. Ganciclovir, 5 mg per kilogram, was administered intravenously. Intravenous vancomycin and cefepime and oral azithromycin were begun to treat a possible bacterial pneumonia, and metronidazole was included as coverage for possible diverticulitis. The patient was admitted to the hospital.

A diagnostic procedure was performed.

DIFFERENTIAL DIAGNOSIS

Dr. Robert H. Rubin: This patient presented with cough, hemoptysis, fever, and abdominal pain six months after he underwent bilateral lung transplantation. His pretransplantation course had in-

Table 1. Laboratory Values on the Day of Admission.*

Variable	Value	Variable	Value
White-cell count (per mm^3)	6,400	Phosphorus (mg/dl)	4.4
Red-cell count (per mm^3)	3,110,000	Magnesium (mmol/liter)	1.5
Hemoglobin (g/dl)	9.8	Bilirubin (mg/dl)	
Hematocrit (%)	30.3	Total	0.3
Platelet count (per mm^3)	193,000	Direct	0.1
Differential count (%)		Protein (g/dl)	
Neutrophils	91	Total	6.6
Lymphocytes	6	Albumin	3.7
Monocytes	3	Globulin	2.9
Eosinophils	0	Amylase (U/liter)	61
Basophils	0	Lipase (U/liter)	1.6
Prothrombin time (sec)	20.8	Alanine aminotransferase (U/liter)	11
International normalized ratio	3.0	Aspartate aminotransferase (U/liter)	24
Partial-thromboplastin time (sec)	30.8	Alkaline phosphatase (U/liter)	64
Sodium (mmol/liter)	144	Glycosylated hemoglobin (%)	6.20
Potassium (mmol/liter)	4.6	Tacrolimus (ng/milliliter)	5.0
Chloride (mmol/liter)	100	Cytomegalovirus antigenemia assay	Positive: 185 cells
Carbon dioxide (mmol/liter)	32.3	Urine culture	No growth
Urea nitrogen (mg/dl)	46	Rectal vancomycin-resistant enterococcus	Negative
Creatinine (mg/dl)	2.4		
Glucose (mg/dl)	97		
Calcium (mg/dl)	9.6		

* To convert the value for urea nitrogen to millimoles per liter, multiply by 0.357. To convert the value for creatinine to micromoles per liter, multiply by 88.4. To convert the value for glucose to millimoles per liter, multiply by 0.05551. To convert the value for calcium to millimoles per liter, multiply by 0.250. To convert the value for phosphorus to millimoles per liter, multiply by 0.3229. To convert the values for total and direct bilirubin to micromoles per liter, multiply by 17.1.

cluded surgical removal of a poorly differentiated squamous-cell carcinoma of the left lower lobe and the discovery of biopsy-proven *M. avium* complex infection. He had not had a recurrence of the cancer in eight years, and there had been no evidence of *M. avium* complex infection since the excisional biopsy; antimycobacterial therapy had been maintained. Other clinical problems before the current admission included possible transplant rejection and a right popliteal deep venous thrombosis. May we see the radiology studies?

Dr. Matthew D. Gilman: The baseline chest radiographic study, performed two months before admission, showed subsegmental atelectasis in the lung bases and mild bilateral pleural thickening or pleural effusions, findings frequently seen in patients who have received lung transplants. Chest radiography performed on the day of admission revealed a new right upper-lobe consolidation (Fig. 1A). The remainder of the chest radiograph was unchanged. Images from a chest CT scan with lung windows revealed a focal consolidation in the posterior segment of the right upper lobe, correlating with the findings in the chest radiograph (Fig. 1B). There was no evidence of hilar or mediastinal lymphadenopathy. The abdominal CT scan showed mild diverticulosis.

Dr. Rubin: The most likely diagnosis of the pulmonary infiltrate, in view of the hemoptysis and prolonged prothrombin time, is hemorrhage, with fluid overload, aspiration, and infection being other possibilities. However, the fever and abdomi-

nal pain suggest an infectious process — and as we consider a possible infection in this patient with a lung transplant, two principles need to be kept in mind.[1,2] First, the signs and symptoms of infection may have been greatly muted by the immunosuppressive therapy; because early diagnosis and treatment are essential in cases involving immunosuppression, the clinician must evaluate minor signs and symptoms, and the approach taken must be more invasive (e.g., biopsy of innocuous-looking lesions that are unexplained). Second, the occurrence of infection in a lung-transplant recipient is due to the interaction of three factors: anatomical or technical problems that are associated with the surgical procedure, particularly the bronchial anastomosis; environmental exposure to pathogens in the air or water; and the net state of immunosuppression (Table 2). All these factors need to be considered in the case of this patient.

This patient's illness developed six months after he underwent transplantation. Although infections can occur at any time in the post-transplantation period, the causes are very different at different times.[1,2] The post-transplantation course can be divided into three parts: the first month after transplantation, one to six months after transplantation, and more than six months after transplantation. In the first month, the three primary causes of infectious conditions are infection from the donor that travels with the allograft, pretransplantion infection in the recipient, and infections that result from technical or anatomical problems at the time of the transplantation.[1,2]

From one to six months after transplantation, the net state of immunosuppression (Table 2) is high because of sustained immunosuppressive therapy and the effects of immunomodulating viruses; viral infections are common, particularly CMV, Epstein–Barr virus (EBV), human herpesvirus-6, hepatitis viruses B and C, and the human immunodeficiency virus; opportunistic infections may occur, such as those with *Pneumocystis carinii*, *Listeria monocytogenes*, and a variety of fungi. CMV accounts for more than 50 percent of febrile episodes in transplant recipients during this time period.[1,2]

More than six months after transplantation, the majority of patients have had a good result, are receiving minimal immunosuppression, and are primarily at risk from respiratory pathogens.[1-3] At this point in their course, a minority of transplant recipients have had a poor result, with too much exposure to immunosuppressive therapy because of ongoing rejection, chronic viral infection, or residual effects of previous infections and anatomical abnormalities, and are at high risk for late opportunistic infections.[1-3]

This patient was on the border between two risk groups, the group that is from one to six months after transplantation and the group that is more than six months after transplantation.

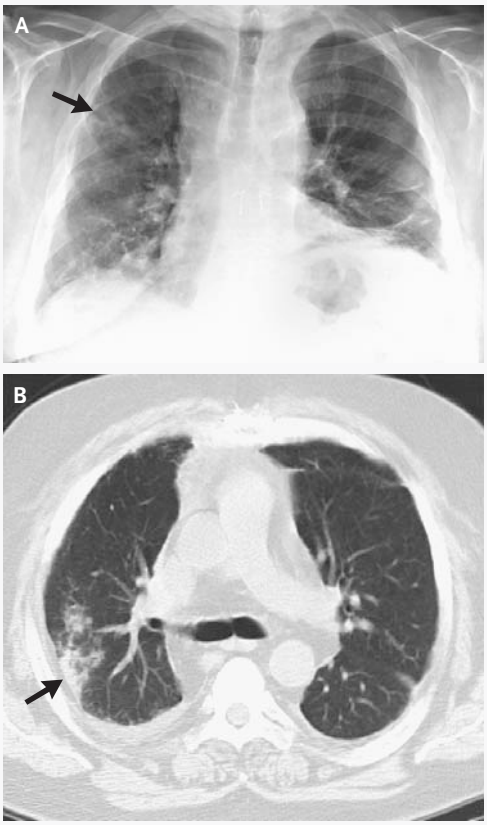


Figure 1. Radiologic Images of the Chest.

A posterior-anterior view of the chest obtained on hospital admission (Panel A) reveals a new focus of consolidation in the right upper lobe (arrow). Sternal wires, subsegmental atelectasis, and mild elevation of the left hemidiaphragm are present and are unchanged, as compared with prior examinations. CT scanning of the chest (Panel B) performed one day after admission shows consolidation in the posterior segment of the right upper lobe (arrow). A small right pleural effusion is present, but decreased in size, as compared with earlier chest CT scans.

Table 2. Factors That Determine the Net State of Immunosuppression.

Host-defense deficits engendered by the underlying disease
Dose, duration, and temporal sequence of immunosuppressive drugs
Neutropenia
Metabolic derangements (possible protein–calorie malnutrition, anemia, hyperglycemia)
Viral infection (infection with cytomegalovirus, Epstein–Barr virus, hepatitis B and C viruses, human immunodeficiency virus)
Age
Underlying immune deficiency

There was no evidence of anatomical or technical problems, he had no early opportunistic or other serious infections, and an episode of rejection responded to treatment. He was almost past the window of opportunity in which a new or reactivated CMV infection should occur. Thus, he fell into the group that has had a good result since the transplantation and should be primarily at risk for community-acquired respiratory infection. However, although this patient had been receiving therapy to prevent CMV infection, the titer of CMV antigen had recently increased. An incomplete protective strategy against CMV will prolong the incubation period but not prevent disease, and therefore, infection with CMV may occur six or more months after transplantation, rather than in the more limited window of one to six months after transplantation.[1,2]

The possible causes of this patient's problems can be divided into the following categories: a recurrence of the squamous-cell carcinoma that he had before transplantation or his proven *M. avium* complex infection; CMV infection with CMV as the sole pathogen; and CMV infection plus a second process, such as an angioinvasive fungus (e.g., *Aspergillus fumigatus*). Although he had a very stormy course before the transplantation, his problems were aggressively identified and treated and there was no evidence of either cancer or mycobacterial infection at the time of bilateral pneumonectomy and transplantation. Recurrence of either of these processes is unlikely to have been responsible for the patient's difficulties.

The cough and pulmonary infiltrates could be signs of infection, pneumonia related to either CMV or another pathogen, or both. The patient had been vomiting, and in an immunosuppressed host, there is a risk for aspiration during any vomiting episode. Given the history of repeated hemoptysis, pulmonary hemorrhage was a likely component of the infiltrate. Pulmonary hemorrhage would be an unusual manifestation of CMV pneumonia and would suggest the presence of a second process, such as angioinvasive fungal infection or even post-transplantation lymphoproliferative disease (PTLD), as well as pulmonary emboli in a patient with a history of thrombophlebitis in his legs. However, in the context of anticoagulant therapy, pulmonary hemorrhage can occur without the presence of a second major process.

PTLD must be considered, and it would be of interest to know if the patient harbors replicating EBV and the level of the viral load. At 64 years of age, he was assuredly EBV-seropositive, and reactivation of endogenous virus at some stage in the post-transplantation course would be expected. The presence of CMV antigenemia is associated with an increase by a factor of 7 to 10 in the incidence of PTLD, and the condition can occur in the allograft. Although PTLD would be an unusual cause of this patient's clinical syndrome, I cannot rule it out.[1]

There are at least four potential causes of the patient's abdominal pain. Diverticulitis is a clinically important problem in transplant recipients, which can be occult, usually requires surgery, and has to be a primary concern until an abdominal CT scan, preferably with rectal contrast material, rules it out (as in this case). Other considerations include an adverse effect of mycophenolate mofetil, an intercurrent bacterial enteritis (e.g., salmonellosis, campylobacter infection, listeriosis), or CMV-related enterocolitis.[4] Although bacteriologic cultures were indicated, CMV was a particularly strong consideration in this patient.

For many years, the interaction of CMV with the gut has been controversial, because immunofluorescence examination of pathological specimens has revealed relatively few cells infected with the virus. It is now apparent that even though they are few, these infected cells produce large amounts of inflammatory cytokines, particularly interleukin-1, which have profound effects on the uninfected tissue. It is now accepted that, in addition to ileus, CMV can have a variety of functional and structural effects on the gut, including

decreased gastric motility, as well as clinically significant enterocolitis.[1,2]

The diagnosis of CMV infection and disease has improved considerably in recent years. The semiquantitative antigenemia assay, as used in this case, and quantitative polymerase-chain-reaction measurements of virus in the blood are major advances, providing useful indicators of viral load. In this case, we are told that over the course of a two-week period, the results of the antigenemia assay went from negative to 185 cells on two slides. Such a rapid and marked elevation is likely to be associated with clinical disease. The critical host defense against CMV is mediated by major-histocompatibility-complex–restricted, virus-specific, cytotoxic T cells. This patient had primary CMV infection, because we know that the organ donor was seropositive and the recipient was seronegative. Thus, it is likely that the patient's immune response was still rather weak, particularly since antiviral prophylaxis was administered, which would have decreased viral replication. Viral replication, so-called endogenous vaccination, may be necessary to mount the potent, cytotoxic T-cell response that is needed for sustained control.

In summary, with the exception of the hemoptysis (and probable pulmonary hemorrhage), infection with CMV could easily be responsible for all of the clinical abnormalities in this patient. However, CMV also predisposes a patient to potentially lethal superinfection with a variety of pathogens. In this case, the particular concern would be an angioinvasive fungal infection, such as *A. fumigatus.* There would be three consequences of invasive aspergillosis in this immunocompromised host: hemorrhage, infarction, and metastases. Particularly in the lung, this secondary infection could have been present, and that possibility illustrates the need for precise diagnosis. I suspect the two procedures carried out were colonoscopy and bronchoscopy with bronchoalveolar lavage.

Dr. Nancy Lee Harris (Pathology): Dr. Ginns, will you describe your thoughts and the diagnostic procedure?

Dr. Leo Ginns (Pulmonary Medicine): I thought that this patient had CMV disease and colitis, and I concluded that the pulmonary process was probably related to aspiration. The diagnostic procedure was colonoscopy with biopsy, followed by bronchoscopy with lavage and transbronchial biopsy.

CLINICAL DIAGNOSIS

Primary CMV infection.

Possibly aspiration pneumonitis.

DR. ROBERT H. RUBIN'S DIAGNOSIS

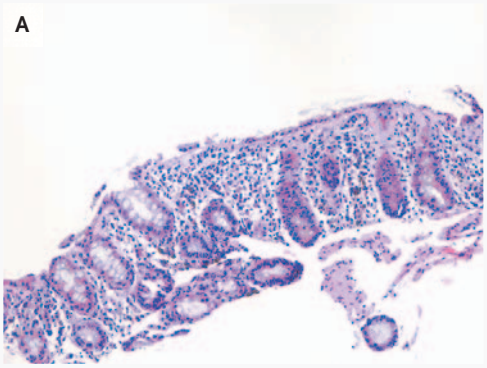
Primary CMV infection, with viremia and colitis.

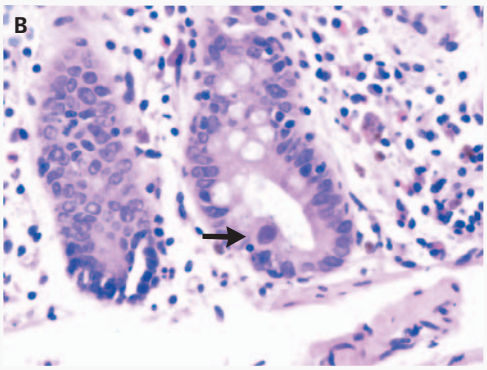
Pulmonary hemorrhage due to anticoagulation therapy.

PATHOLOGICAL DISCUSSION

Dr. Richard L. Kradin: At colonoscopy, diverticula were seen, but there were no mucosal abnormalities. A biopsy specimen of the right colon shows surface injury, edematous mucosa with mild irregularity of the colonic crypts, and a small amount of chronic inflammatory infiltrate (Fig. 2A). There were a few abnormal epithelial cells in the colonic crypts with cytomegalic features. One cell had a large amphophilic nuclear inclusion, characteristic of CMV infection (Fig. 2B). The histopathological features of CMV infection include cytomegaly, an enlarged amphophilic-to-basophilic nuclear inclusion with a surrounding halo, and intracytoplasmic inclusions that stain with periodic acid–Schiff and Gomori methenamine silver preparations. Immunohistochemical staining for CMV early antigens revealed large numbers of infected cells (Fig. 2C), mostly macrophages, in the colonic mucosa. Bronchoscopic lavage performed the day after the colonoscopy showed alveolar macrophages with no evidence of CMV infection. A biopsy of the lung several days later showed edematous bronchial mucosa with a slight increase in tissue eosinophils but no CMV-infected cells. No lung parenchyma was sampled.

CMV is a betaherpesvirus[5] that targets epithelial cells lining the respiratory or gastrointestinal tract in primary infection; macrophages and endothelial cells harbor latent infection. CMV replication causes direct cellular injury, but viral infection also modulates immune responses by promoting the induction of chemokines and by the induction of cytokines. CMV-infected patients





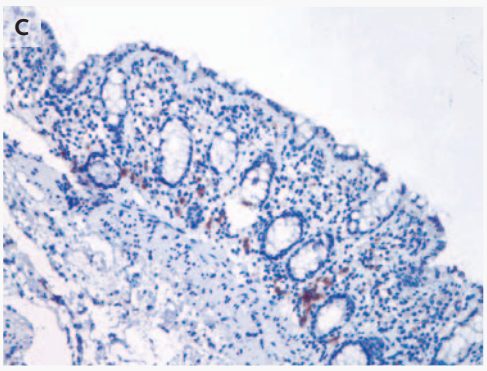


Figure 2. Biopsy Specimen of the Colon.
There is mild mucosal inflammation and irregularity of the colonic crypts (Panel A). An intranuclear inclusion can be seen within an epithelial cell (Panel B, arrow) that also shows cytomegalic changes. With an immunohistochemical stain that reacts to CMV antigen (Panel C), numerous cells are shown to be infected. (Panels A and B, hematoxylin and eosin; Panel C, immunoperoxidase stain.)

may produce a range of autoantibodies that could potentially contribute to tissue damage, including antinuclear antibodies, rheumatoid factor, and anti–smooth-muscle antibodies. CMV has profound effects on the immune response and amplifies preexisting levels of immunosuppression. The greatest effects appear to be on T-cell–mediated immunity.

The diagnosis of CMV[6] requires evidence of active colitis and the presence of activated CMV. The colonic mucosa may be mildly inflamed, as in the present case, but frequently it is also ulcerated because of microvascular injury. Pseudomembranous colitis[7] can be seen, and in severe cases toxic megacolon[8] with peritonitis has been reported. These changes rarely occur in immunocompetent patients. The diagnosis of CMV is confirmed by biopsy with viral culture, immunohistochemical analysis, in situ hybridization, or ultrastructural examination.

Dr. Harris: Dr. Gilman, may we see the follow-up chest radiograph?

Dr. Gilman: A chest radiograph obtained four days after admission shows clearing of the right-upper-lobe consolidation and return to baseline.

Dr. Rubin: This finding strongly suggests that the pulmonary infiltrate and hemoptysis were due to pulmonary hemorrhage, rather than infection.

Dr. Harris: Dr. Ginns, would you give us the follow-up information?

Dr. Ginns: The patient responded well to the combination of intravenous ganciclovir therapy and a reduction in his immunosuppressive therapy. As Dr. Rubin predicted, he did present again approximately one year later with abdominal pain, which on this occasion was due to diverticulitis with a ruptured diverticulum and abscess, and he underwent surgical resection of his colon.

ANATOMICAL DIAGNOSIS

Cytomegalovirus colitis.

Dr. Rubin reports having served as a consultant to Novartis and Merrimack and having received grant support from Pfizer and Merck. No other potential conflict of interest relevant to this article was reported.

REFERENCES

1. Rubin RH. Infection in the organ transplant recipient. In: Rubin RH, Young LS, eds. Clinical approach to infection in the compromised host. 4th ed. New York: Kluwer Academic/Plenum, 2002: 573-9.
2. Van Der Meer JWM, Kullberg BJ. Defects in host defense mechanisms. In: Rubin RH, Young LS, eds. Clinical approach to infection in the compromised host. 4th ed. New York: Kluwer Academic/Plenum, 2002:5-47.
3. Johnson RA, Sober A. Mucocutaneous infections in the immunocompromised host. In: Rubin RH, Young LS, eds. Clinical approach to infection in the compromised host. 4th ed. New York: Kluwer Academic/Plenum, 2002:49-110.
4. Rubin RH. Gastrointestinal infectious disease complications following transplantation and their differentiation from immunosuppressant-induced gastrointestinal toxicities. Clin Transplant 2001;15:Suppl 4:11-22.
5. Soderburg-Naucler C, Nelson JA. Immunomodulation of cytomegalovirus. In: Cunningham MW, Fujinami RJ, eds. Effects of microbes on the immune system. Philadelphia: Lippincott Williams & Wilkins, 2000:399-418.
6. Woods GL, Schnadig VJ, Walker DH, Winn WC Jr, eds. Infectious disease pathology: clinical cases. Boston: Butterworth-Heinemann, 2000.
7. Battaglino MP, Rockey DC. Cytomegalovirus presenting with an endoscopic appearance of pseudomembranous colitis. Gastrointest Endosc 1999;50:697-700.
8. Mukhopadhya A, Samal SC, Patra S, et al. Toxic megacolon in a renal allograft recipient with cytomegalovirus colitis. Indian J Gastroenterol 2001;20:114-5.

EDITORIALS

Optimizing ACE-Inhibitor Therapy for Chronic Kidney Disease

Lee A. Hebert, M.D.

In this issue of the *Journal*, Hou and colleagues[1] present data indicating that the use of benazepril, an angiotensin-converting–enzyme (ACE) inhibitor, is feasible and beneficial in patients with advanced chronic kidney disease, a point that has been much debated. This clinical trial in China, which confirms pilot data from the same group,[2] provides some clarity, demonstrating that ACE inhibitors can be administered in generous doses, even in patients with stage 4 chronic kidney disease, as defined by a glomerular filtration rate (GFR) of 15 to 29 ml per minute per 1.73 m^2 and a serum creatinine level of approximately 3.0 to 5.0 mg per deciliter (265 to 442 μmol per liter). Their findings also indicate that ACE inhibitors may provide renal benefit even if a patient's serum creatinine level continues to increase. Their conclusions may come as news to the many clinicians who avoid or abandon the use of ACE inhibitors or angiotensin-receptor blockers in their patients with stage 4 chronic kidney disease, fearing that this approach merely increases risk (especially of hyperkalemia) without providing benefit (e.g., slowing the decline in GFR). Although their results indicate that it may be time to change our practice, a number of caveats should be considered before we do so.

Hou et al. used a daily dose of 20 mg of benazepril, half the maximal recommended dose for patients with chronic kidney disease. This choice is remarkable because almost all studies that have demonstrated a renal-protective effect of ACE inhibitors used about 15 to 25 percent of the maximal recommended dose,[3] and the patients in those studies had GFRs that were 50 to 100 percent higher than the rates in the patients studied by Hou et al.[4,5] Another important difference is that Hou et al. used a twice-daily dose, a regimen that provides little opportunity for nocturnal recovery from any hyperkalemia that might have developed during the day as a result of dietary intake. Thus, it is surprising that serious hyperkalemia was not a major problem in the study. The study patients were divided into two groups on the basis of their creatinine levels: group 1 had a serum creatinine level of 1.5 to 3.0 mg per deciliter (133 to 265 μmol per liter), and group 2 had a level of 3.1 to 5.0 mg per deciliter (274 to 442 μmol per liter).

The absence of serious hyperkalemia might be explained by three factors. First, during the eight-week run-in period, about 5 percent of the patients in group 2 were excluded because of the development of hyperkalemia or an inordinate increase in serum creatinine levels or because of an adherence rate of less than 80 percent. Second, potassium intake in these Chinese patients may have been substantially lower than that of most Western patients, a fact suggested by the low baseline protein intake in the study subjects — about 0.5 g per kilogram of body weight per day, estimated from 24-hour urea excretion and the body-mass index, as compared with about 1.0 to 1.5 g per kilogram per day in Western patients with chronic kidney disease.[4,5] Third, more than 80 percent of the patients in group 2 received a diuretic during follow-up. In contrast, in the Modification of Diet in Renal Disease study, only about 40 percent of patients received diuretic therapy.[5] Diuretic use protects against hyperkalemia by shifting sodium reabsorption to the distal nephron, where increased sodium–potassium exchange occurs, resulting in increased renal potassium excretion. Also noteworthy is that group 2 had a low salt intake (approximately 100 meq daily), which, by itself, promotes hyperkalemia. Thus, the use of

a diuretic in the great majority of the patients in group 2 was likely an important hedge against hyperkalemia.

In the light of these observations and the conditions of the study, applying the results more widely would have certain implications, particularly with respect to the use of "generous" doses of ACE inhibitors in patients with stage 4 chronic kidney disease. Before ACE-inhibitor therapy is initiated, it is important to assess a patient's dietary potassium intake by determining 24-hour urinary potassium excretion, ideally on at least two representative days. If the potassium content of a 24-hour urine collection is 40 meq per day or less, it is impractical to lower dietary potassium intake further.[3] In such a patient, concomitant therapy with sodium bicarbonate and furosemide may be a consideration if hyperkalemia develops.[4,5]

The initial dose of an ACE inhibitor should be conservative — for instance, 15 to 25 percent of the maximal recommended dose. Furthermore, the dose should be increased only if the initial dose is well tolerated, and then only with caution. Once-daily morning dosing should potentially be considered, since it is likely to result in reduced ACE inhibition in the evening hours and thus permit increased nocturnal excretion of renal potassium to avoid hyperkalemia.

The results of Hou et al. suggest that it is worthwhile to continue ACE-inhibitor therapy in patients with chronic kidney disease even if their serum creatinine level continues to climb. The benefit of such an approach is revealed by extrapolating the creatinine clearance trend lines in Figure 3 of the article. Doing so indicates that end-stage renal disease might take twice as long to develop in the group given benazepril as in the control cohort: 7 years instead of 3.5 years.

Whether other types of ACE inhibitors can achieve the results reported by Hou et al. with benazepril is not clear. ACE inhibitors have potentially important differences in plasma protein binding, their affinity for ACE, and pharmacokinetics.[5] However, there is no convincing evidence that generic long-acting ACE inhibitors (such as lisinopril and enalapril) are inferior to brand-name ACE inhibitors.[4,5] Captopril, however, would not be recommended over benazepril, because its shorter half-life necessitates thrice-daily dosing, increasing the likelihood of missed doses and undertreatment.[6]

Other evidence-based measures that might improve the efficacy of ACE-inhibitor therapy in patients with chronic kidney disease should be considered. One such measure is achieving a target systolic blood pressure, measured while a patient is seated, of 120 mm Hg or less, if tolerated.[4,5,7] Furthermore, controlling dietary salt intake is important during therapy with an ACE inhibitor or an angiotensin-receptor blocker, because a high-salt diet can override the antiproteinuric effects of these agents.[4,5] For the bulk of patients with chronic kidney disease, the recommended salt intake (documented by periodic 24-hour urine testing) is approximately 90 to 120 meq per day (approximately 2 to 3 g of sodium per day).[4,5] To achieve this level of salt intake without sacrificing taste, the patient should prepare a low-salt meal (2 g of sodium, or 88 meq), and then add salt to each portion just before eating it, so that the salt is on the surface of the food, permitting direct contact with the taste buds.[4] An appropriate daily allotment of surface salt is one third of a teaspoon (28 meq of sodium)

Adding an angiotensin-receptor blocker to an ACE inhibitor appears to be beneficial in patients with stage 3 chronic kidney disease (as defined by a GFR of 30 to 59 ml per minute per 1.73 m^2). For example, in the Combination Treatment of Angiotensin-II Receptor Blocker and Angiotensin-Converting–Enzyme Inhibitor in Non-diabetic Renal Disease study, in which the mean baseline GFR was about 38 ml per minute per 1.73 m^2, this combination slowed the progression of kidney disease more than did either drug alone.[8] However, it is not yet clear whether patients with stage 4 chronic kidney disease will benefit from this approach, since similar studies have yet to be performed in this population.[9]

Another point to consider is that there is evidence that controlling hypertension by adding a dihydropyridine calcium-channel blocker (DHCCB) to ACE-inhibitor therapy may not reduce proteinuria or slow the decline in GFR. Thus, arguably, this approach should be avoided unless it is required to achieve the blood-pressure goal. The rationale for this viewpoint comes from the recent Ramipril Efficacy in Nephropathy 2 (REIN-2) study, which added DHCCB to ramipril therapy in patients with chronic kidney disease and proteinuria to achieve a blood-pressure level below the usual goal. Paradoxically, achieving the low blood-pressure goal neither reduced proteinuria nor slowed

the decline in GFR as compared with the results in the control group.[10] This implies that DHCCB therapy sustained glomerular hypertension even though the systemic blood pressure had decreased substantially. Other options to achieve the goal blood pressure and reduce proteinuria have been discussed elsewhere.[5]

Finally, the use of medications to treat other conditions that arise in patients with chronic kidney disease may also help slow the progression of the renal failure itself. For example, there are some data indicating that adding a statin to an ACE inhibitor may enhance the antiproteinuric and renal-protective effects of ACE inhibitors.[11]

The study by Hou et al. does not clarify when in the course of advancing chronic kidney disease should treatment with an ACE inhibitor be stopped. However, common sense mandates that an ACE inhibitor (or an angiotensin-receptor blocker) should certainly be discontinued in the presence of uncontrollable hyperkalemia. Such agents should also be halted to see whether an increase in GFR will ensue, possibly averting the need for dialysis until vascular access is adequate or preemptive kidney transplantation can be performed. By demonstrating that ACE inhibitors can be used successfully in patients with advanced chronic kidney disease, Hou et al. suggest that abandoning treatment with an ACE inhibitor (or angiotensin-receptor blocker) when chronic kidney disease progresses to stage 3 or 4 is not necessary and hastens the onset of end-stage renal disease.

No potential conflict of interest relevant to this article was reported.

From Ohio State University Medical Center, Columbus.

1. Hou FF, Zhang X, Zhang GH, et al. Efficacy and safety of benazepril for advanced chronic renal insufficiency. N Engl J Med 2006;354:131-40.
2. Zhang GH, Hou FF, Zhang X, Liu QF. Can angiotensin-converting enzyme inhibitor be used in chronic kidney disease patients with serum creatinine level greater than 266 micromol/L? Zhonghua Nei Ke Za Zhi 2005;44:592-6. (In Chinese.)
3. Hebert LA. Target blood pressure for antihypertensive therapy in patients with proteinuric renal disease. Curr Hypertens Rep 1999;1:454-60.
4. Hebert LA, Wilmer WA, Falkenhain ME, Ladson-Wofford SE, Nahman NS Jr, Rovin BH. Renoprotection: one or many therapies? Kidney Int 2001;59:1211-26.
5. Wilmer WA, Rovin BH, Hebert CJ, Rao SV, Kumor K, Hebert LA. Management of glomerular proteinuria: a commentary. J Am Soc Nephrol 2003;14:3217-32.
6. Hebert LA, Rovin BH, Hebert CJ. Response to Dr. Brenner's comments. J Am Soc Nephrol 2004;15:1356-7.
7. Hebert CJ, Shidham G, Hebert LA. Should the target for blood pressure control specify both a systolic and a diastolic component? Curr Hypertens Rep 2005;7:360-2.
8. Nakao N, Yoshimura A, Morita H, Takada M, Kayano T, Ideura T. Combination treatment of angiotensin-II receptor blocker and angiotensin-converting-enzyme inhibitor in non-diabetic renal disease (COOPERATE): a randomised controlled trial. Lancet 2003;361:117-24. [Erratum, Lancet 2003;361:1230.]
9. Wolf G, Ritz E. Combination therapy with ACE inhibitors and angiotensin II receptor blockers to halt progression of chronic renal disease: pathophysiology and indications. Kidney Int 2005;67:799-812.
10. Ruggenenti P, Perna A, Loriga G, et al. Blood-pressure control for renoprotection in patients with non-diabetic chronic renal disease (REIN-2): multicentre, randomised controlled trial. Lancet 2005;365:939-46.
11. Bianchi S, Bigazzi R, Caiazza A, Campese VM. A controlled, prospective study of the effects of atorvastatin on proteinuria and progression of kidney disease. Am J Kidney Dis 2003;41:565-70. [Erratum, Am J Kidney Dis 2004;43:193.]

Inhaled Cyclosporine — A Breath of Fresh Air?

Malcolm M. DeCamp, Jr., M.D.

During the past two decades, lung transplantation has evolved from a new investigational intervention performed in fewer than 20 patients per year at one institution to an accepted, albeit complex, therapy for a wide variety of end-stage lung diseases that is performed in more than 1700 patients annually at nearly 150 centers worldwide.[1] Early graft failures and deaths of patients were attributable to an amalgam of technical complications, opportunistic infections, and acute rejection. Refinements in surgical technique, enhanced graft preservation, and the routine use of contemporary monitoring and prophylactic and preemptive regimens for opportunistic viruses, fungi, and protozoan organisms have led to improved early survival after lung transplantation (Fig. 1). Newer drugs for the induction and maintenance of immunosuppression have lessened the severity and frequency of acute rejection.[2,3] However, beyond the first year after engraftment, the rate of death among lung-transplant recipients has essentially remained unchanged between the 1980s and the present. At the five-year follow-up, nearly 50 percent of recipients are dead.[1,4]

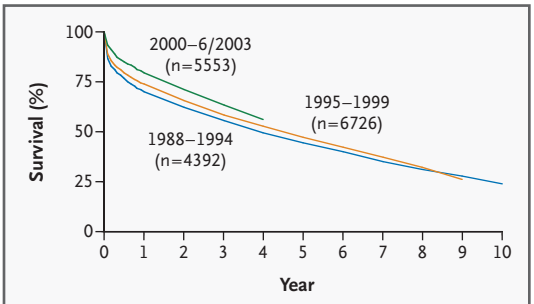


Figure 1. Differential Kaplan–Meier Analysis of Survival after Lung Transplantation, According to Era, 1988 through 2003.

Survival declines rapidly in the first year after transplantation. During the past 15 years, improved treatments for both donors and recipients have steadily driven the early-survival curves upward. In contrast, the rate of declining survival beyond the first year (slope of the curves) that is attributable mostly to chronic rejection (obliterative bronchiolitis) remains unchanged in each era. P=0.01 for a survival comparison of 1988–1994 with 1995–1999; P<0.001 for 1988–1994 as compared with 2000–2003; and P<0.001 for 1995–1999 as compared with 2000–2003. The median survival for 1988–1994 was 3.9 years, as compared with 4.5 years for 1995–1999. Data are from Trulock.[1]

Chronic rejection after lung transplantation is recognized histologically as obliterative bronchiolitis and has consistently been the leading cause of death among recipients who survive the first year.[1,5] Whereas the mechanism of acute rejection in solid-organ transplantation is well understood as an inflammatory response to alloantigen stimulation mediated by T lymphocytes, neither the triggers nor the mechanisms of chronic rejection are known. Obliterative bronchiolitis is thought to be the consequence of some combination of immune, ischemic, and infectious injuries. Strategies to limit ischemia, minimize viral infections by matching the cytomegalovirus (CMV) serologic status between donor and recipient, and specific immune therapies to prevent obliterative bronchiolitis have been either impractical or unsuccessful.[6] Moreover, therapeutic interventions short of retransplantation for established obliterative bronchiolitis have been disappointing.[7] In this issue of the *Journal*, Iacono and colleagues provide the first efficacy data from a randomized, placebo-controlled trial of an intervention both to reduce chronic rejection and to enhance survival in lung-transplant recipients.[8] As would be expected, there are some methodologic shortcomings in this small study of an intervention in a complex population of 58 patients, in which randomization cannot hope to balance equally all the confounding variables. Nevertheless, many of these variables would have been expected to negate any benefit of treatment, which makes the results all the more intriguing.

Lung transplantation lags behind other solid-organ transplantation in terms of medium-term and long-term survival. The explanation for this fact is both immunologic and physiological. Because the lung is in direct contact with the environment through respiration, it has a larger lymphocyte mass than do most other transplantable organs. Therefore, lung-transplant recipients usually require higher maintenance levels of immune suppression than do recipients of heart, liver, or kidney transplants.[9,10] This demand produces a clinical conundrum for the lung-transplantation physician in the form of pulmonary infection, since this common consequence of chronic illness is even more common in an immunocompromised host. Unfortunately, dyspnea, cough, hypoxemia, fever, and pulmonary infiltrates are symptoms, signs, and findings associated with both pneumonia and rejection. Lung recipients are the only population of transplant recipients in which most of the serious infectious complications after transplantation occur in the graft itself.

Iacono and his colleagues from Pittsburgh exploited the unique interface of the lung with our world by delivering augmented immune therapy directly to the graft through the airways. They were able to do this without engendering an increase in pulmonary infection and without the risk of detectable systemic absorption and potential nephrotoxicity from larger amounts of calcineurin inhibitors. Previous attempts to achieve this effect with inhaled corticosteroids were unsuccessful.[11] It would appear that inhaled cyclosporine might help in at least delaying the onset of obliterative bronchiolitis and pushing the late-survival curves of lung-transplant recipients upward toward those of heart, liver, and kidney recipients.

The clinical-trials purist will evaluate the study of Iacono and colleagues and see that with regard to the authors' primary end point (the rate of histologic acute rejection), the study must be considered a "negative" trial. Such a perspective, however, places a methodologic tree ahead of the forest. In addition, the trial fell far short of its

prospectively defined accrual goal of 136 patients. Despite generating only 43 percent of the projected study sample, having only 50 percent treatment compliance, and having some substantial imbalances between treatment and placebo groups, there were very few complications of active treatment, and the clinical benefits of the inhaled drug remained statistically significant.

These results should be received enthusiastically by lung-transplant physicians and surgeons but need to be confirmed in a more broadly inclusive multicenter trial. Such trials have been woefully lacking in the lung-transplantation world, in which 78 percent of centers perform fewer than 20 transplantations per year.[1] There is no consensus regionally, nationally, or internationally regarding such important treatment issues as the use of induction immunosuppression, the optimal maintenance combinations of immunosuppressive drugs, strategies for reducing the use of corticosteroids and limiting calcineurin renal toxicity, the question of when to schedule biopsies and when and how to treat acute rejection, and the optimal prophylactic regimen for CMV, pneumocystis, and fungal infections. Lung transplantation remains a low-volume, high-cost intervention. Without a mechanism for sharing experiences, studying new therapies and techniques, and critically analyzing pooled outcomes, the lung-transplantation community will never establish a set of best practices. Instead, it will be doomed to re-create a series of anecdotal experiences. Perhaps the inhaled-cyclosporine story will be that breath of fresh air that brings this community together.

From the Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Boston.

1. Trulock EP, Edwards LB, Taylor DO, Boucek MM, Keck BM, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: twenty-second official adult lung and heart-lung transplant report — 2005. J Heart Lung Transplant 2005;24:956-67.
2. Palmer SM, Miralles AP, Lawrence CM, Gaynor JW, Davis RD, Tapson VF. Rabbit antithymocyte globulin decreases acute rejection after lung transplantation: results of a randomized, prospective study. Chest 1999;116:127-33.
3. McCurry KR, Iacono A, Zeevi A, et al. Early outcomes in human lung transplantation with Thymoglobulin or Campath-1H for recipient pretreatment followed by posttransplant tacrolimus near-monotherapy. J Thorac Cardiovasc Surg 2005;130:528-37.
4. Barr ML, Bourge RC, Orens JB, et al. Thoracic organ transplantation in the United States, 1994-2003. Am J Transplant 2005;5:934-49.
5. Bando K, Paradis IL, Similo S, et al. Obliterative bronchiolitis after lung and heart-lung transplantation: an analysis of risk factors and management. J Thorac Cardiovasc Surg 1995;110:4-14.
6. Scott AI, Sharples LD, Stewart S. Bronchiolitis obliterans syndrome: risk factors and therapeutic strategies. Drugs 2005; 65:761-71.
7. Date H, Lynch JP, Sundaresan S, Patterson GA, Trulock EP. The impact of cytolytic therapy on bronchiolitis obliterans syndrome. J Heart Lung Transplant 1998;17:869-75.
8. Iacono AT, Johnson BA, Grgurich WF, et al. A randomized trial of inhaled cyclosporine in lung-transplant recipients. N Engl J Med 2006;354:141-50.
9. Zhang JH, Dong ZJ, Zhou RB, Luo de M, Wei HM, Tian ZG. Isolation of lymphocytes and their innate immune characterizations from liver, intestine, lung and uterus. Cell Mol Immunol 2005;2:271-80.
10. Sester U, Gartner BC, Wilkens H, et al. Differences in CMV-specific T-cell levels and long-term susceptibility to CMV infection after kidney, heart and lung transplantation. Am J Transplant 2005;5:1483-9.
11. Whitford H, Walters EH, Levvey B, et al. Addition of inhaled corticosteroids to systemic immunosuppression after lung transplantation: a double-blind, placebo-controlled trial. Transplantation 2002;73:1793-9.

SOUNDING BOARD

Reform of Drug Regulation — Beyond an Independent Drug-Safety Board

Wayne A. Ray, Ph.D., and C. Michael Stein, M.B., B.Ch.

Recent withdrawals from the market of high-profile drugs have led to a reexamination of the process of drug regulation and have stimulated concern that the current process is inadequate for protection of the public health.[1,2] The perceived inability of the Food and Drug Administration (FDA) and the pharmaceutical industry to evaluate drugs impartially and to respond adequately to signals indicating potential drug-safety problems has created extensive support for the establishment of an independent drug-safety board.[3] However, because the limitations of the current process go well beyond these conflicts of interest, we propose more extensive reforms designed to protect the public health better.

LIMITATIONS OF THE PRESENT REGULATORY SYSTEM

The current regulatory process does not have systematic provisions for obtaining important data needed to guide clinical practice (Table 1). Planned data collection occurs almost exclusively during premarketing testing.[4] The FDA approves medications on the basis of studies of limited duration that include relatively small numbers of patients who are often healthier than the target population for the new drug.[3,5-7] Although many important effects of a new medication almost certainly will be unknown at the time of licensing, there are no systematic provisions for post-marketing studies. Thus, there often is insufficient information on several topics: the safety of widely used drugs,[1] the effects of long-term exposure,[8] the frequency of rare adverse effects,[9,10] the effects in special populations[6,11,12] or for indications[13-15] not studied before marketing, the efficacy of a new drug with respect to clinical (as opposed to surrogate) end points,[16-19] and the efficacy of the new drug relative to others for the same indication.[20,21]

The present system of drug regulation is susceptible to the influence of conflicts of interest (Table 1). The Prescription Drug User Fee Act (PDUFA) has led to substantially decreased review times for new drug applications.[22] However, PDUFA's strict timetables and detailed requirements for communication with manufacturers are controversial[2,18,22-25] because they may promote hasty approvals, divert resources from post-marketing surveillance, and foster the perception that the pharmaceutical industry is the FDA's customer. Conflicts of interest created by the obvious economic motivations of the manufacturer and the FDA's potential reluctance to reverse a previous drug-approval decision[3] may impede the response to signs of potential adverse drug effects,[26] such as those indicated by the results of the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial of rofecoxib.[27] There is evidence of bias in the design and conduct of post-marketing studies funded by the pharmaceutical industry,[6,17,28] as well as evidence of selective reporting and selective publication of data.[2,17,29]

Current procedures for effective and impartial communication of the available data to practitioners and patients are inadequate (Table 1). At present, the drug label is the primary route that is officially recognized for providing information to physicians.[13,30] However, several factors limit its influence on clinical practice.[31] The label is drafted by the manufacturer, and its modification requires extensive and often time-consuming negotiations between the manufacturer and the FDA. Its organization does not allow for effective communication, and its content can be undermined by other promotional activities of the manufacturer. The pharmaceutical industry devotes a considerable amount of resources to influencing practice, but this communication may be biased.[26] Thus, the prescription of new medi-

Table 1. Principal Limitations of the Present Drug-Regulation Process.*

Limitation	Drug and Example of Resulting Problem
No systematic provisions for obtaining important data to guide clinical practice	
Premarketing trials are powered inadequately to determine safety of widely used drugs	Coxibs: increased risk of serious cardiovascular disease confirmed 5 years after introduction and after use by millions of patients
Safety of long-term therapy is unknown	HRT: prolonged use increases risk of breast cancer
No systematic surveillance is conducted after marketing to detect rare adverse effects	Terfenadine: causes torsades de pointes when used with other drugs that inhibit its metabolism
Special populations are underrepresented in premarketing studies	ACE inhibitors: risk of angioedema increased for blacks
Off-label use is not studied	Fenfluramine–phentermine: this off-label combination found to cause primary pulmonary hypertension and damage to cardiac valves after use by 6 million patients
Relative efficacy is unknown	Coxib vs. NSAID plus proton-pump inhibitor: no adequately powered studies of the relative gastrointestinal safety of these two therapies have been conducted
Surrogate end points are the only outcomes studied	Cerivastatin: clinical benefit unproven when launched
Conflicts of interest in decision making and collection and reporting of data	
New drugs may be approved too rapidly because of PDUFA requirements	Troglitazone: approved despite scientific doubts about efficacy and safety
Response to signs indicating possible adverse effects is inadequate	Rofecoxib: evidence of increased cardiovascular risk in VIGOR trial did not lead to cardiovascular safety trials
Study design is suboptimal	Cerivastatin: safety with regard to rhabdomyolysis was assessed in a study with serious limitations
Reporting of data is incomplete	SSRIs: data suggesting suicidal thinking and behavior in adolescents were not fully reported
Failure to effectively communicate data to practitioners and patients	
Label is difficult to change and thus often out of date	Rofecoxib: lag of >2 years between clinical trial (VIGOR) showing increased risk of acute myocardial infarction and change to the label noting this fact
Contraindications are not adhered to	Cisapride: 20 percent of use was contraindicated because it was in patients with increased risk of cardiac arrhythmias
Directions for use are not followed	Troglitazone: liver-function tests required, as stated on label, but often not performed
Dose is not optimal	Rofecoxib: 17 percent of long-term use was at the daily 50-mg dose, despite no greater efficacy than at lower doses and despite data suggesting higher rates of adverse effects

* Coxib denotes selective inhibitor of cyclooxygenase-2, HRT hormone-replacement therapy, ACE angiotensin-converting enzyme, NSAID nonsteroidal antiinflammatory drug, PDUFA Prescription Drug User Fee Act, SSRI selective serotonin-reuptake inhibitor, and VIGOR Vioxx Gastrointestinal Outcomes Research.

cations is often suboptimal (Table 1), involving contraindicated use,[32] failure to follow directions for use,[33] and use of inappropriate doses.[34]

The limitations of the present regulatory system are in part the consequence of the narrow focus of the FDA's enabling legislation on premarketing testing. After drug approval, the FDA's statutory authority is limited to requesting changes to the label, negotiating restrictions to distribution with the manufacturer, or petitioning for the withdrawal of the drug.[35] There is no defined process for answering the many questions

that cannot be addressed in premarketing studies. The system is susceptible to influence from the economic interests of manufacturers, and even the limited amount of data available often does not influence clinical practice. Medications approved on the basis of studies of a few thousand patients are rapidly marketed to millions of patients, setting the stage for "drug disasters."[2,3,17,26] Subsequent litigation generates billion of dollars in legal fees and settlements that ultimately become part of the cost of new medications. This haphazard process does not benefit the public health, the medical profession, or the pharmaceutical industry.

A REFORMED REGULATORY PROCESS

The emerging consensus in favor of an independent drug-safety board[1,3,4] is motivated by the potential for biased decision making that is inherent in the present regulatory process.[2] However, by itself, this board would not address other important limitations of the present process (Table 1). The Drug Safety Oversight Board, announced by Department of Health and Human Services after rofecoxib's withdrawal,[4] is even less likely to be effective because it lacks authority, resources, and independence from the FDA.[36]

We thus propose a reformed regulatory authority with three distinct functions (Table 2): new-drug approval, post-marketing studies, and drug information. We envision three independent but cooperative centers within a unified agency, although other administrative structures are possible. The new centers would be funded by a tax on pharmaceutical sales. For the reforms to work, the centers must be provided with adequate statutory authority, sufficient funding, and protection from inappropriate political pressure.[37] Although we describe the reforms in the context of drugs, they also should apply to biologic products and devices.

The Center for New Drug Approval would regulate the initial licensing of new drugs. Changes from the present review process would include replacement of PDUFA funding, as well as registration and complete reporting of all studies conducted under the auspices of this and the other two centers. Studies to provide the additional data not currently required would be deferred until after a drug is approved, which would preserve the ability of the present system to act rapidly.

The Center for Post-marketing Studies would specify and oversee mandatory postapproval studies tailored to address the potentially unique questions associated with each new medication. These studies would include adequately powered safety studies, long-term studies of drugs for chronic diseases, epidemiologic investigations of rare adverse effects and special populations, and randomized trials that assess relative efficacy and clinical end points. When appropriate, the release of the new medication would occur in phases in order to limit the number of patients who are exposed before more data are collected. The center also could require new studies in response to emerging data, such as signals from case reports or evidence of widespread off-label use. To ensure that the data are unbiased, post-marketing studies would be conducted by independent investigators.

The Center for Drug Information would coordinate the communication of accurate, unbiased information to practitioners and patients that promotes the use of drugs in accordance with the best available data. It would write and update as needed an accurate label designed to be more useful to practitioners[31] and, when appropriate, employ more active methods of communication such as academic detailing.[38] The center would prospectively review and regulate promotional activities directed at physicians and patients. Because of the inherent uncertainties about the safety of all new drugs, direct-to-consumer advertising would be prohibited for the first three years after marketing. The center would closely monitor use for unapproved indications. This would include updating the label to reflect the supporting evidence, more effectively regulating promotion of off-label use,[39] and requesting additional studies as needed.

CONTROVERSIAL ELEMENTS OF THE PROPOSED REFORMS

There is now considerable agreement that several of these fundamental regulatory changes are needed, such as enhancing the stature and independence of the FDA,[22,37] reducing the potential for conflicts of interest,[2,3,40] separating further the new-drug approval and post-marketing functions,[1,2] and requiring more post-marketing studies of safety and clinical end points.[1,2,41] Other elements of the proposed reforms are controversial, however, and thus merit further analysis.

Table 2. Central Elements of the Proposed Reforms.
Center for New Drug Approval
PDUFA funding replaced by unconditional funding from a tax on drug sales*
Complete registration and reporting of all studies
Center for Post-marketing Studies
Mandatory post-marketing studies, including relative-efficacy studies, tailored to individual drugs
Phased release of selected medications
Monitoring of emerging data and, when appropriate, requirement of additional studies
Independent conduct of post-marketing studies
Center for Drug Information
Writing and updating of drug labels for effective communication with physicians and consumers
Prospective review of promotional activities
Prohibition of direct-to-consumer advertising for three years after approval
Tracking of off-label use, summaries of evidence on label, and recommendation of further studies
When appropriate, use of more active methods to communicate with physicians

* PDUFA denotes Prescription Drug User Fee Act.

RELATIVE-EFFICACY STUDIES

For patients and physicians, the most important information about a new medication is its efficacy and safety relative to the best available alternative. Although much criticism of the present regulatory system focuses on the inadequacy of safety data, whether or not the risk associated with a new drug is acceptable cannot be determined in the absence of data on its relative benefits. Despite the obvious clinical need for these data, they are not required for drug approval. Once a drug is approved, it usually is difficult to obtain funding for comparative efficacy studies from drug manufacturers or research-funding agencies.[6] The United Kingdom has a systematic program for determining the relative effectiveness of a new drug by performing secondary data analyses[42]; however, since there are often no studies that directly compare the drugs of interest, the validity of such analyses is controversial.

The absence of a regulatory requirement for the most clinically relevant data seems paradoxical, but it may be due in part to the complexity of relative-efficacy studies.[6] Comparator therapies must be chosen from the several that are available, sample sizes are generally larger than those in placebo-controlled studies, and methods must be of extremely high quality to guard against false findings of clinical equivalence. Because comparative-efficacy studies are often time-consuming, under our proposed reforms they would not be mandatory for initial drug approval but, rather, are considered to be one of several categories of post-approval studies that may be required by the Center for Post-marketing Studies.

PHASED RELEASE OF NEW DRUGS

The present regulatory system is highly focused on the rapid approval of new drugs, which reduces delays in the availability of new therapies. Systematic post-marketing studies would address the main disadvantage of rapid approval: the ultimate clinical benefit of the new medication is often incompletely understood when the drug is launched. A related problem, however, is that the intensive promotion of new drugs can lead to use by millions of patients in a matter of months. By the time the benefits and risks of the new medication are better defined, it often becomes clear that these patients were exposed to unnecessary risks.

Phased release of new medications — that is, restriction of use pending completion of key post-marketing studies — would protect patients while uncertainties about drug effects were resolved. Good candidates for phased release are medications that are approved on the basis of surrogate end points (e.g., cerivastatin), those with no clear

advantage over existing therapies (e.g., troglitazone), and those for which premarketing safety data are limited (e.g., selective inhibitors of cyclooxygenase-2 [coxibs]). The use of drugs under phased release would be restricted to patients who are participating in post-marketing studies or for whom the best available data provide strong evidence of probable benefit. For example, the use of a new lipid-lowering drug, approved only on the basis of surrogate end points, would be limited to clinical trials comparing it with one of the many existing drugs that have been shown to improve clinical outcomes or to patients for whom other drugs were not suitable. Once the post-marketing studies were complete, the Center for Drug Information would incorporate the findings into the label, and except in unusual circumstances, the restrictions on use would be lifted.

Could phased release do more harm than good by inordinately delaying patients' access to new medications? In order to avoid this potential problem, we have proposed selective phased release. Medications for which there was convincing evidence of a unique health benefit to patients would not undergo phased release. Patients' interests would be best protected by restricting access to medications for which there is no such convincing evidence until better data become available.

In some cases, a phased-release program might improve access to drugs. Limiting the use of a new medication until the population that would benefit from it is better defined might permit a drug that otherwise would have been withdrawn to be kept on the market. For example, cisapride was widely thought to be beneficial for patients with diabetic gastroparesis.[43] If physicians had not become accustomed to prescribing this drug for millions of patients with dyspepsia, its drastically curtailed availability might not have been necessary.

Would phased release impose an unfair financial burden on the pharmaceutical industry? For some medications, phased release would constitute a de facto extension of the drug-development process. To compensate manufacturers, an extension of the patent period for a drug could be granted under certain circumstances.

Phased release would alter the financial incentives for drug development. It would become less profitable to develop "me-too" drugs,[23] because studies comparing these with existing products could be required before the drug's unrestricted use. However, incentives for the development of truly innovative therapies should ultimately benefit the public health.

INDEPENDENT CONDUCT OF POST-MARKETING STUDIES

A truly independent drug-safety board[3] could reduce the conflicts of interest that occur in interpreting and acting on post-marketing data. Unfortunately, some of the most pernicious biases are those related to conducting studies (including the choice of population and comparator, study end points, power, quality of data, and methods of analysis).[6,44,45] Because many of these methodologic flaws can lead to incorrect inferences of clinical equivalence,[44,45] the FDA's drug-approval process relies extensively on trials that demonstrate the superiority of the new drug to a placebo,[44,45] ensuring that the drug manufacturer has incentives to conduct studies of high quality. Post-marketing studies, however, often assess relative safety or efficacy. Thus, the manufacturer's short-term economic interests could favor low-quality studies that incorrectly report that a new drug is equivalent to comparator therapies.

To prevent this conflict of interest, the Center for Post-marketing Studies would specify the studies needed and conduct peer-reviewed competitions for contracts. The quality of the study and subsequent publication of its results would be important factors for future awards. The independent researchers would have the same strong incentives that are now present for National Institutes of Health grantees to conduct high-quality studies.

To be effective, the reforms would require that careful attention be paid to potential conflicts of interest among investigators who submit proposals and have other funding from the pharmaceutical industry. To ensure public confidence, conflict-of-interest requirements that go beyond disclosure, such as those proposed for FDA advisory committee members, may be necessary.[40]

COMMUNICATION OF DRUG INFORMATION

One of the most frustrating limitations of the present system is the difficulty of translating important new information into clinical practice. This lack of communication can lead to the well-recognized problem of frequent suboptimal use for many widely prescribed medications (Table 1). It

also makes keeping a drug on the market difficult when the new information substantially reduces the number of patients for whom the drug is appropriate.[31] An effective program of communicating with physicians could facilitate such risk management,[46] making it possible to retain drugs that otherwise would have been removed from the market.

Historically, regulatory agencies have had a very limited role in seeking to improve clinical practice. They have tacitly relied on journal articles and other passive methods of diffusing new information. However, these methods often work slowly, particularly in the context of intensive marketing programs developed by industry. Third parties, such as academia, payers, and extraregulatory government programs, can improve practice. Lacking a mandate and funding, however, they are incapable of doing so in a systematic way.

The reformed regulatory system will generate a dynamic flow of data for each new drug. These data will fully benefit the public health only if there are corresponding provisions that ensure that the data have a timely effect on clinical practice. Thus, effective communication of information should become a central responsibility of regulators.

A redesigned drug label would be the initial means of communication. The effectiveness of the label is limited, however, after suboptimal prescribing becomes entrenched.[38,47] Thus, the Center for Drug Information would have the authority to use more effective methods to communicate with physicians when necessary. The center could be guided by the experience of the Quality Improvement Organizations of the Centers for Medicare and Medicaid Services[48] and could use available, proven approaches (including the use of academic detailing,[47] peer leaders,[49] and computerized provider order-entry systems[50]). The center could collaborate with existing programs focused on practice improvement, including the Centers for Education and Research on Therapeutics,[7,51] the Evidence-Based Practice Centers,[52] and the Task Force on Community Preventive Services.[52]

Greater involvement of regulators in communication with physicians should, in conjunction with the other reforms, reduce the frequency of litigation related to drugs. Important bases for these lawsuits have been that the manufacturer failed to communicate available data accurately to physicians or conducted biased studies.[2,17] If the reforms can ensure a strong and independent agency for drug regulation, manufacturers that are fully compliant with the regulatory process could then be provided with limited protection from lawsuits concerning liability.

CONCLUSIONS

The current system of drug regulation has serious flaws. Many of these have been recognized and reforms to correct them have been proposed,[1,2,4-6,11,23,41,53] but only minor changes have occurred since 1962. Proposals for an independent drug-safety board[3] seek to reform the system by reducing the influence of conflicts of interest in the evaluation of post-marketing data. However, by itself, a drug-safety board will not ensure the availability of the data on which rational therapeutics and policy must be based, nor fully correct the excessive influence of manufacturers, nor ensure that clinicians become aware of new data. Thus, we have proposed a more comprehensive plan for reforming the process of drug regulation, which addresses its main structural flaws.

In the United States, important changes in the system of drug regulation have occurred only in response to "drug disasters." The elixir sulfanilamide scandal in 1937 led to the passage of the Food, Drug, and Cosmetic Act of 1938, which established the requirement that basic studies of toxicity be conducted before new products were marketed.[54] The thalidomide tragedy stimulated the enactment in 1962 of the Kefauver–Harris amendments that are the basis of the requirement for premarketing clinical trials.[54] In a like manner, the controversy caused by the unexpected cardiotoxicity of the coxibs has created a valuable opportunity for change.

Implementing the proposed reforms will not be easy, but the costs of inaction are unacceptable. Each new "drug disaster" causes preventable morbidity and mortality, undermines the credibility of the public health infrastructure, erodes patients' confidence in physicians, and fuels costly litigation. Prompt and effective reform is essential.

Dr. Ray reports having served as a consultant to Pfizer and Bristol-Myers Squibb, having received research funding from Pfizer, and having provided expert testimony for litigation involving cerivastatin, fenfluramine derivatives, and rofecoxib.

Dr. Stein reports having received research funding from Pfizer. No other potential conflict of interest relevant to this article was reported.

From the Center for Education and Research on Therapeutics (W.A.R., C.M.S.); the Geriatric Research, Education and Clinical Center, Veterans Affairs Medical Center (W.A.R.); and the Departments of Medicine and Pharmacology, Divisions of Rheumatology and Clinical Pharmacology (C.M.S.), Vanderbilt University School of Medicine — all in Nashville.

1. Psaty BM, Furberg CD. COX-2 inhibitors — lessons in drug safety. N Engl J Med 2005;352:1133-5.
2. Fontanarosa PB, Rennie D, DeAngelis CD. Postmarketing surveillance — lack of vigilance, lack of trust. JAMA 2004; 292:2647-50.
3. Wood AJ, Stein CM, Woosley R. Making medicines safer — the need for an independent drug safety board. N Engl J Med 1998;339:1851-4.
4. Okie S. Safety in numbers — monitoring risk in approved drugs. N Engl J Med 2005;352:1173-6.
5. Joint Commission on Prescription Drug Use. Final report. Washington, D.C.: Government Printing Office, 1980.
6. Ray WA, Griffin MR, Avorn J. Evaluating drugs after their approval for clinical use. N Engl J Med 1993;329:2029-32.
7. Woosley RL. Centers for education and research in therapeutics. Clin Pharmacol Ther 1994;55:249-55.
8. Chlebowski RT, Hendrix SL, Langer RD, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. JAMA 2003;289:3243-53.
9. Woosley RL. Cardiac actions of antihistamines. Annu Rev Pharmacol Toxicol 1996;36:233-52.
10. Woosley RL, Chen Y, Freiman JP, Gillis RA. Mechanism of the cardiotoxic actions of terfenadine. JAMA 1993;269:1532-6.
11. Ray WA. Population-based studies of adverse drug effects. N Engl J Med 2003;349:1592-4.
12. Brown NJ, Ray WA, Snowden M, Griffin MR. Black Americans have an increased rate of angiotensin converting enzyme inhibitor-associated angioedema. Clin Pharmacol Ther 1996;60: 8-13.
13. Committee on Drugs, American Academy of Pediatrics. Uses of drugs not described in the package insert (off-label uses). Pediatrics 2002;110:181-3.
14. Connolly HM, Crary JL, McGoon MD, et al. Valvular heart disease associated with fenfluramine–phentermine. N Engl J Med 1997;337:581-8. [Erratum, N Engl J Med 1997;337:1783.]
15. Abenhaim L, Moride Y, Brenot F, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. N Engl J Med 1996;335:609-16.
16. Fleming TR, DeMets DL. Surrogate end points in clinical trials: are we being misled? Ann Intern Med 1996;125:605-13.
17. Psaty BM, Furberg CD, Ray WA, Weiss NS. Potential for conflict of interest in the evaluation of suspected adverse drug reactions: use of cerivastatin and the risk of rhabdomyolysis. JAMA 2004;292:2622-31.
18. Gale EA. Lessons from the glitazones: a story of drug development. Lancet 2001;357:1870-5.
19. Graham DJ, Staffa JA, Shatin D, et al. Incidence of hospitalized rhabdomyolysis in patients treated with lipid-lowering drugs. JAMA 2004;292:2585-90.
20. Lanza FL. A guideline for the treatment and prevention of NSAID-induced ulcers. Am J Gastroenterol 1998;93:2037-46.
21. Hawkey CJ, Karrasch JA, Szczepanski L, et al. Omeprazole compared with misoprostol for ulcers associated with nonsteroidal antiinflammatory drugs. N Engl J Med 1998;338:727-34.
22. Okie S. What ails the FDA? N Engl J Med 2005;352:1063-6. [Erratum, N Engl J Med 2005;352:2563.]
23. Angell M. The truth about the drug companies. New York: Random House, 2004.
24. Slater EE. Today's FDA. N Engl J Med 2005;352:293-7.
25. Effect of user fees on drug approval times, withdrawals, and other agency activities. Washington, D.C.: General Accounting Office, 2002. (Document no. GAO-02-958.)
26. Topol EJ. Failing the public health — rofecoxib, Merck, and the FDA. N Engl J Med 2004;351:1707-9.
27. Bombardier C, Laine L, Reicin A, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med 2000;343:1520-8.
28. Landefeld CS. Commercial support and bias in pharmaceutical research. Am J Med 2004;117:876-8.
29. Nissen SE. High-dose statins in acute coronary syndromes: not just lipid levels. JAMA 2004;292:1365-7.
30. Meadows M. The FDA's drug review process: ensuring drugs are safe and effective. FDA Consum 2002;36(4):19-24.
31. Woosley RL. Drug labeling revisions — guaranteed to fail? JAMA 2000;284:3047-9.
32. Smalley W, Shatin D, Wysowski DK, et al. Contraindicated use of cisapride: impact of Food and Drug Administration regulatory action. JAMA 2000;284:3036-9.
33. Graham DJ, Drinkard CR, Shatin D, Tsong Y, Burgess MJ. Liver enzyme monitoring in patients treated with troglitazone. JAMA 2001;286:831-3.
34. Griffin MR, Stein CM, Graham DJ, Daugherty JR, Arbogast PG, Ray WA. High frequency of use of rofecoxib at greater than recommended doses: cause for concern. Pharmacoepidemiol Drug Saf 2004;13:339-43.
35. Meadows M. Why drugs get pulled off the market. FDA Consum 2002;36(1):11-7. [Erratum, FDA Consum 2002;36(3):3.]
36. Harris G. F.D.A. to create advisory board on drug safety. The New York Times. February 16, 2005:A1.
37. Wood AJJ, Drazen JM, Greene MF. A sad day for science at the FDA. N Engl J Med 2005;353:1197-9.
38. Soumerai SB, Avorn J. Principles of educational outreach ('academic detailing') to improve clinical decision making. JAMA 1990;263:549-56.
39. 'Off-label' promo allowed as FDA appeal is dismissed. Are drug firms protected? Medical Marketing and Media 2000; 35(3):6.
40. Steinbrook R. Financial conflicts of interest and the Food and Drug Administration's advisory committees. N Engl J Med 2005;353:116-8.
41. Griffin MR, Stein CM, Ray WA. Postmarketing surveillance for drug safety: surely we can do better. Clin Pharmacol Ther 2004;75:491-4.
42. Rawlins MD. 5 NICE years. Lancet 2005;365:904-8.
43. Wiseman LR, Faulds D. Cisapride: an updated review of its pharmacology and therapeutic efficacy as a prokinetic agent in gastrointestinal motility disorders. Drugs 1994;47:116-52.
44. Temple RJ. Government viewpoint of clinical trials of cardiovascular drugs. Med Clin North Am 1989;73:495-509.
45. *Idem.* When are clinical trials of a given agent vs. placebo no longer appropriate or feasible? Control Clin Trials 1997;18:613-20.
46. Campbell WH, Califf RM. Improving communication of drug risks to prevent patient injury: proceedings of a workshop. Pharmacoepidemiol Drug Saf 2003;12:183-94.
47. Avorn J, Soumerai SB. Improving drug-therapy decisions through educational outreach: a randomized controlled trial of academically based "detailing." N Engl J Med 1983;308:1457-63.
48. Bradley EH, Carlson MD, Gallo WT, Scinto J, Campbell MK, Krumholz HM. From adversary to partner: have quality improvement organizations made the transition? Health Serv Res 2005; 40:459-76.
49. Soumerai SB, McLaughlin TJ, Gurwitz JH, et al. Effect of local medical opinion leaders on quality of care for acute myo-

cardial infarction: a randomized controlled trial. JAMA 1998; 279:1358-63.
50. Bates DW, Gawande AA. Improving safety with information technology. N Engl J Med 2003;348:2526-34.
51. Califf RM. The need for a national infrastructure to improve the rational use of therapeutics. Pharmacoepidemiol Drug Saf 2002;11:319-27.
52. Briss PA. Evidence-based: US road and public-health side of the street. Lancet 2005;365:828-30.
53. Avorn J. Powerful medicines: the benefits, risks, and costs of prescription drugs. New York: Alfred A. Knopf, 2004.
54. Silverman M, Lee PR. Pills, profits, and politics. Berkeley: University of California Press, 1974.

POSTING PRESENTATIONS AT MEDICAL MEETINGS ON THE INTERNET

Posting an audio recording of an oral presentation at a medical meeting on the Internet, with selected slides from the presentation, will not be considered prior publication. This will allow students and physicians who are unable to attend the meeting to hear the presentation and view the slides. If there are any questions about this policy, authors should feel free to call the *Journal*'s Editorial Offices.

CORRESPONDENCE

Bacteria Associated with Bacterial Vaginosis

TO THE EDITOR: Fredricks and colleagues (Nov. 3 issue)[1] reported complex communities of bacteria and a high level of species diversity in vaginal secretions from women with bacterial vaginosis. However, the study of vaginal fluid alone provides an incomplete picture of disease pathogenesis, since infections also may affect the vaginal epithelium. Fluorescence in situ hybridization of vaginal-biopsy specimens with the use of bacteria-specific probes demonstrates that a clinical diagnosis of bacterial vaginosis is highly associated with the development of a bacterial biofilm on the epithelial surface.[2] Gardnerella and atopobium species together constitute more than 90 percent of the biofilm mass. The biofilm also contains numerous, less abundant bacterial species, in agreement with the findings of Fredricks and others.[1,3] Formation of the biofilm allows bacteria to reach higher concentrations than can be achieved in vaginal fluid alone. Desquamation of epithelial cells coated with biofilm in situ results in the clue cells that are diagnostic of bacterial vaginosis. Given such complex microbiota, it is clear that identification of single pathogens, as was introduced by Koch and Pasteur more than 100 years ago, is inadequate for explaining the pathogenesis of bacterial vaginosis.

Laura P. Hale, M.D., Ph.D.
Duke University Medical Center
Durham, NC 27710
laura.hale@duke.edu

Alexander Swidsinski, M.D., Ph.D.
Charité Hospital of Humboldt University
10098 Berlin, Germany

Werner Mendling, M.D.
Vivantes Clinic for Obstetrics and Gynecology
10967 Berlin, Germany

1. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 2005;353:1899-911.
2. Swidsinski A, Mendling W, Loening-Baucke V, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol 2005;106:1013-23.
3. Verhelst R, Verstraelen H, Claeys G, et al. Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between Atopobium vaginae, Gardnerella vaginalis and bacterial vaginosis. BMC Microbiol 2004;4:16.

THE AUTHORS REPLY: Dr. Hale and colleagues highlight their study showing that adherent bacterial biofilms commonly were detected in vaginal-biopsy specimens from subjects with bacterial vaginosis, suggesting that biofilms may play a role in the pathophysiology of this syndrome.[1] We agree that it is important to study both the vaginal epithelial surface and free vaginal fluid to determine how bacterial communities may lead to disease. Fluorescence in situ hybridization is an excellent method for studying the spatial relationships and composition of bacteria in these compartments. We also agree that no single bacterium

THIS WEEK'S LETTERS

is likely to be the cause of bacterial vaginosis and that Koch's postulates for disease causation are inadequate for describing potential causal relationships in this syndrome. Bacterial vaginosis probably results from infection with complex communities of bacteria that consist of metabolically interdependent (syntrophic) species. Diseases caused by uncultivated microbes or communities of microbes are not amenable to the application of Koch's postulates in their original formulation[2]; therefore, we must build a case for causation on the basis of a concordance of scientific evidence.[3]

David N. Fredricks, M.D.
Fred Hutchinson Cancer Research Center
Seattle, WA 98109
dfredric@fhcrc.org

Jeanne M. Marrazzo, M.D., M.P.H.
University of Washington
Seattle, WA 98195

1. Swidsinski A, Mendling W, Loening-Baucke V, et al. Adherent biofilms in bacterial vaginosis. Obstet Gynecol 2005;106:1013-23.
2. Evans AS. Causation and disease: a chronological journey. New York: Plenum Medical Book, 1993:238.
3. Fredricks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev 1996;9:18-33.

Azithromycin versus Penicillin for Early Syphilis

TO THE EDITOR: In their study on the treatment of early syphilis, Riedner et al. (Sept. 22 issue)[1] concluded that the wider use of oral azithromycin should be encouraged as part of syphilis-control programs in developing countries. Whereas this conclusion would appear to be rational on the basis of the authors' results, we believe that there are other factors that should be considered before opting for such a strategy.

Although the authors acknowledged the potential for the emergence of azithromycin-resistant *Treponema pallidum*, ongoing monitoring for such resistance, as they suggested, requires molecular-sequencing techniques,[2] which are unavailable in most developing countries. More important, the inability of azithromycin to cross the placenta[3] limits its use in the prevention of congenital disease. Treatment of seropositive mothers with oral azithromycin, after routine antenatal screening, could result in declining maternal titers on the rapid plasma reagin test without affecting the potential for fetal infection.

Since the prevention of congenital syphilis remains a major objective of control programs and is a current focus for global elimination activities,[4] we believe that azithromycin has only a limited role in the management of syphilis in resource-constrained settings.

Ronald C. Ballard, Ph.D.
Stuart M. Berman, M.D., Sc.M.
Kevin A. Fenton, M.D., Ph.D., M.F.P.H.
Centers for Disease Control and Prevention
Atlanta, GA 30333
rballard@cdc.gov

1. Riedner G, Rusizoka M, Todd J, et al. Single-dose azithromycin versus penicillin G benzathine for the treatment of early syphilis. N Engl J Med 2005;353:1236-44.
2. Lukehart SA, Godornes C, Molini BJ, et al. Macrolide resistance in *Treponema pallidum* in the United States and Ireland. N Engl J Med 2004;351:154-8.
3. Heikkinen T, Laine K, Neuvonen PJ, Ekblad U. The transplacental transfer of the macrolide antibiotics erythromycin, roxithromycin and azithromycin. BJOG 2000;107:770-5.
4. Maternal and congenital syphilis. Bull World Health Organ 2004;82:399-478.

TO THE EDITOR: Riedner and colleagues report that azithromycin is equivalent to penicillin G benzathine in treating early syphilis and may be useful in developing countries in which use of penicillin G benzathine is problematic, and they alert us about the cases of azithromycin-resistant *T. pallidum*. In Brazil, we struggle even with inexpensive drugs, such as penicillin G benzathine; azithromycin is not widely available and can be 10 times as expensive as penicillin G benzathine. We believe that it is not wise to change from a known, inexpensive drug with few cases of resistance after a half century of use[1] to a more expensive, unfamiliar drug that has already shown resistance after a few years of use.[2] Thus, the implementation of azithromycin in developing countries remains prohibitive because of the cost and because of the possibility of resistance, and this drug should not be used as a first choice yet.

Ricardo F. Savaris, M.D.
Alberto M. Abeche, M.D.
Universidade Federal do Rio Grande do Sul
90035-003 Porto Alegre, Brazil
rsavaris@hcpa.ufrgs.br

1. Stapleton JT, Stamm LV, Bassford PJ Jr. Potential for development of antibiotic resistance in pathogenic treponemes. Rev Infect Dis 1985;7:Suppl 2:S314-S317.
2. Azithromycin treatment failures in syphilis infections — San Francisco, California, 2002–2003. MMWR Morb Mortal Wkly Rep 2004;53:197-8.

TO THE EDITOR: Riedner and colleagues demonstrated the successful treatment of early syphilis with azithromycin. Holmes's accompanying editorial laments the absence of prospective data on patients treated for early syphilis with azithromycin and the influence of molecularly defined azithromycin-resistant *T. pallidum* on treatment outcomes.[1]

In San Francisco, where an estimated 56 percent of circulating strains of *T. pallidum* were resistant to azithromycin in 2004,[2] we conducted a randomized, controlled trial of azithromycin (1 g given orally as a single dose) as compared with penicillin G benzathine (2.4 million units intramuscularly) in sexual contacts of persons with infectious syphilis; our aim was to compare the efficacy of the two drugs for the treatment of incubating syphilis. A data safety monitoring board (DSMB) supervised the study.

After two treatment failures in the 12 patients receiving azithromycin as compared with none in 13 patients receiving penicillin, the DSMB terminated the study (P=0.18, by Fisher's exact test). Although it was a small study sample (n=25), our data suggest that azithromycin was inferior to penicillin in the presence of high community levels of azithromycin-resistant *T. pallidum*. Although we have feasible methods to monitor macrolide resistance in *T. pallidum*, routine surveillance is not currently supported by federal agencies.

Jeffrey D. Klausner, M.D., M.P.H.
Robert P. Kohn, M.P.H.
Charlotte K. Kent, M.P.H.
San Francisco Department of Public Health
San Francisco, CA 94103
jeff.klausner@sfdph.org

Dr. Klausner reports having received honoraria from King Pharmaceuticals and a research grant from Pfizer.

1. Holmes KK. Azithromycin versus penicillin G benzathine for early syphilis. N Engl J Med 2005;353:1291-3.
2. Klausner JD, Mitchell SJ, Lukehart SA, Gordones C, Engelman J. Rapid and large increase in azithromycin resistance in syphilis whilst low steady resistance in gonorrhea 2000-2004. In: Program and abstracts of the 16th Biennial Meeting of the International Society for Sexually Transmitted Diseases Research, Amsterdam, July 10–13, 2005. abstract.

THE AUTHORS REPLY: We agree with Ballard et al. that further studies are needed before azithromycin can be recommended for the treatment of syphilis in pregnancy, although studies in women undergoing cesarean section have shown that azithromycin does cross the placenta.[1]

Resistance is clearly a concern in view of the high proportion of strains of *T. pallidum* found among men who have sex with men in the United States and Ireland that contain mutations that may confer resistance to macrolides. The clinical significance of this mutation has not been definitively established, although the small study by Klausner et al.[2] provides some support for such a link. The results of our trial suggest that azithromycin resistance is not currently a clinically significant problem among heterosexual patients in Tanzania. We recognize that most laboratories in Africa do not have the facilities to identify mutations in local strains of *T. pallidum*. In view of the considerable advantages that would be conferred by a single-dose oral treatment for syphilis, however, we believe further studies are warranted to study the geographic distribution and clinical significance of strains bearing this mutation.

We do not agree with Savaris and Abeche that azithromycin is too expensive to be used for the treatment of syphilis in developing countries. Generic supplies of the drug, made in India, have been available for some years at a cost of approximately $1.20 for a 2-g dose.[3] Azithromycin came off patent in the United States in November 2005. Although penicillin G benzathine is an inexpensive drug, the cost of administering it has to include the cost of the needle and syringe.

Despite the issues raised by the correspondents, we consider that single-dose azithromycin may have a place in the treatment of early syphilis and in the management of genital-ulcer disease at the primary health care level in developing countries.

Gabriele Riedner, M.D., Ph.D.
London School of Hygiene and Tropical Medicine
London WC1E 7HT, United Kingdom
riednerg@emro.who.int
Heiner Grosskurth, M.D., Ph.D.
Uganda Virus Research Institute
Entebbe, Uganda
Richard Hayes, D.Sc.
London School of Hygiene and Tropical Medicine
London WC1E 7HT, United Kingdom

1. Ramsey PS, Vaules MB, Vasdev GM, Andrews WW, Ramin KD. Maternal and transplacental pharmacokinetics of azithromycin. Am J Obstet Gynecol 2003;188:714-8.
2. Klausner JD, Mitchell SJ, Lukehart SA, Gordones C, Engelman J. Rapid and large increase in azithromycin resistance in syphilis whilst low steady resistance in gonorrhea 2000-2004. In: Program and abstracts of the 16th Biennial Meeting of the International Society for Sexually Transmitted Diseases Research, Amsterdam, July 10–13, 2005. abstract.
3. Pepin J, Mabey D. Sexually transmitted infections in Africa: single dose treatment is now affordable. Sex Transm Infect 2003;79:432-4.

THE EDITORIALIST REPLIES: It is striking that the first randomized trial demonstrating azithromycin's efficacy for early syphilis in Africa was conducted virtually simultaneously with the emergence of azithromycin-resistance mutations in *T. pallidum* in all five cities where such mutations were sought in the United States and Ireland. This may represent a world record for the adaptation of a pathogen to an antimicrobial agent newly proved effective to treat it — and this by an organism not previously known for its propensity to develop resistance to other antimicrobial agents. Reservations about the use of azithromycin for the treatment of early syphilis are clearly warranted.

Fortunately, *T. pallidum* remains fully susceptible to penicillin G benzathine worldwide, and the forthcoming 2006 Sexually Transmitted Disease Guidelines from the Centers for Disease Control and Prevention will correctly recommend that "penicillin G, administered parenterally, is the preferred drug for treatment of all stages of syphilis" and that the recommended regimen for adults with primary, secondary, or early latent syphilis is "benzathine penicillin G 2.4 million units IM [intramuscular] in a single intramuscular dose."

King K. Holmes, M.D., Ph.D.
University of Washington
Seattle, WA 98195

Cost-Effectiveness of ICDs

TO THE EDITOR: The cost-effectiveness of implantable cardioverter–defibrillator (ICD) therapy reported by Sanders et al. (Oct. 6 issue)[1] is overly optimistic, because it does not fully account for several factors that raise the costs and lower the effectiveness of this therapy. The authors assumed a constant benefit of the ICD during the patient's lifetime, whereas in previous investigations, the benefit declined, with a convergence of survival curves by seven to eight years.[2] The assumed probability of lead-related complications (2.4 percent over 20 months) underestimates the spectrum and frequency of serious complications (up to 14 percent in the Sudden Cardiac Death in Heart Failure Trial [SCD-HeFT]).[3] The high frequency of recalls of devices and the consequent interventions are not accounted for.[4] The base-case assumption of an equivalent quality of life among patients who received an ICD and the control patients does not account for the adverse effect of ICD shocks (31 percent in SCD-HeFT)[3] or for the discomfort, inconvenience, and the loss of time and income due to the implantation procedure and the need for replacement of the device, the checking and programming of the device before and after many forms of surgery, and, because of the presence of the device, the exclusion of several types of diagnostic procedures, treatments, employment, and recreation. The inclusion of these factors, in addition to those noted by Goldman in his editorial,[5] would unfavorably affect the cost-effectiveness of ICDs.

Kelley P. Anderson, M.D.
Marshfield Clinic
Marshfield, WI 54449
kpand@att.net

1. Sanders GD, Hlatky MA, Owens DK. Cost-effectiveness of implantable cardioverter–defibrillators. N Engl J Med 2005;353:1471-80.
2. Weiss JP, Saynina O, McDonald KM, McClellan MB, Hlatky MA. Effectiveness and cost-effectiveness of implantable cardioverter defibrillators in the treatment of ventricular arrhythmias among Medicare beneficiaries. Am J Med 2002;112:519-27.
3. Bardy GH, Lee KL, Mark DB, et al. Amiodarone or an implantable cardioverter–defibrillator for congestive heart failure. N Engl J Med 2005;352:225-37. [Erratum, N Engl J Med 2005;352:2146.]
4. Maisel WH, Sweeney MO, Stevenson WG, Ellison KE, Epstein LM. Recalls and safety alerts involving pacemakers and implantable cardioverter-defibrillator generators. JAMA 2001;286:793-9.
5. Goldman L. Cost-effectiveness in a flat world — can ICDs help the United States get rhythm? N Engl J Med 2005;353:1513-5.

TO THE EDITOR: The study by Sanders et al. of the cost-effectiveness of ICDs exemplifies the skilled analyses that are crucial as the choices become

explicit between technologies and between technology and disease management, which is traditionally judged more harshly according to cost savings. However, the conclusions of the study may be less applicable to patients with heart failure, who are most often considered for ICDs, than to those who do not have heart failure, because the assumptions of stable risks and quality of life for seven years extend beyond the median survival of patients with symptomatic heart failure. Future models should include patients with New York Heart Association (NYHA) class III symptoms of heart failure and could use a Markov model similar to that previously used to study candidates for heart transplantation.[1] Patients with class III symptoms of heart failure face a higher risk of both death from any cause, death from causes other than arrhythmias, and diminished quality of life,[2] with utility closer to 0.50 than the 0.88 year used for this analysis. Patients with NYHA class III heart failure in SCD-HeFT had no survival benefit with ICDs, and the benefit attributable to ICDs in the Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) trial ended after two quality-adjusted life-years (QALYs) gained.[3] The transition rate to class III heart failure may be a critical determinant of cost-effectiveness, making ICDs acceptably cost-effective only when follow-up after implantation is linked with management of heart failure that is focused on preventing disease progression.

Lynne W. Stevenson, M.D.
William G. Stevenson, M.D.
Brigham and Women's Hospital
Boston, MA 02115

Dr. L.W. Stevenson reports having received consulting fees and honoraria from Medtronic; and Dr. W.G. Stevenson, honoraria from Medtronic, Guidant, and St. Jude Medical.

1. Stevenson LW, Warner SL, Hamilton MA, et al. Modeling distribution of donor hearts to maximize early candidate survival. Circulation 1992;86:Suppl II:II-224–II-230.
2. Lewis EF, Johnson PA, Johnson W, Collins C, Griffin L, Stevenson LW. Preferences for quality of life or survival expressed by patients with heart failure. J Heart Lung Transplant 2001; 20:1016-24.
3. Bristow MR, Saxon LA, Boehmer J, et al. Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. N Engl J Med 2004;350:2140-50.

TO THE EDITOR: In their analysis, Sanders et al. found that prophylactic implantation of an ICD has a cost-effectiveness ratio below $100,000 per QALY gained in populations in which a significant reduction in device-related mortality has been demonstrated. It may be disappointing to note that the cost-effectiveness of the ICD seems to decrease from the first studies to the later ones: $34,000 per QALY gained in the Multicenter Automatic Defibrillator Implantation Trial (MADIT), $54,100 in MADIT II, and $70,200 in the SCD-HeFT. The more recent studies defined high risk more broadly (entirely on the basis of a decrease in left ventricular ejection fraction). It is a challenge for future studies to identify properly subgroups of patients who may derive a greater benefit from a prophylactic ICD (with the use of quantitative electrocardiography, for example), particularly those with heart failure in whom there are competing risks between death as a result of worsening pump failure and sudden death.[1,2] Such definition of a subgroup is likely to improve markedly the cost-effectiveness of the prophylactic ICD.

Laurent Fauchier, M.D.
Dominique Babuty, M.D.
Centre Hospitalier Universitaire Trousseau
37044 Tours, France

1. McClellan MB, Tunis SR. Medicare coverage of ICDs. N Engl J Med 2005;352:222-4.
2. Kadish A. Prophylactic defibrillator implantation — toward an evidence-based approach. N Engl J Med 2005;352:285-7.

THE AUTHORS REPLY: We thank Dr. Anderson for his careful review of our model's structure and assumptions. We believe that these assumptions reflect the best available current evidence, but we also checked to see whether changes in the assumptions would have affected the cost-effectiveness of the ICD. We did find that the cost-effectiveness of the ICD became much less favorable if its efficacy ceased after a short time period (Fig. 4A in our report).[1] We found that the ICD cost less than $100,000 per QALY as long as it reduced mortality for no less than 7 years. Lead complications had less effect on the cost-effectiveness of the ICD. Increasing this rate to 14 percent over the median 45.5 months follow-up, as Dr. Anderson suggests, would change the cost-effectiveness of the ICD in the SCD-HeFT population from $70,200 per QALY to $74,300 per QALY. Our model captured the effects of complications that resulted in therapy crossover by adopting an intention-to-treat analysis of the efficacy of the ICD. We agree that the quality of life is important to consider (as done in Fig. 3C of our article).[1] Quality-of-life outcomes may vary among individual

patients, however, so we used the anticipated effect on the quality of life in the entire population of patients.

Drs. Stevenson and Stevenson raise important points about the patient populations considered for prophylactic ICD implantation and emphasize that patients with severe heart failure were not generally enrolled in the randomized, controlled trials we studied. We agree, and we caution readers not to extrapolate our results beyond the populations of patients in whom the ICD has been proved to be effective by clinical trials.

Drs. Fauchier and Babuty accurately point out the influence of risk stratification among patients on the cost-effectiveness of the ICD. We agree that there is a need to identify patients who are at increased risk for sudden death but whose competing risks of death would allow them to realize the greatest benefit from receipt of an ICD.[1] The effectiveness and cost-effectiveness of the ICD will improve if further studies can identify the clinical characteristics or diagnostic tests that will enable clinicians to select patients who will gain the most benefit from the ICD.

Gillian D. Sanders, Ph.D.
Duke University
Durham, NC 27715
gillian.sanders@duke.edu

Mark A. Hlatky, M.D.
Stanford University
Stanford, CA 94305

Douglas K. Owens, M.D.
Veterans Affairs Palo Alto Health Care System
Palo Alto, CA 94304

1. Owens DK, Sanders GD, Heidenreich PA, McDonald KM, Hlatky MA. Effect of risk stratification on cost-effectiveness of the implantable cardioverter defibrillator. Am Heart J 2002;144:440-8.

Medicare and Cost-Effectiveness Analysis

TO THE EDITOR: Shortly after reading the commentary by Neumann et al. (Oct. 6 issue),[1] I met with several residents in internal medicine to discuss economic evaluation in health care. I was struck by their lack of knowledge not only about the methods and uses of cost-effectiveness analysis but also about the structure and financing of the health care system.

Any "campaign to educate policymakers and the public"[1] about the rationale for cost-effectiveness analysis should include efforts to educate our medical students and ourselves. In the crowded medical curriculum, when there is a choice between adding more course work in the basic sciences and introducing issues of social and health policy, we give a consistent nod to the basic sciences — to the detriment of the public.

The lack of systematic efforts to integrate knowledge of health systems and economic evaluation into the medical curriculum is part of our larger failure to teach clinical skills that are grounded in evidence-based medicine. We cannot expect policymakers and the public to take these topics seriously without expecting the same of the medical profession.

Kevin A. Schulman, M.D.
Duke University
Durham, NC 27705

1. Neumann PJ, Rosen AB, Weinstein MC. Medicare and cost-effectiveness analysis. N Engl J Med 2005;353:1516-22.

TO THE EDITOR: The article by Neumann et al. and the accompanying editorial by Goldman[1] further enshrine the specious concept of cost-effectiveness that is now on display in the medical literature. Who, in fact, accepted the $50,000-per-year threshold for quality-adjusted life-years (QALYs) gained? An extrapolation from the hemodialysis situation proves unpersuasive; patients who are withdrawn from hemodialysis generally die within the month. Extrapolating this cost-effectiveness standard to such tests as screening colonoscopies and bone-density measurements benefits our profession more than it does the welfare of American society. If every American actually obtained every procedure now deemed cost-effective, the price tag would approach the gross national product.

Opportunity cost represents a sounder economic concept: money once spent on medical technology cannot be spent on education and job training. Better-paying jobs and improved education correlate more powerfully with good health and longevity than do implanted defibrillators. We ought to reevaluate the ethics of our economic priorities before a wiser president one day

warns Americans to beware the medical–industrial complex.

Mitchell T. Smolkin, M.D.
University of Virginia Health Services Foundation
Staunton, VA 24401
mts8s@virginia.edu

1. Goldman L. Cost-effectiveness in a flat world — can ICDs help the United States get rhythm? N Engl J Med 2005;353:1513-5.

TO THE EDITOR: We endorse the call of Neumann et al. for systematic use of cost-effectiveness analysis. Evidence-based comparisons of safety and effectiveness, including advantages and disadvantages in specific subpopulations, should form the foundation for choices among alternative medical technologies. Informed by good-quality economic analyses, decisions to allocate resources can be made more efficiently. Decision makers, however, must ensure the integrity and fairness of analyses.

Since the potential for bias, such as assumptions about class effects and projections beyond trial results, exists regardless of sponsorship and always requires critical evaluation, organizations conducting cost-effectiveness analyses should engage diverse stakeholders to provide feedback throughout the entire process, ensuring reasonableness and transparency of assumptions and methods.[1] In addition, economic findings must be integrated with local concerns and values; otherwise winner-take-all decisions regarding coverage may discourage innovation. We welcome a constructive dialogue with the Centers for Medicare and Medicaid Services (CMS) and other stakeholders to incorporate comparative and cost-effectiveness analyses into health policy decisions.

Marc Berger, M.D.
Peter Honig, M.D.
Ian Spatz, J.D.
Merck
West Point, PA 19486
marc_berger@merck.com

1. Berger ML, Teutsch SM. Cost-effectiveness analysis: from science to application. Med Care 2005;43:Suppl II:49-53.

THE AUTHORS REPLY: We appreciate the endorsement of Berger et al. of the systematic use of cost-effectiveness analysis, and we are particularly heartened by their call for a constructive dialogue with the CMS and other stakeholders about the use of comparative and cost-effectiveness analysis in health policy decisions.

Smolkin argues that our article "enshrines" the notion that $50,000 per QALY represents reasonable value for medicine. In fact, nowhere in our article did we state or suggest that figure, though we acknowledge that it has been used elsewhere. We do agree with him that there would be an inherent problem if every American received every procedure deemed to be cost-effective without a concomitant attempt to withdraw interventions that do not provide good value for the money. We emphasized in our commentary that cost-effectiveness analysis is a guide that must be combined with other dimensions that decision makers believe are important. Smolkin also asserts that better-paying jobs and improved education correlate more powerfully with good health and longevity than do implanted defibrillators. This could be true, though he provides no evidence on the matter. To the extent possible, nonmedical interventions should also be subject to formal cost-effectiveness analysis.

Finally, we agree with Schulman that educational efforts regarding cost-effectiveness analysis would do well to include medical students and the medical profession in general. Our institutions have training programs for physicians regarding cost-effectiveness analysis and medical decision science, and we applaud the initiatives of many of our sister institutions in this regard. As we noted, getting the United States to move beyond its anxiety about cost-effectiveness analysis will require leadership in the academic community as well as among policymakers in both the public and private sectors and will probably take time and require further education. The entire medical community needs to be a key part of any change.

Peter J. Neumann, Sc.D.
Harvard School of Public Health
Boston, MA 02115
Allison B. Rosen, M.D., Sc.D.
University of Michigan Health System
Ann Arbor, MI 48109
Milton C. Weinstein, Ph.D.
Harvard School of Public Health
Boston, MA 02115

THE EDITORIALIST REPLIES: For Dr. Smolkin to dismiss the entire science of cost-effectiveness analysis because of his unhappiness about appropriate thresholds is as specious as avoiding all statistical analysis because of disagreement about which P value should be considered significant or eliminating all taxes because of debates about the appropriate rates. Recognition of the critical

roles played by statistics, taxes, and in my opinion, cost-effectiveness analysis helps refine methods and promote a civilized, even if never perfect, consensus. As emphasized in my editorial, the effort that is needed to achieve such results is warranted because the alternative, which is uninformed chaos, is far worse.

Lee Goldman, M.D.
University of California, San Francisco
San Francisco, CA 94143

Case 26-2005: Loss of Consciousness while Jogging

TO THE EDITOR: We fear that the discussion of "syncope" in the Case Records, as in Binder et al. (Aug. 25 issue),[1] may confuse readers. Seizures, hypoglycemia, and psychiatric conditions are considered causes of syncope (as listed in Table 1 of the article), but these are incompatible with the later statement that "all forms of syncope result from a sudden decrease in cerebral blood flow." The authors seem to use syncope in two different meanings, as occurs commonly in the literature.[2] The first use (as in their Table 1) appears to mean transient loss of consciousness, a temporary, self-limited, and short-term loss of consciousness not due to mechanical trauma.[3,4] We doubt the wisdom of this use, as few physicians would use the word syncope to identify epileptic seizures. The second meaning restricts the use of syncope to transient loss of consciousness due to cerebral hypoperfusion, conforming to the European Society of Cardiology guidelines.[3,4] We suggest that the word be used in this sense only, and that specific terms such as syncope or seizure be used when the cause is clear; if not, the use of the more general phrase "transient loss of consciousness" forces physicians to keep an open mind regarding the diagnosis.[3-5]

J. Gert van Dijk, M.D.
Leiden University Medical Centre
2300 RC Leiden, the Netherlands
j.g.van_dijk.neur@lumc.nl

David G. Benditt, M.D.
University of Minnesota Medical School
Minneapolis, MN 55455

Michele Brignole, M.D.
Ospedali del Tigullio
16033 Lavagna, Italy

1. Case Records of the Massachusetts General Hospital (Case 26-2005). N Engl J Med 2005;353:824-32.
2. Thijs RD, Benditt DG, Mathias CJ, et al. Unconscious confusion — a literature search for definitions of syncope and related disorders. Clin Auton Res 2005;15:35-9.
3. Brignole M, Alboni P, Benditt DG, et al. Guidelines on management (diagnosis and treatment) of syncope — update 2004: executive summary. Eur Heart J 2004;25:2054-72.
4. Brignole M, Alboni P, Benditt DG, et al. Guidelines on management (diagnosis and treatment) of syncope — update 2004. Europace 2004;6:467-537.
5. Thijs RD, Wieling W, Kaufmann H, van Dijk G. Defining and classifying syncope. Clin Auton Res 2004;14:Suppl 1:4-8.

Primary Prevention of Sudden Death in Patients with Lamin A/C Gene Mutations

TO THE EDITOR: Lamin A/C gene mutations are associated with various disorders,[1,2] including cardiac abnormalities characterized by atrial fibrillation, conduction-system disturbances, sudden death, and heart failure.[3,4] In retrospective analyses, we have previously investigated the high incidence of sudden death among carriers of such mutations.[4,5] Although there is consensus regarding the efficacy of implantable cardioverter–defibrillators (ICDs) in the secondary prevention of sudden death in persons with cardiovascular disease and also in primary prevention in patients with a reduced ejection fraction (<35 percent), it is not yet known whether ICD therapy is efficacious as primary prevention in carriers of a lamin A/C mutation.

We prospectively studied patients with genetically proven lamin A/C gene mutations; between March 1999 and January 2004, all patients referred to a participating center for permanent cardiac pacing were systematically offered the implantation of an ICD. A history of ventricular tachyarrhythmia, spontaneous or inducible by programmed ventricular stimulation, was not a selection criterion, and patients were enrolled solely on the basis of the presence of lamin A/C mutations associated with cardiac conduction defects. Indications for pacemaker implantation

were progressive conduction block and sinus block. All the ICDs used in the study had storage capabilities, and events were scored as inappropriate or appropriate by two investigators. The study protocol was reviewed and approved by the ethics committee of each participating center, and all patients provided written informed consent.

The study included 19 patients whose clinical characteristics are listed in Table 1. During a mean follow-up period of 33.9±21.0 months, eight of the patients (42 percent) received appropriate ICD therapy. Six patients received ICD shocks for ventricular fibrillation (cycle length, <240 msec), two patients received ICD shocks for ventricular tachycardia (cycle length, >240 msec), and one patient received antitachycardia pacing for ventricular tachycardia. One patient received an inappropriate shock. The mean (±SD) left ventricular ejection fraction (LVEF) in the overall study population was 58±12 percent. No factor — including LVEF, spontaneous or inducible ventricular tachycardia or ventricular fibrillation, or drug therapy — was found to be related to the occurrence of ventricular arrhythmias. The LVEF was not depressed in patients receiving appropriate ICD therapy, indicating a high risk of sudden death before the development of cardiac failure.

Table 1. Baseline Clinical Characteristics of the 19 Patients.*

Characteristic	Value
Sex (no.)	
Male	14
Female	5
Age (yr)	41.7±13.4
Muscular phenotype (no.)	
Emery–Dreifuss	9
Limb-girdle	1
DCM plus conduction system disease	8
Shoulder-muscle amyotrophy	1
Gene mutation (no.)	
c.28ins, p.Thr10delinsThrfsX30	2
c.1_356Del, p.Met1 (Del exon 1)	3
c.778_780delAAG, p.Lys260del	1
c.16C>T, p.Gln6X	5
c.746G>A, p.Arg249Gln	1
c.1129 C>T, p.Arg377Cys	2
c.1130 G>A, p.Arg377His	1
c.1357 C>T, p. Arg453Trp	1
c.149 G>C, p.Arg50Pro	1
c.178 C>G, p.Arg60Gly	1
c.976 T>A, p.Ser326Thr	1
Supraventricular arrhythmias (no.)†	
None	4
Atrial premature complexes in runs	4
Paroxysmal atrial fibrillation	10
Permanent atrial fibrillation	1
Ventricular arrhythmias (no.)†	
None	8
Isolated premature ventricular beats	8
Ventricular premature complexes in runs	2
Ventricular tachycardia	1
Left ventricular ejection fraction (%)	58±12
Inducible ventricular tachyarrhythmia (no.)‡	
Yes	3
No	12

* Plus–minus values are means ±SD. DCM denotes dilated cardiomyopathy.
† Arrhythmias were monitored with the use of 24-hour electrocardiography.
‡ Fifteen patients were included in the analysis.

In conclusion, ICD implantation in patients with a lamin A/C mutation who are in need of a pacemaker is effective in treating possibly lethal tachyarrhythmias. The implantation of an ICD, rather than a pacemaker, should be considered for these patients.

Christophe Meune, M.D.
Paris V University Hospital Cochin
75014 Paris, France

Jop H. Van Berlo, M.D.
University Hospital Maastricht
6229 HX Maastricht, the Netherlands

Frédéric Anselme, M.D.
Charles Nicolle Hospital
76000 Rouen, France

Gisèle Bonne, M.D., Ph.D.
INSERM Unité 582
75013 Paris, France

Yigal M. Pinto, M.D., Ph.D.
Interuniversity Cardiology Institute
3501 DG Utrecht, the Netherlands

Denis Duboc, M.D., Ph.D.
Paris V University Hospital Cochin
75014 Paris, France
denis.duboc@cch.ap-hop-paris.fr

1. Ben Yaou R, Muchir A, Arimura T, et al. Genetics of laminopathies. Novartis Found Symp 2005;264:81-90.
2. Bonne G, Di Barletta MR, Varnous S, et al. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. Nat Genet 1999;21:285-8.
3. Taylor MR, Fain PR, Sinagra G, et al. Natural history of dilated cardiomyopathy due to lamin A/C gene mutations. J Am Coll Cardiol 2003;41:771-80. [Erratum, J Am Coll Cardiol 2003;42:590.]
4. van Berlo JH, de Voogt WG, van der Kooi AJ, et al. Meta-analysis of clinical characteristics of 299 carriers of LMNA gene mutations: do lamin A/C mutations portend a high risk of sudden death? J Mol Med 2005;83:79-83.
5. Bécane HM, Bonne G, Varnous S, et al. High incidence of sudden death with conduction system and myocardial disease due to lamins A and C gene mutation. Pacing Clin Electrophysiol 2000;23:1661-6.

BOOK REVIEWS

TARGETING BIOTERRORISM

BIOLOGICAL WEAPONS: FROM THE INVENTION OF STATE-SPONSORED PROGRAMS TO CONTEMPORARY BIOTERRORISM

By Jeanne Guillemin. 258 pp. New York, Columbia University Press, 2005. $27.95. ISBN 0-231-12942-4.

BIOLOGICAL WEAPONS DEFENSE: INFECTIOUS DISEASES AND COUNTERBIOTERRORISM

(Infectious Disease.) Edited by Luther E. Lindler, Frank J. Lebeda, and George W. Korch. 597 pp., illustrated. Totowa, N.J., Humana Press, 2005. $145. ISBN 1-58829-184-7.

MEDICAL RESPONSE TO TERRORISM: PREPAREDNESS AND CLINICAL PRACTICE

Edited by Daniel C. Keyes, Jonathan L. Burstein, Richard B. Schwartz, and Raymond E. Swienton. 449 pp., illustrated. Philadelphia, Lippincott Williams & Wilkins, 2005. $99. ISBN 0-7817-4986-7.

JEANNE GUILLEMIN, A PROFESSOR OF SOCIOLOGY at Boston College and the author of *Anthrax: The Investigation of a Deadly Outbreak* (Berkeley: University of California Press, 1999), a definitive study of the 1979 anthrax attack by terrorists in the former Soviet Union, wrote *Biological Weapons* to "bring historical context to present concerns about biological weapons and the potential for bioterrorism." She has far surpassed that goal. The scholarship and the clarity of the writing are remarkable.

In recent years bioterrorism has become a potent subject that invokes fear in our society. Guillemin brings light to the history of biologic weapons as she traces the checkered history of the United States in relation to control of weapon-grade pathogens. She tracks the rise of state-sponsored programs for the development of offensive biologic weapons since the end of World War I and details the horrible story of General Ishii Shiro, the Japanese military biologist who developed and deployed plague-infected fleas and bacterial pathogens to contaminate food and water in "total war" attacks on Chinese civilians in Manchuria. The rise of biologic-warfare programs in the United Kingdom, the United States, and the former Soviet Union after World War II is succinctly documented and annotated. Guillemin pulls no punches in blasting the covert development of biologic weapons, which the Soviets began immediately after they had signed the Biological Warfare Convention in 1972.

But Guillemin also notes that the United States failed to ratify the Geneva Protocol of 1925, the original international agreement prohibiting the development of such weapons. President Richard Nixon gets full credit for the United States's decision to abandon work on offensive biologic warfare at the end of 1969 and to sign the Biological Warfare Convention, but Guillemin points out that the United Kingdom was the first nation to renounce such weapons, 10 years earlier. She dryly comments that the United States finally ratified the Geneva Protocol in 1975 but recently withdrew from international attempts to strengthen the accord by adding inspection and verification of conformity to it.

The U.S. government argues that our military and commercial interests must be protected from outside access. Guillemin's unveiled concern is that U.S. secrecy only promotes doubt among other nations about this country's intentions that may well lead to a biologic arms race and would exclude our allies from information and access that are important for their own protection. She pushes for creating more transparency in our defense programs, arguing that failure to do so will be interpreted as evidence that we have something to hide — such as the development of offensive biologic weapons. *Biological Weapons* deserves to be read widely, not just by those directly involved in research on or responses to biologic attacks by terrorists.

Matthew Meselson, who long has championed the elimination of chemical and biologic weapons, makes a further plea for transparency in his fore-

Robert Spencer, AP/Wide World Photos.

Disaster Response Drill in Brooklyn, New York, 2003.

word to *Biological Weapons Defense,* a collection of review articles that document recent work by scientists at the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick, Maryland, and their academic collaborators. Detailed chapters are devoted to the pathogenesis of, molecular taxonomy of, and genomic variability among several major bacterial pathogens. The innate immune system and its possible future use as broad-spectrum emergency prevention and therapy for infections after a terrorist attack are carefully described. There is an excellent chapter on the range of agents that may emerge as new threats, not the least of which are potential hybrid pathogens intentionally created by sophisticated enemies.

Unstated, but evident to me, is that the United States has a very long road to travel before it can be said to be well prepared to deal with biologic weapons as they might be deployed against civilian populations. Diagnostic technology (especially for hemorrhagic fever viruses) is far from ready for final validation exercises; protocols for real-time surveillance of infectious disease are blizzards of government and commercial acronyms and include no coordination of effort and no evident path for development and decisions with regard to national implementation. This book, too, is not well prepared in terms of references, which are from 2001 or earlier, almost without exception. A CD-ROM version of the book is included with each copy, providing convenient access for scientists, journalists, and administrators.

The book presents virtually nothing, however, concerning the United States's expensive air-sampling program, which was initiated by the Department of Defense and then transferred to the Department of Homeland Security. It could be argued that information about sites, agents, and methods of sampling should be classified, to keep terrorists in the dark. But leaks to the public about false positive results for aerosols or even true positive results for natural aerosols (e.g., *Francisella tularensis* in New England) send mixed messages about the effectiveness and extent of coverage that urban areas of our country do or do not enjoy. Scientifically validated, sensitive, and specific air sampling is crucial, so that drugs or vaccines, or both, when available, can be administered after exposure. Secrecy has a history of thoughtless use, as well as use in hiding from the public the fact that things are simply not working. Meselson's warning against secrecy should be heeded.

Medical Response to Terrorism takes up the disparate challenges involved in coping with a terrorist assault on innocent civilians. Tightly written, this book has a standard, detailed outline, numerous summary tables, and a short multiple-choice quiz at the end of each chapter. It is clearly a textbook for the many groups of individuals and organizations that will be called into action after an attack — whether chemical, biologic, or explosive — or even a hit on the computerized systems that run hospitals and communication networks.

This book is probably destined to be the basic textbook for training sessions supported by the Department of Homeland Security and various state agencies, and it would help immensely if it, like *Biological Weapons Defense,* were available as a CD-ROM, for immediate reference. Because chemical and biologic attacks are not likely to become everyday events, it also might be valuable to create separate, terse summary documents for those in the medical and general communities who respond in such emergencies. These documents should be readily available by computer, so that a quick refresher is at hand if suddenly needed. I recall that in 1976, colleagues from other countries, who worked with us at the Centers for Disease Control and Prevention during the initial Ebola epidemic in what was then Zaire, promised readiness to respond to any new outbreak of that virulent virus. We had a roster of names, many units of immune plasma obtained from patients during convalescence stored in three locations, and boxes of other supplies just hours from central Africa. But because the next Ebola outbreak didn't occur until 19 years later, completely new personnel, supplies, and field protocols had to be organized.

These three books, each with a distinct content and focus, remind us all of the huge investment the United States has made (and will continue to make) to prevent a biologic attack by terrorists on our citizens. Taken together, they provide a powerful portrayal of where we've been and where we are likely to go in the relatively near future. Today, many in government who have access to classified information are convinced that a biologic attack is not a matter of if, but a matter of when. This prophecy will surely drive a search for answers for years to come.

Karl M. Johnson, M.D.

10 Calle Final
Placitas, NM 87043

CORRECTIONS

Exhaled Nitric Oxide and Asthma (August 18, 2005;353:732-3). In the letter by Smith and Taylor, in the first full paragraph of the right-hand column on page 733, lines 3, 10, and 18 should have read "long-acting beta-agonists," rather than "long-acting $beta_2$-agonists" and "long-acting $beta_2$-antagonists," as printed. We regret the error.

Cerebral Folate Deficiency Syndrome (August 18, 2005;353:740). In the letter by Willemsen et al., author Marcel M. Verbeek's name was misspelled.

Low HDL Cholesterol Levels (September 22, 2005;353:1252-60). On page 1257, in the left-hand column, line 6 of the first full paragraph should have read, ". . . high-dose niacin (2 to 4 g per day) . . .," rather than ". . . (2 to 4 mg per day) . . .," as printed. Also, lines 13 through 16 of the second full paragraph should have read, "Medial thickness of the carotid intima significantly increased in the placebo group . . . but not in the niacin group," rather than ". . . but not in the placebo group," as printed. We regret the error.

NOTICES

Notices submitted for publication should contain a mailing address and telephone number of a contact person or department. We regret that we are unable to publish all notices received. Notices also appear on the Journal*'s Web site (www.nejm.org/meetings). The listings can be viewed in their entirety or searched by location, month, or key word.*

SEIZE THE MOMENT: REACHING EXCELLENCE IN PATIENT SAFETY

The conference will be held in Boston, Feb. 6 and 7.

Contact CRICO/RMF, 101 Main St., Cambridge, MA 02142; or see http://www.rmf.harvard.edu/conference2006; or call (617) 679-1390.

LEROY D. VANDAM, M.D.: AN ANESTHESIA JOURNEY

Leroy Vandam was a universal man: surgeon, artist, scientist, writer, and anesthesiologist. The Department of Anesthesiology at Boston University Medical Center and Anaesthesia Associates of Massachusetts have published a DVD featuring Dr. Leroy Vandam's narration of his illustrious career and the evolution of anesthesia equipment. Dr. Vandam provides an eloquent and intimate account of his professional experiences and the very nature of anesthesiology in the 20th century. Many historical photographs and film clips of equipment, people, and places accompany the program. The DVD, directed by Rafael Ortega, M.D., honors Dr. Vandam's memory and is distributed free of charge. You may e-mail maureen.omalley@bmc.org to request a complimentary copy.

AMERICAN HEADACHE SOCIETY

The following meetings will be held: "2006 Winter Headache Symposium" (Henderson, Nev., Jan. 27–29); "48th Annual Scientific Meeting" (Los Angeles, June 22–25); and "2006 Scottsdale Headache Symposium" (Scottsdale, Ariz., Nov. 10–12).

Contact American Headache Society, 19 Mantua Rd., Mount Royal, NJ 08061; or call (856) 423-0043; or fax (856) 423-3420; or e-mail ahsmtgs@talley.com.

SOUL SPEAK: PLAIN TALK ABOUT HEALTH LITERACY AND THE PHYSICIAN-PATIENT PARTNERSHIP

The conference will be held in Jackson Hole, Wyo., Feb. 8–11. It is presented by the University of Tennessee Graduate School of Medicine and College of Medicine.

Contact the University of Tennessee Graduate School of Medicine, 1924 Alcoa Highway, D-116, Knoxville, TN 37920-6999; or call (865) 544-9190; or e-mail afjohnso@mc.utmck.edu; or see http://www.tennessee.edu/cme/healthliteracy.

HIGHLIGHTS OF ASH 2005

The annual meeting of the American Society of Hematology will be held in Miami, Feb. 10 and 11.

Contact the American Society of Hematology, 1900 M. St., Suite 200, Washington, DC, 22036; or call (202) 776-0544; or see http://www.hematology.org/meetings/highlights/index.cfm; or e-mail cme@hematology.org.

NETHERLANDS INSTITUTE FOR HEALTH SCIENCES

The following courses will be offered in Rotterdam, the Netherlands, unless otherwise indicated: "Epidemiology of Infectious Diseases/CE05" (Amsterdam, Feb. 13–17); "Advanced Diagnostic Research/CE10" (Utrecht, the Netherlands, Feb. 27–March 3); "Principles of Epidemiologic Data Analysis/EP15" (Lunteren, the Netherlands, Feb. 27–March 3); "Quantitative Models for Evaluation of Tropical Disease Control/HS06" (March 6–10); "Bayesian Statistics/CE09" (March 13–15); "Addiction and Substance Use/HS13" (March 20–24); "Medical Demography/HS04" (April 3–7); "Prognostic Research/CE11" (Utrecht, the Netherlands, April 3–7); "Psychiatric Epidemiology/EP12" (Groningen, the Netherlands, April 10–13); "Clinical Trials and Drug Risk Assessment/CE04" (Utrecht, the Netherlands, April 24–28); "Planning and Evaluation of Screening/HS05" (May 8–12); "Cancer Epidemiology/EP13" (Amsterdam, May 15–19); and "Operational Research Applied to Health Services/HS07" (May 17–19).

Contact the Netherlands Institute for Health Sciences, P.O. Box 1738, 3000 DR Rotterdam, the Netherlands; or call (31) 10 408 8149; or e-mail info@nihes.nl; or see http://www.nihes.nl.

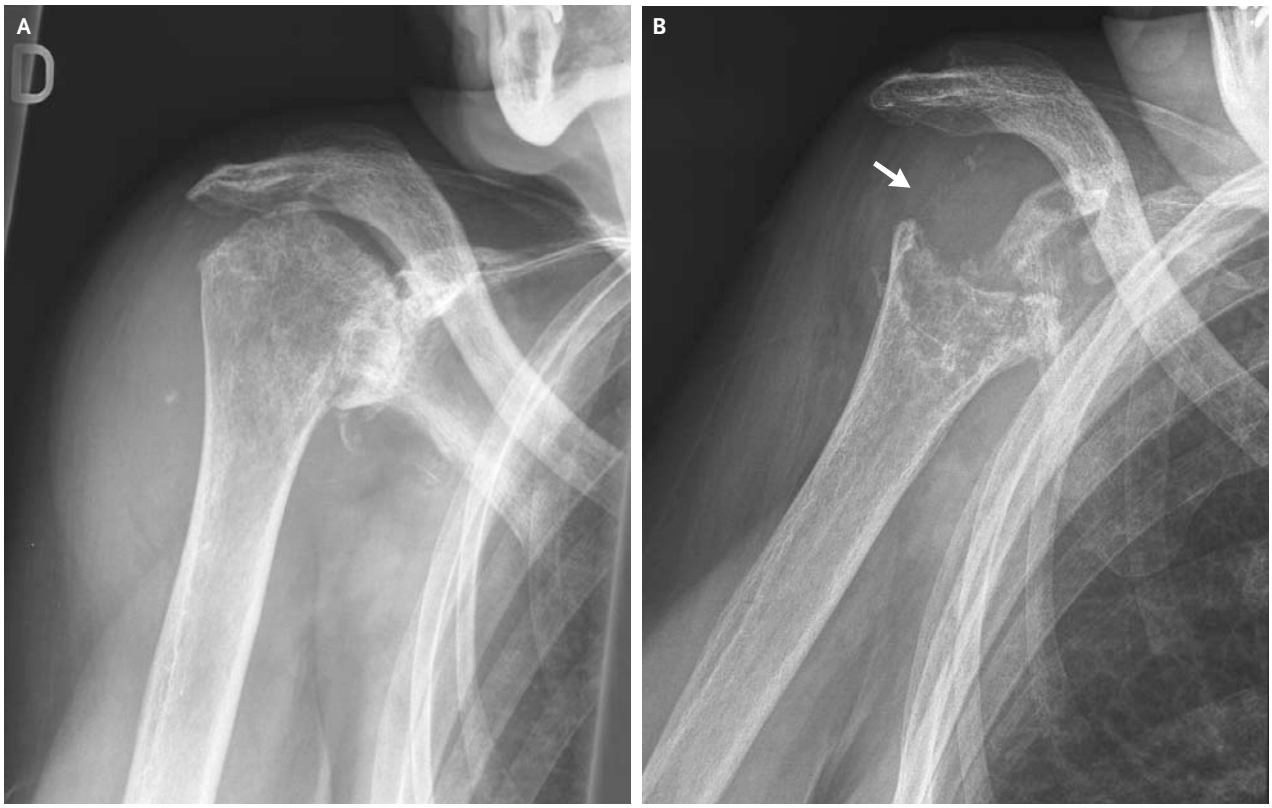
25TH ANNUAL SQUAW VALLEY RETINAL SYMPOSIUM

The symposium will be held in Squaw Valley, Calif., Feb. 9–12.

Contact Dr. Robert Wendel or Laura Wendel, 3939 J St., Suite 106, Sacramento, CA 95819; or call (916) 483-6299; or fax (916) 483-6297; or e-mail squawvalleyretina@comcast.net; or see http://www.squawvalleyretina.com.

IMAGES IN CLINICAL MEDICINE

Milwaukee Shoulder



Marcia S. Genta, M.D.
Cem Gabay, M.D.

Geneva University Hospital
1211 Geneva 14, Switzerland

An 85-year-old woman presented with right shoulder pain and swelling and a large hematoma extending into the chest wall. Active range of motion was severely limited, and passive mobility was painful but largely unrestricted. She had had no recent trauma to the shoulder area. Arthrocentesis yielded a hemorrhagic noninflammatory fluid; no crystals were seen on polarized microscopy. Alizarin red staining of the synovial fluid showed rare hydroxyapatite crystals. All cultures were negative, and cancer was not detected by cytologic analysis. Radiography of the right shoulder was suggestive of degenerative arthritis and lateral distention of the subacromial bursa (Panel A). Three months later, the patient presented with similar clinical findings, although at this time radiography showed complete destruction of the humeral head (Panel B, arrow). Milwaukee shoulder, a rare arthropathy that mainly affects elderly women, was diagnosed, and the patient was treated conservatively. A year later, the patient presented with similar clinical symptoms and radiographic findings in the left shoulder and continues to have severely limited active and passive range of motion.

Milwaukee shoulder is characterized by intraarticular or periarticular hydroxyapatite crystals and rapid destruction of the rotator cuff and the glenohumeral joint.