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Perspective

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The Big Chill — Inserting the DEA into End-of-Life Care

Timothy E. Quill, M.D., and Diane E. Meier, M.D.

On October 5, 2005, the U.S. Supreme Court heard oral arguments in *Gonzales v. Oregon*. On the surface, this case is about the legitimacy of physicians' prescribing of medications under Oregon's Death with Dignity Act and whether the federal government can overrule the states in defining "legitimate medical practice." Just beneath the surface, however, lies the risk of empowering agents of the Drug Enforcement Agency (DEA) — whose traditional role is to prevent drug abuse and diversion — to evaluate the end-of-life practices of physicians whose patients die while receiving prescribed opioids or barbiturates. A finding in favor of the Justice Department would not only nullify the Death with Dignity Act, permitting the DEA to penalize physicians for providing medications to hasten the deaths of terminally ill patients, but also have a chilling effect on physicians' willingness to treat patients' terminal symptoms.

Uncontrolled pain and other distressing symptoms are the primary concerns and greatest fears of patients facing serious illness.[1] More than 90 percent of the pain associated with severe illness can be relieved if physicians adhere to well-established guidelines and seek help, when necessary, from experts in pain management or palliative care. For the infrequent instances in which all palliative care alternatives have been exhausted without providing adequate relief from the symptoms of advanced terminal disease, there is a growing consensus that sedation to the point of comfortable sleep is permissible.[2] Despite the efficacy of opioids and a commitment by the medical profession to treat pain, abundant evidence suggests that patients' fears of undertreatment of distressing symptoms are justified.[1] Although a lack of proper training and overblown fears of addiction contribute to such undertreatment, physicians' fears of regulatory oversight and disciplinary action remain a central stumbling block.[3]

Several initiatives have lessened the adverse effects of regulatory constraints on symptom management.[4] Many legislatures and regulatory boards have adopted model pain statutes that encourage compliance with established standards for the prescribing of pharmacologic agents for pain and other symptoms and that protect physicians who observe these guidelines from regulatory intrusion and possible prosecution. Other states have simplified or eliminated special prescribing

rules (such as those requiring the use of triplicate prescription pads) that were designed to control and monitor prescribing but that had the (presumably unintended) effect of discouraging all prescribing of controlled substances. California now requires training in pain management and palliative care as a condition of licensure.

Two cases in California highlight the legal consequences of physicians' undertreatment of pain, providing a counterweight to the fear of legal vulnerability for the prescribing of controlled substances.[5] In 2001, in *Bergman v. Chin*, a jury found that a dying patient had received inadequate pain management and convicted the treating physician under the state's elder-abuse statute, awarding the patient's family $1.5 million. In 2003, in *Tomlinson v. Bayberry Care Center*, charges of inadequate pain management were brought successfully against both the treating physician and the patient's nursing home. Both cases demonstrate that, in addition to representing an unacceptably poor quality of care, the undertreatment of pain may carry legal risks and consequences.

Nevertheless, physicians continue to believe that regulatory oversight translates into a high risk of disciplinary action for prescribing opioids and other controlled substances. Consider the following cases.

Patient 1, a young man, became acutely ill with an aggressive but highly treatable cancer that caused severe acute chest pain. Since he had to make quick and extremely difficult decisions about his treatment options, he sought advice and pain medication from his trusted primary care physician — only to learn that his physician, wishing to be spared any possibility of regulatory suspicion, had never applied for prescribing privileges for strong opioids. At this critical juncture, the patient, who is himself a physician, had to find a new doctor in order to receive standard pain treatment.

This directive might open to investigation every instance of prescribing of a controlled substance for a dying patient.

Patient 2, a middle-aged woman with progressive cancer that had metastasized to bone, had accelerating pain requiring increasing doses of morphine. She ran out of pain medicine earlier than anticipated, but her physician refused to refill her prescription for fear that she was using it too much and that he might be reviewed for overprescribing. When she went to the emergency department with a pain crisis, a palliative care consultant recognized that her worsening pain and increased morphine requirements were caused by the progression of cancer. With a moderate increase in her dose, satisfactory pain control was achieved, and the patient went home to live out her final months in relative comfort.

Patient 3 had advanced metastatic lung cancer and had been receiving opioids at home when he was admitted to the hospital with new metastases to his thoracic spine. He was confused, could not move his legs, had difficulty breathing, and was in excruciating pain — screaming whenever he moved and grimacing with each breath. He was near death, and the primary goal of medical care was to control pain, agitation, and dyspnea. He was given a subcutaneous infusion of opioids at an equianalgesic dose 30 percent higher than his usual dose, and the nurses were instructed to give him another dose, equal to 10 percent of the total daily dose, "as needed" every half hour if he appeared to be in pain (the proper approach, according to standard guidelines). But several nurses and physicians refused to give the "as needed" doses, despite evidence of continuing distress, because they feared hastening his death. Ethics and palliative care consultants were called in, and they refocused the team on the professional obligation to relieve pain and suffering. The patient died hours after receiving the additional doses, and some staff members remained unsettled about whether they might have been legally liable for "causing" his death.

For better or for worse, the DEA sets the tone and drives physicians' perceptions about the legal risk associated with prescribing Schedule 2 drugs (potentially addictive drugs with critical medical uses)

for seriously ill and dying patients. Concerns about regulatory oversight have led some physicians, such as Patient 1's provider, to avoid prescribing opioids entirely and have rendered others, such as the physicians of Patients 2 and 3, fearful or hesitant. It is likely that such physicians will be further intimidated if the role of the DEA is expanded as the federal government proposes — and the risk of the inadequate management of symptoms during serious illness will increase.

Two other attempts by the federal government to invalidate Oregon's Death with Dignity Act preceded *Gonzales* v. *Oregon.* The first was the Lethal Drug Abuse Prevention Act, which a year later was repackaged as the Pain Relief Promotion Act (PRPA) of 1999. The PRPA contained some valuable provisions that would have encouraged education and research in pain management and palliative care, but the primary purpose of both acts was to make prescribing controlled substances under the Oregon law a violation of the Controlled Substances Act.

Although the regulation of medical practice is the legal province of the states, the PRPA would have allowed the federal government to undermine state law by making it a crime for physicians to provide medications that humanely hasten death. Furthermore, the PRPA would have empowered the DEA to investigate whether or not such a violation had occurred, raising the specter of DEA oversight of every death of a patient who had received barbiturates or opioids. After an outcry from both advocates and opponents of assisted suicide, all of whom recognized the danger such legislation posed to the practice of pain management and palliative care, the PRPA died in committee.

Then, in November 2001, U.S. Attorney General John Ashcroft issued a directive suggesting that the prescription of Schedule 2 medications under the Oregon law violates the Controlled Substances Act, since "assisting in a suicide is not a 'legitimate medical purpose.'" The State of Oregon and several interested parties challenged this directive, arguing that the definition of legitimate medical practice is a responsibility of the states, not a function of the Controlled Substances Act. If passed, this directive would allow the federal government to overrule established state law, empower the DEA to investigate whether a violation had occurred, and potentially open to investigation every instance of prescribing of a controlled substance for a dying patient. The U.S. Court of Appeals for the Ninth Circuit supported the arguments made by the State of Oregon, and the case was recently heard by the Supreme Court. The Court has not yet announced its decision.

This type of DEA involvement in medical practice would adversely affect far more patients than those few who seek assistance with a hastened death in Oregon. If the government thus oversteps its legitimate role and expertise, allowing DEA agents, trained only to combat criminal substance abuse and diversion, to dictate to physicians what constitutes acceptable medical practice for seriously ill and dying persons, it will undermine palliative care and pain management for the much larger number of seriously ill patients in all states. Physicians may become hesitant to prescribe the best available medications to manage the pain, agitation, and shortness of breath that sometimes accompany the end stages of illness. As a result, they may, in essence, abandon patients and their families in their moment of greatest need.

An interview with Dr. Quill and Dr. Meier can be heard at www.nejm.org.

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1. Field MJ, Cassel CK, eds. Approaching death: improving care at the end of life. Washington, D.C.: National Academy Press, 1997.
2. Lo B, Rubenfeld GD. Palliative sedation in dying patients: "we turn to it when everything else hasn't worked." JAMA 2005;294:1810-6.
3. Drayer RA, Henderson J, Reidenberg M. Barriers for better pain control in hospitalized patients. J Pain Symptom Manage 1999;17:440.
4. Gilson AM, Mauer MA, Joranson DE. State policy affecting pain management: recent improvements and the positive impact of regulatory health policies. Health Policy 2005;74:192-204.
5. Tucker KL. The debate on elder abuse for undertreated pain. Pain Med 2004;5:214-7.

Searching for the Right Search — Reaching the Medical Literature

Robert Steinbrook, M.D.

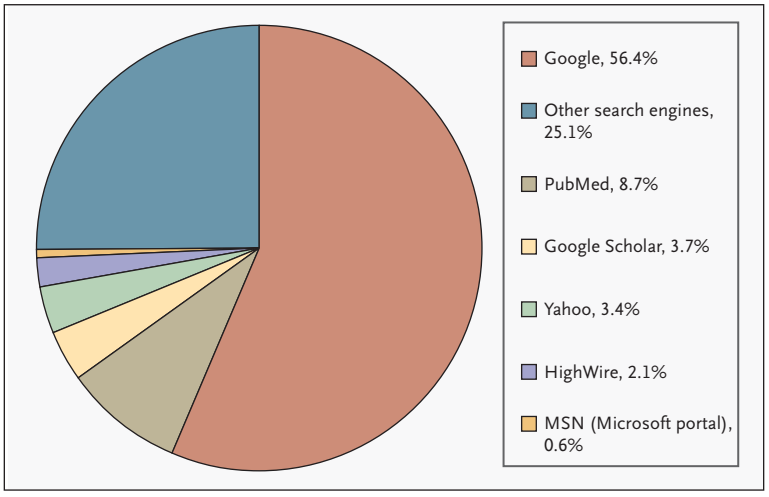
Web-based search engines are transforming our use of the medical literature. Although we continue to read the print issues of journals and to browse current issues online, we are now using links from Google — the flagship search engine of the Mountain View, California, company of the same name — and other search engines, as well as citation links in other articles, to gain direct access to the articles we want. For example, by quickly searching by the title of an article, an author, or a specific topic, we can often link to a bibliographic citation, the abstract, or the online version of the article itself. In addition, an increasing number of biomedical libraries and medical institutions can link from search results to their online subscriptions to journals, their electronic catalogue of print holdings, publishers' Web sites, and other full-text digital archives.

The ongoing changes are part of a broader trend in society. According to a May 2005 report from the Pew Internet and American Life Project, 8 in 10 Internet users have looked for health information online, showing increasing interest in diet, fitness, drugs, health insurance, experimental treatments, and particular doctors and hospitals.[1] About three quarters of scholarly journals are now online. The Web sites of most biomedical journals continue to see "marked increases in usage, with no end in sight," according to John Sack, the director of HighWire Press, a division of the Stanford University Libraries (highwire.stanford.edu). HighWire hosts the Web sites of about 918 scholarly publications (including that of the *Journal*) and maintains a large archive of full-text articles. Articles found by searching the Web (see box) are a logical evolution from the photocopies of articles that medical students and residents have pulled out of their backpacks for decades.[2]

"What readers see now are articles," Sack said recently. "They don't see articles bound in the context of issues or in the context of well-known journals. This has been happening for a while, but it has been greatly accelerated by the Internet and by Google and other search engines that are indexing everything that is out there."

There are many search engines and many ways to gain access to the online medical literature. At the moment, however, Google is the most widely used. Other widely used search tools are PubMed, a federal government portal that offers access to the enormous database of citations and abstracts at the National Library of Medicine; Google Scholar, which specifically searches the scholarly literature; and Yahoo, the search engine of the Sunnyvale, California, company of the same name. These search engines are available to anyone who has an Internet connection; none require registration, and searching is free of charge.

The rapid changes are illustrated by data compiled by HighWire Press. In June 2005, Google provided the majority (56.4 percent) of the referrals from search engines to articles in HighWire-hosted journals (see pie chart). PubMed accounted for 8.7 percent, Google Scholar 3.7 percent, and Yahoo 3.4 percent. Google



Referrals from Search Engines to Web Sites of 844 Journals Hosted by HighWire Press.

Data are for June 2005 and are from HighWire Press.

Scholar has been available only since late 2004, and many people remain unaware of it.

When he first saw similar data earlier in the year, Sack recalled, he was "surprised that Google had greatly surpassed PubMed and that a new product such as Google Scholar had approached half of PubMed's referrals within a few months." He added, "The data indicate that readers' habits for finding information are shifting. New readers are using the new search tools, not the old ones." In September 2005, the percentage of referrals from Google Scholar to HighWire-hosted journals surpassed the percentage from PubMed. By November 2005, there were 35.7 percent more referrals from Google Scholar than from PubMed. The reason is that although the total percentage of referrals from either Google or Google Scholar is about the same, more people are using Google Scholar. The percentage of referrals from Yahoo has also increased, but not as rapidly.

In the past, searching has often started with PubMed, which was launched in June 1996. The largest component of PubMed is the Medical Literature Analysis and Retrieval System Online, more commonly known as Medline, which covers more than 4800 biomedical journals published in the United States and 70 other countries, primarily from 1966 to the present. Medline contains

Choosing among Search Engines

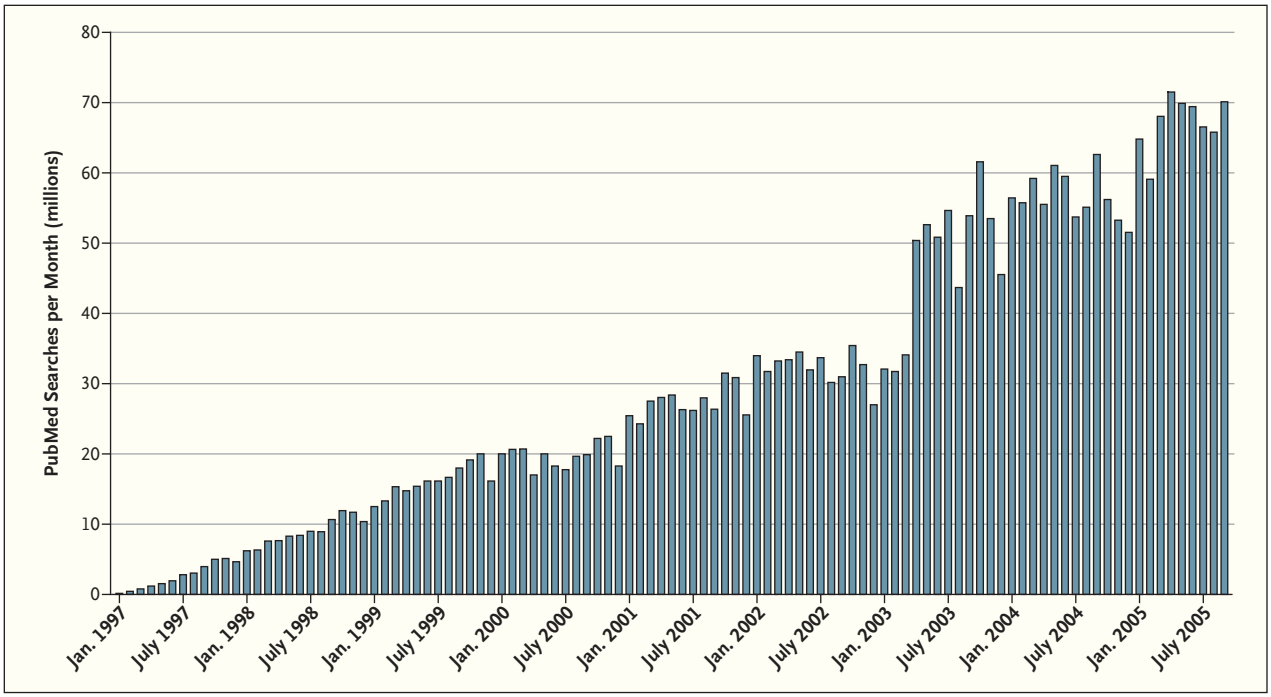
Whether one should search using PubMed (www.ncbi.nlm.nih.gov/entrez/query.fcgi), Google (www.google.com), Google Scholar (scholar.google.com/), Yahoo (www.yahoo.com), or another search engine depends on the information one desires, one's personal preference, and the search engine's strengths and weaknesses. Google, Google Scholar, and Yahoo are easy to use and provide breadth, but the results will vary widely depending on the search terms that are chosen. These search engines index the contents of PubMed as well as the online content of many scholarly publishers. Google searches have "lots of hits and lots of misses," according to Dr. Daniel Masys, chair of the Department of Biomedical Informatics at Vanderbilt University Medical Center. PubMed can provide more depth but may require more effort to use; training may help searchers to obtain the best results. In many instances, the choice will not matter, because several approaches will work. For more in-depth searching, the use of several search engines may provide complementary results.

Google and Yahoo are general search engines. PubMed and Google Scholar primarily find journal articles, the former from the life sciences and related fields, the latter from all areas of research. PubMed can sort results according to date, author, or journal but not according to the number of citations. According to Dr. Mohammad Al-Ubaydli, a visiting research fellow at the National Library of Medicine, who has studied the characteristics of various search engines, results with Google and Google Scholar can be narrowed with the use of more specific search terms — by entering "myocardial infarction thrombolysis," for instance, rather than simply "myocardial infarction." Google Scholar orders the results according to how relevant to the query it deems references to be, taking into account the full text of the article, the author, the publication in which it appeared, and how often it has been cited in other scholarly publications. Searches can be limited to an author, a publication, or a range of dates. Widely cited and important papers in a given field will often appear at the top of the results, with newer papers and others with fewer citations appearing near the end. Google Scholar also includes a "cited by" feature that links an article to the others that have cited it.

PubMed searches define databases that have extensive indexing and quality control. In addition to searching the text for particular words, it uses a controlled-vocabulary thesaurus of medical subject headings, known as MeSH. This feature permits searching with various degrees of specificity. However, "most human beings on the planet who are not librarians don't know anything about how to search with MeSH," said John Sack, the director of HighWire Press. Clinical searches through PubMed can be limited to one of four study categories (therapy, diagnosis, etiology, or prognosis), studies conducted in humans, or studies with particular types of research methods, such as systematic reviews, among other options.

Google Scholar is more difficult to focus than PubMed — but it may find papers, theses, books, preprints, abstracts, and technical reports that are not in National Library of Medicine databases. However, Google Scholar does not identify the sources that it has — or has not — indexed. Thus, there is no way to know what may be missing. Google Scholar is separate from Google Book Search, which searches the full text of books and which is related to Google's widely publicized project to digitize most of the books from several major libraries.

There are many proprietary medical resources and databases that are currently not publicly searchable by means of any Internet search engine, although subscribers may use them through the Web. Publishers have control over access to their articles. A search engine can index only the material that it identifies, "crawls," and processes. Google will index papers with access restrictions only if all users of its search tools are offered at least an abstract or an extract. The situation, however, is in flux. For example, Yahoo has a feature that searches content — with the permission of the source — that is not normally accessible. Access to the content, however, usually requires a subscription to the publication.



PubMed Searches per Month, January 1997 through September 2005.

Data are from the National Center for Biotechnology Information at the National Library of Medicine. The increase in the number of searches in the spring of 2003 reflects changes in the Web-log accounting systems; previously, the number of searches was slightly undercounted.

more than 12 million citations. PubMed contains additional citations and journals, including about 2 million citations to articles published between 1950 and 1965, and searches can extend to other databases at the National Library of Medicine, such as GenBank, PubChem, and PubMed Central (www.pubmedcentral.nih.gov), the National Institutes of Health's (NIH's) archive of biomedical and life-sciences literature.

The number of searches performed with PubMed has increased steadily to about 70 million per month (see bar graph). Yet at the same time, an increasing number of people are finding their way to citations and abstracts in PubMed through searches that begin with Google — the largest single source of referrals to PubMed — or with Google Scholar or Yahoo.

Many articles are available through Web sites maintained by journals, although there may be charges or registration requirements. Some are also available without charge through nonjournal Web sites — sometimes with the permission of the publisher, sometimes without.[3] Such sites may be personal ones established by an author or online repositories maintained by the author's institution or another institution. Archiving through nonjournal sites is incomplete, however, and it is more likely to be available for basic research articles than for clinical research articles. Some journals and publishers — as well as Web sites and Web-based links — come and go. And search engines do not store content and make it available to readers. Rather, they provide links to the actual sources of content, and they can identify only content that they have successfully indexed (see box).

Because of the limits of other online sources, central electronic repositories of journals and articles serve a critical archival function, according to Dr. David Lipman, the director of the National Center for Biotechnology Information at the National Library of Medicine, home to PubMed and PubMed Central. Within the year, PubMed Central is expected to contain between 700,000 and 800,000 reports, including many articles from back issues of about 180 journals.[4] Central repositories can also store supplemental data and may permit more detailed searches and a greater ability to retrieve and manipulate the underlying information than is possible with papers that may be archived in different formats

at different sites. "Biomedical research has changed," noted Lipman. "Every paper has more and more data. People are not just reading these papers. Researchers want to compute on the underlying data."

The NIH is seeking to expand public access to the research it sponsors and to increase the usefulness of PubMed Central. As of May 2, 2005, the NIH has asked the investigators it supports to submit voluntarily to PubMed Central an electronic copy of any scientific report, on acceptance for publication, and to specify when the article should become publicly available through the repository.[4] According to the policy, posting for public accessibility "is requested and strongly encouraged as soon as possible (and within 12 months of the publisher's official date of final publication)." However, the initial response to the voluntary policy has been slow.

With 100 percent participation, about 5500 peer-reviewed manuscripts that have been accepted but not yet published — equivalent to about 10 percent of the articles indexed monthly by PubMed — would be submitted to PubMed Central each month, according to Lipman. As of July 9, 2005, 340 such unpublished manuscripts (or about 165 per month) had been submitted — a participation rate of only 3 percent. There are no signs that the participation rate for unpublished manuscripts is increasing — in August, September, and October of 2005, it was between 2.2 and 2.7 percent. In December 2005, Senators Joseph Lieberman (D-Conn.) and Thad Cochran (R-Miss.) introduced legislation that would require the public posting of all NIH-funded peer-reviewed manuscripts at PubMed Central within six months of their publication. Failure to comply could result in the loss of public funding for federal employees or grantees.

Physicians and researchers have extremely diverse information needs. Meeting these needs requires diverse resources. Search engines and the Internet are not only changing the medical literature. They are also challenging the traditional economics of scholarly publishing and fueling heated debate about the extent to which the biomedical literature should be accessible online and available without charge to the user.[4,5] As search engines and the online medical literature itself continue to evolve, the pace of change is likely to increase.

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

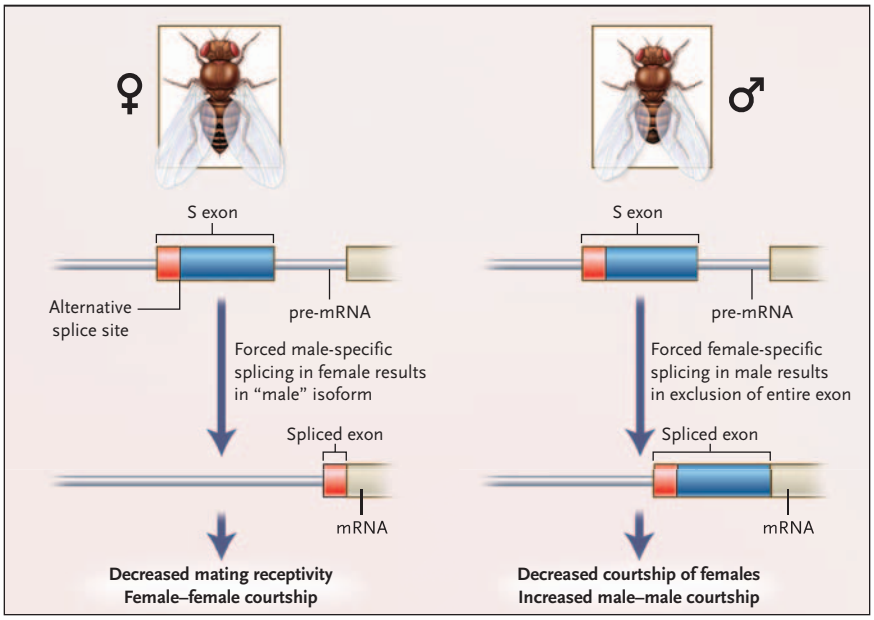
1. Fox S. Health information online. Washington, D.C.: Pew Internet & American Life Project, May 17, 2005. (Accessed December 14, 2005, at http://www.pewinternet.org/PPF/r/156/report_display.asp.)
2. Sack J. HighWire Press: ten years of publisher-driven innovation. Learned Publ 2005; 18:131-42.
3. Wren JD. Open access and openly accessible: a study of scientific publications shared via the Internet. BMJ 2005;330:1128-31.
4. Steinbrook R. Public access to NIH-funded research. N Engl J Med 2005;352:1739-41.
5. Wysocki B. Scholarly journals' premier status is diluted by Web. Wall Street Journal. May 23, 2005:A1.

Is Our Behavior Written in Our Genes?

Dennis Drayna, Ph.D.

Scientists recently reached an important milestone in the understanding of genetic contributions to behavior. A new study demonstrated the role of a single gene in specifying sexual behavior in the fruit fly *Drosophila melanogaster*.[1] The findings prompt provocative thinking about the contribution of genetic factors to sexual orientation in humans, as well as about genes that might underlie a broader spectrum of human behaviors.

The investigators in the fruit-fly study, Demir and Dickson, focused on a gene called *fruitless* that has long been known to have strong effects on mating, fertility, and reproduction in fruit flies. The messenger RNA product of this gene (see figure) encodes a transcription factor that is essential for development and that can occur in any of several variously spliced forms. Two of these forms are sex-specific, one being unique to male flies and the other to female flies. Demir and Dickson used genetic manipulation to produce anatomically female flies that carried only the male form of the gene (see figure). The resulting flies exhibited courtship and mating behavior toward females that is normally engaged in only by male flies. Whereas previous studies have shown that the male form of the *fruitless* gene is necessary for male courtship, the new study shows that it is sufficient to produce this behavior, even in females — making it the first single gene to be identified as both necessary and sufficient for specifying a complex behavior in a higher-level organism.



Splicing Sexual Behavior.

A study by Demir and Dickson[1] showed that a single gene is sufficient to specify behavior in the fruit fly. The authors generated female flies that spliced the *fruitless (fru)* gene in a male-specific manner, and male flies that spliced the *fru* gene in a female-specific manner. The modified females showed a reduction in receptivity to mating and were likely to court other females; the modified males showed a disinclination to court females and were more likely to court other males than were control males. S denotes sex-specific, and mRNA messenger RNA.

What other forms of behavior with such complex manifestations might prove to have such a simple origin? Is it conceivable that complex behaviors in humans could be specified by a single gene? Could these results deepen our understanding of human sexual orientation or sexual behavior?

Behavioral genetics has long been hampered by the fact that a vast array of structures and functions in the human body are required to produce a behavior, and the failure of any one of them can render that behavior impossible for a given person. Thus, it is not difficult to show that a gene is necessary for behavior, but such a demonstration is often not very informative. Mutations that result in defects in the bones of the arm may prevent humans from playing the violin, for example, but what we would really like to know about are the neural functions that underlie humans' apparently unique ability to produce and appreciate music.

The scientific issues surrounding the general problem of the influence of genetic factors on behavior have been laid out,[2] and researchers have found particular behaviors in several different model organisms that seem likely to be determined by single genes — for instance, the foraging behavior of drosophila larvae and the social behavior of nematodes. Among higher-level organisms, it is known that genetic factors specify the nature and quantity of provisions that parrots gather for their nests and the types of nests that mice build. However, the sophisticated genetic manipulations we can undertake in fruit flies cannot yet be performed in these other organisms, so we do not have unequivocal proof of the role of any particular gene.

Key characteristics of these genetically influenced types of behavior are that they are highly instinctive and consist of a series of programmed actions that directly affect the survival and reproduction of the organism. As such, these behaviors can be directly affected by natural selection. Indeed, since natural selection acts by affecting the genes of a species, it would not be surprising to find a strong influence of genetic factors in generating this class of behaviors.

Humans have highly developed cortical functions that control behavior by integrating many different sensory inputs and motivations; moreover, these functions are highly plastic and susceptible to modification by experience. Most human behavior seems likely to be insulated from the effects of natural selection and therefore is unlikely to be associated with the action of one gene or a few genes.

Nevertheless, humans do display some simple reflex behaviors, such as the hand-grasping (Darwinian reflex) and startle (Moro reflex) responses of infants. The other hallmark of single-gene behavioral control in lower-level organisms is that the gene controls a program of actions carried out by structures such as neural circuits that are specified by other genes and already in place. Dedicated neural circuits have been identified for simple muscle reflexes in a number of systems, and such circuits may also exist for some human behaviors. Beyond simple motor reflexes, other types of behavior that occur in all persons in a recurring, programmed fashion may have strong genetic influences. Such

behaviors often have important health consequences — they may, for instance, include some activities associated with food intake, sleep and wakefulness, and even tobacco use.

Despite being variable and subject to strong cultural influences, human sexual and reproductive behavior has some components that are probably instinctive. Together with existing evidence that human sexual orientation has a genetic component, this instinctive element raises the question of whether sexual orientation or aspects of sexual behavior in humans could be determined by the action of one or a few genes — a provocative hypothesis, but one that is not addressed by the results of Demir and Dickson. The fruit fly has no neural functions comparable to those of the human cerebral cortex (which has a large role in most human sexual behavior). There is evidence that male sexual orientation in humans — in particular, male homosexual orientation — has some characteristics of an instinct. The sexual orientation of the human male is a consistent feature that is under neural control, that generally leads to specific behaviors, and that is thought to have a strong biologic basis.[3] However, detailed genetic studies of male sexual orientation have produced conflicting results. The sum of the data suggests a role for specific genes on specific chromosomes, but no individual genes have been identified.

Human genes are not subject to experimental manipulation, and there can be strong political resistance to certain types of research into human sexual behavior. As a result, it may take some time to accumulate evidence that any particular gene is necessary and sufficient to specify sexual orientation or a particular sexual behavior in humans. More generally, human behavior is an exceedingly complex phenomenon and cannot be viewed as the product of a set of genes. Nevertheless, our behaviors that are instinctive and crucial to survival and reproduction are likely to be subject to simple genetic control. Such behaviors might include those necessary to maintain homeostasis — such as eating, drinking, excreting, and thermal regulation — and those associated with mating and the maternal care of infants.

Dr. Drayna is the acting chief of the Section on Systems Biology of Communication Disorders, National Institute on Deafness and Other Communication Disorders, Rockville, Md.

1. Demir E, Dickson BJ. *Fruitless* splicing specifies male courtship behavior in *Drosophila*. Cell 2005;121:785-94.

2. Baker BS, Taylor BJ, Hall JC. Are complex behaviors specified by dedicated regulatory genes? Reasoning from *Drosophila*. Cell 2001; 105:13-24.

3. Mustanski BS, Chivers ML, Bailey JM. A critical review of recent biological research on human sexual orientation. Annu Rev Sex Res 2002;12:89-140.

THIS WEEK *in the* JOURNAL

ORIGINAL ARTICLE

Efficacy and Safety of a G1P[8] Rotavirus Vaccine

In this double-blind trial, two oral doses of a live attenuated G1P[8] rotavirus vaccine were highly efficacious in protecting infants against severe diarrheal disease. During the active surveillance of 63,225 infants, the risk of intussusception was no greater after vaccination than it was with placebo (six cases vs. seven cases).

SEE P. 11; EDITORIAL, P. 75; CME, P. 107

ORIGINAL ARTICLE

Human–Bovine Reassortant Rotavirus Vaccine

In this randomized trial, the clinical efficacy of an oral, live pentavalent human–bovine reassortant vaccine was estimated to be 98.0 percent against severe gastroenteritis due to rotavirus. In the safety study, which included 68,038 infants, the rates of intussusception were similar in the vaccine and placebo groups (relative risk, 0.8; 95 percent confidence interval, 0.3 to 1.8).

SEE P. 23

ORIGINAL ARTICLE

Cisplatin and Paclitaxel in Ovarian Cancer

In a trial of adjuvant chemotherapy for ovarian cancer, a regimen of intravenous paclitaxel plus intraperitoneal cisplatin and paclitaxel was superior to intravenous paclitaxel plus intravenous cisplatin.

SEE P. 34; EDITORIAL, P. 77

CURRENT CONCEPTS

Management of Bacterial Meningitis

This review summarizes recent changes in the treatment of adults with community-acquired bacterial meningitis. It explains the initial assessment and management, the use of adjunctive corticosteroids, and intensive care monitoring. The authors detail the approach to complications such as transtentorial herniation, hydrocephalus, and focal seizures.

SEE P. 44; CME, P. 106

MEDICAL PROGRESS

Autoimmune Hepatitis

Autoimmune hepatitis is a generally progressive, sometimes fluctuating chronic hepatitis of unknown cause that occurs in children and adults of all ages. It is important to distinguish autoimmune hepatitis from other forms of chronic hepatitis, because a high percentage of cases respond to antiinflammatory or immunosuppressive therapy, or both. Appropriate management can prolong survival, improve quality of life, and avoid liver transplantation. This review discusses the pathogenesis, diagnosis, and treatment of this important form of hepatitis.

SEE P. 54; CME, P. 105

CLINICAL PROBLEM-SOLVING

Needle in a Haystack

A 63-year-old man presented to the emergency department with shortness of breath that had begun the evening before, after he had gone to bed, and worsened progressively during the night. He had had no fevers, chills, cough, hemoptysis, chest pain, or peripheral edema and had no history of congestive heart failure. Five months earlier, a pulmonary embolus had been diagnosed, for which he received warfarin maintenance therapy; the results of prothrombin-time testing, expressed as an international normalized ratio, were consistently above 2.0.

SEE P. 68

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Targeting Rheumatoid Arthritis

A kinase inhibitor protected against synovial inflammation and joint destruction in a mouse model of rheumatoid arthritis.

SEE P. 80

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Safety and Efficacy of an Attenuated Vaccine against Severe Rotavirus Gastroenteritis

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ABSTRACT

BACKGROUND

The safety and efficacy of an attenuated G1P[8] human rotavirus (HRV) vaccine were tested in a randomized, double-blind, phase 3 trial.

METHODS

We studied 63,225 healthy infants from 11 Latin American countries and Finland who received two oral doses of either the HRV vaccine (31,673 infants) or placebo (31,552 infants) at approximately two months and four months of age. Severe gastroenteritis episodes were identified by active surveillance. The severity of disease was graded with the use of the 20-point Vesikari scale. Vaccine efficacy was evaluated in a subgroup of 20,169 infants (10,159 vaccinees and 10,010 placebo recipients).

RESULTS

The efficacy of the vaccine against severe rotavirus gastroenteritis and against rotavirus-associated hospitalization was 85 percent ($P<0.001$ for the comparison with placebo) and reached 100 percent against more severe rotavirus gastroenteritis. Hospitalization for diarrhea of any cause was reduced by 42 percent (95 percent confidence interval, 29 to 53 percent; $P<0.001$). During the 31-day window after each dose, six vaccine recipients and seven placebo recipients had definite intussusception (difference in risk, –0.32 per 10,000 infants; 95 percent confidence interval, –2.91 to 2.18; $P=0.78$).

CONCLUSIONS

Two oral doses of the live attenuated G1P[8] HRV vaccine were highly efficacious in protecting infants against severe rotavirus gastroenteritis, significantly reduced the rate of severe gastroenteritis from any cause, and were not associated with an increased risk of intussusception. (ClinicalTrials.gov numbers, NCT00139347 and NCT00263666.)

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Rotavirus is the leading recognized cause of diarrhea-related illness and death among infants and young children.[1-5] Every year, rotavirus is associated with 25 million clinic visits, 2 million hospitalizations, and more than 600,000 deaths worldwide among children younger than five years of age.[6,7] Development of a safe and effective rotavirus vaccine is therefore a high priority, particularly but not exclusively in developing countries, where the burden of disease is highest.[8,9] Since the withdrawal from the market of the tetravalent rhesus–human reassortant vaccine (RotaShield, Wyeth Laboratories) because of an association with intussusception,[10,11] ruling out such a risk has become critical for the licensure and universal use of any new rotavirus vaccine.

A live attenuated human rotavirus (HRV) vaccine containing the RIX4414 strain of G1P[8] specificity[12] has been developed from the parent vaccine strain 89-12.[13-15] Clinical trials with the HRV vaccine in Finnish[16] and Latin American[17] (Brazilian, Mexican, and Venezuelan) infants showed that two doses were well tolerated and immunogenic. In phase 2 clinical trials, the efficacy of the vaccine against severe rotavirus gastroenteritis reached 90 to 100 percent.[16-18] Protection started as early as the first dose, lasted until the subjects were up to two years of age, and was demonstrated against both G1P[8] and G9P[8] rotaviruses.[16-18]

Although the initial trials had included 6670 infants, a larger, multinational, randomized, double-blind, placebo-controlled, phase 3 trial was required to evaluate any potential risk of intussusception within 31 days after administration of each of two oral doses of the HRV vaccine, as well as any other serious adverse events. Other end points were assessed to confirm previously reported evidence that two oral doses of the HRV vaccine are efficacious against severe rotavirus gastroenteritis, to define the effect of vaccination on the burden of severe diarrhea of any cause, and to extend the observations of protection against different circulating strains in infants up to one year of age.

METHODS

STUDY DESIGN AND PARTICIPANTS

Investigators from Argentina, Brazil, Chile, Colombia, the Dominican Republic, Honduras, Mexico, Nicaragua, Panama, Peru, Venezuela, and Finland recruited infants at public pediatric clinics or hospitals for this randomized, double-blind, placebo-controlled, phase 3 trial. The study protocol and informed-consent document were approved by ethics review committees at each center, and the study was conducted in accordance with the Declaration of Helsinki guidelines for good clinical practice.

After a parent or guardian had provided written informed consent, 6-to-13-week-old healthy infants were enrolled. The infants were randomly assigned to receive two oral doses of either the HRV vaccine or placebo — the first dose at visit 1 and the second at visit 2, one to two months later. After the administration of the second dose, the overall cohort was followed for a median duration of 100 days after the first dose for the assessment of any adverse events, including the occurrence of intussusception (the safety cohort, evaluated at visit 3), and a subgroup of infants was followed for 9 to 10 months for the assessment of efficacy (the efficacy cohort, evaluated at visit 4).

Cases of intussusception, severe gastroenteritis, and serious adverse events were the outcomes captured by an active-surveillance system implemented six months before initiation of the study in all medical facilities able to receive infants with these outcomes (as described in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Outcomes were recaptured during the scheduled visits, if missed by the active-surveillance system.

VACCINE

The HRV vaccine (Rotarix, GlaxoSmithKline Biologicals) contained $10^{6.5}$ median cell-culture infective doses of the RIX4414 vaccine strain. The placebo had the same constituents as the active vaccine but without the vaccine virus. After the vaccine or placebo had been reconstituted with 1.3 ml of liquid calcium carbonate buffer, an oral dose was administered in a blinded manner to infants when they were approximately two months of age and again when they were four months of age. Infants received routine immunizations according to local regulations; oral poliovirus vaccination was provided at least two weeks before or after the administration of a dose of the HRV vaccine.

DEFINITION OF CASES

All possible cases of intussusception identified by active surveillance were analyzed by an independent

clinical-events committee. Using the case definition from the Brighton Collaboration Working Group on Intussusception[19] and remaining blinded to study-group assignments, this committee categorized cases of intussusception as definite, probable, or possible, according to the certainty of the diagnosis. A case of definite intussusception required confirmation at surgery or autopsy or with the use of imaging techniques, such as imaging with gas- or liquid-contrast enema or abdominal ultrasonography (as described in the Supplementary Appendix).

The clinical case definition of an episode of severe gastroenteritis was an episode of diarrhea (the passage of three or more loose or watery stools within a 24-hour period), with or without vomiting, that required overnight hospitalization or rehydration therapy equivalent to World Health Organization (WHO) plan B (oral rehydration therapy) or plan C (intravenous rehydration therapy) in a medical facility such as a hospital, clinic, or supervised rural health care center. To quantify the severity of gastroenteritis, the same scale used in the evaluation of the rhesus–human reassortant rotavirus vaccine[20] was implemented. It is a widely used scoring system[21] referred to as the Vesikari scale, with scores ranging from 0 to 20 (where higher scores indicate greater severity). An episode of gastroenteritis with a score of 11 or greater was considered a severe episode.[21]

ASSESSMENT OF SAFETY

Serious adverse events were defined as any new health-related problems that resulted in death, were life-threatening, necessitated hospitalization or prolongation of existing hospitalization, or resulted in disability or incapacity. Thus defined, serious adverse events included intussusception. Investigators asked parents about the occurrence of serious adverse events at each follow-up visit and recorded this information. To standardize the reporting of serious adverse events, medical terms used by investigators were analyzed at two levels, according to the Medical Dictionary for Regulatory Activities (MedDRA)[22]: one level was that of the unique "preferred term," and the other that of the "system organ class," which is a grouping of related preferred terms.

An independent data-monitoring committee of expert clinicians who were not blinded to the study-group assignments and an independent statistician were empowered to stop the trial. They periodically reviewed all serious adverse events, including intussusception. A blinded safety-review committee independently reviewed all cases involving death to assign a primary cause of death and to determine associated secondary diagnoses and other underlying conditions.

LABORATORY ANALYSIS

Stool specimens from each infant with severe gastroenteritis were tested for rotavirus by means of enzyme-linked immunoassay (Rotaclone, Meridian Bioscience)[23-25] at GlaxoSmithKline Biologicals (details are provided in the Supplementary Appendix). Rotavirus serotyping and identification of the vaccine virus were performed by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analysis followed by a reverse hybridization assay at Delft Diagnostic Laboratory.[26] Testing for other enteropathogens was not part of the study protocol and was left to the discretion of the individual investigators or sites.

END POINTS

The primary and secondary safety objectives were to assess the risk of definite intussusception within 31 days after the administration of each vaccine dose and to assess the occurrence of serious adverse events, including intussusception, during the entire study period. The primary efficacy end point was the prevention of severe rotavirus gastroenteritis, according to the case definition, from two weeks after the second dose (i.e., after completion of the full vaccination course) until one year of age. The secondary end points were efficacy against severe rotavirus gastroenteritis defined according to the Vesikari scale, efficacy against gastroenteritis associated with specific circulating rotavirus types, and efficacy against severe rotavirus gastroenteritis occurring after the first dose. Other end points were the prevention of hospitalization due to rotavirus gastroenteritis, of hospitalization for any reason, and of severe gastroenteritis from any cause.

STATISTICAL ANALYSIS

The numbers of infants with intussusception within 31 days after receipt of a dose of vaccine or placebo and during the entire safety-surveillance period were compared between the study groups. The asymptotic standardized 95 percent confidence interval for the difference in risk between the groups and for the relative risk in the vaccine

group as compared with the placebo group was calculated.[27] According to prespecified criteria, the primary safety objective would be met if the two-sided 95 percent confidence interval of the difference in the risk of intussusception within 31 days after vaccination was less than 6 per 10,000 and included zero (as described in the Supplementary Appendix). Serious adverse events, reasons for hospitalization, and primary causes of death were categorized according to the MedDRA classification system and compared between the groups with use of the two-sided asymptotic standardized 95 percent confidence interval for the difference in risk, without adjustment for multiple testing. A two-sided asymptotic score test for the null hypothesis of identical incidence in the two groups (alpha level, 0.05) was used to screen for potential differences between the two groups. All infants who had received at least one dose of the study vaccine or placebo were included in the safety analysis.

Assuming an attack rate of 1.5 percent for severe rotavirus gastroenteritis in the placebo group,[6] a true vaccine efficacy of 70 percent,[11] and a 10 percent withdrawal rate, we calculated that a sample of 20,000 infants would provide at least 80 percent power to detect a lower limit of the 95 percent confidence interval for vaccine efficacy of greater than 50 percent. The number of children enrolled varied among countries and centers because enrollment above a minimum target sample size was competitive.

In the efficacy study, an "according-to-protocol" cohort was used to calculate the efficacy of the vaccine and included participants who completed the full two-dose vaccination course and for whom compliance with the protocol was complete (as shown in Fig. 1 of the Supplementary Appendix). The overall efficacy cohort was used to calculate efficacy beginning at time of administration of the first dose and included all infants who received at least one dose of either vaccine or placebo. For each efficacy end point, the percentages of infants for whom at least one episode was reported were compared between the groups and expressed in terms of the relative risk. Vaccine efficacy was calculated with its 95 percent confidence interval with use of the following formula: (1 − relative risk) × 100. The 95 percent confidence intervals for vaccine efficacy were derived from the exact confidence interval for the Poisson rate ratio.[28] In the analysis of vaccine efficacy according to each G serotype of the virus, infants were counted in each G-type category when more than

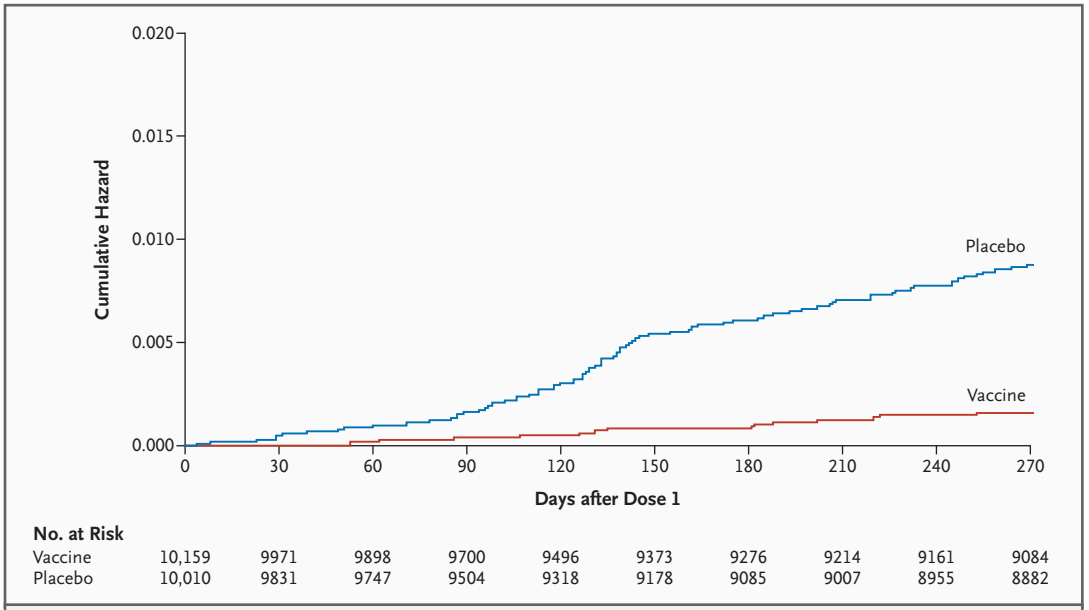

Figure 1. Cumulative Hazard of a First Episode of Severe Gastroenteritis.

The cumulative hazard of a first episode was estimated as a minus-log transformation (of log data to nonlog data) of the Kaplan–Meier survival curve during the period from dose 1 until one year of age among all the infants who received at least one dose of either vaccine or placebo. The difference between receiving placebo and receiving vaccine was significant ($P<0.001$ by the log-rank test).

one G type was isolated for a given episode. The cumulative hazard of a first episode of severe rotavirus gastroenteritis between comparative groups was estimated as a minus-log transformation of the Kaplan–Meier survival curve.

The P value for the cumulative-hazard curve was calculated with the use of the log-rank test. All other reported P values are two-sided for the null hypothesis of equivalence of the two treatment groups. Data analysis was performed with the use of SAS software (version 8.2) and Proc-StatXact 5 with Windows NT 4.0. GlaxoSmithKline Biologicals held the data and performed the analyses, with continuous feedback from the academic authors. The manuscript was written jointly by the company authors and the academic authors, who vouch for the accuracy and completeness of the reported data.

RESULTS

STUDY GROUPS

A total of 63,225 infants were enrolled and were to receive two doses of the HRV vaccine or placebo between August 5, 2003, and March 12, 2004. The entire cohort (the safety cohort) was followed for safety until July 23, 2004, when the last subject completed visit 3. From this cohort, the first 20,169 infants were enrolled in the evaluation of efficacy and were followed until they were one year of age (efficacy cohort) (Fig. 1 of the Supplementary Appendix). In both cohorts, the vaccine and placebo groups were similar in terms of sex distribution and distribution of race or ethnic background and in terms of mean age at the time of each vaccination and at the end of the safety and efficacy follow-up periods (Table 1). The proportions of infants who were withdrawn from the study and the reasons for withdrawal were similar between the groups (Fig. 1 of the Supplementary Appendix).

INTUSSUSCEPTION

In the safety cohort of 63,225 children, 26 cases of intussusception were detected by capture–recapture methods during hospital surveillance or by active follow-up; 25 cases were determined to be definite intussusception.[19] Thirteen cases of definite intussusception were diagnosed within 31 days after administration of either dose, six in the vaccine group and seven in the placebo group (respective incidence rates, 1.89 and 2.21 per 10,000 infants; difference in risk, −0.32 per 10,000 infants; 95 percent confidence interval, −2.91 to 2.18; relative risk in the vaccine group, 0.85; P=0.78) (Table 2). Twelve cases of intussusception, three in the vaccine group and nine in the placebo group, were reported after the 31-day window (difference in risk, −1.91 per 10,000 infants; 95 percent confidence interval, −4.58 to 0.29; P=0.08). Thus, during the entire safety-surveillance period (median duration, 100 days after dose 1), intussusception occurred in 9 vaccine recipients and 16 placebo recipients (incidence

Table 1. Characteristics of the Study Populations, According to Study Group.*

Characteristic	Safety Study		Efficacy Study	
	HRV Vaccine	Placebo	HRV Vaccine	Placebo
Infants — no.	31,673	31,552	10,159	10,010
Male sex — no. (%)	16,105 (50.8)	16,150 (51.2)	5100 (50.2)	5160 (51.5)
Age — wk				
At dose 1	8.2±2.39	8.2±2.39	8.4±2.39	8.4±2.38
At dose 2	15.8±3.75	15.8±3.79	16.3±3.74	16.3±3.78
At end of safety or efficacy follow-up	22.7±5.3	22.7±5.3	50.8±10.4	50.5±10.6
Race or ethnic background — no. (%)†				
Hispanic	25,729 (81.2)	25,648 (81.3)	8776 (86.4)	8651 (86.4)
White	3,488 (11.0)	3,434 (10.9)	780 (7.7)	738 (7.4)
Other‡	2,456 (7.8)	2,470 (7.8)	603 (5.9)	621 (6.2)

* Plus–minus values are means ±SD. HRV denotes human rotavirus.
† Race or ethnic group was determined by the investigators.
‡ Other races and ethnic backgrounds included African, South Asian, Arabic or North African, Aborigine, Afro-Caribbean, Caribbean, mixed race, and Indian.

rates, 2.84 and 5.07 per 10,000 infants; difference in risk, –2.23 per 10,000 infants; 95 percent confidence interval, –5.70 to 0.94; P=0.16) (Table 2).

Of the 25 cases of definite intussusception, 10 occurred after dose 1 (in three vaccinees and seven placebo recipients) and 15 occurred after dose 2 (in six vaccinees and nine placebo recipients). There was no temporal cluster of intussusception cases after either dose. Most of the cases (15 of 25) occurred at four to five months of age. The intussusception was reduced by enema in 6 infants (2 vaccinees and 4 placebo recipients) and by surgery in 19 infants (7 vaccinees and 12 placebo recipients). After hospitalization (mean duration, five days), all the infants had a complete recovery (as described in the Supplementary Appendix).

SERIOUS ADVERSE EVENTS

In the safety cohort, significantly fewer serious adverse events were reported in the vaccine group than in the placebo group (293.0 vs. 331.8 events per 10,000 infants, P=0.005) (Table 2). Serious adverse events related to gastroenteritis, such as diarrhea, vomiting, dehydration, and hypovolemic shock, were reported in fewer vaccinees than placebo recipients. The hospitalization rate was also lower in the vaccine group than in the placebo group (279.7 vs. 317.9 hospitalizations per 10,000 infants, P=0.005) (Table 2). A post hoc exploratory analysis revealed a reduction of 42 percent (95 percent confidence interval, 28.6 to 53.1 percent) in the vaccine group in the need for hospitalization for gastroenteritis or diarrhea of any cause during the 100-day observation period (100 hospitalizations, vs. 179 hospitalizations in the placebo group; P<0.001).

Overall mortality did not differ significantly between the vaccine recipients and the placebo recipients. Fifty-six deaths occurred in the vaccine group, and 43 in the placebo group (P=0.20) (Table 2); 4 and 2, respectively, were related to diarrhea (P=0.41). The causes of diarrhea in those cases were not determined, because stool samples were not available.

Further analysis of the deaths stratified at the level of MedDRA preferred terms suggested that there was a potential imbalance of deaths due to

Table 2. Risk of Definite Intussusception and Other Serious Adverse Events among Infants Receiving Vaccine or Placebo.*

Adverse Event	HRV Vaccine (N=31,673)		Placebo (N=31,552)		Difference in Risk per 10,000 Infants (95% CI)†	Relative Risk (95% CI)‡	P Value§
	No. of Events	Incidence Rate¶	No. of Events	Incidence Rate¶			
Definite intussusception‖							
≤31 Days after either dose‖	6	1.89	7	2.21	–0.32 (–2.91 to 2.18)	0.85 (0.30 to 2.42)	0.78
≤31 Days after dose 1‖	1	0.31	2	0.63	–0.32 (–2.03 to 1.20)	0.50 (0.07 to 3.80)	0.56
≤31 Days after dose 2‖	5**	1.57	5††	1.58	–0.01 (–2.48 to 2.45)	0.99 (0.31 to 3.21)	0.99
Between dose 1 and visit 3‡‡	9	2.84	16	5.07	–2.23 (–5.70 to 0.94)	0.56 (0.25 to 1.24)	0.16
Serious adverse event between dose 1 and visit 3							
Overall§§	928	290.99	1047	331.83	–38.84 (–66.02 to –11.73)	0.88 (0.81 to 0.96)	0.005
Hospitalization	886	279.73	1003	317.89	–38.15 (–64.76 to –11.62)	0.88 (0.81 to 0.96)	0.005
Death	56	17.68	43	13.63	4.05 (–2.15 to 10.40)	1.30 (0.87 to 1.93)	0.20

* HRV denotes human rotavirus, and CI confidence interval.
† The difference in risk is the incidence rate in the HRV-vaccine group minus that in the placebo group.
‡ The relative risk is the risk in the HRV-vaccine group as compared with that in the placebo group.
§ P values are the results of a comparison between the groups by a two-sided asymptotic score test for the null hypothesis of identical incidence in the groups (alpha level, 0.05).
¶ The incidence rate is the number of infants with the specified serious adverse event per 10,000 infants.
‖ The 31-day postvaccination window included the day of vaccination and the 30-day period after the dose.
** Data were available for 29,616 infants.
†† Data were available for 29,465 infants.
‡‡ Visit 3 took place 30 to 90 days after dose 2.
§§ Overall serious adverse events were any untoward medical occurrence that resulted in death, was life-threatening, required hospitalization or prolonging of existing hospitalization, or resulted in disability or incapacity. These events were not necessarily mutually exclusive.

pneumonia among infants receiving the HRV vaccine. This potential imbalance was a further investigated. In 16 vaccine recipients and 6 placebo recipients, the primary cause of death was related to pneumonia (P=0.05). However, the distribution of pneumonia-related deaths within the first 31 days after vaccination did not differ statistically between the two groups (seven cases in the vaccine group and three in the placebo group). An additional analysis showed that there was no difference between the two study groups in terms of the number of serious adverse events related to pneumonia (280 in the vaccine group and 276 in the placebo group), overall pneumonia-related hospitalizations (277 and 273, respectively), or pneumonia-related hospitalizations within 31 days after the first dose (99 and 94), within 31 days after the second dose (49 and 56), or at any other time point. Data on retention within the efficacy cohort are provided in the Supplementary Appendix.

EFFICACY OF THE VACCINE AGAINST SEVERE ROTAVIRUS GASTROENTERITIS

There were 12 children in the vaccine group and 77 in the placebo group with severe rotavirus gastroenteritis according to the clinical definition (2.0 vs. 13.3 children with at least one episode per 1000 infant-years, P<0.001), resulting in a vaccine efficacy of 84.7 percent (P<0.001) against severe rotavirus gastroenteritis from two weeks after dose 2 until one year of age (Table 3). Similar results were obtained with the overall cohort of infants who received at least one dose of vaccine or placebo (vaccine efficacy from dose 1 until one year of age, 81.1 percent; 95 percent confidence interval, 68.4 to 95.3 percent; P<0.001). Hospitalization for at least one night was required for 9 children in the vaccine group and 59 in the placebo group (1.5 vs. 10.2 hospitalizations per 1000 infant-years), for a vaccine efficacy against hospitalization for severe rotavirus gastroenteritis of 85.0 percent (P<0.001) (Table 3).

The cumulative hazard of severe rotavirus gastroenteritis was significantly lower in the vaccine group than in the placebo group, both in the according-to-protocol analysis (data not shown) and in the intention-to-treat analysis (P<0.001 by the log-rank test) (Fig. 1). The difference increased with time and led to an approximately sevenfold risk of severe rotavirus gastroenteritis in the placebo group as compared with the vaccine group at one year of age.

Eleven of 12 children with episodes of severe gastroenteritis in the vaccine group and 71 of 77 in the placebo group had a Vesikari score of 11 or greater, yielding a vaccine efficacy of 84.8 percent (P<0.001). For increasing disease severity with scores between 11 and 20, the efficacy of the vaccine was increasingly higher, reaching 100 percent against more severe rotavirus gastroenteritis, defined as gastroenteritis with a Vesikari score of 19 or 20 (Table 4). Sixteen episodes of severe rotavirus gastroenteritis with a Vesikari score of 11 or greater were reported from dose 1 until dose 2 (6 in the vaccine group and 10 in the placebo group).

The type-specific[29] efficacy of the vaccine against wild-type strains is shown in Table 3. Its efficacy against severe rotavirus episodes with a Vesikari score of 11 or greater caused by type G1P[8] strains, homologous to the vaccine strain, was 90.8 percent (P<0.001). The efficacy of the vaccine against strains sharing only the P[8] antigen (G3P[8], G4P[8], and G9P[8]) was 87.3 percent (P<0.001). The type G2P[4] rotavirus, which does not share either the G or the P antigen with the vaccine strain, was detected in specimens from five infants in the vaccine group and nine in the placebo group, for an efficacy of 41.0 percent (P=0.30).

EFFECT OF THE VACCINE ON THE BURDEN OF DIARRHEAL ILLNESS

The incidence rate of severe gastroenteritis of any cause that required rehydration according to WHO plan B or C was 30.9 per 1000 infant-years in the vaccine group, as compared with 51.7 per 1000 infant-years in the placebo group, for an overall rate reduction of 40.0 percent among vaccine recipients (P<0.001). Likewise, the rate of hospitalization for diarrhea of any cause was significantly reduced, by 42.0 percent, in the vaccine group (P<0.001) (Table 3).

DISCUSSION

In this large, multinational trial conducted in 20,169 infants for efficacy and 63,225 infants for safety, the live attenuated RIX4414 G1P[8] HRV vaccine was highly protective against severe rotavirus gastroenteritis and related hospitalizations. This rotavirus vaccine also proved to be safe with respect to the risk of intussusception.

Within this trial setting, the vaccine was not

Table 3. Efficacy of the HRV Vaccine against Gastroenteritis during the Period from Two Weeks after Dose 2 until One Year of Age.*

Type of Gastroenteritis	HRV Vaccine (N=9009)		Placebo (N=8858)		Relative Risk†	Vaccine Efficacy (95% CI)
	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡		
Severe, according to clinical case definition§						
Rotavirus gastroenteritis						
Severe	12	2.0	77	13.3	0.153	84.7 (71.7 to 92.4)
Hospitalization	9	1.5	59	10.2	0.150	85.0 (69.6 to 93.5)
Gastroenteritis from any cause						
Severe	183	30.9	300	51.7	0.600	40.0 (27.7 to 50.4)
Hospitalization	145	24.5	246	42.4	0.580	42.0 (28.6 to 53.1)
Serotype-specific gastroenteritis						
G1P[8]¶	3‖	0.5	36**	6.2	0.082	91.8 (74.1 to 98.4)
G3P[8], G4P[8], G9P[8]	4††	0.66	31‡‡	5.3	0.126	87.3 (64.1 to 96.7)
G2P[4]	6	1.0	10§§	1.7	0.590	41.0 (−79.2 to 82.4)
Serotype-specific severe rotavirus gastroenteritis with a score of ≥11 on the Vesikari scale¶¶						
G1P[8]¶	3	0.5	32	5.5	0.092	90.8 (70.5 to 98.2)
G3P[8], G4P[8], G9P[8]	4	0.7	30	5.2	0.130	86.9 (62.8 to 96.6)
G2P[4]	5	0.8	9	1.5	0.546	45.4 (−81.5 to 85.6)

* Infants with episodes involving more than one isolated G type were counted in each of the detected type categories. One isolate from the placebo group could not be serotyped because the quantity of the sample was insufficient; one isolate from the placebo group was negative on reverse-transcriptase–polymerase-chain-reaction analysis; and one isolate from the placebo group could not be typed, but the vaccine strain was ruled out. HRV denotes human rotavirus, and CI confidence interval.

† The relative risk is the ratio of the incidence rate among infants in the vaccine group with at least one episode to the incidence rate among infants in the placebo group with at least one episode.

‡ The 1000 infant-year ratio is the number of infants presenting with ≥1 specified episode per 1000 infants per year.

§ The clinical definition of a case, according to the study protocol, was an episode of diarrhea (passage of three or more loose or watery stools within one day) with or without vomiting that required overnight hospitalization or rehydration therapy equivalent to World Health Organization plan B (oral rehydration therapy) or plan C (intravenous rehydration therapy) in a medical facility such as a hospital, clinic, or supervised rural health care center.

¶ All G1 types isolated were wild-type rotavirus.

‖ G1P[8] type alone was isolated from two infants; G1P[8] and G9P[8] types were isolated from one infant.

** G1P[8] type alone was isolated from 34 infants; G1P[8] and G9P[8] types were isolated from 1 infant; and G1, G2, and G9 types were isolated from 1 infant.

†† G3P[8] type alone was isolated from one infant, G4P[8] alone from one infant, and G9P[8] alone from one infant; both G1P[8] and G9P[8] were isolated from one infant.

‡‡ G3P[8] type alone was isolated from 8 infants, G4P[8] alone from 2 infants, and G9P[8] alone from 19 infants; G1P[8] and G9P[8] were isolated from 1 infant and G1P[8], G2P[4], and G9P[8] from 1 infant.

§§ G2P[4] alone was isolated from nine infants, and G1P[8], G2P[4], and G9P[8] were isolated from one infant.

¶¶ Scores on the Vesikari scale range from 0 to 20, with higher scores indicating more severe cases. An episode with a score of 11 or greater was considered severe.

associated with an increased risk of intussusception during a 31-day period after administration of either of the two doses, as compared with placebo. Because the second dose was administered toward the peak age incidence of intussusception in the Latin American population,[30-34] the risk of intussusception associated with the HRV vaccine would have been most apparent. However, cases of intussusception after dose 2 were evenly distributed between the HRV group and the placebo group. Not only is the observed risk estimate of −0.32 per 10,000 infants below the initial risk increase of 4 per 10,000[35,36] that led to the withdrawal of tetravalent rhesus–human

Table 4. Efficacy of the HRV Vaccine against Severe Rotavirus Gastroenteritis with a Vesikari Score between 11 and 20 during the Period from Two Weeks after Dose 2 until One Year of Age.*

Vesikari Score	HRV Vaccine (N = 9009)		Placebo (N = 8858)		Relative Risk†	Vaccine Efficacy (95% CI)
	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡	No. of Infants with ≥1 Episode	1000 Infant-Yr Ratio‡		
≥11	11	1.9	71	12.2	0.152	84.8 (71.1–92.7)
≥15	7	1.2	54	9.3	0.127	87.3 (71.9–95.1)
≥19	0	0	16	2.8	0	100.0 (74.5–100.0)

* Scores on the Vesikari scale range from 0 to 20, with higher scores indicating more severe cases. An episode with a score of 11 or greater was considered severe. CI denotes confidence interval.
† The relative risk is the ratio of the incidence rate among infants in the vaccine group with at least one episode to the incidence rate among infants in the placebo group with at least one episode.
‡ The 1000 infant-year ratio is the number of infants presenting with ≥1 specified episode per 1000 infants per year.

reassortant vaccine; it also is below the subsequent consensus risk estimate of 1 per 10,000[37,38] for that vaccine. By meeting the predefined criteria of a 95 percent confidence interval for a difference in risk that included zero and was below 6 per 10,000 infants, this trial shows that the HRV vaccine, given according to a two-dose vaccination schedule at two and four months of age, is safe with respect to the risk of intussusception. The incidence rate of intussusception observed in the placebo group (51 per 100,000 infants) is consistent with rates previously reported in Latin America.[30-34] In addition, the age at intussusception was similar in infants who had received vaccine and those who had received placebo — in contrast to the post-marketing observation with the tetravalent rhesus–human reassortant vaccine that vaccinees with intussusception tended to be younger than unvaccinated infants.[35] These data suggest that the intussusception problem encountered with the tetravalent rhesus–human reassortant vaccine may have resulted from the use of a rhesus strain, rather than from the oral administration of live rotavirus in general.[35,39] These observations are in agreement with a recent report that wild-type rotavirus infection is not associated with intussusception.[40]

The overall profile of serious adverse events was in favor of the HRV vaccine: fewer vaccinated infants than infants who received placebo had serious adverse events or required hospitalization because of gastrointestinal events. Numerous comparisons of serious adverse events grouped according to MedDRA preferred term (without adjustment for multiplicity) found no risk imbalance attributable to the vaccine that could be supported by clinical review and no plausible temporal or biologic causality. There was no significant difference in overall mortality between the groups. An observed potential imbalance in the number of pneumonia-related deaths among the vaccine recipients was not supported by observation of other pneumonia-related serious adverse events.

The vaccine proved to be highly protective against episodes of rotavirus gastroenteritis measured by a clinical definition for case capture that focused on hospitalization and rehydration, as well as by the validated Vesikari scale,[21] which includes quantifiable outcomes related to diarrhea, vomiting, fever, dehydration, and hospitalization. The efficacies observed with two doses of this HRV vaccine — 85 percent against severe episodes of rotavirus gastroenteritis and 100 percent against more severe episodes — are similar to those found in a previous HRV vaccine study conducted in Brazil, Mexico, and Venezuela[17,18] and to those of a three-dose study of tetravalent rhesus–human reassortant vaccine in Venezuela.[41] The results are also in agreement with data indicating that two wild-type rotavirus infections are fully protective against subsequent episodes of severe disease.[42]

The live attenuated vaccine protected against common serotypes circulating in Latin America and the Caribbean. A high level of protection (vaccine efficacy, 91 percent) was demonstrated against homologous G1P[8] rotaviruses, which have two outer capsid proteins (VP4 and VP7) and one inner capsid protein (VP6) antigenically similar to those in the HRV vaccine.[29] It also protected well against strains sharing only the P[8] genotype (the VP4 antigen) and the VP6 antigen (vaccine effica-

cy, 87 percent). Protection against rotavirus strains not having any of the outer or inner capsid antigens of the HRV vaccine seemed to be lower (vaccine efficacy, 45 percent). However, in a meta-analysis including the results of this study and the two phase 2 studies from Finland[16] and Latin America[17] (all based on identical methods and efficacy criteria), the efficacy of the vaccine against the G2P[4] type was 67 percent (95 percent confidence interval, 15 to 87 percent),[43] indicating that the vaccine can also protect, though to a lesser extent, against strains that do not share G or P epitopes with the vaccine strain.

Of public health importance is the finding that the HRV vaccine conferred 42 percent protection against hospitalization for gastroenteritis of any cause. This represents a significant reduction in the overall burden of gastroenteritis. With rotaviruses having been detected only in 26 percent of the cases of severe gastroenteritis in the control group, the observed 42 percent protection was greater than expected. The additional protection may be explained in part by rotavirus infections undetected by enzyme-linked immunoassay.[42] Therefore, it appears that the burden of disease caused by rotavirus is much greater than that reflected by the incidence of rotavirus-associated hospitalizations calculated by antigen detection in the stool. This discordance between specific and nonspecific results may be further explored by means of RT-PCR, a more sensitive method, to detect rotavirus in stool specimens[26] or by probe studies, as has been reported for other vaccines, such as those against *Haemophilus influenzae* type b and *Streptococcus pneumoniae*.[44]

The observed reduction in the rate of severe gastroenteritis of any cause and the strong protection against severe gastroenteritis due to G1P[8] and non-G1P[8] rotaviruses indicate the potential public health value of the HRV vaccine. Efforts should now be focused on bringing this vaccine to infants as part of routine immunization programs, especially in areas where rotavirus is associated with an important proportion of the burden of illness and childhood death. Wide use of this vaccine will require parallel implementation of post-marketing surveillance, including follow-up investigations of deaths among HRV vaccine recipients, to answer a number of remaining questions. Because it has been shown that initiating the administration of tetravalent rhesus–human reassortant vaccine to infants after the age of 90 days considerably increases the rate of intussusception,[45] one of the relevant issues that will need to be monitored in the future includes intussusception-related safety when the vaccine is used in older children. Another important remaining question is efficacy against possible other new emerging serotypes that do or do not share any capsid antigen with the vaccine virus.[46]

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APPENDIX

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REFERENCES

1. Velazquez FR, Garcia-Lozano H, Rodriguez E, et al. Diarrhea morbidity and mortality in Mexican children: impact of rotavirus disease. Pediatr Infect Dis J 2004;23:Suppl:S149-S155.

2. Guardado JAA, Clara WAW, Turcios RM, et al. Rotavirus in El Salvador: an outbreak, surveillance and estimates of disease burden, 2000-2002. Pediatr Infect Dis J 2004;23:Suppl:S156-S160.

3. Salinas B, Gonzalez G, Gonzalez R, Escalona M, Materan M, Schael IP. Epidemiologic and clinical characteristics of rotavirus disease during five years of surveillance in Venezuela. Pediatr Infect Dis J 2004;23:Suppl:S161-S167.

4. O'Ryan M, Perez-Schael I, Mamani N, et al. Rotavirus-associated medical visits and hospitalizations in South America: a prospective study at three large sentinel hospitals. Pediatr Infect Dis J 2001;20:685-93.

5. Kane EM, Turcios RM, Arvay ML, Garcia S, Bresee JS, Glass RI. The epidemiology of rotavirus diarrhea in Latin America: anticipating rotavirus vaccines. Rev Panam Salud Publica 2004;16:371-7.

6. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003;9:565-72.

7. Parashar U. Session 1: epidemiology and disease burden. In: Proceedings of the Sixth International Rotavirus Symposium, Mexico City, July 7–9, 2004:6.

8. Rotavirus vaccines, an update. Wkly Epidemiol Rec 2003;78:2-3.

9. Vaccine research and development: rotavirus vaccines for developing countries. Wkly Epidemiol Rec 1997;72:35-40.

10. Intussusception among recipients of rotavirus vaccine — United States, 1998–1999. MMWR Morb Mortal Wkly Rep 1999; 48:577-81.

11. Withdrawal of rotavirus vaccine recommendation. MMWR Morb Mortal Wkly Rep 1999;48:1007.

12. Vesikari T, Karvonen A, Korhonen T, et al. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine in adults, toddlers and previously uninfected infants. Vaccine 2004;22:2836-42.

13. Bernstein DI, Smith VE, Sherwood JR, et al. Safety and immunogenicity of live, attenuated human rotavirus vaccine 89-12. Vaccine 1998;16:381-7.

14. Bernstein DI, Sack DA, Rothstein E, et al. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. Lancet 1999;354: 287-90.

15. Bernstein DI, Sack DA, Reisinger K, Rothstein E, Ward RL. Second-year follow-up evaluation of live, attenuated human rotavirus vaccine 89-12 in healthy infants. J Infect Dis 2002;186:1487-9.

16. Vesikari T, Karvonen A, Puustinen L, et al. Efficacy of RIX4414 live attenuated human rotavirus vaccine in Finnish infants. Pediatr Infect Dis J 2004;23:937-43.

17. Salinas B, Schael IP, Linhares AC, et al. Evaluation of safety, immunogenicity and efficacy of an attenuated rotavirus vaccine, RIX4414: a randomized, placebo-controlled trial in Latin American infants. Pediatr Infect Dis J 2005;24:807-16.

18. De Vos B, Vesikari T, Linhares AC, et al. A rotavirus vaccine for prophylaxis of infants against rotavirus gastroenteritis. Pediatr Infect Dis J 2004;23:Suppl:S179-S182.

19. Bines JE, Kohl KS, Forster J, et al. Acute intussusception in infants and children as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine 2004;22:569-74.

20. Santosham M, Moulton LH, Reid R, et al. Efficacy and safety of high-dose rhesus-human reassortant rotavirus vaccine in Native American populations. J Pediatr 1997;131:632-8.

21. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis 1990;22:259-67.

22. MedDRA Maintenance and Support Services Organization home page. Reston, Va.: MedDRA MSSO, 2005. (Accessed December 9, 2005, at http://www.meddramsso.com.)
23. Bresee J, Fang Z-Y, Wang B, et al. First report from the Asian Rotavirus Surveillance Network. Emerg Infect Dis 2004;10:988-95.
24. Griffin DD, Kirkwood CD, Parashar UD, et al. Surveillance of rotavirus strains in the United States: identification of unusual strains. J Clin Microbiol 2000;38:2784-7.
25. Hsu VP, Staat MA, Roberts N, et al. Use of active surveillance to validate International Classification of Diseases code estimates of rotavirus hospitalizations in children. Pediatrics 2005;115:78-82.
26. Pang XL, Joensuu J, Hoshino Y, Kapikian AZ, Vesikari T. Rotaviruses detected by reverse transcription polymerase chain reaction in acute gastroenteritis during a trial of rhesus-human reassortant rotavirus tetravalent vaccine: implications for vaccine efficacy analysis. J Clin Virol 1999;13:9-16.
27. Miettinen O, Nurminen N. Comparative analysis of two rates. Stat Med 1985;4:213-66.
28. Tang ML, Ng HK. Comment on: confidence limits for the ratio of two rates based on likelihood scores: non-iterative method. Stat Med 2004;23:685-92.
29. Linhares AC, Bresee JS. Rotavirus vaccines and vaccination in Latin America. Rev Panam Salud Publica 2000;8:305-31.
30. Lucero Y, Valenzuela MT, O'Ryan M. Clinical and epidemiological profile of intestinal intussusception among infants of metropolitan Santiago. Rev Med Chil 2004;132:565-72. (In Spanish.)
31. O'Ryan M, Lucero Y, Pena A, Valenzuela MT. Two year review of intestinal intussusception in six large public hospitals of Santiago, Chile. Pediatr Infect Dis J 2003;22:717-21.
32. Abate H, Linhares AC, Venegas G, et al. A multi-center study of intussusception in Latin America: first year results. Presented at the International Congress of Pediatrics, Cancun, Mexico, August 15–20, 2004 (paper).
33. Perez-Schael I, Escalona M, Salinas B, Materan M, Perez ME, Gonzalez G. Intussusception-associated hospitalization among Venezuelan infants during 1998 through 2001: anticipating rotavirus vaccines. Pediatr Infect Dis J 2003;22:234-9.
34. Saez-Llorens X, Guevara JN. Intussusception and rotavirus vaccines: what is the background risk? Pediatr Infect Dis J 2004;23:363-5.
35. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344:564-72. [Erratum, N Engl J Med 2001;344:1564.]
36. Kramarz P, France EK, Destefano F, et al. Population-based study of rotavirus vaccination and intussusception. Pediatr Infect Dis J 2001;20:410-6.
37. Murphy BR, Morens DM, Simonsen L, Chanock RM, La Montagne JR, Kapikian AZ. Reappraisal of the association of intussusception with the licensed live rotavirus vaccine challenges initial conclusions. J Infect Dis 2003;187:1301-8.
38. Peter G, Myers MG. Intussusception, rotavirus, oral vaccines: summary of a workshop. Pediatrics 2002;110(6):e67.
39. Heaton PM. Overview: characteristics of the investigational pentavalent human-bovine reassortant rotavirus vaccine (PRV): primary efficacy and safety results of the large-scale Rotavirus Efficacy and Safety Trial (REST). Presented at the meeting of the Advisory Committee on Immunization Practices, Atlanta, February 10–11, 2005.
40. Velázquez FR, Luna G, Cedillo R, Torres J, Muñoz O. Natural rotavirus infection is not associated to intussusception in Mexican children. Pediatr Infect Dis J 2004;23:Suppl:S173-S178.
41. Perez-Schael I, Guntinas MJ, Perez M, et al. Efficacy of the rhesus rotavirus-based quadrivalent vaccine in infants and young children in Venezuela. N Engl J Med 1997;337:1181-7. [Erratum, N Engl J Med 1998;338:1002.]
42. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infection in infants as protection against subsequent infections. N Engl J Med 1996;335:1022-8.
43. Perez-Schael I, Linhares AC, Vesikari T, et al. Two doses of the human attenuated rotavirus vaccine RIX4414 (Rotarix) show heterotypic protection in Latin America and Europe. In: Program and abstracts of the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., December 16–19, 2005 (poster) (in press).
44. Gessner BD, Sutanto A, Linehan M, et al. Incidences of vaccine-preventable Haemophilus influenzae type b pneumonia and meningitis in Indonesian children: hamlet-randomised vaccine-probe trial. Lancet 2005;365:43-52.
45. Simonsen L, Viboud C, Elixhauser A, Taylor RJ, Kapikian AZ. More on RotaShield and intussusception: the role of age at the time of vaccination. J Infect Dis 2005;192:Suppl 1:S36-S43.
46. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 2005;15:29-56.

ORIGINAL ARTICLE

Safety and Efficacy of a Pentavalent Human–Bovine (WC3) Reassortant Rotavirus Vaccine

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ABSTRACT

BACKGROUND

Rotavirus is a leading cause of childhood gastroenteritis and death worldwide.

METHODS

We studied healthy infants approximately 6 to 12 weeks old who were randomly assigned to receive three oral doses of live pentavalent human–bovine (WC3 strain) reassortant rotavirus vaccine containing human serotypes G1, G2, G3, G4, and P[8] or placebo at 4-to-10-week intervals in a blinded fashion. Active surveillance was used to identify subjects with serious adverse and other events.

RESULTS

The 34,035 infants in the vaccine group and 34,003 in the placebo group were monitored for serious adverse events. Intussusception occurred in 12 vaccine recipients and 15 placebo recipients within one year after the first dose including six vaccine recipients and five placebo recipients within 42 days after any dose (relative risk, 1.6; 95 percent confidence interval, 0.4 to 6.4). The vaccine reduced hospitalizations and emergency department visits related to G1–G4 rotavirus gastroenteritis occurring 14 or more days after the third dose by 94.5 percent (95 percent confidence interval, 91.2 to 96.6 percent). In a nested substudy, efficacy against any G1–G4 rotavirus gastroenteritis through the first full rotavirus season after vaccination was 74.0 percent (95 percent confidence interval, 66.8 to 79.9 percent); efficacy against severe gastroenteritis was 98.0 percent (95 percent confidence interval, 88.3 to 100 percent). The vaccine reduced clinic visits for G1–G4 rotavirus gastroenteritis by 86.0 percent (95 percent confidence interval, 73.9 to 92.5 percent).

CONCLUSIONS

This vaccine was efficacious in preventing rotavirus gastroenteritis, decreasing severe disease and health care contacts. The risk of intussusception was similar in vaccine and placebo recipients. (ClinicalTrials.gov number, NCT00090233.)

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Rotavirus is the leading cause of hospitalization and death from acute gastroenteritis among infants and young children worldwide. More than 2 million hospitalizations and nearly half a million deaths are attributed to this infection annually.[1,2] The strategy of preventing rotavirus through vaccination derives from studies demonstrating that wild-type rotavirus infection induces immunity against subsequent rotavirus gastroenteritis.[3-6] Primary rotavirus infection provides substantial protection against gastroenteritis caused by the same serotype and against severe disease regardless of serotype. The four most prevalent serotypes, which account for more than 80 percent of cases of human rotavirus disease worldwide, are G1P[8], G2P[4], G3P[8], and G4P[8].[7,8]

In 1998, a tetravalent rhesus–human reassortant rotavirus vaccine (RRV-TV; RotaShield, Wyeth Laboratories) was licensed and recommended for routine immunization of infants in the United States.[9] Shortly thereafter, an association between the use of the vaccine and intestinal intussusception — an uncommon illness with a background incidence of 18 to 56 cases per 100,000 infant-years during the first year of life — was recognized.[10-13] The risk was greatest during the 3-to-14-day period after the first dose and the 3-to-7-day period after the second dose. Experts estimated that the population attributable risk of RRV-TV–associated intussusception was approximately 1 per 10,000 recipients.[14] RRV-TV was also associated with fever, vomiting, diarrhea, abdominal pain, and bloody stools.[15-18] The vaccine was voluntarily withdrawn from the market in October 1999.[19]

Development of a human–bovine reassortant rotavirus vaccine was continued because of the need for a safe and effective rotavirus vaccine and the importance of such a vaccine to public health.[19-21] In phase 2 clinical trials, various formulations of the human–bovine reassortant vaccine prevented approximately 70 percent of episodes of rotavirus gastroenteritis of any severity and 100 percent of episodes of severe disease.[22,23] In contrast to the findings with RRV-TV, the incidence of fever and gastrointestinal symptoms was generally similar in the vaccine and placebo groups. Further development of the human–bovine reassortant vaccine was also supported by the absence of an apparent association between intussusception and wild-type human rotavirus disease,[24,25] indicating that intussusception was not necessarily associated with all rotaviruses.

We report the results of the Rotavirus Efficacy and Safety Trial (REST), a randomized, placebo-controlled clinical trial of an oral, live pentavalent (G1, G2, G3, G4, and P[8]) human–bovine (WC3) reassortant rotavirus vaccine (RotaTeq, Merck). The trial included an evaluation of the safety of the vaccine with regard to intussusception and other adverse events and its efficacy in preventing rotavirus gastroenteritis and the associated use of health care resources.

METHODS

STUDY DESIGN

The study was a double-blind (with sponsor blinding), placebo-controlled, randomized trial conducted from 2001 to 2004 in 11 countries (as detailed in Part I of the Supplementary Appendix, available with the full text of this article at www.nejm.org). The protocol was approved by the ethics review committees of participating sites, and written informed consent was obtained from each participant's parent or guardian before enrollment. Healthy infants between 6 and 12 weeks of age were eligible. Infants were excluded if oral poliovirus vaccine had been given during the 42-day period preceding the planned first dose or if it was anticipated that oral poliovirus vaccine would be administered during the study. Concomitant administration of other licensed vaccines and breast-feeding were not restricted.

This study was designed to evaluate safety with respect to intussusception. The large sample size also provided us the opportunity to evaluate the efficacy of the vaccine in reducing the need for hospitalization or emergency department care for rotavirus gastroenteritis. Substudies nested within the large-scale study were designed to evaluate safety with respect to all adverse events (the detailed safety substudy) as well as immunogenicity and efficacy against rotavirus gastroenteritis of any severity (the clinical-efficacy substudy) (Fig. 1A of the Supplementary Appendix). Sites for each substudy were prospectively identified.

The trial (Merck protocol V260-006) was designed, managed, and analyzed by the sponsor in conjunction with the external investigators and members of the data and safety monitoring board and safety end-point adjudication committee (listed in Part I of the Supplementary Appendix). The investigators had access to all study data. This report was drafted primarily by Drs. Vesikari, Dallas, DiNubile, and Heaton and was reviewed

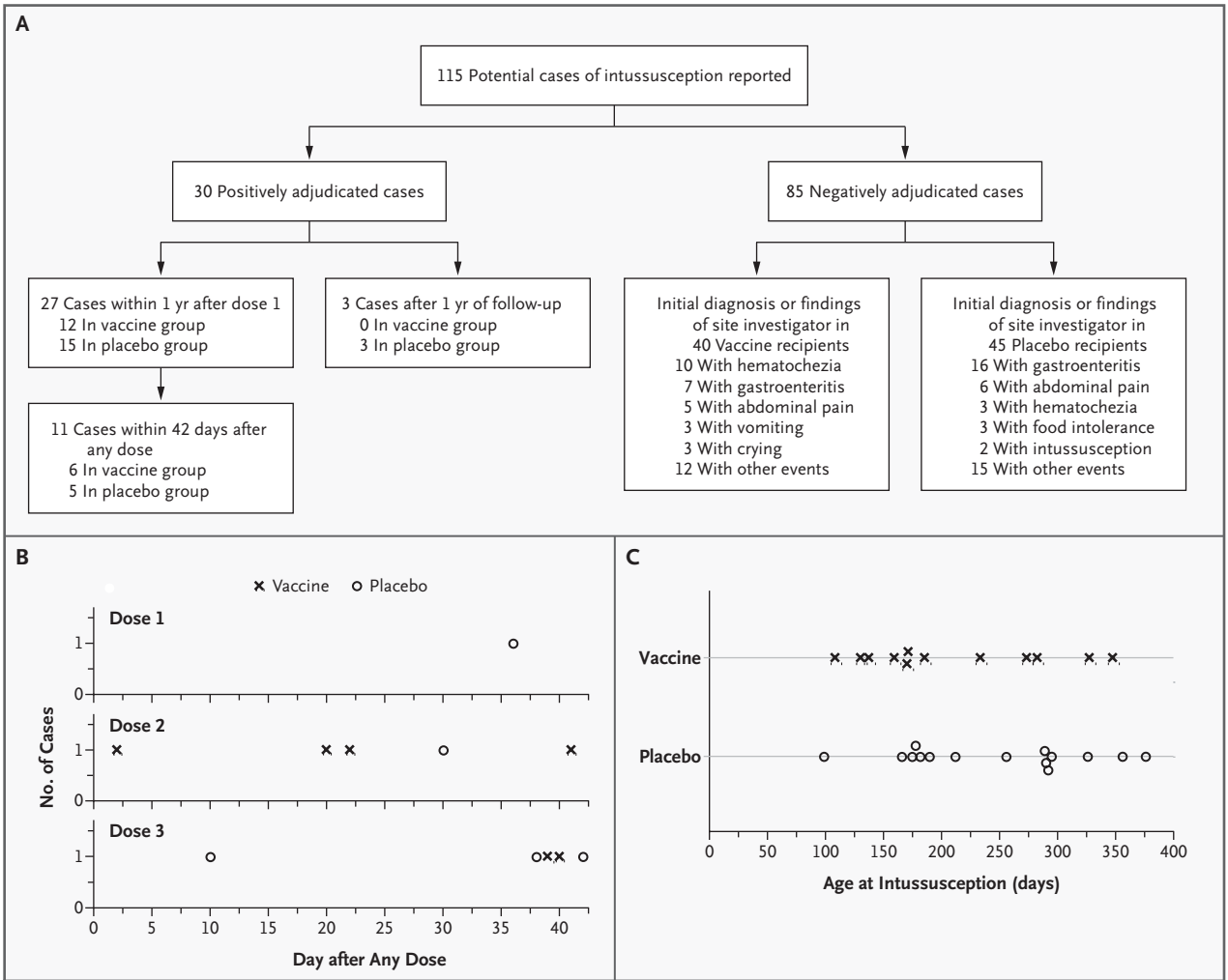

Figure 1. Cases of Intussusception.

Panel A shows potential cases of intussusception reported to the safety end-point adjudication committee before the recommendation of the data safety and monitoring board to discontinue enrollment, and the results of adjudication. One potential case of intussusception that was reported could not be adjudicated because radiographs were not available (owing to equipment malfunction). This case occurred in a placebo recipient 55 days after the second dose. The initial diagnosis or findings of the site investigator are shown for the negatively adjudicated cases (those not confirmed to be cases of intussusception). Panel B shows confirmed cases of intussusception that were reported within 42 days after vaccination, according to dose number and study group (vaccine or placebo). Panel C shows the subjects' ages at the time of the diagnosis of intussusception, according to study group (vaccine or placebo). Subjects already enrolled in the study at the time that enrollment was stopped, as recommended by the data safety and monitoring board, were followed until the end of the study. A single additional case of intussusception, in an eight-month-old boy, occurred 116 days after the third dose of vaccine. The child recovered fully.

and approved by each coauthor. The Indian Health Service approved the protocol but was otherwise uninvolved in the study.

VACCINE

The live pentavalent rotavirus vaccine contained five human–bovine reassortant rotaviruses, each consisting of the WC3 bovine strain with viral surface proteins corresponding to human rotavirus serotypes G1, G2, G3, G4, and P[8].[26] Reassortants were suspended in a liquid sodium citrate and phosphate buffer at an aggregate viral titer of approximately 6.7×10^7 to 12.4×10^7 infectious units per dose. Infants were randomly assigned, in a 1:1 ratio, to receive three 2-ml oral doses of vaccine or visibly indistinguishable placebo, 4 to

10 weeks apart. Doses were administered year round.

EVALUATION OF INTUSSUSCEPTION AND OTHER ADVERSE EVENTS

All subjects were monitored for at least 42 days after each dose for serious adverse events, including intussusception. Vaccine-related serious adverse events, deaths, and instances of intussusception were reported until the end of the study. Active surveillance was used to obtain safety data; parents or legal guardians were contacted on days 7, 14, and 42 after each dose and every six weeks thereafter for one year after the first dose with respect to intussusception and serious adverse events. Safety follow-up was completed for subjects for whom vaccinations were discontinued early. When available, stool specimens from infants with intussusception were tested for rotavirus antigen by enzyme immunoassay.[27] In the detailed safety substudy, parents or guardians were also asked to record their infants' temperature and the number of episodes of vomiting and diarrhea daily for 7 days after each dose and all adverse events for 42 days after each dose. Potential fecal shedding of vaccine strains between four and six days after each dose was monitored in a subgroup of subjects at prespecified sites, regardless of symptoms, by viral culture with use of a plaque assay and RNA electropherotyping.[28]

ADJUDICATION OF CASES OF INTUSSUSCEPTION AND ROLE OF THE DATA AND SAFETY MONITORING BOARD

All suspected cases of intussusception were reported to an independent, blinded adjudication committee, which included a pediatric surgeon, a pediatric radiologist, and a pediatrician with extensive experience in emergency medicine. The committee adjudicated potential cases of intussusception according to a prespecified case definition that required confirmation of the diagnosis by radiography or at surgery or autopsy.

As they were reported, positively adjudicated cases of intussusception were unblinded according to treatment group by the data and safety monitoring board to allow decisions to be made about the continuation of the study. The board's guidelines called for early stopping of the study if a significantly higher risk of intussusception among vaccine recipients than among placebo recipients (lower bound of the 95 percent confidence interval, >1.0) was detected during interim monitoring for the 7-day or 42-day period after any dose.

The data and safety monitoring board also made recommendations regarding completion of overall enrollment according to whether the criteria associated with the primary safety hypothesis that the vaccine would not increase the risk of intussusception within 42 days after any dose had been satisfied. The study used a group-sequential design,[29] with a minimum enrollment of 60,000 subjects and sequential enrollment of groups of 10,000 subjects if statistical criteria for the primary safety hypothesis were not met, to a maximum of 100,000 subjects.

CASE DEFINITION OF ROTAVIRUS GASTROENTERITIS

A case of rotavirus gastroenteritis was defined as the production of three or more watery or looser-than-normal stools within a 24-hour period or forceful vomiting, along with the detection of rotavirus by enzyme immunoassay in a stool specimen obtained within 14 days after the onset of symptoms. G serotypes were identified by one-step reverse-transcriptase–polymerase-chain-reaction analysis followed by sequencing.[30] All rotavirus-positive stools were to be evaluated for vaccine strains by viral culture with the use of a plaque assay and RNA electropherotyping.

EVALUATION OF EFFICACY IN TERMS OF HOSPITALIZATION AND EMERGENCY DEPARTMENT CARE FOR ROTAVIRUS GASTROENTERITIS

All subjects in the study were followed with respect to hospitalizations and emergency department visits for acute gastroenteritis. Parents or guardians were questioned about health care contacts for gastroenteritis at the same time that they were asked about intussusception and other adverse events. Lost work time was assessed for parents or guardians of subjects with confirmed rotavirus gastroenteritis.

EVALUATION OF CLINICAL EFFICACY AGAINST ROTAVIRUS GASTROENTERITIS

The clinical-efficacy substudy enrolled subjects from Finland and the United States (including subjects from the Navajo Nation and the White Mountain Apache Tribe). Parents or guardians were asked to report any episodes of acute gastroenteritis in their infants after the first dose. Active surveillance for all episodes of gastroenteritis, in-

cluding office visits to a physician for gastroenteritis, was conducted by contacting parents or guardians every two weeks. The rotavirus season was prospectively determined from historical epidemiologic data.[31-33] Most subjects were followed for one full rotavirus season after vaccination; however, some subjects were enrolled early enough to allow follow-up through a second full season. For subjects enrolled during a rotavirus season, surveillance was continued for the remainder of that season and through the next full rotavirus season.

To determine whether an episode of acute gastroenteritis satisfied the definition of a case of rotavirus gastroenteritis and to assess its clinical severity, parents or guardians were asked to complete diary cards and record symptoms daily until the illness resolved. An established clinical scoring system based on the intensity and duration of fever, vomiting, diarrhea, and changes in behavior was used to categorize episodes of rotavirus gastroenteritis on a 24-point severity scale; scores greater than 16 were considered to indicate severe disease (details are provided in Part II of the Supplementary Appendix).[22,34]

EVALUATION OF IMMUNOGENICITY

Immune responses to vaccination were assessed in a subgroup of subjects in the clinical-efficacy substudy. Serum samples were collected before the first dose and approximately 14 days after the third dose for measurement of antirotavirus IgA titers[35] and neutralizing antibodies against the G1, G2, G3, G4, and P[8] serotypes.[35] Seroconversion was defined as an increase in the antibody titer by a factor of 3 or more from baseline.

STATISTICAL ANALYSIS

Intussusception and Other Adverse Events

All subjects receiving at least one dose who had follow-up evaluations were included in the safety analyses according to the treatment actually received. The primary safety hypothesis was that the vaccine, relative to placebo, would not increase the risk of intussusception within 42 days after any dose. To satisfy this hypothesis, two criteria were prespecified, as follows. First, during the study, there could not be a significantly increased risk of intussusception among vaccine recipients as compared with placebo recipients within 7 days and 42 days after any dose. Second, at the end of the study, the upper bound of the 95 percent confidence interval for the relative risk of intussusception within 42 days after any dose had to be 10 or less, representing vaccine-to-placebo case ratios for intussusception of 2 or less based on the total number of expected cases, with such ratios considered to indicate a clinically acceptable relative risk of an uncommon event. This hypothesis was tested with the use of an exact binomial procedure based on the proportion of subjects with intussusception who received vaccine. The P value, point estimate, and confidence limits were appropriately adjusted for the group-sequential design of the study.[29] The relative risk was also assessed for the 7-day, 14-day, and 60-day periods after any dose and for the 365-day period after the first dose.

The power to detect an increased risk of intussusception during the study and to satisfy the primary safety hypothesis at the end of the study was estimated with the use of Monte Carlo simulation, with the assumption that cases of intussusception would accrue at a rate of 50 per 100,000 infant-years. If the risk of intussusception after vaccination was not increased, the probability that the primary safety hypothesis would be satisfied was approximately 94 percent. If the risk was similar to that reported for the RRV-TV,[14] the probability that the study would be stopped early was 85 to 91 percent.

Use of Health Care Resources

Use of health care resources because of rotavirus gastroenteritis occurring 14 or more days after the completion of the three-dose series for up to 2 years was assessed in the per-protocol population, which consisted of subjects for whom there was no protocol violation. Poisson regression with generalized estimating equations was used to estimate the reduction in the rate of use of health care resources and lost days of parents' or guardians' work in the vaccine group as compared with the placebo group.

Clinical Efficacy

The primary efficacy hypothesis specified that the vaccine would be efficacious in preventing wild-type G1–G4 rotavirus gastroenteritis occurring 14 or more days after completion of the three-dose series through the first full rotavirus season after vaccination. Subjects with multiple episodes meeting the case definition were counted only once. The statistical analysis was based on the total

number of subjects with rotavirus gastroenteritis from both groups, such that the number of subjects with rotavirus gastroenteritis in the vaccine group followed a binomial distribution. Exact inference was used. To permit the conclusion that the vaccine was efficacious, the lower bound of the two-sided 95 percent confidence interval had to be greater than 35 percent.

The primary efficacy analysis was based on the per-protocol population from the clinical-efficacy substudy, with use of the protocol case definition for G1–G4 rotavirus gastroenteritis occurring 14 or more days after the third dose. In secondary analyses of the per-protocol population, efficacy against severe G1–G4 rotavirus gastroenteritis and efficacy through a second rotavirus season after vaccination were examined. Another efficacy analysis was based on an intention-to-treat population, which consisted of all subjects (regardless of protocol violations) who received at least one dose and in which all cases of G1–G4 rotavirus meeting the protocol case definition and occurring at any time after the first dose were counted.

RESULTS

SUBJECTS

After 60,000 subjects had been monitored for 42 days after their last dose, the data and safety monitoring board reviewed the intussusception data with respect to treatment assignment and recommended the enrollment of 10,000 additional subjects because the criteria for stopping enrollment associated with the primary safety hypothesis had not yet been satisfied. After the additional subjects had been enrolled and followed, the board advised stopping enrollment because the prespecified criteria had been met. The analyses in this report are based on data available when the board members made their recommendation to stop enrollment.

In total, 70,301 subjects were enrolled, and data for 69,274 randomly assigned subjects were available in the clinical database. Overall, 68,038 subjects (98.2 percent) received at least one dose of vaccine or placebo; 59,210 (85.5 percent) received three doses and were followed for safety for 42 days after the third dose; and 56,310 (81.3 percent) were followed for 1 year after the first dose (Fig. 1B of the Supplementary Appendix). Among the subjects who received at least one dose, 67,756 (99.6 percent) were followed for 42 days after their last dose. The demographic characteristics of the subjects in the vaccine and placebo groups were generally similar (Table 1). The median age of the subjects at the time of entry was 10 weeks.

INTUSSUSCEPTION

A confirmed case of intussusception occurred within one year after the first dose in 12 vaccine recipients and 15 placebo recipients (relative risk, 0.8; 95 percent confidence interval, 0.3 to 1.8) (Fig. 1A). A confirmed case of intussusception occurred within the 42-day period after any dose in six vaccine recipients and five placebo recipients (multiplicity-adjusted relative risk, 1.6; 95 percent confidence interval, 0.4 to 6.4) — a result that satisfied the primary safety hypothesis (Fig. 1B). In no case did intussusception occur in a vaccine recipient within 42 days after the first dose.

Of the 27 confirmed cases of intussusception occurring within one year after the first dose, 16 (59 percent) involved boys. At the time of intussusception, the vaccine recipients were not younger than the placebo recipients (Fig. 1C). One death from postoperative sepsis occurred in a vaccine recipient in whom intussusception had been diagnosed 98 days after dose 3. Five stool specimens available from subjects with a confirmed case of intussusception at the time of diagnosis tested negative for rotavirus antigen.

OTHER ADVERSE EVENTS

Serious adverse events were reported in 803 of 34,035 vaccine recipients (2.4 percent) and 859 of 34,003 placebo recipients (2.5 percent). Overall, 44 deaths occurred during the study, 24 among vaccine recipients (<0.1 percent) and 20 among placebo recipients (<0.1 percent). The most common cause of death in both groups was sudden infant death syndrome, which occurred in seven vaccine recipients and eight placebo recipients. No deaths were attributed to vaccination by investigators blinded to treatment assignment.

Among the 9605 subjects in the detailed safety substudy (4806 in the vaccine group and 4799 in the placebo group), the rates of fever, vomiting, and diarrhea within 42 days after any dose were similar among vaccine recipients and placebo recipients (Fig. 2). The overall incidence of hematochezia within 42 days after any dose was 0.6 percent in each group. Among those with nega-

Table 1. Baseline Demographic Characteristics of the Subjects.*

Variable	Large-Scale Study		Detailed Safety Substudy		Clinical-Efficacy Substudy	
	Vaccine Group	Placebo Group	Vaccine Group	Placebo Group	Vaccine Group	Placebo Group
Randomly assigned to study group — no.	34,644	34,630	4826	4821	2841	2845
Sex — no. (%)						
Male	17,586 (50.8)	17,529 (50.6)	2482 (51.4)	2491 (51.7)	1462 (51.5)	1467 (51.6)
Female	17,058 (49.2)	17,101 (49.4)	2344 (48.6)	2330 (48.3)	1379 (48.5)	1378 (48.4)
Age at entry — wk						
Mean	9.8±1.4	9.8±1.4	9.7±1.4	9.7±1.4	9.7±1.6	9.7±1.5
Median	10	10	10	10	10	10
Range	3–13	1–16	3–13	4–13	3–13	4–13
Race or ethnic group — no. (%)†						
White	23,772 (68.6)	23,788 (68.7)	3052 (63.2)	3031 (62.9)	1854 (65.3)	1,885 (66.3)
Hispanic	4,963 (14.3)	4,911 (14.2)	499 (10.3)	486 (10.1)	282 (9.9)	251 (8.8)
Black	2,908 (8.4)	2,941 (8.5)	209 (4.3)	237 (4.9)	49 (1.7)	58 (2.0)
Multiracial	1,815 (5.2)	1,817 (5.2)	305 (6.3)	304 (6.3)	126 (4.4)	143 (5.0)
Asian	536 (1.5)	552 (1.6)	221 (4.6)	237 (4.9)	18 (0.6)	12 (0.4)
Native American	531 (1.5)	514 (1.5)	512 (10.6)	493 (10.2)	510 (18.0)	492 (17.3)
Other	119 (0.3)	107 (0.3)	28 (0.6)	33 (0.7)	2 (0.1)	4 (0.1)

* Plus–minus values are means ±SD.
† Race or ethnic group was determined by the investigator according to prespecified categories.

tively adjudicated cases of intussusception, hematochezia occurred more frequently in the vaccine group (10 subjects) than in the placebo group (3 subjects). With the exception of dermatitis (which was more common among vaccine recipients than among placebo recipients), adverse events were reported with similar frequency in the two groups.

During the four-to-six-day period after the administration of the first dose, fecal shedding of vaccine strains was detected in 17 of 134 vaccine recipients (12.7 percent). None of 109 vaccine recipients shed vaccine strains from four to six days after dose 2 and none of 99 did so after dose 3.

USE OF HEALTH CARE RESOURCES FOR ROTAVIRUS GASTROENTERITIS

In the large-scale study, 28,646 and 28,488 subjects in the vaccine and placebo groups, respectively, were included in the per-protocol analysis of the efficacy of the vaccine in reducing the need for hospitalization or emergency department care for rotavirus gastroenteritis (Fig. 1C of the Supplementary Appendix). Overall, 204 subjects (13 in the vaccine group and 191 in the placebo group) visited emergency departments and 144 subjects (6 in the vaccine group and 138 in the placebo group) were hospitalized for G1–G4 rotavirus gastroenteritis. The vaccine reduced the combined incidence of hospitalization or emergency department care for G1–G4 rotavirus gastroenteritis by 94.5 percent (95 percent confidence interval, 91.2 to 96.6 percent), with a 95.8 percent reduction in the rate of hospitalization (95 percent confidence interval, 90.5 to 98.2 percent) and a 93.7 percent reduction in the rate of emergency department visits (95 percent confidence interval, 88.8 to 96.5 percent). The numbers of hospitalizations and emergency department visits are shown according to serotype in Table 2.

The efficacy of the vaccine against all gastroenteritis-related hospitalizations after the first dose was 58.9 percent (95 percent confidence interval, 51.7 to 65.0 percent). There also was an 86.6 percent reduction (95 percent confidence interval, 78.0 to 91.9 percent) in the number of lost workdays associated with G1–G4 rotavirus gas-

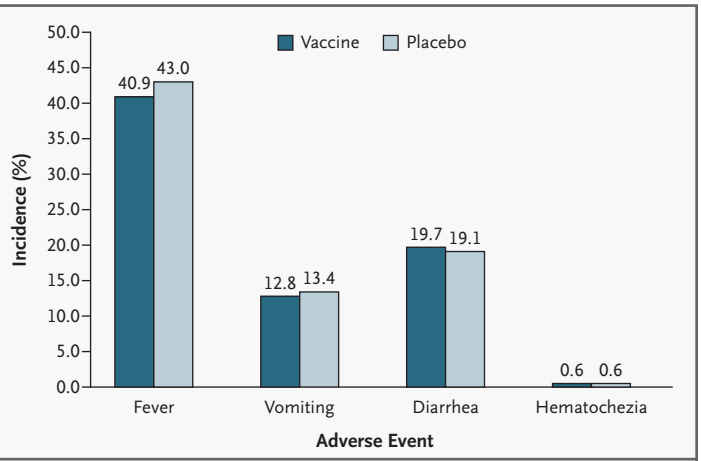

Figure 2. Percentage of Subjects in the Detailed Safety Substudy with Fever, Vomiting, Diarrhea, or Hematochezia within 42 Days after Any Dose, According to Study Group.

Fever refers to all reported episodes of fever.

Table 2. Reduction in the Numbers of Hospitalizations and Emergency Department Visits in the Per-Protocol Population of the Large-Scale Study, According to G Serotype Identified in the Subject's Stool.*

Serotype	No. of Cases of Rotavirus Gastroenteritis		Percent Rate Reduction (95% CI)
	Vaccine Group (N = 34,035)	Placebo Group (N = 34,003)	
G1	16	328	95.1 (91.6–97.1)
G2	1	8	87.6 (<0–98.5)
G3	1	15	93.4 (49.4–99.1)
G4	2	18	89.1 (52.0–97.5)
G9	0	13	100.0 (67.4–100.0)
G12	0	1	100.0 (<0–100.0)

* The number of subjects in each group is the number who received at least one dose. Some subjects had more than one event. CI denotes confidence interval.

troenteritis: the parents or guardians of vaccine recipients lost 65 workdays, whereas the parents or guardians of placebo recipients lost 487 workdays. In the clinical-efficacy substudy, the vaccine reduced office or clinic visits for G1–G4 rotavirus gastroenteritis by 86.0 percent (95 percent confidence interval, 73.9 to 92.5 percent).

CLINICAL EFFICACY AGAINST ROTAVIRUS GASTROENTERITIS, ACCORDING TO SEVERITY

The clinical-efficacy substudy included 5673 vaccinated subjects (Fig. 1D of the Supplementary Appendix). Among 4512 subjects (2207 in the vaccine group and 2305 in the placebo group) whose data could be evaluated in the per-protocol efficacy analysis, 397 cases of rotavirus gastroenteritis (82 and 315, respectively) caused by G1–G4 serotypes (G1 in 358, G2 in 23, G3 in 7, and G4 in 9) occurred 14 or more days after the third dose during the first full rotavirus season. The efficacy of the vaccine against G1–G4 rotavirus gastroenteritis of any severity was 74.0 percent (95 percent confidence interval, 66.8 to 79.9 percent) and that against severe G1–G4 rotavirus gastroenteritis was 98.0 percent (95 percent confidence interval, 88.3 to 100 percent). Only one case of severe rotavirus gastroenteritis occurred among vaccine recipients during the first full rotavirus season after vaccination. The mean severity score for cases in vaccine recipients was 9.1 (range, 1 to 17), as compared with 12.9 (range, 2 to 21) for cases in placebo recipients. Serotype-specific results are presented in Table 3. In a modified intention-to-treat analysis that included all subjects who received at least one dose and in which per-protocol cases occurring anytime during the first full rotavirus season after the first dose were counted, the efficacy of the vaccine was 60.0 percent (95 percent confidence interval, 51.5 to 67.1 percent) against G1–G4 rotavirus gastroenteritis of any severity.

During the second rotavirus season after vaccination, there were 36 G1–G4 cases among 813 vaccine recipients with data that could be evaluated and 88 G1–G4 cases among 756 placebo recipients that could be evaluated. Second-season efficacy against G1–G4 rotavirus gastroenteritis of any severity was 62.6 percent (95 percent confidence interval, 44.3 to 75.4 percent) and that against severe disease (which occurred in 2 vaccine recipients and 17 placebo recipients) was 88.0 percent (95 percent confidence interval, 49.4 to 98.7 percent).

IMMUNOGENICITY

Antibody responses were measured in a subgroup of subjects from whom serum samples had been obtained, according to a predetermined schedule, before the first dose and approximately two weeks after the third dose (Fig. 3). Seroconversion rates for serum neutralizing antibody to each human rotavirus serotype in the vaccine were significantly higher in the vaccine group than in the placebo group. A higher proportion of vaccine recipients whose data could be evaluated had seroconversion

to G1, G4, and P[8] than to G2 or G3. Seroconversion rates for serum antirotavirus IgA were 95.2 percent (95 percent confidence interval, 91.2 to 97.8 percent) among 189 vaccine recipients whose data could be evaluated, as compared with 14.3 percent (95 percent confidence interval, 9.3 to 20.7 percent) among 161 placebo recipients that could be evaluated.

DISCUSSION

The results of our study provide a high level of confidence in the safety of the pentavalent human–bovine (WC3) reassortant rotavirus vaccine and demonstrate its potential benefit in preventing rotavirus gastroenteritis and the associated use of health care resources. Active surveillance did not detect a significantly increased risk of intussusception in vaccine recipients at any time during the study, and the primary safety hypothesis was satisfied at the end of the study. The relative risk of intussusception among vaccine recipients, as compared with placebo recipients, was 1.6 (95 percent confidence interval, 0.4 to 6.4) during the 42-day period after any dose — a result that met prespecified criteria for an acceptable safety profile. Cases of intussusception occurred sporadically, without evidence of increased risk among vaccine recipients during the 7-day and 14-day periods after each dose — the periods of greatest risk with RRV-TV.[10-14] In contrast to observations with RRV-TV,[15-18] the rates of fever, vomiting, diarrhea, and hematochezia were similar among vaccine and placebo recipients in our study. These data are consistent with the results of the early-phase clinical trials of the human–bovine (WC3) reassortant vaccine, in which only a single case of intussusception was reported (in a seven-month-old boy) among the 2470 vaccine recipients.[22,23]

The pentavalent rotavirus vaccine was highly efficacious against severe rotavirus gastroenteritis and provided substantial protection against rotavirus gastroenteritis of any severity. Its efficacy, especially against severe rotavirus disease, persisted through a second rotavirus season. These data are consistent with the considerable protection induced by wild-type rotavirus infection against mild-to-moderate rotavirus gastroenteritis and the virtually complete immunity induced by wild-type infection against severe disease.[3,5] Our study confirms the results of phase 2 trials of the pentavalent vaccine and its predecessors, in which efficacy was 68 percent to 75 percent against rotavirus gastroenteritis of any severity and 100 percent against severe disease.[22,23]

The large sample size in our trial provided an opportunity to quantify the effect of vaccination on health care outcomes related to rotavirus gastroenteritis in a prelicensure setting. The vaccine significantly reduced the need for hospitalization, emergency department visits, and office visits associated with rotavirus gastroenteritis, underscoring the potential public health benefit of a universal vaccination program if the efficacy

Table 3. Clinical Efficacy against Rotavirus Gastroenteritis of Any Severity in the Per-Protocol Population of the Clinical-Efficacy Substudy, According to G Serotype Identified in the Subject's Stool.*

Serotype	No. of Cases of Rotavirus Gastroenteritis		Percent Efficacy (95% CI)
	Vaccine Group (N=2834)	Placebo Group (N=2839)	
G1	72	286	74.9 (67.3–80.9)
G2	6	17	63.4 (2.6–88.2)
G3	1	6	82.7 (<0–99.6)
G4	3	6	48.1 (<0–91.6)
G9	1	3	65.4 (<0–99.3)

* The number of subjects in each group is the number who received at least one dose. CI denotes confidence interval.

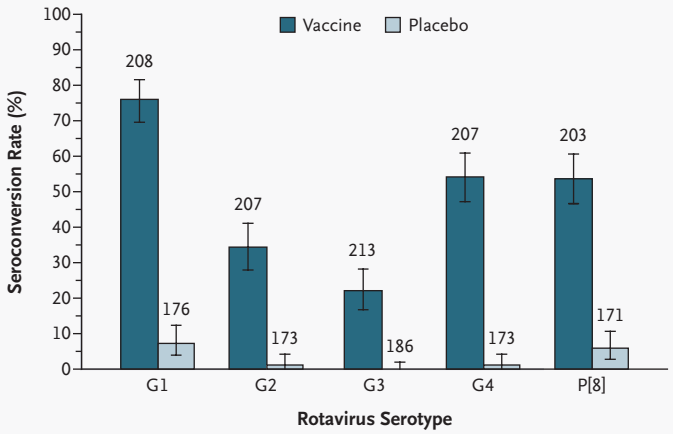

Figure 3. Seroconversion Rates for Serum Neutralizing Antibodies against Human Serotypes Included in the Vaccine.

Seroconversion was defined as an increase by a factor of 3 or more in the serum titer of neutralizing antibodies against the specified rotavirus serotype between baseline and approximately 14 days after the third dose. The number of subjects tested in each group is given above the corresponding bar. I bars represent the 95 percent confidence interval for the point estimates.

observed in our trial is reproduced in clinical practice. Vaccination could also have indirect benefits to society by reducing lost workdays for parents or guardians of young children.

The immunologic mechanism by which rotavirus vaccines protect against rotavirus gastroenteritis is unclear.[35,36] Primary wild-type rotavirus infection induces immunity that is predominantly serotype-specific.[4,5,37] Serotype-specific efficacy could be assessed in the present study only for the strains circulating during the study period. The efficacy of the vaccine could be demonstrated against serotypes G1 through G4 and in a small number of G9 cases, as evidenced by reductions in the incidence of gastroenteritis or the rate of use of health care resources, or both, associated with these serotypes.

For the most part, we enrolled healthy infants between 6 and 12 weeks of age who were from developed countries. Given that the vaccine is administered orally, additional studies to confirm efficacy in children who are malnourished or infected with multiple enteric pathogens are warranted. Along with the apparent absence of an association between intussusception and wild-type rotavirus disease,[24,25] the results of this large trial are reassuring in indicating that not all rotavirus vaccines are associated with intussusception. Because intussusception is an uncommon event, continued monitoring is appropriate. Our results also confirmed the efficacy of the vaccine against rotavirus gastroenteritis through two rotavirus seasons after vaccination. The vaccine markedly decreased the rotavirus-associated use of health care resources. Widespread administration of a safe and effective vaccine could substantially reduce the morbidity and mortality associated with this global childhood disease.

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The views expressed in this article are those of the authors and do not necessarily reflect those of the Indian Health Service.

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REFERENCES

1. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003;9:565-72.
2. Bresee JS, Glass RI, Ivanoff B, Gentsch JR. Current status and future priorities for rotavirus vaccine development, evaluation and implementation in developing countries. Vaccine 1999;17:2207-22.
3. Moulton LH, Staat MA, Santosham M, Ward RL. The protective effectiveness of natural rotavirus infection in an American Indian population. J Infect Dis 1998;178:1562-66.
4. O'Ryan ML, Matson DO, Estes MK, Pickering LK. Anti-rotavirus G type-specific and isotype-specific antibodies in children with natural rotavirus infections. J Infect Dis 1994;169:504-11.
5. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infections in infants as protection against subsequent infections. N Engl J Med 1996;335:1022-8.
6. Velazquez FR, Matson DO, Guerrero ML, et al. Serum antibody as a marker of protection against natural rotavirus infection and disease. J Infect Dis 2000;182:1602-9.
7. Gentsch JR, Woods PA, Ramachandran M, et al. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. J Infect Dis 1996;174:Suppl 1:S30-S36.
8. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 2005;15:29-56.
9. Rotavirus vaccine for the prevention of rotavirus gastroenteritis among children: recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 1999;48(RR-2):1-20.
10. Intussusception among recipients of rotavirus vaccine — United States, 1998–1999. MMWR Morb Mortal Wkly Rep 1999;48:577-81.
11. Kramarz P, France EK, Destefano F, et al. Population-based study of rotavirus vaccination and intussusception. Pediatr Infect Dis J 2001;204:10-6.
12. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344:564-72.
13. Parashar UD, Holman RC, Cummings KC, et al. Trends in intussusception-associated hospitalizations and deaths among US infants. Pediatrics 2000;106:1413-21.
14. Peter G, Myers MG. Intussusception, rotavirus, and oral vaccines: summary of a workshop. Pediatrics 2002;110(6):e67.
15. Haber P, Chen RT, Zanardi LR, Mootrey GT, English R, Braun MM. An analysis of rotavirus vaccine reports to the vaccine adverse event reporting system: more than intussusception alone? Pediatrics 2004;113(4):e353-e359.
16. Joensuu J, Koskenniemi E, Pang XL, Vesikari T. Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. Lancet 1997;350:1205-9.
17. Joensuu J, Koskenniemi E, Vesikari T. Symptoms associated with rhesus-human reassortant rotavirus vaccine in infants. Pediatr Infect Dis J 1998;17:334-40.
18. Rennels MB, Glass RI, Dennehy PH, et al. Safety and efficacy of high-dose rhe-

sus-human reassortant rotavirus vaccines — report of the National Multicenter Trial: United States Rotavirus Vaccine Efficacy Group. Pediatrics 1996;97:7-13.
19. Withdrawal of rotavirus vaccine recommendation. MMWR Morb Mortal Wkly Rep 1999;48:1007.
20. Clark HF, Offit PA. Vaccines for rotavirus gastroenteritis universally needed for infants. Pediatr Ann 2004;33:536-43.
21. Glass RI, Bresee JS, Parashar UD, Jiang B, Gentsch J. The future of rotavirus vaccines: a major setback leads to new opportunities. Lancet 2004;363:1547-50.
22. Clark HF, Bernstein DI, Dennehy PH, et al. Safety, efficacy, and immunogenicity of a live, quadrivalent human-bovine reassortant rotavirus vaccine in healthy infants. J Pediatr 2004;144:184-90.
23. Vesikari T, Clark HF, Offit PA, et al. The effect of dose and composition of a pentavalent rotavirus vaccine (RotaTeq) upon safety, efficacy, and immunogenicity in healthy infants. Presented at the 22nd annual meeting of the European Society for Pediatric Infectious Diseases (ESPID), Tampere, Finland, May 26–28, 2004.
24. Chang EJ, Zangwill KM, Lee H, Ward JI. Lack of association between rotavirus infection and intussusception: implications for use of attenuated rotavirus vaccines. Pediatr Infect Dis J 2002;21:97-102.
25. Rennels MB, Parashar UD, Holman RC, Le CT, Chang HG, Glass RI. Lack of an apparent association between intussusception and wild or vaccine rotavirus infection. Pediatr Infect Dis J 1998;17:924-5.
26. Heaton PM, Goveia MG, Miller JM, Offit P, Clark HF. Development of a pentavalent rotavirus vaccine against prevalent serotypes of rotavirus gastroenteritis. J Infect Dis 2005;192:Suppl 1:S17-S21.
27. Gilchrist MJ, Bretl TS, Moultney K, Knowlton DR, Ward RL. Comparison of seven kits for detection of rotavirus in fecal specimens with a sensitive, specific enzyme immunoassay. Diagn Microbiol Infect Dis 1987;8:221-8.
28. Dolan KT, Twist EM, Horton-Slight P, et al. Epidemiology of rotavirus electropherotypes determined by a simplified diagnostic technique with RNA analysis. J Clin Microbiol 1985;21:753-8.
29. Jennison C, Turnbull BW. Group sequential methods with applications to clinical trials. Boca Raton, Fla.: Chapman & Hall/CRC, 2000.
30. DiStefano DJ, Kraiouchkine N, Mallette L, et al. Novel rotavirus VP7 typing assay using a one-step reverse transcriptase PCR protocol and product sequencing and utility of the assay for epidemiological studies and strain characterization, including serotype subgroup analysis. J Clin Microbiol 2005;43:5876-80.
31. Laboratory-based surveillance for rotavirus — United States, July 1996–June 1997. MMWR Morb Mortal Wkly Rep 1997; 46:1092-4.
32. LeBaron CW, Lew J, Glass RI, Weber JM, Ruiz-Palacios GM. Annual rotavirus epidemic patterns in North America: results of a 5-year retrospective survey of 88 centers in Canada, Mexico, and the United States. JAMA 1990;264:983-8.
33. Vesikari T, Rautanen T, Von Bonsdorff CH. Rotavirus gastroenteritis in Finland: burden of disease and epidemiological features. Acta Paediatr Suppl 1999;88:24-30.
34. Duffy LC, Byers TE, Riepenhoff-Talty M, La Scolea LJ, Zielezny M, Ogra PL. The effects of infant feeding on rotavirus-induced gastroenteritis: a prospective study. Am J Public Health 1986;76:259-63.
35. Ward RL, Knowlton DR, Zito ET, Davidson BL, Rappaport R, Mack ME. Serologic correlates of immunity in a tetravalent reassortant rotavirus vaccine trial: US Rotavirus Vaccine Efficacy Group. J Infect Dis 1997;176:570-7.
36. Coffin SE, Moser CA, Cohen S, Clark HF, Offit PA. Immunologic correlates of protection against rotavirus challenge after intramuscular immunization of mice. J Virol 1997;71:7851-6.
37. Rodriguez WJ, Kim HW, Brandt CD, et al. Sequential enteric illnesses associated with different rotavirus serotypes. Lancet 1978;2:37.

ORIGINAL ARTICLE

Intraperitoneal Cisplatin and Paclitaxel in Ovarian Cancer

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ABSTRACT

BACKGROUND

Standard chemotherapy for newly diagnosed ovarian cancer is a platinum–taxane combination. The Gynecologic Oncology Group conducted a randomized, phase 3 trial that compared intravenous paclitaxel plus cisplatin with intravenous paclitaxel plus intraperitoneal cisplatin and paclitaxel in patients with stage III ovarian cancer.

METHODS

We randomly assigned patients with stage III ovarian carcinoma or primary peritoneal carcinoma with no residual mass greater than 1.0 cm to receive 135 mg of intravenous paclitaxel per square meter of body-surface area over a 24-hour period followed by either 75 mg of intravenous cisplatin per square meter on day 2 (intravenous-therapy group) or 100 mg of intraperitoneal cisplatin per square meter on day 2 and 60 mg of intraperitoneal paclitaxel per square meter on day 8 (intraperitoneal-therapy group). Treatment was given every three weeks for six cycles. Quality of life was assessed.

RESULTS

Of 429 patients who underwent randomization, 415 were eligible. Grade 3 and 4 pain, fatigue, and hematologic, gastrointestinal, metabolic, and neurologic toxic effects were more common in the intraperitoneal-therapy group than in the intravenous-therapy group ($P \leq 0.001$). Only 42 percent of the patients in the intraperitoneal-therapy group completed six cycles of the assigned therapy, but the median duration of progression-free survival in the intravenous-therapy and intraperitoneal-therapy groups was 18.3 and 23.8 months, respectively ($P=0.05$ by the log-rank test). The median duration of overall survival in the intravenous-therapy and intraperitoneal-therapy groups was 49.7 and 65.6 months, respectively ($P=0.03$ by the log-rank test). Quality of life was significantly worse in the intraperitoneal-therapy group before cycle 4 and three to six weeks after treatment but not one year after treatment.

CONCLUSIONS

As compared with intravenous paclitaxel plus cisplatin, intravenous paclitaxel plus intraperitoneal cisplatin and paclitaxel improves survival in patients with optimally debulked stage III ovarian cancer.

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*The Gynecologic Oncology Group member institutions that participated in this study are listed in the Appendix.

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Ovarian cancer is the leading cause of death from a gynecologic cancer in the United States.[1] In most cases, the high death rate is due to tumor that has spread beyond the ovary at the time of diagnosis.[2] In the United States, the standard chemotherapy for the initial treatment of ovarian cancer is a combination of a platinum analogue with paclitaxel.[3,4] With modern surgical interventions and contemporary chemotherapy, most patients attain complete clinical remission.[3,5] The majority of them, however, will eventually have a relapse and die of the disease.

The peritoneal cavity is the principal site of disease in ovarian cancer.[2,6] Although the intensity of intravenous chemotherapy is limited mainly by myelotoxicity, several active drugs can be administered directly into the peritoneal cavity. The rationale for intraperitoneal therapy in ovarian cancer is that the peritoneum, the predominant site of tumor, receives sustained exposure to high concentrations of antitumor agents while normal tissues, such as the bone marrow, are relatively spared.

Two randomized, phase 3 intergroup trials have compared intraperitoneal with intravenous chemotherapy in advanced, low-volume ovarian cancer.[7,8] The first demonstrated a statistically significant survival advantage among patients treated with intraperitoneal chemotherapy, but the regimen did not include paclitaxel.[7] The second trial showed a significant difference in progression-free survival, but the difference in overall survival was of borderline significance (P=0.05). Furthermore, the intraperitoneal-therapy group included two cycles of moderately intensive intravenous carboplatin, which complicated the interpretation of results and added to the toxicity of the treatment.[8] Neither of these trials led to widespread acceptance of intraperitoneal treatment. The reluctance of clinicians to embrace intraperitoneal therapy is due to multiple factors, including its high cost and toxicity and clinicians' lack of familiarity with peritoneal administration and catheter-placement techniques. The possibility that improved outcomes with newer forms of therapy could replace intraperitoneal treatment has also been a consideration.[9,10]

We report the results of a randomized, phase 3 trial in which a regimen of six cycles of treatment with intravenous paclitaxel followed by intravenous cisplatin was compared with six cycles of intravenous paclitaxel followed by intraperitoneal cisplatin and intraperitoneal paclitaxel in women with previously untreated stage III ovarian cancer.

METHODS

PATIENTS

Eligible patients had stage III epithelial ovarian or peritoneal carcinoma with no residual mass greater than 1.0 cm in diameter after surgery, a Gynecologic Oncology Group (GOG) performance status of 0 to 2 (with 0 being fully active and 4 completely disabled), normal blood counts, and adequate renal and hepatic function. All cases were centrally reviewed by the GOG to confirm patients' surgical and pathological eligibility for enrollment. This review was not strictly blinded. However, pathology reports, operative notes, and eligibility information were collected before registration. Patients who had undergone prior chemotherapy or radiation for ovarian cancer were not eligible. All patients gave written informed consent according to institutional and federal guidelines before enrollment. Approval was granted by the institutional review board at each participating site.

At registration, participants decided whether they would undergo a second-look laparotomy at the completion of chemotherapy. At study entry and before each treatment, a physical examination was performed and medical history taking, complete blood count, blood chemical measurements, and measurement of serum ovarian cancer antigen 125 were carried out. This evaluation was repeated at the completion of therapy, every 3 months for 24 months, and then every 6 months. Quality-of-life assessment, with use of the Functional Assessment of Cancer Therapy — Ovarian (FACT-O) instrument,[11] was performed four times: at registration, before cycle 4, 3 to 6 weeks after cycle 6, and 12 months after the completion of therapy. All patients were followed for clinical progression and death.

TREATMENT PLAN

Patients were randomly assigned to receive either 135 mg of intravenous paclitaxel per square meter of body-surface area over a 24-hour period on day 1 followed by 75 mg of intravenous cisplatin per square meter on day 2 (intravenous-therapy group) or 135 mg of intravenous paclitaxel per square meter over a 24-hour period on day 1 followed by 100 mg of intraperitoneal cisplatin per

square meter on day 2 and 60 mg of intraperitoneal paclitaxel per square meter on day 8 (intraperitoneal-therapy group). Standard premedication was given to prevent hypersensitivity reactions to paclitaxel. Hydration and antiemetic agents were given before cisplatin was administered. For intraperitoneal therapy, paclitaxel or cisplatin was reconstituted in 2 liters of warmed normal saline and infused as rapidly as possible through an implantable peritoneal catheter. Treatments were administered every three weeks for six cycles.

Before they could receive a subsequent cycle of therapy, patients were required to have an absolute neutrophil count of 1500 cells per cubic millimeter or greater, a platelet count of 100,000 cells per cubic millimeter or greater, and a creatinine level of 2.0 mg per deciliter or less. Treatment modifications for hematologic toxic effects included cycle delay, dose reduction, and the addition of granulocyte colony-stimulating factor (in that sequence). There was no dose modification if the nadir of leukopenia was not accompanied by fever. Treatment was postponed in the case of grade 3 or 4 peripheral neuropathy, a creatinine level greater than 2.0 mg per deciliter, or a creatinine clearance of less than 50 ml per minute. Patients in whom treatment was delayed for more than three weeks were removed from the study.

Among patients in the intraperitoneal-therapy group, the dose of intraperitoneal drug was reduced if there was grade 2 abdominal pain. Patients with grade 3 abdominal pain, recurrent grade 2 abdominal pain after a dose reduction, or complications involving the intraperitoneal catheter that prohibited further intraperitoneal therapy received intravenous chemotherapy for the remaining cycles. The dose of cisplatin was reduced if there was grade 2 peripheral neuropathy. Women in either group who had a cisplatin-related toxic effect requiring discontinuation of the protocol treatment received intravenous therapy, with carboplatin substituted for cisplatin.

If second-look assessment was elected at registration, it was performed within 8 weeks after the last cycle of chemotherapy and no later than 29 weeks after study entry. Categories of pathological response were defined as follows: negative (i.e., there was a complete response), positive with microscopic disease only, or positive with grossly visible persistent disease.

STATISTICAL ANALYSIS

The GOG Statistical and Data Center randomly assigned patients to one of the two treatment groups, with stratification according to residual disease (grossly visible disease vs. no visible disease) and the second-look surgery option (selected vs. declined), with use of a permuted block containing three assignments for each regimen. A sample size of 384 eligible patients was set, with sufficient follow-up to observe 208 recurrences (and 208 deaths) before final testing of the primary hypothesis, which was based on the following research question: Does the use of intraperitoneal cisplatin and paclitaxel improve progression-free and overall survival as compared with intravenous cisplatin and paclitaxel? This sample size provided 90 percent statistical power with the use of a one-sided log-rank test,[12] an alpha level of 0.05, and a hazard ratio (for intravenous vs. intraperitoneal administration) of 1.5.[13] Projections indicated that 61 percent of the patients in the intravenous-therapy group would have died by the time of the final analysis.

The primary study end points — progression-free survival and overall survival — were measured from the date of randomization. Survival was measured up to the date of death or, for living patients, the date of last contact. The duration of progression-free survival was the time until progression, death, or the date of last contact, whichever came first. The planned analyses of overall survival and progression-free survival included only eligible patients (on the basis of the intention-to-treat principle). All causes of death were used in the calculation of overall survival. Estimates of the cumulative proportions of survival were based on the Kaplan–Meier procedure.[14] Estimates of the relative risk and confidence intervals for treatment effects with respect to progression and death were generated with use of the Cox model.[15] Primary unadjusted estimates were calculated with use of the two stratification factors as covariates. Adjusted estimates were based on two previously identified additional covariates (age and histologic features).[16]

Eligible women who received at least one cycle of treatment were assessed for toxic effects. Patients in the intraperitoneal-therapy group who had complications related to the intraperitoneal catheter were assessed for toxic effects, regardless

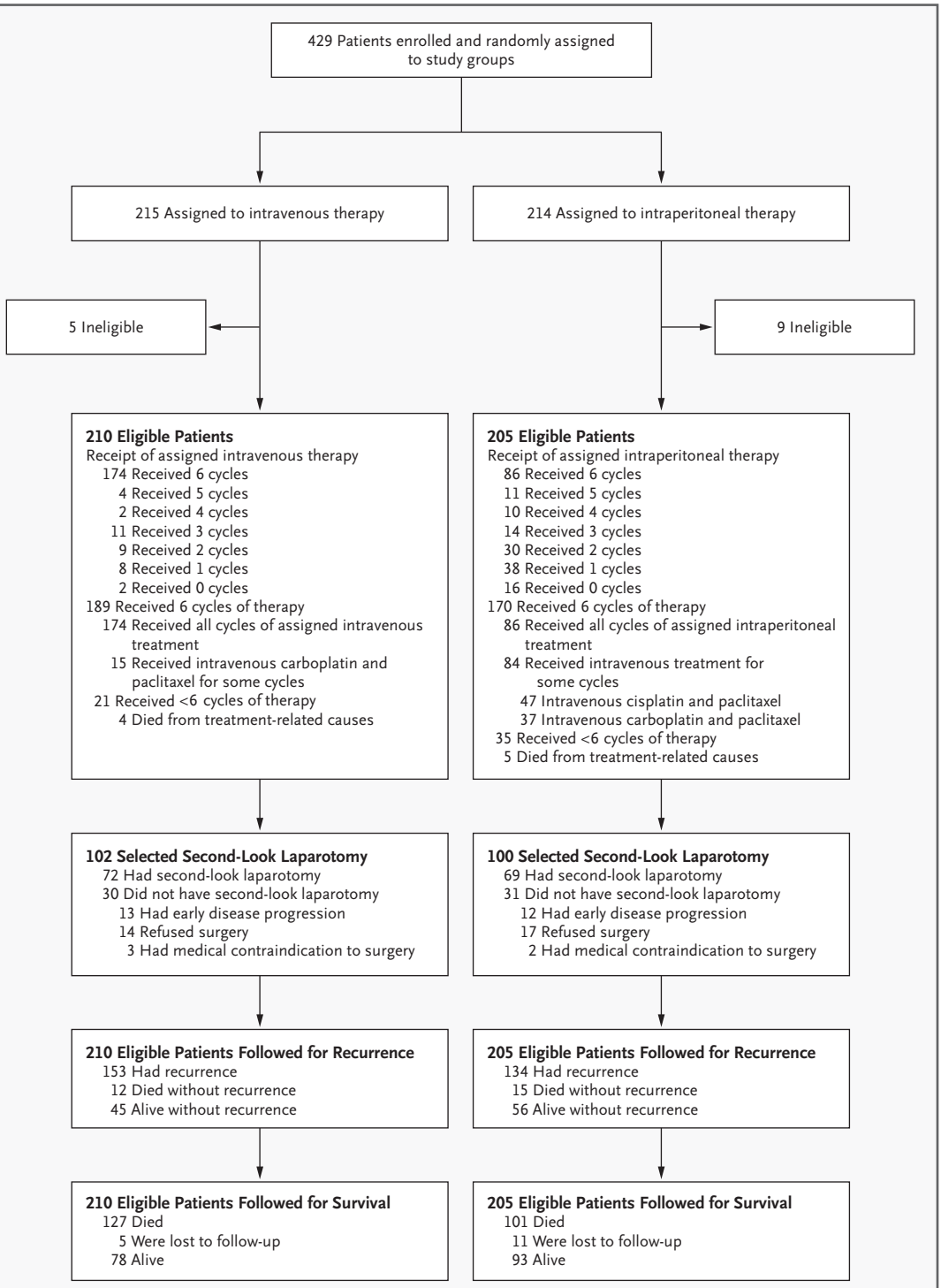


Figure 1. Study Patients.

Table 1. Characteristics of the Patients.*

Characteristic	Intravenous-Therapy Group (N=210)	Intraperitoneal-Therapy Group (N=205)
	no. (%)	
Second-look laparotomy		
Not elected	108 (51)	105 (51)
Elected	102 (49)	100 (49)
Age at diagnosis		
21–30 yr	0	4 (2)
31–40 yr	15 (7)	8 (4)
41–50 yr	43 (20)	52 (25)
51–60 yr	74 (35)	62 (30)
61–70 yr	56 (27)	53 (26)
71–80 yr	19 (9)	24 (12)
>80 yr	3 (1)	2 (1)
Race or ethnic group†		
Hispanic	9 (4)	9 (4)
Asian or Pacific Islander	9 (4)	4 (2)
Black	4 (2)	7 (3)
White	187 (89)	185 (90)
Other	1 (<1)	0
GOG performance status		
0	90 (43)	91 (44)
1	112 (53)	99 (48)
2	8 (4)	15 (7)
Histologic type		
Serous adenocarcinoma	170 (81)	158 (77)
Endometrioid adenocarcinoma	12 (6)	17 (8)
Mixed epithelial carcinoma	11 (5)	14 (7)
Clear-cell carcinoma	9 (4)	11 (5)
Other type	8 (4)	5 (2)
Histologic grade‡		
1	18 (9)	25 (12)
2	83 (40)	72 (35)
3	106 (50)	106 (52)
Gross residual disease		
No	75 (36)	78 (38)
Yes	135 (64)	127 (62)
Disease		
Ovarian cancer	183 (87)	184 (90)
Primary peritoneal cancer	27 (13)	21 (10)

* Because of rounding, not all percentages total 100.
† Race or ethnic group was determined by the investigator or was self-reported at each site.
‡ Five cases were not graded.

of their ability to receive treatment. The Wilcoxon rank-sum test was used to test the independence of the risk of severe and life-threatening toxic effects (grade 0, 1, or 2 vs. grade 3 vs. grade 4) from the assigned treatment.[17]

Quality-of-life assessments from baseline to follow-up (conducted before the fourth cycle, 3 to 6 weeks after the sixth cycle, and 12 months after the sixth cycle) were analyzed with linear models with an unstructured covariance matrix. Patients' age, performance status at randomization, and baseline assessment scores were potential covariates. The restricted maximum likelihood was used to estimate the covariance parameters. Quality of life was a secondary end point. All P values are two-sided.

RESULTS

PATIENTS

Between March 1998 and January 2001, 429 women were randomly assigned to the intravenous-therapy group (215 patients) or the intraperitoneal-therapy group (214 patients) (Fig. 1). Fourteen patients were ineligible (five in the intravenous-therapy group and nine in the intraperitoneal-therapy group) for the following reasons: stage other than optimal stage III (three patients), the presence of a second primary cancer (one patient), a nonepithelial cell type (five patients), a primary cancer other than ovarian or peritoneal carcinoma (one patient), inadequate surgery (two patients), or a tumor with low malignant potential (two patients). Table 1 shows the characteristics of the 415 eligible patients whose data form the basis of this report.

TOXICITY

Of the 210 eligible patients assigned to the intravenous-therapy group, 189 (90 percent) completed six cycles of chemotherapy, and 174 (83 percent) received all six cycles of the assigned intravenous therapy (Fig. 1). Of the 205 eligible patients assigned to the intraperitoneal-therapy group, 170 (83 percent) completed six cycles of chemotherapy, and 86 (42 percent) received all six cycles of the assigned intraperitoneal therapy. For patients in either group who had intolerable toxic effects related to cisplatin, that drug was switched to intravenous carboplatin. The primary reason for discontinuation of intraperitoneal therapy was catheter-related complications.[18] There were

nine treatment-related deaths, four in the intravenous-therapy group and five in the intraperitoneal-therapy group. All nine treatment-related deaths were attributed to infection. Of the five treatment-related deaths in the intraperitoneal-therapy group, three were also partially attributed to the tumor.

Table 2 lists adverse events. Significantly more patients in the intraperitoneal-therapy group than in the intravenous-therapy group had severe or life-threatening (grade 3 or 4) fatigue, pain, or hematologic, gastrointestinal, metabolic, or neurologic toxic effects (P≤0.001).

Table 2. Frequency of Grade 3 or 4 Adverse Events.

Adverse Event	Intravenous-Therapy Group (N=210)	Intraperitoneal-Therapy Group (N=201)*	P Value†
	no. (%)		
Leukopenia‡	134 (64)	152 (76)	<0.001
Platelet count <25,000/mm^3	8 (4)	24 (12)	0.002
Other hematologic event	190 (90)	188 (94)	0.87
Gastrointestinal event	51 (24)	92 (46)	<0.001
Renal or genitourinary event	5 (2)	14 (7)	0.03
Pulmonary event	5 (2)	7 (3)	0.50
Cardiovascular event	10 (5)	19 (9)	0.06
Neurologic event	18 (9)	39 (19)	0.001
Cutaneous change	2 (1)	2 (1)	0.96
Event involving lymphatic system	0	3 (1)	0.07
Fever	8 (4)	19 (9)	0.02
Infection	12 (6)	33 (16)	0.001
Fatigue	9 (4)	36 (18)	<0.001
Metabolic event	15 (7)	55 (27)	<0.001
Pain	3 (1)	23 (11)	<0.001
Hepatic event	1 (<1)	6 (3)	0.05
Other	1 (<1)	6 (3)	0.05

* Four patients did not receive any protocol-based therapy.
† P values were calculated by the Wilcoxon rank-sum test (grades 0, 1, and 2 vs. grades 3 and 4).
‡ A white-cell count below 1000 per cubic millimeter was considered to indicate leukopenia.

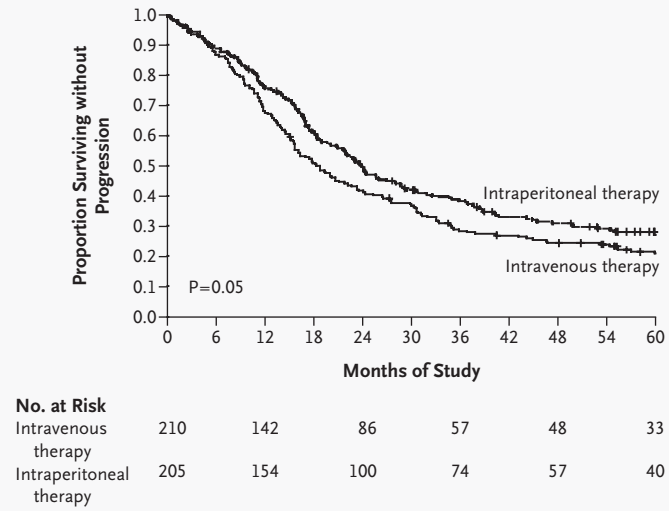
PATHOLOGICAL RESPONSES AT SECOND-LOOK LAPAROTOMY

Second-look laparotomy after the completion of therapy was not mandatory, and the results of second-look surgery were not an end point of this study. Of the 415 eligible patients, 202 (49 percent) registered for second-look surgery. The frequency of refusal and the rate of medical contraindication to the procedure were similar in the two groups. The rate of complete pathological response was 41 percent in the intravenous group (35 of 85 patients had such a response) and 57 percent in the intraperitoneal group (46 of 81 patients).

SURVIVAL

The median duration of follow-up was 48.2 months in the intravenous-therapy group and 52.6 months in the intraperitoneal-therapy group, with 5 and 11 patients, respectively, lost-to-follow-up. The median progression-free survival was 18.3 months in the intravenous-therapy group and 23.8 months in the intraperitoneal-therapy group (Fig. 2A and Table 3). The median overall survival was 49.7 and 65.6 months, respectively (Fig. 2B and Table 3). Table 3 lists relative risks, 95 percent confidence intervals, and P values for progression-free and overall survival in the two groups. The adjusted estimates of the relative risk of recurrence and death (0.77 and 0.73, respectively, in the intraperitoneal-therapy group as compared with the intravenous-therapy group) were similar to the primary estimates (0.80 and 0.75, respectively). There was no statistical difference in the risk reduction associated with intraperitoneal therapy between the subgroup with gross visible residual disease and the subgroup with no visible residual disease at initial surgery (Table 3). An analysis that included all randomly assigned patients (eligible and ineligible) yielded negligible changes in the relative-risk estimates.

Before randomization, patients in the intraperitoneal-therapy group reported lower FACT-O (quality-of-life) scores than those in the intravenous group. After adjustments were made for age, performance status, and the baseline FACT-O score, patients receiving intraperitoneal therapy reported worse quality of life before cycle 4 (P<0.001) and three to six weeks after treatment (P=0.009). There were no significant quality-of-life differences between the groups one year after treatment (Table 4). Differences in neurotoxic effects and abdominal discomfort between the two groups have been reported elsewhere.[19,20]



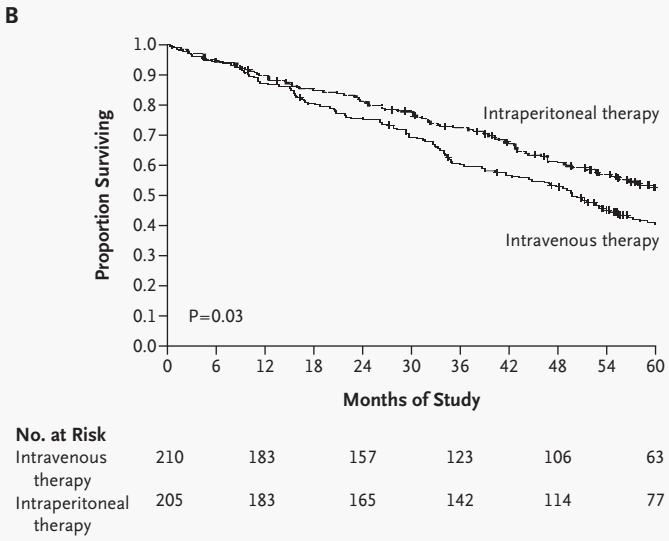


Figure 2. Progression-free and Overall Survival.

Panel A shows progression-free survival and Panel B overall survival among the 415 eligible patients with stage III ovarian cancer who were randomly assigned to treatment with intravenous paclitaxel and cisplatin or to treatment with intravenous paclitaxel, intraperitoneal cisplatin, and intraperitoneal paclitaxel. Eighty-five percent of the patients either died or were followed for five years. As shown in Panel A, treatment failed in 165 patients in the intravenous-therapy group: 153 (73 percent) had a recurrence, and 12 died without a documented recurrence. Forty-five patients in the intravenous-therapy group had no evidence of disease. Treatment failed in 149 patients in the intraperitoneal-therapy group: 134 (65 percent) had a recurrence, and 15 died without a documented recurrence. Fifty-six patients in the intraperitoneal group had no evidence of disease. As shown in Panel B, in the intravenous-therapy group, 127 patients (60 percent) died and 5 were lost to follow-up. Seventy-eight patients in the intravenous-therapy group were alive. In the intraperitoneal-therapy group, 101 patients (49 percent) died and 11 were lost to follow-up. Ninety-three patients in the intraperitoneal-therapy group were alive.

DISCUSSION

An intensive regimen of intravenous paclitaxel followed by intraperitoneal cisplatin and paclitaxel significantly improved progression-free survival (P=0.05) and overall survival (P=0.03) among women with newly diagnosed, optimally debulked stage III ovarian cancer. As compared with the intravenous-therapy group, women who received intraperitoneal treatment had a 25 percent reduction in the risk of death. Among all randomized phase 3 trials conducted by the GOG among patients with advanced ovarian cancer, the current trial yielded the longest median survival: 65.6 months, in the group of patients who received intraperitoneal therapy.

Ovarian cancer commonly spreads within the peritoneal cavity; there is a reduced likelihood of substantial hematogenous or lymphatic dissemination. Successful tumor cytoreduction with modern surgical approaches allows chemotherapy to be administered in the setting of low-volume residual disease within the peritoneal cavity. The rationale for intraperitoneal administration is supported by preclinical and pharmacokinetic data and, with this study, a growing body of clinical data. In a previous GOG study, doubling the dose of intravenous cisplatin and cyclophosphamide did not improve survival.[21] Furthermore, the strategy of increasing the dose density or dose intensity of systemic platinum agents is limited by the nonhematologic toxicity of cisplatin and the lack of a reliable platelet growth factor to overcome carboplatin-related thrombocytopenia. These limitations can be overcome, in part, by intraperitoneal administration.

Patients in the intraperitoneal-therapy group had more toxic events than women in the intravenous-therapy group. These toxic events may be attributed to the higher dose of cisplatin in the intraperitoneal-therapy group. The rationale for increasing the cisplatin dose is that capillary uptake of cisplatin from peritoneal surfaces is slow and incomplete, resulting in systemic exposure that is prolonged but lower than that with intravenous administration.[22] The dose of intraperitoneal cisplatin used in this study has previously been given in combination with intravenous paclitaxel[8] and with intravenous cyclophosphamide[7] and in a phase 2 trial of the same regimen[23] with acceptable toxicity. Alternatively, the increased incidence of toxic events

Table 3. Summary of Comparisons between the Treatment Groups.

Variable	Median Duration		No. of Events*		Relative Risk (95% CI)†	P Value
	Intravenous-Therapy Group	Intraperitoneal-Therapy Group	Intravenous-Therapy Group	Intraperitoneal-Therapy Group		
	mo					
Progression-free survival	18.3	23.8	165	149	0.80 (0.64–1.00)	0.05
Gross residual disease	15.4	18.3	115	105	0.81 (0.62–1.05)	0.97‡
No visible residual disease	35.2	37.6	50	44	0.80 (0.54–1.21)	
Overall survival	49.7	65.6	127	101	0.75 (0.58–0.97)	0.03
Gross residual disease	39.1	52.6	95	77	0.77 (0.57–1.04)	0.72‡
No visible residual disease	78.2	NA§	32	24	0.69 (0.41–1.17)	

* Events were a recurrence of disease or death without documented recurrence in the analysis of progression-free survival and death regardless of cause in the analysis of overall survival.
† The relative risk is the risk of recurrence or death in the intraperitoneal-therapy group as compared with that in the intravenous-therapy group. The primary estimate for the entire study group included the covariates of residual-disease status and the second-look surgery option.
‡ The P value was calculated by a test for the homogeneity of relative risk between the two categories of residual-disease status.
§ NA denotes not applicable because the medians for survival had not yet been reached.

in the intraperitoneal-therapy group may be due to the intraperitoneal paclitaxel. Paclitaxel persists in the peritoneum for one week after intraperitoneal administration, suggesting that peritoneal clearance is very slow.[24] Nevertheless, with the dose used in this study, paclitaxel is detectable in the plasma after intraperitoneal administration.[24] It is possible that peritoneal clearance of paclitaxel is altered when the drug is given after intraperitoneal cisplatin, as it was in this study, or that even low blood levels of paclitaxel one week after the administration of intravenous paclitaxel and intra-peritoneal cisplatin can increase toxicity. Careful monitoring of toxicity and the use of contemporary supportive care measures might improve the tolerability of the regimen we used. However, it is not known whether altering the intraperitoneal regimen to decrease toxicity will affect its efficacy.

Given the increased toxicity associated with intraperitoneal therapy, an important secondary outcome of this study was the quality of life. Patients in the intraperitoneal-therapy group reported worse quality of life before cycle 4 and three to six weeks after treatment was completed than did those in the intravenous-therapy group. These differences were not observed one year after treatment was completed, at which time quality-of-life scores had improved relative to baseline in both groups.

A substantial portion of patients in the intraperitoneal-therapy group had toxic effects and treatment intolerance related to the catheter required for intraperitoneal administration. In this group, 48 percent received three or fewer cycles of intraperitoneal treatment, and only 42 percent received all six assigned cycles of intraperitoneal therapy. The type of catheter and the timing of catheter placement were not specified in the study design. A separate, detailed evaluation of intraperitoneal catheter–related outcomes in this study showed that patients who had a left colonic or rectosigmoid resection at the time of initial surgery were less likely to receive all planned doses of intraperitoneal therapy.[18] The single-lumen venous-access catheter attached to an implanted subcutaneous port has been reported to be superior to the fenestrated catheter designed for intraperitoneal use, with minimal fibrous-sheath formation and a markedly reduced risk of small-bowel obstruction or perforation.[25] Thus, standardization of the device to be used and the technique and timing of port implantation could improve the success of intraperitoneal therapy.

Although fewer than half the patients assigned to the intraperitoneal group received six cycles of intraperitoneal treatment, the group as a whole had a significant improvement in survival as compared with the intravenous group. It is possible that most of the benefit of intraperitoneal therapy occurs early, during the initial cycles, or that

Table 4. Mean FACT-O Quality-of-Life Scores in the Two Groups at Each Assessment Point.*

Assessment Point	Intravenous-Therapy Group		Intraperitoneal-Therapy Group		Mean Difference (95% CI)†	P Value
	No. of Patients	Score	No. of Patients	Score		
Before randomization	201	111.9±19.3	198	106.4±20.5	5.0 (1.2 to 8.8)	0.03‡
Before fourth cycle	172	114.7±18.6	148	103.3±19.2	8.9 (5.3 to 12.5)	<0.001§
3–6 Wk after sixth cycle	171	118.4±19.2	159	110.5±21.0	5.2 (1.3 to 9.1)	0.009§
12 Mo after sixth cycle	140	127.2±19.1	139	125.5±19.2	1.2 (–5.1 to 2.8)	0.56§

* Plus–minus values are means ±SD. Lower Functional Assessment of Cancer Therapy — Ovarian (FACT-O) scores (ranging from 0 to 156) indicate poorer quality of life. CI denotes confidence interval.
† The mean difference is the estimated adjusted mean value in the intravenous-therapy group minus the corresponding mean value in the intraperitoneal-therapy group.
‡ The P value was calculated with use of the general linear model, with adjustment for age and performance status at randomization.
§ The P value was calculated with use of the linear mixed model, with adjustment for age, performance status, and baseline FACT-O score.

the benefit of intraperitoneal therapy may be greater if more patients can successfully complete six cycles of treatment. This study was not designed to address the effect of the duration of treatment on clinical outcome, and retrospective analysis of this variable has the potential for bias. Possible means of improving the tolerability of intraperitoneal treatment include identification and exclusion of patients at risk for poor tolerance, modification of the dose of drug used, alteration of the administration schedule, and use of less toxic chemotherapeutic agents. Studies of intraperitoneal carboplatin,[26] of weekly intraperitoneal paclitaxel, and of combinations of intravenous paclitaxel and intraperitoneal docetaxel may identify regimens with improved tolerance. Since modifications that improve tolerability may decrease antitumor efficacy, these approaches will require rigorous testing in randomized trials before they can be recommended.

Including this study, there are now three randomized trials showing that intraperitoneal chemotherapy has a clinical advantage in the treatment of ovarian cancer. Although this advantage comes at the expense of increased toxicity and reduced quality of life during treatment, these results should encourage the use of intraperitoneal chemotherapy in patients with advanced ovarian cancer.

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APPENDIX

The following Gynecologic Oncology Group member institutions participated in this study: the University of Alabama at Birmingham, Duke University Medical Center, Abington Memorial Hospital, Walter Reed Army Medical Center, Wayne State University, the University of Minnesota Medical School, the University of Mississippi Medical Center, the Colorado Foundation for Medical Care, the University of California Medical Center at Los Angeles, the University of Washington Medical Center, the Hospital of the University of Pennsylvania, the Milton S. Hershey School of Medicine of the Pennsylvania State University, the University of Cincinnati College of Medicine, the University of North Carolina School of Medicine, the University of Iowa Hospitals and Clinics, the University of Texas Southwestern Medical Center at Dallas, Indiana University School of Medicine, Wake Forest University School of Medicine, the University of California, Irvine, Medical Center, Tufts New England Medical Center, Rush–Presbyterian-St. Luke's Medical Center, the University of Kentucky, National Cancer Institute–Community Clinical Oncology Program, the Cleveland Clinic Foundation, State University of New York at Stony Brook, Washington University School of Medicine, Columbus Cancer Council, the University of Massachusetts Medical Center, the Women's Cancer Center of California, University of Oklahoma, the University of Virginia, the University of Chicago, Tacoma General Hospital, Thomas Jefferson University Hospital, the Mayo Clinic, Case Western Reserve University, Tampa Bay Cancer Consortium, North Shore University Hospital, Brookview Research, and Ellis Fischel Cancer Center.

REFERENCES

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. CA Cancer J Clin 2005; 55:10-30. [Erratum, CA Cancer J Clin 2005; 55:259.]
2. Cannistra SA. Cancer of the ovary. N Engl J Med 2004;351:2519-29.
3. McGuire WP, Hoskins WJ, Brady MF, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N Engl J Med 1996;334:1-6.
4. Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol 2003;21:3194-200.
5. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol 2002; 20:1248-59.
6. Thigpen T. The if and when of surgical debulking for ovarian carcinoma. N Engl J Med 2004;351:2544-6.
7. Alberts DS, Liu PY, Hannigan EV, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 1996;335:1950-5.
8. Markman M, Bundy BN, Alberts DS, et al. Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 2001;19:1001-7.
9. McGuire WP. Intraperitoneal therapy for ovarian cancer: a sacrifice bunt. J Clin Oncol 2001;19:921-3.
10. Ozols RF, Gore M, Trope C, Grenman S. Intraperitoneal treatment and dose-intense therapy in ovarian cancer. Ann Oncol 1999; 10:Suppl 1:59-64.
11. Basen-Engquist K, Bodurka-Bevers D, Fitzgerald MA, et al. Reliability and validity of the Functional Assessment of Cancer Therapy — Ovarian. J Clin Oncol 2001; 19:1809-17.
12. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163-70.
13. Schoenfeld D. Sample-size formula for the proportional-hazards regression model. Biometrics 1983;39:499-503.
14. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.
15. Cox DR. Regression models and life tables. J R Stat Soc [B] 1972;34:187-220.
16. Greer BE, Bundy BN, Ozols RF, et al. Implications of second-look laparotomy in the context of optimally resected stage III ovarian cancer: a non-randomized comparison using an explanatory analysis: a Gynecologic Oncology Group study. Gynecol Oncol 2005;99:71-9.
17. Hollander M, Wolfe DA. Nonparametric statistical methods. 2nd ed. New York: John Wiley, 1999.
18. Walker JL, Armstrong DK, Huang HQ, et al. Intraperitoneal catheter outcomes in a phase 3 trial of intravenous vs. intraperitoneal chemotherapy in optimal stage III ovarian and primary peritoneal cancer: a Gynecologic Oncology Group study. Gynecol Oncol 2006;100:27-32.
19. Wenzel LB, Huang H, Armstrong D, Walker J, Cella D. Quality of life (QOL) results of a randomized study of intravenous (IV) paclitaxel and cisplatin vs intravenous paclitaxel, intraperitoneal (intraperitonal) cisplatin and intraperitonal paclitaxel in optimal stage III epithelial ovarian cancer (OC): a Gynecologic Oncology Group trial. Proc Am Soc Clin Oncol 2004;23:454. abstract.
20. *Idem.* Validation of a FACT/GOG-Abdominal Discomfort (AD) subscale: a Gynecologic Oncology Group (GOG) study. Proc Am Soc Clin Oncol 2005;23:754. abstract.
21. McGuire WP, Hoskins WJ, Brady MF, et al. Assessment of dose-intensive therapy in suboptimally debulked ovarian cancer: a Gynecologic Oncology Group study. J Clin Oncol 1995;13:1589-99.
22. Schneider JG. Intraperitoneal chemotherapy. Obstet Gynecol Clin North Am 1994;21:195-212.
23. Rothenberg ML, Liu PY, Braly PS, et al. Combined intraperitoneal and intravenous chemotherapy for women with optimally debulked ovarian cancer: results from an intergroup phase II trial. J Clin Oncol 2003;21:1313-9.
24. Francis P, Rowinsky E, Schneider J, Hakes T, Hoskins W, Markman M. Phase I feasibility and pharmacologic study of weekly intraperitoneal paclitaxel: a Gynecologic Oncology Group pilot study. J Clin Oncol 1995;13:2961-7.
25. Alberts DS, Markman M, Armstrong D, Rothenberg ML, Muggia F, Howell SB. Intraperitoneal therapy for stage III ovarian cancer: a therapy whose time has come! J Clin Oncol 2002;20:3944-46.
26. Fujiwara K, Sakuragi N, Suzuki S, et al. First-line intraperitoneal carboplatin-based chemotherapy for 165 patients with epithelial ovarian carcinoma: results of long-term follow-up. Gynecol Oncol 2003;90: 637-43. [Erratum, Gynecol Oncol 2003;91: 662.]

REVIEW ARTICLE

CURRENT CONCEPTS

Community-Acquired Bacterial Meningitis in Adults

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Bacterial meningitis is a medical, neurologic, and sometimes neurosurgical emergency that requires a multidisciplinary approach. Bacterial meningitis has an annual incidence of 4 to 6 cases per 100,000 adults (defined as patients older than 16 years of age), and *Streptococcus pneumoniae* and *Neisseria meningitidis* are responsible for 80 percent of all cases.[1,2] A diagnosis of bacterial meningitis is often considered, but the disease can be difficult to recognize.[1-8] Recommendations for antimicrobial therapy are changing as a result of the emergence of antimicrobial resistance. In this review we summarize the current concepts of the initial approach to the treatment of adults with bacterial meningitis, highlighting adjunctive dexamethasone therapy and focusing on the management of neurologic complications.

INITIAL APPROACH

In adults presenting with community-acquired acute bacterial meningitis, the sensitivity of the classic triad of fever, neck stiffness, and altered mental status is low (44 percent), but almost all such patients present with at least two of four symptoms — headache, fever, neck stiffness, and altered mental status (as defined by a score below 14 on the Glasgow Coma Scale).[1] Lumbar puncture is mandatory in any patient in whom bacterial meningitis is suspected, although the procedure can be hazardous (Fig. 1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Reports have emphasized the risk of brain herniation as a complication of diagnostic lumbar puncture in patients with meningitis.[9-13] Patients with expanding masses (e.g., subdural empyema, brain abscess, or necrotic temporal lobe in herpes simplex encephalitis) may present with symptoms that appear to be identical with those of bacterial meningitis, and in these patients as well, lumbar puncture may be complicated by brain herniation.[5,9] The withdrawal of cerebrospinal fluid reduces counterpressure from below, thereby adding to the effect of compression from above, increasing the brain shift that may already be present (Fig. 1).[9] Neuroimaging — either cranial computed tomography (CT) or magnetic resonance imaging (MRI) — to detect brain shift is recommended as a precaution in selected patients before lumbar puncture.[9,10] A prospective study involving 301 adults with suspected meningitis confirmed that clinical features can be used to identify patients who are unlikely to have abnormal findings on cranial CT (41 percent of the patients in this study).[10] Of 235 patients who underwent cranial CT, in only 5 patients (2 percent) was bacterial meningitis confirmed, reflecting the heterogeneity of the study group with clinically suspected bacterial meningitis. Cranial imaging should precede lumbar puncture in patients who have new-onset seizures, an immunocompromised state, signs that are suspicious for space-occupying lesions, or moderate-to-severe impairment of consciousness.[9,10,13] When these

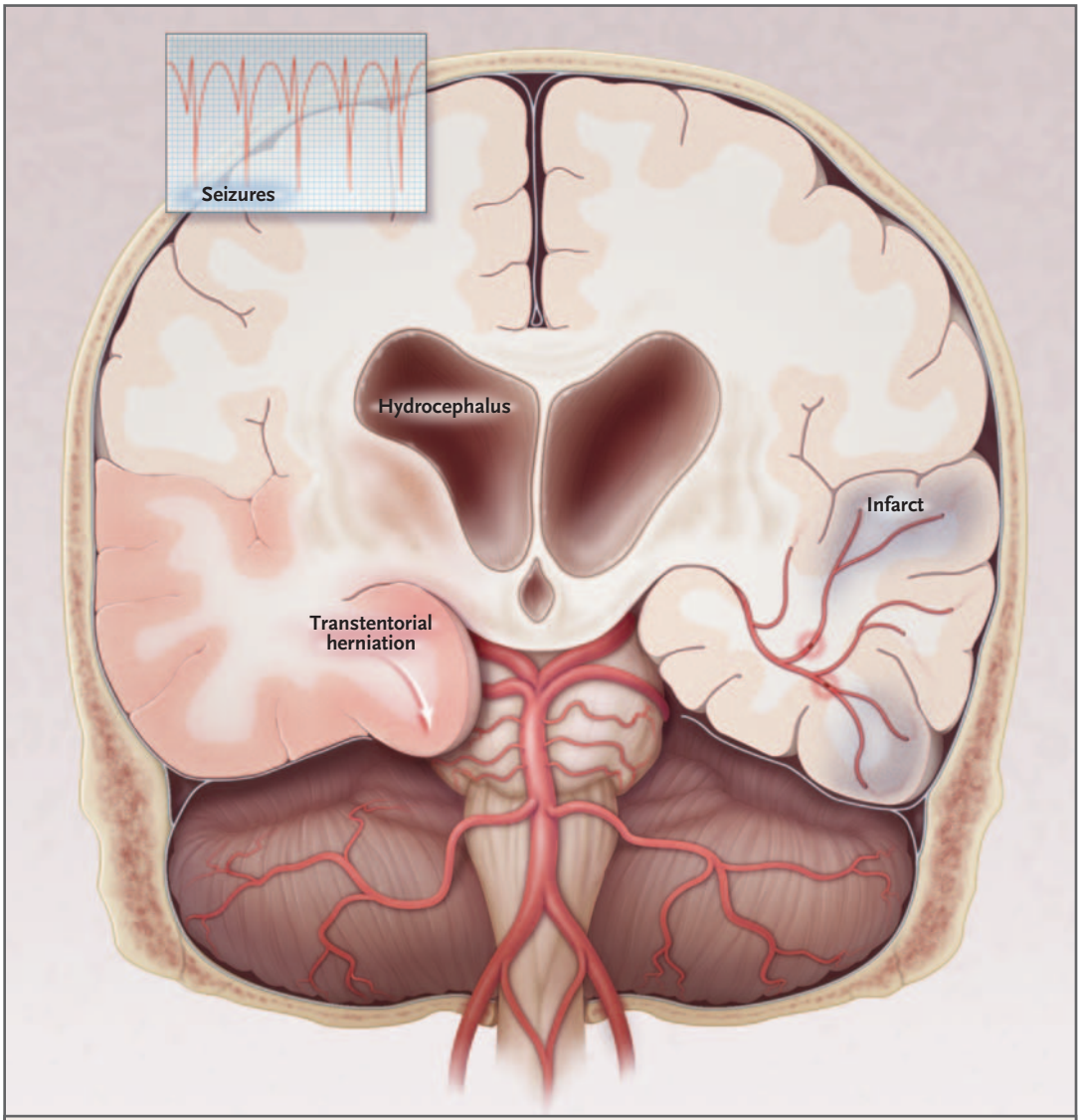

Figure 1. Major Intracranial Complications in Bacterial Meningitis in Adults.

Transtentorial herniation is caused by diffuse swelling of the brain, or hydrocephalus; the herniation may be asymmetrical when lateral focal lesions are present. Hydrocephalus results from basal obstruction of the cerebrospinal fluid. Infarcts are caused by inflammatory occlusion of the basal arteries. Seizures, as indicated on encephalograms, are caused by cortical inflammation.

criteria are met, indications for cranial imaging before lumbar puncture are present in about 45 percent of patients with bacterial meningitis confirmed by cerebrospinal fluid culture.[1] Lumbar puncture may also be harmful in patients with coagulopathy, because of the chance of needle-induced subarachnoid hemorrhage or of the development of spinal subdural and epidural hematomas.[14] When a diagnosis of bacterial meningitis is probable but neuroimaging is not available, lumbar puncture should be given preference in patients with moderate-to-severe impairment of consciousness or in an immunocompromised state. However, when warning signs of a space-occupying lesion (e.g., new-onset seizure, papilledema, or evolving signs of brain tissue shift) are present, lumbar puncture should not be performed until after neuroimaging has been performed.

Delay in the initiation of antimicrobial therapy can result in poor outcome in this disease. In a retrospective study, patients were stratified as having a low, intermediate, or high risk of adverse

outcome, according to baseline prognostic factors.[15] The median delay between the time of arrival at the emergency department and the administration of antibiotics was four hours. Among patients whose condition worsened while they were in the emergency department to a category with a higher risk of having an adverse outcome, an association was found between the time of the start of antimicrobial therapy and outcome. Another retrospective study found an association between delays in administering antibiotics longer than six hours after arrival in the emergency department and death.[16] Delay was most frequently due to the performance of cranial imaging before diagnostic lumbar puncture and the transfer of patients to another hospital. If imaging is performed before lumbar puncture, therapy should be initiated before the patient is sent for neuroimaging (Fig. 1 in the Supplementary Appendix). In patients who have not undergone prior imaging and in whom disease progression is apparent, therapy should be started directly after lumbar puncture, as well as in all patients with cloudy cerebrospinal fluid (suggesting the diagnosis of bacterial meningitis).[14]

The opening pressure of the cerebrospinal fluid is elevated in most patients with bacterial meningitis.[1,5] In a prospective cohort study, 40 percent of the patients had very high opening pressures (>400 mm, as measured with the use of a water manometer), which were associated with lower levels of consciousness but not with adverse outcome.[1] Cerebrospinal fluid findings are important in the differential diagnosis of patients with suspected meningitis.[17] In this disease, pleocytosis (100 to 10,000 white cells per cubic millimeter), elevated protein levels (>50 mg per deciliter [0.5 g per liter]), and decreased cerebrospinal fluid glucose levels (<40 percent of simultaneously measured serum glucose) are usually present.[1,4-8,17] There is usually a predominance of neutrophils (range, 80 to 95 percent) in the cerebrospinal fluid, but a predominance of lymphocytes can occur.[1,4-8] Normal or marginally elevated cerebrospinal fluid white-cell counts occur in 5 to 10 percent of patients and are associated with an adverse outcome.[1]

Gram's staining of cerebrospinal fluid permits the rapid identification of the causative organism (sensitivity, 60 to 90 percent; specificity, ≥97 percent).[1,8] Bacterial antigen tests have a limited sensitivity, but they may be helpful in patients with findings consistent with bacterial meningitis and negative Gram's staining and cultures of cerebrospinal fluid.[8] New molecular techniques for detecting bacteria in the cerebrospinal fluid by polymerase chain reaction (PCR) have emerged as powerful tools in the diagnosis of patients with negative cultures of cerebrospinal fluid; such tools have high sensitivity and specificity, although further refinements are needed before PCR can be routinely recommended.[18,19]

Table 1. Recommendations for Empirical Antimicrobial Therapy in Adults with Community-Acquired Bacterial Meningitis.*

Predisposing Factor	Common Bacterial Pathogen	Antimicrobial Therapy
Age		
16–50 yr	*Neisseria meningitidis, Streptococcus pneumoniae*	Vancomycin plus a third-generation cephalosporin†‡
>50 yr	*S. pneumoniae, N. meningitidis, Listeria monocytogenes,* aerobic gram-negative bacilli	Vancomycin plus a third-generation cephalosporin plus ampicillin‡§
Presence of a risk factor¶	*S. pneumoniae, L. monocytogenes, Haemophilus influenzae*	Vancomycin plus a third-generation cephalosporin plus ampicillin‡§

* For additional information, including alternative antimicrobial therapies, see Table 1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org.
† Only in regions with very low rates of penicillin resistance (<1 percent), monotherapy with penicillin may be considered, although many experts recommend combination therapy for all patients until the results of in vitro susceptibility testing are available.
‡ Cefotaxime and ceftriaxone are the third-generation cephalosporins recommended.
§ Only in regions with very low rates of penicillin resistance and cephalosporin resistance, combination therapy with amoxicillin (ampicillin) and a third-generation cephalosporin may be considered.
¶ Risk factors include alcoholism and altered immune status.

The choice of initial antimicrobial therapy is based on the most common bacteria causing the disease according to the patient's age and the clinical setting and on patterns of antimicrobial susceptibility (Table 1).[20,21] After the results of culture and susceptibility testing are available, antimicrobial therapy can be modified for optimal treatment (Table 1 in the Supplementary Appendix).[20,21] With the worldwide increase in the prevalence of penicillin-resistant pneumococci, combination therapy with vancomycin plus a third-generation cephalosporin (either ceftriaxone or cefotaxime) has become the standard approach to empirical antimicrobial therapy.[22,23] Although clinical data on the efficacy of rifampin in patients with pneumococcal meningitis are lacking, some authorities would use this agent in combination with a third-generation cephalosporin, with or without vancomycin, in patients with pneumococcal meningitis caused by bacterial strains that, on the basis of local epidemiology, are likely to be highly resistant to penicillin or cephalosporin. Such patients should also receive adjunctive dexamethasone therapy.

Respiratory isolation for 24 hours is indicated for patients with suspected meningococcal infection. Isolation is not required for those with signs of pneumococcal infection (otitis or pneumonia) or with bacteria other than *N. meningitidis* identified by Gram's staining. Persons who have close contact with the patient must receive chemoprophylaxis to eradicate meningococcal carriage (Table 1 in the Supplementary Appendix).[8,23]

ADJUNCTIVE DEXAMETHASONE THERAPY

A recent randomized, placebo-controlled trial involving 301 adults with suspected meningitis in combination with cloudy cerebrospinal fluid, bacteria in the cerebrospinal fluid on Gram's staining, or a cerebrospinal fluid leukocyte count of more than 1000 per cubic millimeter showed that adjunctive treatment with dexamethasone before or with the first dose of antimicrobial therapy reduced the risk of unfavorable outcome from 25 percent to 15 percent (number needed to treat, 10 patients).[24] Mortality was reduced from 15 percent to 7 percent. The benefit was greatest in patients with intermediate disease severity, as defined by a score on the Glasgow Coma Scale on admission of 8 to 11 (scores can range from 3 to 15, with 15 indicating a normal level of consciousness), and in those with pneumococcal meningitis, in whom unfavorable outcomes declined from 52 percent to 26 percent (number needed to treat, four). In patients with pneumococcal meningitis, mortality was reduced from 34 percent to 14 percent. This benefit was a result of reduced mortality from systemic causes.[25] In addition, the benefits of dexamethasone were not offset by any apparent side effects of treatment with dexamethasone.

What are the practical implications of the results of this study?[24] First, in all patients whose condition fulfills the inclusion criteria of the study, dexamethasone (at a dose of 10 mg) should be initiated before or with the first dose of antibiotics and continued for four days (at a dose of 10 mg every six hours). Second, in patients with suspected meningitis, the results of the study support the administration of adjunctive dexamethasone with or before the first dose of empirical antibiotics, although the study did not specifically address this issue. This course may result in the unnecessary treatment of patients who do not have bacterial meningitis, but the potential benefits outweigh any potential risks associated with dexamethasone therapy. Therapy should be discontinued if the patient is found not to have bacterial meningitis. Third, dexamethasone should be continued for four days in patients with bacterial meningitis, regardless of microbial cause or clinical severity. The absence of a significant clinical benefit in some subgroups of patients does not rule out a beneficial effect of dexamethasone in these subgroups, because the study was not powered to analyze all subgroups of interest. Some experts, however, would discontinue dexamethasone if the meningitis is found to be caused by a bacterium other than *S. pneumoniae*.[21]

In a recent quantitative review of this topic that included the results of five clinical trials,[26] treatment with corticosteroids was associated with a significant reduction in mortality and neurologic sequelae. In the subgroup of patients with meningococcal meningitis, mortality (relative risk, 0.9; 95 percent confidence interval, 0.3 to 2.1) and neurologic sequelae (relative risk, 0.5; 95 percent confidence interval, 0.1 to 1.7) were both reduced, although the results were not statistically significant.

For some adults with suspected meningitis, however, adjunctive dexamethasone can be harmful (Fig. 1 in the Supplementary Appendix). Pa-

tients with septic shock and adrenal insufficiency benefit from corticosteroid therapy in physiologic doses and for longer than four days; however, when there is no evidence of relative adrenal insufficiency, therapy with corticosteroids may be detrimental.[27,28] There are no controlled studies of the effects of corticosteroid therapy in a substantial number of patients with both meningitis and septic shock, and therefore corticosteroid therapy cannot be unequivocally recommended for such patients, although the use of low doses, as used by Annane et al.[27] (hydrocortisone, 50 mg every 6 hours, and fludrocortisone, 50 μg daily), seems reasonable at present. Starting corticosteroids before or with the first dose of parenteral antimicrobial therapy appears to be more effective than starting corticosteroids after the first dose of antimicrobial therapy.[29] In experimentally induced pneumococcal meningitis in animals, bacterial concentrations in the cerebrospinal fluid at the start of therapy appeared to be a more important factor influencing the antimicrobial-induced inflammatory response than the time dexamethasone therapy was initiated.[30] There is a point after the first (parenteral) administration of an antimicrobial agent beyond which dexamethasone loses its effectiveness, but this point has not been clearly defined.

INTENSIVE CARE MANAGEMENT

Monitoring in a neurologic–neurosurgical intensive care unit is recommended in order to recognize changes in the patient's consciousness and the development of new neurologic signs, monitor for subtle seizures, and treat severe agitation effectively.[31] Practical recommendations and admission criteria are given in Table 2 (and Fig. 1 in the Supplementary Appendix). Bacterial meningitis is often associated with septic shock, which is an important predictor of outcome.[1,32] Patients with meningitis and septic shock may require insertion of a Swan–Ganz catheter, to measure cardiac output, the cardiac index, systemic vascular resistance, and pulmonary wedge pressures in order to assess intravascular volume and cardiac function.[31] Adrenocorticoid insufficiency in patients with septic shock must be treated with low doses of corticosteroids.[27] Care should be taken to estimate and replace imperceptable fluid loss through the skin and lungs in patients who are febrile.[31]

Patients with bacterial meningitis are at risk of acute hyponatremia, although most cases are mild.[1,8,32] Hyponatremia may be a result of cerebral salt wasting, the syndrome of inappropriate antidiuretic hormone secretion, or exacerbation by aggressive fluid resuscitation.[31,33,34] This lack of clarity about the mechanism has resulted in the clinical dilemma with regard to whether intravenous fluids should be restricted in bacterial meningitis.[34] In children with bacterial meningitis, fluid restriction does not improve either brain edema or outcome.[33,35] Therefore, adult patients with meningitis should be treated with the goal of a normovolemic state. A core body temperature of more than 40°C probably would need to be treated with techniques of cooling by conduction or antipyretic agents to avoid excessive fluid loss. In experimentally induced meningitis in animals, moderate hypothermia ameliorates inflammatory changes, although no clinical studies have been performed.[36,37]

DECLINE IN CONSCIOUSNESS

For patients with a decline in consciousness, or those whose condition fails to improve after the initiation of appropriate antimicrobial therapy, brain imaging is indicated. The indication for repeated imaging is often arbitrarily based on the clinical status of the patient, the time between the decline in consciousness and the initiation of adequate therapy, and the results of previous imaging studies.

A common cause of a decline in consciousness in bacterial meningitis is clinical evidence of meningoencephalitis (Table 3). The release of proinflammatory mediators in the subarachnoid space leads to an inflammatory response in the central nervous system that contributes to an increased permeability of the blood–brain barrier, cerebral edema, and increased intracranial pressure.[38] On neuroimaging, early signs of brain edema are the disappearance of sylvian fissures and a narrowing of ventricular size. In patients with an advanced stage of brain edema and raised intracranial pressure, basal cisterns and sulci may become obliterated. Several supportive therapies have been described, although no therapy has been proved to have clinical efficacy.[8,39-43]

A recent study in Sweden reported findings on the use of measurements of continuous intracranial pressure and cerebral perfusion pressure

Table 2. Management of Bacterial Meningitis in Adults in the Intensive Care Unit.

Neurocritical care
In patients with a high risk of brain herniation, consider monitoring intracranial pressure and intermittent administration of osmotic diuretics (mannitol [25%] or hypertonic [3%] saline) to maintain an intracranial pressure of <15 mm Hg and a cerebral perfusion pressure of ≥60 mm Hg
Initiate repeated lumbar puncture, lumbar drain, or ventriculostomy in patients with acute hydrocephalus
Electroencephalographic monitoring in patients with a history of seizures and fluctuating scores on the Glasgow Coma Scale*
Airway and respiratory care
Intubate or provide noninvasive ventilation in patients with worsening consciousness (clinical and laboratory indicators for intubation include poor cough and pooling secretions, a respiratory rate of >35 per minute, arterial oxygen saturation of <90% or arterial partial pressure of oxygen of <60 mm Hg, and arterial partial pressure of carbon dioxide of >60 mm Hg)
Maintain ventilatory support with intermittent mandatory ventilation, pressure-support ventilation, or continuous positive airway pressure
Circulatory care
In patients with septic shock, administer low doses of corticosteroids (if there is a poor response on corticotropin testing, indicating adrenocorticoid insufficiency, corticosteroids should be continued)
Initiate inotropic agents (dopamine or milrinone) to maintain blood pressure (mean arterial pressure, 70–100 mm Hg)
Initiate crystalloids or albumin (5%) to maintain adequate fluid balance
Consider the use of a Swan–Ganz catheter to monitor hemodynamic measurements
Gastrointestinal care
Initiate nasogastric tube feeding of a standard nutrition formula
Initiate prophylaxis with proton-pump inhibitors
Other supportive care
Administer subcutaneous heparin as prophylaxis against deep venous thrombosis
Maintain normoglycemic state (serum glucose level, <150 mg per deciliter), with the use of sliding-scale regimens of insulin or continuous intravenous administration of insulin
In patients with a body temperature of >40°C, use cooling by conduction or antipyretic agents

* Scores on the Glasgow Coma Scale can range from 3 to 15, with 15 indicating a normal level of consciousness.

in the treatment of patients with severe bacterial meningitis.[43] In this observational study, intracranial pressure was successfully lowered in most patients by a broad range of measures and with the use of an unconventional volume-targeted ("Lund concept"[43]) intracranial pressure management protocol. The mean intracranial pressure was significantly higher and cerebral perfusion pressure was markedly decreased in patients who did not survive (in spite of treatment). Management of intracranial pressure is not routine in bacterial meningitis, and randomized comparative studies of various treatment regimens should be performed. Nevertheless, in patients with impending cerebral herniation, monitoring of intracranial pressure may be considered, but the outcome is expected to be poor. The use of osmotic diuretics to control intracranial pressure may be an option, although there are no definitive data on the efficacy of this approach.[31,43]

Seizures and acute hydrocephalus are other frequent causes of deteriorating consciousness.[1,4,5] Patients with seizures or a clinical suspicion of prior seizure should receive anticonvulsant therapy, but the low incidence of this complication does not justify prophylaxis.[1] A rare cause of the deterioration of consciousness in meningitis is nonconvulsive status epilepticus.[44] If seizures have occurred and the patient does not awaken or consciousness waxes and wanes, an electroencephalogram is indicated. In patients with acute hydrocephalus, a lumbar puncture can allow the measurement of cerebrospinal fluid pressure.[14] Repeated lumbar puncture or the placement of a

Table 3. Complications during the Clinical Course and Outcomes in Adults with Bacterial Meningitis.*

Complications	Frequency (%)	Outcome	Frequency (%)
Systemic complications		Score on Glasgow Outcome Scale	
Cardiorespiratory failure	29	1 (death)	21
Hyponatremia	26	2 (vegetative state)	<1
Disseminated intravascular coagulation	8	3 (severe disability)	3
Arthritis	2–6	4 (moderate disability)	10
Endocarditis/myocarditis	<1	5 (mild or no disability)	66
Deterioration of consciousness		Focal neurologic abnormalities	
Clinical evidence of meningoencephalitis	15–20	Cranial-nerve palsies	
Seizures	15–23	Third nerve	1
Brain edema	6–10	Sixth nerve	3
Hydrocephalus	3–8	Seventh nerve	1
Focal neurologic abnormalities		Eighth nerve	14
Cerebrovascular complications	15–20	Aphasia	2
Arterial infarction or vasculitis	10–15	Hemiparesis	4
Venous infarction	3–5	Quadriparesis	1
Hemorrhage	<1	Late effects	
Hearing loss	14–20	Cognitive impairment	10
Subdural empyema	<1		
Brain abscess	<1		
Myelitis	<1		

* Frequencies are for patients who are not routinely treated with early dexamethasone therapy; if routine dexamethasone therapy is provided, complications and the sequelae are expected to decline.

temporary lumbar drain may effectively reduce intracranial pressure; performing a ventriculostomy may also be considered.[31] In patients with mild enlargement of the ventricular system with no clinical deterioration, a spontaneous resolution may occur, and invasive procedures are therefore withheld. Cerebral infarcts may involve large vascular territories and may cause brain swelling and a mass effect, which may result in a decline in consciousness.[1,31]

FOCAL NEUROLOGIC ABNORMALITIES

In meningitis, focal cerebral abnormalities (hemiparesis, monoparesis, or aphasia) are most commonly caused by stroke, seizures, or a combination of the two (Table 3).[1,4,32,45,46] Signs of cerebral infarction and cytotoxic edema on cranial imaging suggest septic arteritis or endarteritis obliterans, venous thrombophlebitis, or thromboembolic events (Fig. 2).[4,45-47] Activation of inflammation and coagulation are closely related and interdependent.[48] The possibility of cerebral venous thrombophlebitis should be considered in patients with deterioration of consciousness, seizures, fluctuating focal neurologic abnormalities, and stroke with nonarterial distribution[31,49]; MRI with venous-phase studies confirms the diagnosis.[49] Treatment of cerebral thrombophlebitis in bacterial meningitis is directed toward the infection.

In a patient with rapid deterioration, subdural empyema should be considered.[50] Clues to the diagnosis are the presence of sinusitis and mastoiditis (and recent surgery for either of these disorders). Seizures and epilepsia partialis continua are relatively common in patients with subdural empyema.[50] In most patients with subdural empyema, contrast-enhanced CT shows hypodense collections.[31,50] However, MRI may be needed to image the localization of the subdural empyema

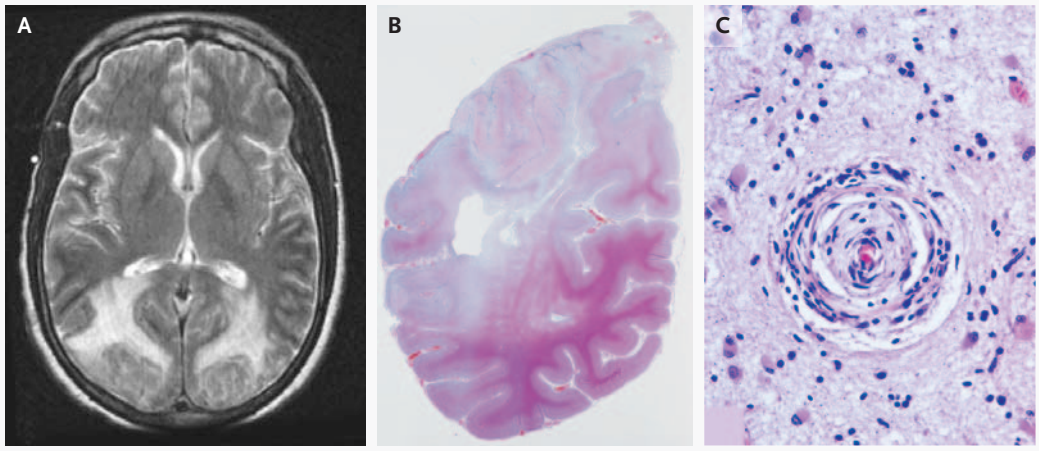

Figure 2. Cerebrovascular Complications in Bacterial Meningitis.

In Panel A, T_2-proton-density–weighted magnetic resonance imaging of the brain shows a transverse view of a hyperintense signal of the posterior lobes that indicates cerebral edema. In Panel B, a postmortem coronal view of the left posterior lobe of the same patient shows large areas of confluent necrosis involving the upper part of the hemisphere, as indicated by the loss of staining for hematoxylin and eosin. In Panel C, the microscopic substrate in the posterior lobe of this patient shows a small, almost completely obstructed vessel in the cortex with perivascular lymphocytic infiltration (endarteritis obliterans) that is surrounded by gliosis.

at the convexity.[31] In the majority of patients with subdural empyema, surgical drainage by craniotomy is indicated.[50]

Abnormalities of the cranial nerves are caused by the meningeal inflammatory process or by an increase in cerebrospinal fluid pressure.[8] The most frequent cranial-nerve abnormality is the involvement of the eighth cranial nerve, which is reflected in a hearing loss in 14 percent of patients.[1] A cochlear implant may eventually be needed by some severely affected patients.[51]

REPEATED LUMBAR PUNCTURE

The analysis of the cerebrospinal fluid should be repeated only in patients whose condition has not responded clinically after 48 hours of appropriate antimicrobial therapy. Repeated lumbar puncture is especially essential in the treatment of patients with pneumococcal meningitis caused by penicillin-resistant or cephalosporin-resistant strains and who receive adjunctive dexamethasone therapy and vancomycin.[21,26] Dexamethasone reduces inflammation of the cerebrospinal fluid and therefore may decrease the permeability of selected antimicrobial agents across the blood–brain barrier and, as a consequence, impede the penetration of vancomycin into the subarachnoid space.[26,52] Treatment failures have been reported in adults who received vancomycin and adjunctive dexamethasone.[53] If lumbar puncture is repeated, Gram's staining and culture of the cerebrospinal fluid should be negative after 24 hours of appropriate antimicrobial therapy.

OUTCOME

Community-acquired meningitis caused by *S. pneumoniae* has high case fatality rates, reported as from 19 to 37 percent.[1,4-8,31,46] In up to 30 percent of survivors, long-term neurologic sequelae develop, including hearing loss and other focal neurologic deficits.[1,4-8,32,46] The mortality and morbidity for meningococcal meningitis are lower than those for pneumococcal meningitis, with case fatality rates of 3 to 13 percent and morbidity rates of 3 to 7 percent.[1,4-6] The strongest risk factors for an unfavorable outcome are those indicative of systemic compromise, impaired consciousness, low white-cell count in the cerebrospinal fluid, and infection with *S. pneumoniae*.[1] All cohort studies were performed before dexamethasone was routinely administered; now that routine dexamethasone therapy has been implemented, complications and sequelae are expected to decline.[1,24,26] Cognitive impairment occurs fre-

quently after bacterial meningitis.[54] In one prospective study, cognitive impairment was detected in 27 percent of adults who had a good recovery from pneumococcal meningitis. Cognitive impairment consisted mainly of cognitive slowness, which was related to lower scores on questionnaires measuring the quality of life.[54]

FUTURE DIRECTIONS

Recent advances in experimentally induced bacterial meningitis in animals include the role of oxygen–glucose deprivation of hippocampal neurons as a complication of meningitis, the role of cytokines, and the protective roles of nuclear factor-κB1 and brain-derived neurotrophic factor.[37,54-58] Although such advances are promising, it is unlikely that additional adjunctive therapies will be studied in controlled trials in patients with bacterial meningitis. Progress is more likely to come from investigations into preventive measures, especially the use of available vaccines and the development of new vaccines.[59] Meningitis caused by *Haemophilus influenzae* type B has been nearly eliminated in the Western world since routine vaccination with the *H. influenzae* type B conjugate vaccine was initiated.[59] The introduction of conjugate vaccines against *S. pneumoniae* may substantially reduce the burden of childhood pneumococcal meningitis and may even produce herd immunity among adults.[60] The approval in 2005 of a conjugate meningococcal vaccine against serogroups A, C, Y, and W135 is also an important advance that may decrease the incidence of this devastating infection.[61]

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REFERENCES

1. van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M. Clinical features and prognostic factors in adults with bacterial meningitis. N Engl J Med 2004;351:1849-59. [Erratum, N Engl J Med 2005;352:950.]
2. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. N Engl J Med 1997;337:970-6.
3. Attia J, Hatala R, Cook DJ, Wong JG. The rational clinical examination: does this adult patient have acute meningitis? JAMA 1999;282:175-81.
4. Dodge PR, Swartz MN. Bacterial meningitis — a review of selected aspects. II. Special neurologic problems, postmeningitic complications and clinicopathological correlations. N Engl J Med 1965;272:954-60.
5. Durand ML, Calderwood SB, Weber DJ, et al. Acute bacterial meningitis in adults: a review of 493 episodes. N Engl J Med 1993;328:21-8.
6. Sigurdardottir B, Bjornsson OM, Jonsdottir KE, Erlendsdottir H, Gudmundsson S. Acute bacterial meningitis in adults: a 20-year overview. Arch Intern Med 1997;157:425-30.
7. Hussein AS, Shafran SD. Acute bacterial meningitis in adults: a 12-year review. Medicine (Baltimore) 2000;79:360-8.
8. Tunkel AR. Bacterial meningitis. Philadelphia: Lippincott Williams & Wilkens, 2001.
9. van Crevel H, Hijdra A, de Gans J. Lumbar puncture and the risk of herniation: when should we first perform CT? J Neurol 2002;249:129-37.
10. Hasbun R, Abrahams J, Jekel J, Quagliarello VJ. Computed tomography of the head before lumbar puncture in adults with suspected meningitis. N Engl J Med 2001;345:1727-33.
11. Oliver WJ, Shope TC, Kuhns LR. Fatal lumbar puncture: fact versus fiction — an approach to a clinical dilemma. Pediatrics 2003;112:174-6.
12. Winkler F, Kastenbauer S, Yousry TA, Maerz U, Pfister HW. Discrepancies between brain CT imaging and severely raised intracranial pressure proven by ventriculostomy in adults with pneumococcal meningitis. J Neurol 2002;249:1292-7.
13. van de Beek D, de Gans J. Prognostic factors in adults with bacterial meningitis. N Engl J Med 2005;352:514.
14. Fishman RA. Cerebrospinal fluid in diseases of the nervous system. Philadelphia: Saunders, 1992.
15. Aronin SI, Peduzzi P, Quagliarello VJ. Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. Ann Intern Med 1998;129:862-9.
16. Proulx N, Frechette D, Toye B, Chan J, Kravcik S. Delays in the administration of antibiotics are associated with mortality from adult acute bacterial meningitis. QJM 2005;98:291-8.
17. Spanos A, Harrell FE Jr, Durack DT. Differential diagnosis of acute meningitis: an analysis of the predictive value of initial observations. JAMA 1989;262:2700-7.
18. Rådström P, Bäckman A, Qian N, Kragsbjerg P, Påhlson C, Olcén P. Detection of bacterial DNA in cerebrospinal fluid by an assay for simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and streptococci using a seminested PCR strategy. J Clin Microbiol 1994;32:2738-44.
19. Schuurman T, de Boer RF, Kooistra-Smid AM, van Zwet AA. Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting. J Clin Microbiol 2004;42:734-40.
20. van de Beek D, de Gans J, Spanjaard L, Vermeulen M, Dankert J. Antibiotic guidelines and antibiotic use in adult bacterial meningitis in The Netherlands. J Antimicrob Chemother 2002;49:661-6.
21. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis 2004;39:1267-84.
22. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant Streptococcus pneumoniae in the United States. N Engl J Med 2000;343:1917-24.
23. Fraser A, Gafter-Gvili A, Paul M, Leibovici L. Antibiotics for preventing meningococcal infections. Cochrane Database Syst Rev 2005;1:CD004785.
24. de Gans J, van de Beek D. Dexamethasone in adults with bacterial meningitis. N Engl J Med 2002;347:1549-56.
25. van de Beek D, de Gans J. Dexamethasone and pneumococcal meningitis. Ann Intern Med 2004;141:327.
26. van de Beek D, de Gans J, McIntyre P, Prasad K. Steroids in adults with bacterial meningitis: a systematic review. Lancet Infect Dis 2004;4:139-43.
27. Annane D, Sebille V, Charpentier C,

et al. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 2002;288:862-71.
28. Cooper MS, Stewart PM. Corticosteroid insufficiency in acutely ill patients. N Engl J Med 2003;348:727-34.
29. van de Beek D, de Gans J, McIntyre P, Prasad K. Corticosteroids in acute bacterial meningitis. Cochrane Database Syst Rev 2003;3:CD004305.
30. Lutsar I, Friedland IR, Jafri HS, et al. Factors influencing the anti-inflammatory effect of dexamethasone therapy in experimental pneumococcal meningitis. J Antimicrob Chemother 2003;52:651-5.
31. Wijdicks EFM. The clinical practice of critical care neurology. 2nd ed. New York: Oxford University Press, 2003.
32. Pfister HW, Feiden W, Einhaupl KM. Spectrum of complications during bacterial meningitis in adults: results of a prospective clinical study. Arch Neurol 1993; 50:575-81.
33. Oates-Whitehead R, Maconochie I, Baumer H, Stewart M. Fluid therapy for acute bacterial meningitis. Cochrane Database Syst Rev 2005;3:CD004786.
34. Harrigan MR. Cerebral salt wasting syndrome: a review. Neurosurgery 1996; 38:152-60.
35. Täuber MG, Sande E, Fournier MA, Tureen JH, Sande MA. Fluid administration, brain edema, and cerebrospinal fluid lactate and glucose concentrations in experimental *Escherichia coli* meningitis. J Infect Dis 1993;168:473-6.
36. Angstwurm K, Reuss S, Freyer D, et al. Induced hypothermia in experimental pneumococcal meningitis. J Cereb Blood Flow Metab 2000;20:834-8.
37. Deng H, Han HS, Cheng D, Sun GH, Yenari MA. Mild hypothermia inhibits inflammation after experimental stroke and brain inflammation. Stroke 2003;34:2495-501.
38. Koedel U, Scheld WM, Pfister HW. Pathogenesis and pathophysiology of pneumococcal meningitis. Lancet Infect Dis 2002;2:721-36.
39. Lorenzl S, Koedel U, Pfister HW. Mannitol, but not allopurinol, modulates changes in cerebral blood flow, intracranial pressure, and brain water content during pneumococcal meningitis in the rat. Crit Care Med 1996;24:1874-80.
40. Möller K, Skinhoj P, Knudsen GM, Larsen FS. Effect of short-term hyperventilation on cerebral blood flow autoregulation in patients with acute bacterial meningitis. Stroke 2000;31:1116-22.
41. Paul R, Koedel U, Pfister HW. Reduction of intracranial pressure by nimodipine in experimental pneumococcal meningitis. Crit Care Med 2000;28:2552-6.
42. Grande PO, Myhre EB, Nordstrom CH, Schliamser S. Treatment of intracranial hypertension and aspects on lumbar dural puncture in severe bacterial meningitis. Acta Anaesthesiol Scand 2002;46:264-70.
43. Lindvall P, Ahlm C, Ericsson M, Gothefors L, Naredi S, Koskinen LO. Reducing intracranial pressure may increase survival among patients with bacterial meningitis. Clin Infect Dis 2004;38:384-90.
44. Mitchell WG. Status epilepticus and acute repetitive seizures in children, adolescents, and young adults: etiology, outcome, and treatment. Epilepsia 1996;37: Suppl 1:S74-S80.
45. Weststrate W, Hijdra A, de Gans J. Brain infarcts in adults with bacterial meningitis. Lancet 1996;347:399.
46. Kastenbauer S, Pfister HW. Pneumococcal meningitis in adults — spectrum of complications and prognostic factors in a series of 87 cases. Brain 2003;126:1015-25.
47. DiNubile MJ, Boom WH, Southwick FS. Septic cortical thrombophlebitis. J Infect Dis 1990;161:1216-20.
48. Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. Circulation 2004; 109:2698-704.
49. Stam J. Thrombosis of the cerebral veins and sinuses. N Engl J Med 2005;352: 1791-8.
50. Dill SR, Cobbs CG, McDonald CK. Subdural empyema: analysis of 32 cases and review. Clin Infect Dis 1995;20:372-86.
51. Gates GA, Miyamoto RT. Cochlear implants. N Engl J Med 2003;349:421-3.
52. Klugman KP, Friedland IR, Bradley JS. Bactericidal activity against cephalosporin-resistant Streptococcus pneumoniae in cerebrospinal fluid of children with acute bacterial meningitis. Antimicrob Agents Chemother 1995;39:1988-92.
53. Viladrich PF, Gudiol F, Linares J, et al. Evaluation of vancomycin for therapy of adult pneumococcal meningitis. Antimicrob Agents Chemother 1991;35:2467-72.
54. van de Beek D, Schmand B, de Gans J, et al. Cognitive impairment in adults with good recovery after bacterial meningitis. J Infect Dis 2002;186:1047-52.
55. Jiang X, Mu D, Manabat C, et al. Differential vulnerability of immature murine neurons to oxygen-glucose deprivation. Exp Neurol 2004;190:224-32.
56. Kastenbauer S, Koedel U, Weih F, Ziegler-Heitbrock L, Pfister HW. Protective role of NF-kappaB1 (p50) in experimental pneumococcal meningitis. Eur J Pharmacol 2004;498:315-8. [Erratum, Eur J Pharmacol 2004;504:235.]
57. Bifrare YD, Kummer J, Joss P, Tauber MG, Leib SL. Brain-derived neurotrophic factor protects against multiple forms of brain injury in bacterial meningitis. J Infect Dis 2005;191:40-5.
58. Kim KS. Pathogenesis of bacterial meningitis: from bacteraemia to neuronal injury. Nat Rev Neurosci 2003;4:376-85.
59. Swartz MN. Bacterial meningitis — a view of the past 90 years. N Engl J Med 2004;351:1826-8.
60. Whitney CG, Farley MM, Hadler J, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med 2003;348:1737-46.
61. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2005; 54(RR-7):1-21.

***JOURNAL* INDEX**

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REVIEW ARTICLE

MEDICAL PROGRESS

Autoimmune Hepatitis

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AUTOIMMUNE HEPATITIS IS A GENERALLY PROGRESSIVE, CHRONIC HEPatitis of unknown cause that occurs in children and adults of all ages. Occasionally, it has a fluctuating course, with periods of increased or decreased activity. The diagnosis is based on histologic abnormalities, characteristic clinical and biochemical findings, and abnormal levels of serum globulins, including autoantibodies. Since the first descriptions of this disorder more than 50 years ago,[1] many labels have been applied, but "autoimmune hepatitis" has been accepted as the most appropriate and least redundant term.[2,3] Variant, overlapping, or mixed forms of autoimmune hepatitis that share features with other putative autoimmune liver diseases, primary biliary cirrhosis, and primary sclerosing cholangitis occur as well. The distinctions among these disorders at present are necessarily descriptive.

It remains important to distinguish autoimmune hepatitis from other forms of chronic hepatitis, because a high percentage of cases respond to antiinflammatory or immunosuppressive therapy, or both. Although appropriate management can prolong survival, improve the quality of life, and avoid the need for liver transplantation, considerable therapeutic challenges remain in the treatment of this disorder.[4]

PATHOGENESIS

A conceptual framework for the pathogenesis of autoimmune hepatitis postulates an environmental agent that triggers a cascade of T-cell–mediated events directed at liver antigens in a host genetically predisposed to this disease, leading to a progressive necroinflammatory and fibrotic process in the liver.

POTENTIAL TRIGGERS

The environmental agents assumed to induce autoimmune hepatitis have not been delineated but include viruses. The finding of molecular mimicry by cross-reactivity between epitopes of viruses and certain liver antigens adds credence to a hypothesis of virally triggered disease. Because the trigger or triggers of autoimmune hepatitis may be part of a so-called hit-and-run phenomenon, in which induction occurs many years before overt autoimmune disease, identifying an infectious agent may prove impossible. There has been evidence implicating measles virus, hepatitis viruses, cytomegalovirus, and Epstein–Barr virus as initiators of the disease; the most convincing evidence is related to hepatitis viruses.[5-7]

Certain drugs, including oxyphenisatin, methyldopa, nitrofurantoin, diclofenac, interferon, pemoline, minocycline, and atorvastatin, can induce hepatocellular injury that mimics autoimmune hepatitis.[8-12] It has also been suggested that herbal agents such as black cohosh and dai-saiko-to might trigger autoimmune hepatitis. Whether drugs and herbs unmask or induce autoimmune hepatitis or simply cause a drug-induced hepatitis with accompanying autoimmune features is unclear. Minocycline[10,11]

and atorvastatin, which induce other autoimmune syndromes, have been implicated most recently as potential triggering agents of this disease.

GENETIC SUSCEPTIBILITY

Most knowledge concerning the genetics of autoimmune hepatitis comes from studies of the HLA genes that reside in the major histocompatibility complex (MHC), located on the short arm of chromosome 6. The MHC is a genetic system with extensive polymorphism. Although multiple genes are probably involved, HLA genes appear to play the dominant role in a predisposition to autoimmune hepatitis.[13,14]

Type 1 autoimmune hepatitis, characterized by circulating antinuclear antibodies (ANA), smooth-muscle antibodies, antiactin antibodies, atypical perinuclear antineutrophilic cytoplasmic antibodies (pANCA), and autoantibodies against soluble liver antigen and liver–pancreas antigen (SLA/LP), is associated with the HLA-DR3 serotype (found in linkage disequilibrium with HLA-B8 and HLA-A1), particularly among white patients. There is an association with HLA-DR4 among patients who are HLA-DR3–negative. HLA-DR3–associated disease is more common in the early-onset, severe form of autoimmune hepatitis, which often occurs in girls and young women. In comparison, the association with HLA-DR4 is more common in adults and may be associated with an increased incidence of extrahepatic manifestations, milder disease, and a better response to corticosteroid therapy. In Japan, where HLA-DR3 is rare, the most common associated HLA locus is HLA-DR4.

The results of serotyping studies have been confirmed with the use of genotyping for HLA-DRB, DQA, and DQB with polymerase-chain-reaction techniques. A high frequency of the HLA-DRB1*0301DRB3*0101DQAl*0501DQB1*0201 haplotype (the first two elements correspond to the serologic determinants DR3 and DR52) and the HLA-DRB1*0401 allele have been observed in association with autoimmune hepatitis. In South American populations, an increased frequency of the HLA-DRB1*1301 allele was reported,[15,16] whereas in Japan, autoimmune hepatitis has been associated with the DRB1*0405DQB1*0401 haplotype.[17] In children, type 1 autoimmune hepatitis is commonly associated with the HLA-DRB1*03 and HLA-DRB1*13 alleles.

Type 2 autoimmune hepatitis, a rare disorder characterized by antibodies against liver–kidney microsome 1 (LKM-1) and liver cytosol 1 (ALC-1), has been associated with the HLA-DRB1 and HLA-DQB1 alleles.[18] HLA-DR2 appears to be protective in white northern Europeans, and a study of white Argentineans suggested that the HLA-DRB1*1302 allele is protective.[14,15]

Susceptibility to autoimmune hepatitis has been reported to be associated with tumor necrosis factor (TNF) genes, the loci of which are in the class III region of the MHC, although this finding has been disputed.[19,20] A polymorphism at position 308 of the TNF-α gene has been associated with susceptibility to type 1 autoimmune hepatitis in both European and North American patients, but it may simply represent linkage disequilibrium with HLA-DRB1*0301. There were no significant differences in the response to therapy between those with and those without the 308 polymorphism.[19] Furthermore, this association was not present in Japanese or Brazilian patients with autoimmune hepatitis.[17,20] Similar associations of susceptibility with polymorphisms of cytotoxic T-lymphocyte antigen 4 observed in northern European patients were not seen in Brazilian patients.[21,22] Potential associations with loci in other chromosomes are under investigation.[23,24]

MECHANISMS OF ABERRANT AUTOREACTIVITY

Knowledge concerning autoantigens responsible for initiating the cascades of events in autoimmune hepatitis is still rudimentary. A leading candidate for many years has been the asialoglycoprotein receptor, a liver-specific membrane protein with high levels of expression in periportal hepatocytes. Information based on the identification of SLA/LP autoantibodies and the cloning and characterization of the SLA/LP antigen, which shares some amino acid sequences with the asialoglycoprotein receptor, suggests that this 50-kD cytosolic protein may represent a relevant antigen in at least some patients with type 1 autoimmune hepatitis.[25,26]

Evidence of an autoimmune process in the type 2 form of the disease is more compelling. The presence of immunodominant B-cell epitopes of cytochrome P-450 2D6 (CYP2D6) and evidence of cross-reactivity with homologues of different viruses suggest that relevant antigens exist within CYP2D6.[27]

The identification of CD4+ regulatory T cells has reinvoked the concept that failure of or escape

from normal suppression of reactivity against the self has an essential role in the development of autoimmune disease. The hypothesis that this escape phenomenon occurs in autoimmune hepatitis has remained attractive and is based on early studies of immune regulation.[28-31] Recent experimental evidence suggests that immunoregulatory dysfunction characterized by decreased numbers of CD4+CD25+ regulatory T cells and decreased levels of scurfin, the protein product of the *FOXP3* gene that is a member of the forkhead family of transcription factors, may occur in autoimmune hepatitis.[32] Such observations suggest that a decrease in the number of regulatory T cells and their ability to expand may lead to autoimmune liver disease.

CLINICAL CHARACTERISTICS

Autoimmune hepatitis is more common among women than men, but it occurs globally in children and adults of both sexes in diverse ethnic groups.[16-18,33-41] Since chronic viral hepatitis appears to be very common, the prevalence of autoimmune hepatitis may be higher than reported because of concomitant chronic hepatitis C or B or both.[38]

PRESENTATION

The presentation of autoimmune hepatitis is heterogeneous, and the clinical course may be characterized by periods of decreased or increased activity; thus, clinical manifestations are variable. The spectrum of presentation ranges from no symptoms to debilitating symptoms and even fulminant hepatic failure.

Patients may present with nonspecific symptoms of varying severity, such as fatigue, lethargy, malaise, anorexia, nausea, abdominal pain, and itching. Arthralgia involving small joints is common. Physical examination may reveal no abnormalities, but it may also reveal hepatomegaly, splenomegaly, jaundice, and signs and symptoms of chronic liver disease.

Patients with severe or fulminant symptoms accompanied by profound jaundice and a prolonged prothrombin time may have aminotransferase levels in the thousands.[42] Many patients with an acute presentation have histologic evidence of chronic disease on liver biopsy, indicating that they probably have had subclinical disease for a long time. Long periods of subclinical disease may also occur after presentation.

Autoimmune hepatitis may first become evident during pregnancy or in the early postpartum period. Furthermore, postpartum exacerbations may occur in patients whose condition improved during pregnancy.[43-45]

One clue to diagnosing autoimmune hepatitis is the presence of other diseases with autoimmune features, commonly thyroiditis, ulcerative colitis, type 1 diabetes, rheumatoid arthritis, and celiac disease.[46,47] Occasionally, circulating antiendomysial antibodies, antigluten antibodies, and anti–tissue transglutaminase antibodies may be found in patients with autoimmune hepatitis; this finding generally reflects the coexistence of celiac sprue and autoimmune hepatitis.

LABORATORY ABNORMALITIES

In general, aminotransferase elevations are more striking than abnormalities in bilirubin and alkaline phosphatase levels in patients with autoimmune hepatitis. Some cases, however, are characterized by cholestasis, with high levels of conjugated bilirubin and alkaline phosphatase. In such circumstances, extrahepatic obstruction and cholestatic forms of viral hepatitis, drug-induced disease, primary biliary cirrhosis, primary sclerosing cholangitis, and variant syndromes must be considered.

One characteristic laboratory feature of autoimmune hepatitis, although not invariant, is a generalized elevation of serum globulins, in particular, gamma globulin and IgG, which are generally 1.2 to 3.0 times normal. The characteristic circulating autoantibodies seen in autoimmune hepatitis include ANA, smooth-muscle antibody, antiactin antibody, SLA/LP autoantibodies, pANCA, anti–LKM-1, and anti–LC-1. Antimitochondrial antibodies are sometimes present in patients with autoimmune hepatitis. It should be noted, however, that autoantibodies are found in various liver diseases, and their presence, by itself, is not diagnostic of autoimmune hepatitis. There is little evidence that autoantibodies play a part in its pathogenesis.

CLASSIFICATION AND AUTOANTIBODIES

Classification of autoimmune hepatitis on the basis of autoantibody patterns has been helpful to clinicians (Table 1). Although the distinction was

initially based on circulating antibodies alone, other differences have become apparent. The main serologic markers of type 1 autoimmune hepatitis are ANA and smooth-muscle antibody. Titers of at least 1:80 are generally accepted as positive,[3] but results vary, depending on the assays used; lower titers may signify a positive response in children. Antiactin antibodies are more specific for type 1 autoimmune hepatitis.[48] Anti–LKM-1 and anti–LC-1 characterize type 2 disease.[18,33,34]

The identification of other circulating autoantibodies, in particular SLA/LP autoantibodies[25,26,49] and atypical pANCA,[50-52] are sometimes helpful in diagnosing type 1 disease. SLA/LP autoantibodies are the most specific autoantibody identified in type 1 autoimmune hepatitis but is found in only 10 to 30 percent of cases. Atypical pANCA is frequently present, and on rare occasions, it occurs as an isolated autoantibody.[52]

Anti–LKM-1 and anti–LC-1 can occur alone or together in type 2 autoimmune hepatitis.[18,33,34,53] Anti–LKM-1, which is directed at CYP2D6, can occur in chronic hepatitis C, though the antibody response to immunodominant epitopes differs.[27] Anti–LC-1 generally occurs in conjunction with anti–LKM-1, but it may be the sole autoantibody.[34] It recognizes formiminotransferase cyclodeaminase, a liver-specific 58-kD metabolic enzyme.[54]

COMPLICATIONS

The complications of autoimmune hepatitis are the same as in any progressive liver disease. Primary hepatocellular carcinoma is a known consequence of autoimmune hepatitis; in some patients, chronic hepatitis progresses to cirrhosis and, ultimately, to carcinoma. However, carcinoma occurs in association with autoimmune hepatitis less frequently than does chronic viral hepatitis.

HISTOLOGIC APPEARANCE

The histologic appearance of autoimmune hepatitis is the same as that of chronic hepatitis, and although certain changes are characteristic, no findings are specific for autoimmune hepatitis.[55] The histologic differential diagnosis of chronic hepatitis is provided in Table 2. Advances in virologic studies and refinements in cholangiographic methods have made it easier to rule out other clinical entities.

Table 1. Classification of Autoimmune Hepatitis.

Variable	Type 1 Autoimmune Hepatitis	Type 2 Autoimmune Hepatitis
Characteristic autoantibodies	Antinuclear antibody* Smooth-muscle antibody* Antiactin antibody† Autoantibodies against soluble liver antigen and liver–pancreas antigen‡ Atypical perinuclear antineutrophil cytoplasmic antibody	Antibody against liver–kidney microsome 1* Antibody against liver cytosol 1*
Geographic variation	Worldwide	Worldwide; rare in North America
Age at presentation	Any age	Predominantly childhood and young adulthood
Sex of patients	Female in approximately 75% of cases	Female in approximately 95% of cases
Association with other autoimmune diseases	Common	Common§
Clinical severity	Broad range	Generally severe
Histopathologic features at presentation	Broad range	Generally advanced
Treatment failure	Infrequent	Frequent
Relapse after drug withdrawal	Variable	Common
Need for long-term maintenance	Variable	Approximately 100%

* The conventional method of detection is immunofluorescence.
† Tests for this antibody are rarely available in commercial laboratories.
‡ This antibody is detected by enzyme-linked immunosorbent assay.
§ Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy is seen only in patients with type 2 disease.[47]

Table 2. Histologic Differential Diagnosis of Chronic Hepatitis.

Disease	Distinguishing Features*
Autoimmune hepatitis	Conspicuous plasma-cell infiltrates
Primary biliary cirrhosis	Lymphocytic and granulomatous infiltrates of bile ducts; ductopenia
Primary sclerosing cholangitis	Fibrous obliterative cholangitis; ductopenia
Autoimmune cholangitis†	Lymphocytic and granulomatous infiltrates of bile ducts; ductopenia
Chronic viral hepatitis	Ground-glass hepatocytes; immunoperoxidase staining for hepatitis B surface and core antigens in patients with chronic hepatitis B; nodular-appearing infiltrates characteristic in patients with chronic hepatitis C; steatosis possible in patients infected with hepatitis C virus genotype 3
Chronic drug-induced hepatitis	No helpful distinguishing histologic features
$Alpha_1$-antitrypsin deficiency	Intracytoplasmic globules
Wilson's disease	Heavy copper deposition
Granulomatous hepatitis	Conspicuous and frequent granulomas
Graft-versus-host disease	Lymphocytic and granulomatous infiltrates of bile ducts; ductopenia
Alcoholic steatohepatitis	Steatosis; central inflammation and fibrosis; Mallory bodies
Nonalcoholic steatohepatitis	Glycogenated nuclei; steatosis; central inflammation and fibrosis; Mallory bodies

* These histologic features may be helpful in distinguishing among the causes of chronic hepatitis. Differences in histopathological findings among the diseases may be more apparent depending on the grade and stage of disease.[55]
† There is still debate as to whether this entity is antimitochondrial-antibody–negative primary biliary cirrhosis.[56]

Autoimmune hepatitis is generally characterized by a mononuclear-cell infiltrate invading the limiting plate — that is, the sharply demarcated hepatocyte boundary that surrounds the portal triad and permeates the surrounding parenchyma (periportal infiltrate, also called piecemeal necrosis or interface hepatitis that progresses to lobular hepatitis). There may be an abundance of plasma cells, a finding that in the past led to the use of the term "plasma-cell hepatitis." Eosinophils are frequently present. The portal lesion generally spares the biliary tree. Fibrosis is present in all but the mildest forms of autoimmune hepatitis. In advanced disease, the fibrosis is extensive, and with the distortion of the hepatic lobule and the appearance of regenerative nodules, it results in cirrhosis.[55] Occasionally, centrizonal lesions occur.[42,57]

The findings in patients with acute-onset autoimmune hepatitis differ somewhat from those with an insidious presentation. Patients presenting with fulminant hepatic failure tend to have interface and lobular hepatitis, lobular disarray, and hepatocyte, central, and submassive necrosis. However, they have less fibrosis than patients who present with a more chronic course.[42] Steatosis occurs in a minority of patients,[55] although nonalcoholic fatty liver disease may occur in conjunction with autoimmune hepatitis. The various histologic appearances are depicted in Figure 1.

In patients who have a spontaneous or pharmacologically induced remission, the histologic findings may revert to normal or inflammation may be confined to portal areas. In this setting, cirrhosis may become inactive and fibrosis may diminish or disappear.[55,59-61]

DIAGNOSIS

In the presence of a compatible histologic picture, the diagnosis of autoimmune hepatitis is based on characteristic clinical and biochemical findings, circulating autoantibodies, and abnormal levels of serum globulins. Circulating antibodies are absent in about 10 percent of patients. A scoring system proposed and subsequently revised by the International Autoimmune Hepatitis Group[3] to standardize the diagnosis for clinical trials and population studies has had limited

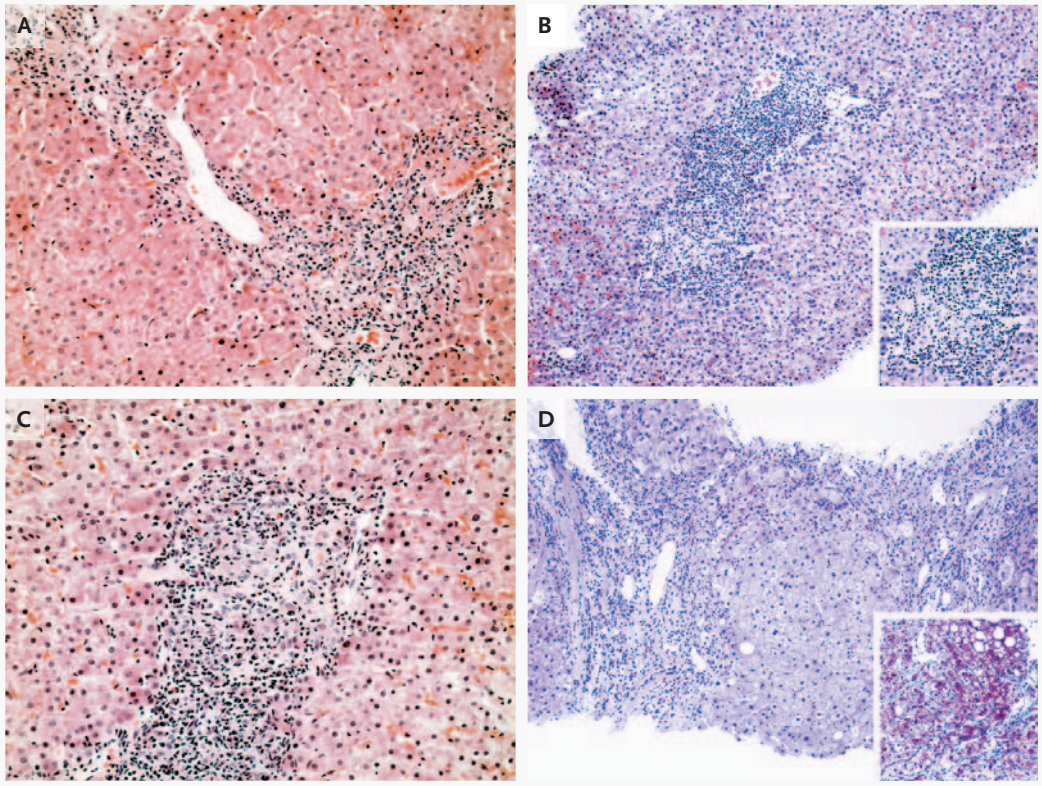

Figure 1. Photomicrographs of Liver-Biopsy Specimens from Four Patients with Autoimmune Hepatitis.

Panel A shows portal and interface hepatitis, with a mixed inflammatory infiltrate composed of lymphocytes, plasma cells, and eosinophils (hematoxylin and eosin). This specimen was obtained from a 16-year-old girl in whom autoimmune hepatitis developed while she was taking minocycline for acne. A test for antinuclear antibody was positive; tests for smooth-muscle antibody and antibodies against liver–kidney microsome 1 (LKM-1) were negative, and the IgG level was 2180 mg per deciliter. After treatment with prednisone for only two months, her aminotransferase levels became normal and prednisone was discontinued. A subsequent exacerbation was treated with prednisone for nine months. The patient completed the therapy and has remained in remission for 15 months. Panel B shows interface hepatitis, with a mixed inflammatory infiltrate composed of lymphocytes and plasma cells (hematoxylin and eosin). A granuloma, which is commonly seen in primary biliary cirrhosis but occurs occasionally in autoimmune hepatitis, is present (inset, hematoxylin and eosin). This specimen was obtained from a 44-year-old woman, who weighed 95.3 kg, with a body-mass index (the weight in kilograms divided by the square of the height in meters) of 36. A test for smooth-muscle antibody was positive; tests for antinuclear antibody and antimitochondrial antibody were negative; the gamma globulin level was 3.2 g per deciliter. No steatosis was present in the biopsy specimen, although diabetes mellitus subsequently developed during treatment with prednisone. Panel C shows interface hepatitis with a mixed inflammatory infiltrate composed of lymphocytes, plasma cells, and eosinophils, as well as diffuse ballooning degeneration of the hepatocytes (hematoxylin and eosin). The bile ducts were heterochromatic but normal in number and not infiltrated. No granulomas were present. This specimen was obtained from a 50-year-old woman with autoimmune hepatitis of acute onset. A test for antimitochondrial antibody was positive; tests for antinuclear antibody, smooth-muscle antibody, and anti–LKM-1 were negative. The IgG level was 2580 mg per deciliter, and the peak bilirubin level was 11.3 mg per deciliter (193.2 μmol per liter). The alkaline phosphatase level was 224 U per liter, the alanine aminotransferase level was 3400 U per liter, and the aspartate aminotransferase level was 2200 U per liter. The patient's response to prednisone and subsequently to azathioprine therapy was typical of that seen in patients with autoimmune hepatitis. Now called "antimitochondrial-antibody–positive autoimmune hepatitis," it is also known as the "overlap syndrome."[58] Panel D shows cirrhosis with interface hepatitis characteristic of autoimmune hepatitis (hematoxylin and eosin). Steatosis and "chickenwire" pericentral fibrosis (inset, trichrome stain) are characteristic of nonalcoholic steatohepatitis. This specimen was obtained from a 78-year-old woman with hyperlipidemia who weighed 56.7 kg and had a body-mass index of 28. Tests for antinuclear antibody and antimitochondrial antibody were negative; a test for smooth-muscle antibody was positive. The total globulin level was 4.7 g per milliliter, and the gamma globulin level was 1.7 g per milliliter.

value and may be inaccurate when applied to individual patients, especially children. Attempts are under way to devise a less complicated and more accurate system.[62]

VARIANT SYNDROMES

Although we have long known that the clinical, histologic, and serologic profiles of so-called overlap, mixed, or variant syndromes differ from the classic features of autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis, no consensus regarding categorization has been reached. Terms such as "overlap syndrome," "antimitochondrial-antibody–negative primary biliary cirrhosis," "the hepatic form of primary biliary cirrhosis," "autoimmune cholangitis," "autoimmune cholangiopathy," "chronic autoimmune cholestasis," "immunocholangitis," "immune cholangiopathy," and "combined hepatitic/cholestatic syndrome" have all been used to describe patients with features of both autoimmune hepatitis and primary biliary cirrhosis. The presentation of putative coincidental diseases, consecutive diseases, and evolution from one disease to another have highlighted the complexity of this issue.[56,58,63-66]

One approach is to consider the variant syndromes of autoimmune hepatitis and primary biliary cirrhosis as part of a continuum that extends from classic autoimmune hepatitis to classic primary biliary cirrhosis. Examination of a biopsy specimen with histologic features of autoimmune hepatitis but serologic findings characteristic of primary biliary cirrhosis, such as an isolated antimitochondrial antibody directed toward enzymes in the 2-oxo acid dehydrogenase family, would be indicative of the overlap syndrome,[58] or antimitochondrial-antibody–positive autoimmune hepatitis (Table 3). The clinical course and response to therapy in this syndrome appear to be identical to those in classic autoimmune hepatitis.

There is disagreement as to whether the variant most commonly called autoimmune cholangitis[56,58] merely represents antimitochondrial-antibody–negative primary biliary cirrhosis (Table 3). Immunoblotting and enzyme-linked immunosorbent assays for antimitochondrial antibodies and primary biliary cirrhosis–specific antinuclear antibodies (anti-Sp100 and anti-gp210) have yielded different autoantibody profiles for the two conditions, underscoring the heterogeneity of these syndromes.[66]

Identifying and classifying autoimmune hepatitis–primary sclerosing cholangitis overlap syndromes is also difficult, particularly in children.[53,67-72] "Autoimmune sclerosing cholangitis" is the term applied to this disease in affected children and could arguably be applied to that in adults as well. Although primary sclerosing cholangitis can evolve to autoimmune hepatitis, autoimmune hepatitis more commonly evolves to autoimmune sclerosing cholangitis.[72] Autoimmune sclerosing cholangitis cannot be diagnosed in the absence of cholangiographic abnormalities. Patients suspected of having autoimmune hepatitis who also have histologic bile-duct abnormalities, cholestatic laboratory changes (e.g., elevations of alkaline phosphatase, γ-glutamyltransferase, or both), pruritus, inflammatory bowel disease, or loss of response to antiinflammatory or immunosuppressive therapy may have autoimmune sclerosing cholangitis.

Table 3. Characteristics of Autoimmune Hepatitis–Primary Biliary Cirrhosis Variant Syndromes.

Characteristic	Overlap Syndrome*	Autoimmune Cholangitis†
Antinuclear antibody	Absent	Generally present
Smooth-muscle antibody	Absent	Generally present
Antimitochondrial antibody	Present	Absent
Biochemical cholestasis†	Absent	Present
Histologic evidence of bile-duct abnormalities	Absent	Present
Cholangiographic abnormalities	Absent	Absent
Responsiveness to immunosuppression	Present	Variable

* This syndrome is also called antimitochondrial-antibody–positive autoimmune hepatitis.[58] There is debate as to whether autoimmune cholangitis and antimitochondrial-antibody–negative primary biliary cirrhosis represent different entities.[56,63-66]

† This condition is characterized by elevated levels of serum alkaline phosphatase, γ-glutamyltransferase, or both.

TREATMENT

In the 1970s, evidence that mercaptopurine and azathioprine were effective in treating autoimmune diseases, together with controlled studies of corticosteroids, led to the opinion that autoimmune hepatitis is a treatable disease. Antiinflammatory or immunosuppressive therapy has been a mainstay in the treatment of both type 1 and type 2 disease. Depending on the definition of a response, therapy is reported to be successful in 65 to 80 percent of cases, which indicates that a substantial percentage of patients require therapy beyond standard treatment. Current response rates appear better than those in early trials, presumably because earlier trials involved more patients with severe disease and antedated the present ability to test for chronic viral hepatitis B and C. Ten-year survival rates (with the end point being death or transplantation) among treated patients are now considered to exceed 90 percent; but the 20-year survival rate may be less than 80 percent among patients without cirrhosis and less than 40 percent among those with cirrhosis at presentation.[73] Once the disease is in remission, maintenance therapy with azathioprine alone is successful in approximately 80 percent of patients.[74]

Response to treatment is helpful in establishing the diagnosis of autoimmune hepatitis, but the response rate to standard therapy is not 100 percent. Thus, a lack of response cannot rule out this diagnosis. Moreover, not all patients receive treatment, and the prescribed doses of prednisone and azathioprine or mercaptopurine vary. In addition, other diseases, including some variant syndromes, may respond to corticosteroids.

Progress in the medical management of autoimmune hepatitis has been slow. Considerable challenges still exist in the areas of initial and maintenance regimens, management of relapse, management of a lack of response to therapy, drug toxicity and intolerance, noncompliance, and treatment during pregnancy. Although guidelines for the treatment of autoimmune hepatitis have been published by the American Association for the Study of Liver Diseases, these are meant to be flexible.[75] The heterogeneity of autoimmune hepatitis underscores the need for individualized therapy in adults and children.[4,75,76]

STANDARD TREATMENT

Initial treatment with prednisone (or prednisolone) alone or in combination with azathioprine should be instituted in nearly all patients in whom the histologic findings include interface hepatitis, with or without fibrosis or cirrhosis. The magnitude of aminotransferase and gamma globulin elevations does not necessarily correlate with the histologic extent of injury and provides little help with respect to the initiation of treatment. In patients with only portal inflammation, the decision to treat is often determined on the basis of the levels of aminotransferase, gamma globulin, or both; the symptoms; or the combination of levels and symptoms. Asymptomatic patients and those with portal inflammation without fibrosis may be followed without treatment, but their clinical status, including the findings on liver biopsy, should be monitored carefully for evidence of progression of disease, since the activity of autoimmune hepatitis sometimes fluctuates. Initial treatment consists of combination therapy in order to avoid or mitigate the side effects of corticosteroid treatment. An alternative approach is to wait until remission is achieved before corticosteroid-sparing treatment with azathioprine or mercaptopurine is initiated (Table 4).

Adverse effects or intolerance of azathioprine, mercaptopurine, or both is an issue of particular concern.[79,80] Azathioprine is a prodrug of mercaptopurine. The methylation of mercaptopurine and 6-thioguanosine 5′-monophosphate is catalyzed by thiopurine methyltransferase, which is encoded by highly polymorphic genes. Patients who are homozygous for a mutation of thiopurine methyltransferase associated with low enzyme activity are at high risk for severe complications, including death. Patients who are heterozygous for a mutation of thiopurine methyltransferase probably are at intermediate risk. Given these findings, some investigators have suggested performing thiopurine methyltransferase genotyping before prescribing azathioprine or mercaptopurine. However, some patients who cannot tolerate azathioprine appear to be able to tolerate mercaptopurine without side effects, indicating that azathioprine-induced toxicity is not simply due to a deficiency of thiopurine methyltransferase.[81] Despite the availability of reliable methods for genoptying thiopurine methyltransferase and determining levels of mercaptopurine metabolites, their use in the clinical management of autoimmune hepatitis is not established.[79,80]

In general, a patient's progress is followed by monitoring levels of serum aminotransferases

Table 4. Drugs Used in the Treatment of Autoimmune Hepatitis in Adults and Children.

Drug	Initial Therapy	Maintenance Therapy	Comments
Prednisone or prednisolone	Used as monotherapy in adults (20–60 mg/day) and children (1–2 mg per kilogram of body weight/day); also used in combination therapy in adults (15–30 mg/day) and children (1–2 mg/kg/day) with azathioprine or mercaptopurine	Used as monotherapy in adults (5–15 mg/day) and children (1 mg/kg/day); also used in combination therapy in adults (5–10 mg/day) and children (0.5–1.0 mg/kg/day) with azathioprine or mercaptopurine	Relatively contraindicated in patients with osteoporosis, diabetes mellitus, glaucoma, cataracts, arterial hypertension, major depression, and femoral avascular necrosis; reduced doses may work; use of budesonide under investigation[77]
Azathioprine	Used in combination with prednisone or prednisolone in adults (50–100 mg/day) and children (1.5–2.0 mg/kg/day)	Used as monotherapy in adults (50–200 mg/day) and children (1.5–2.0 mg/kg/day); also used in combination therapy in adults (50–150 mg/day) and children (1.5–2.0 mg/kg/day)	Contraindicated in patients with homozygous thiopurine methyltransferase deficiency; relatively contraindicated in patients with heterozygous thiopurine methyltransferase deficiency, cancer, or cytopenia, and pregnant patients
6-Mercaptopurine	May be substituted for azathioprine in combination therapy in adults (25–100 mg/day) and children (0.75–1.0 mg/kg/day)	Used as monotherapy in adults (25–100 mg/day) and children (0.75–1.0 mg/kg/day); also used in combination therapy in adults (25–100 mg/day) and children (0.5–1.0 mg/kg/day)	Contraindicated in patients with homozygous thiopurine methyltransferase deficiency; relatively contraindicated in patients with heterozygous thiopurine methyltransferase deficiency, cancer, or cytopenia, and pregnant patients
Cyclosporine	Sometimes used as monotherapy in children[78]; sometimes used as an alternative drug in adults with treatment-refractory disease	Sometimes used as an alternative drug in adults with treatment-refractory disease	Once remission achieved in children, maintenance therapy initiated with a combination of prednisone and azathioprine[78]; role of tacrolimus in place of cyclosporine not established
Mycophenolate mofetil	Sometimes used in patients with treatment-refractory disease or in patients with adverse drug reactions to or intolerance of azathioprine, mercaptopurine, or both	Sometimes used in patients with treatment-refractory disease or in patients with adverse drug reactions to or intolerance of azathioprine, mercaptopurine, or both	Role of mycophenolate mofetil, methotrexate, and cyclophosphamide not established
Ursodiol	Sometimes used in combination with prednisone, azathioprine, or both	Sometimes used in combination with prednisone, azathioprine, or both	Role of ursodiol not established

and circulating globulins (total or gamma globulin, or both, with or without IgG). The histologic response typically lags behind the biochemical response, and a clinical remission does not necessarily mean that there is histologic evidence of resolution. Reasonable intervals for repeated liver biopsy appear to be one year after levels of aspartate aminotransferase and alanine aminotransferase have become normal or approximately two years after presentation.

Although some patients remain in remission after drug treatment is withdrawn, most require long-term maintenance therapy. In general, patients with milder disease have a better response. Adults and children with cirrhosis at the time of the initial biopsy, particularly children with type 2 disease, rarely stay in remission when treatment is withdrawn. Thus, lifelong maintenance therapy is generally indicated in such cases. The wisdom of the administration of azathioprine alone or as a corticosteroid-sparing agent should be approached by weighing the side effects of long-term corticosteroid use against those of long-term azathioprine use; patients treated with azathioprine alone frequently have arthralgia.[74]

In the presence of severe side effects from the

use of corticosteroids, partial control of the autoimmune hepatitis in patients who have multiple relapses may be preferable and can be achieved with doses of prednisone lower than conventional doses.[82] Some patients remain in remission for months or years before the disease flares. These patients may not need antiinflammatory therapy for long periods, but their condition should still be monitored every three to six months, so that therapy can be reinstated if the disease becomes active.

OTHER THERAPY

Decisions regarding the use of other medications must be based on meager data obtained from case reports and series of small numbers of patients. Cyclosporine appeared effective in a group of adult patients who were corticosteroid-resistant.[83] A regimen of cyclosporine for six months followed by the administration of prednisone and azathioprine was reported as successful in inducing remission in children.[78] Limited data are available concerning the use of tacrolimus,[84] methotrexate,[85,86] cyclophosphamide,[87] ursodiol,[88] budesonide,[77] and mycophenolate mofetil[89] (Table 4).

TREATMENT OF VARIANT SYNDROMES

No trials have been performed that could provide a basis for the treatment of variant syndromes. The treatment for antimitochondrial-antibody–positive autoimmune hepatitis is identical to that outlined for classic autoimmune hepatitis. Reports concerning the effectiveness of corticosteroid therapy in other autoimmune hepatitis–primary biliary cirrhosis variant syndromes have been conflicting. Although ursodiol, the mainstay of treatment for primary biliary cirrhosis,[90] may reduce levels of liver enzymes, it is not known whether the drug mitigates the necroinflammatory process or retards the progression of disease in these variant syndromes.[63,65] A therapeutic trial of corticosteroids with or without ursodiol, especially in patients with few cholestatic features, no or minimal bile-duct changes on biopsy, or both, may be required before a long-term regimen can be devised.

Limited success has been achieved with variant forms of autoimmune hepatitis–primary sclerosing cholangitis in adults with use of a regimen combining corticosteroids, azathioprine, and ursodiol.[69] Present therapeutic options include immunosuppression, ursodiol, or both, but data regarding efficacy are conflicting.[67-69]

LIVER TRANSPLANTATION

Liver transplantation is required in patients who are refractory to or intolerant of immunosuppressive therapy and in whom end-stage liver disease develops. The survival rate among patients and grafts 5 years after liver transplantation is approximately 80 to 90 percent, the 10-year survival rate is approximately 75 percent, and the recurrence rate has been reported to be as high as 42 percent.[91-95] Histologic evidence of recurrence may precede clinical and biochemical evidence of recurrence.[95] Recurrence may be related to the immunosuppressive regimen used after transplantation.

Autoimmune hepatitis has been reported after liver transplantation for other diseases in adults and children,[96-100] although the use of the term in this setting has been questioned. It has been suggested that alternative nomenclature such as "post-transplant immune hepatitis" or "graft dysfunction mimicking autoimmune hepatitis" may be more appropriate.[97] This entity, however, appears to respond well to corticosteroid treatment, thus avoiding graft rejection and the need for another transplantation and improving long-term survival.[99]

SUMMARY

Autoimmune hepatitis is a generally progressive, chronic disease with occasionally fluctuating activity that occurs worldwide in children and adults. Although the cause of autoimmune hepatitis is unknown, aberrant autoreactivity is thought to have a role in its pathogenesis. The diagnosis is based on histologic changes, characteristic clinical and biochemical findings, circulating autoantibodies, and abnormal levels of serum globulins. Variant forms of autoimmune hepatitis share features with other putative autoimmune liver diseases, primary biliary cirrhosis, and primary sclerosing cholangitis. Despite its clinical heterogeneity, autoimmune hepatitis generally responds to antiinflammatory or immunosuppressive treatment, or both. Lifetime maintenance therapy may be required, especially for patients with type 2 autoimmune hepatitis and those who have cirrhosis at presentation. Liver transplantation has been

successful in patients who have no response to medical management.

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REFERENCES

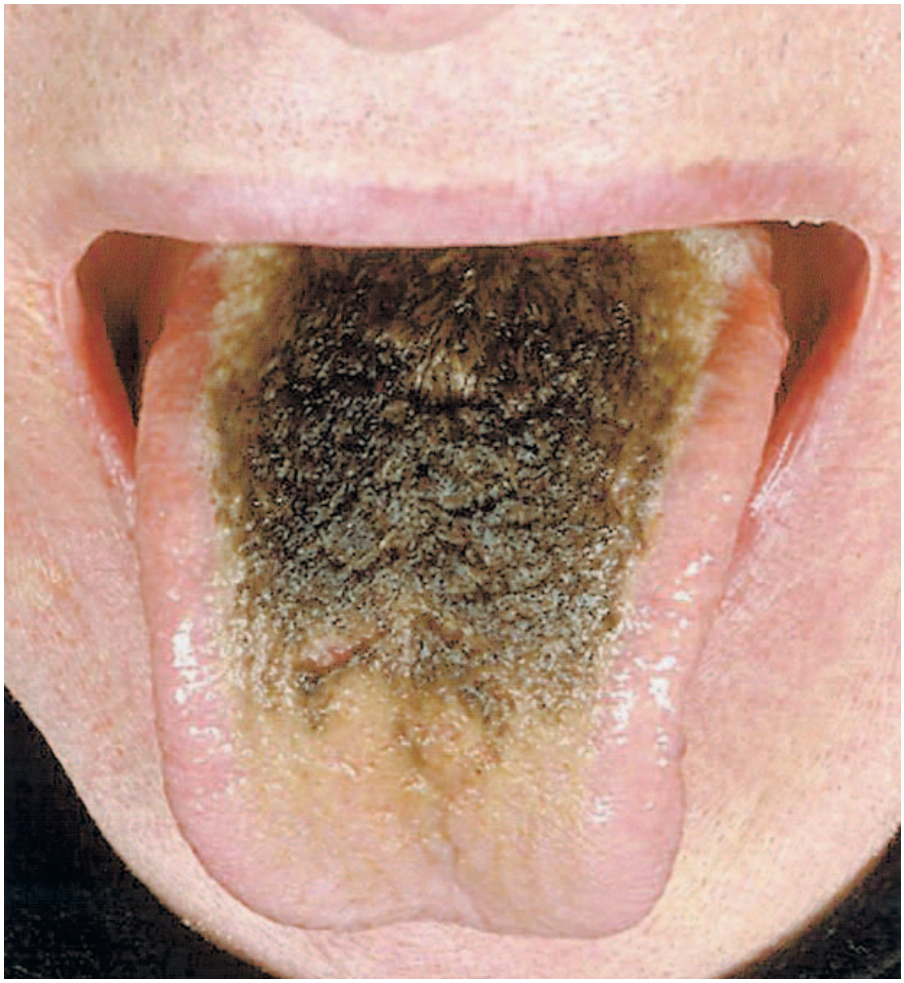
1. Reuben A. A sheep in wolf's clothing. Hepatology 2003;38:1596-601.
2. Mackay IR, Weiden S, Hasker J. Autoimmune hepatitis. Ann N Y Acad Sci 1965; 124:767-80.
3. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31: 929-38.
4. Czaja AJ, Bianchi FB, Carpenter HA, et al. Treatment challenges and investigational opportunities in autoimmune hepatitis. Hepatology 2005;41:207-15.
5. Huppertz H-I, Treichel U, Gassel AM, Jeschke R, Meyer zum Buschenfelde KH. Autoimmune hepatitis following hepatitis A virus infection. J Hepatol 1995;23:204-8.
6. Skoog SM, Rivard RE, Batts KP, Smith CI. Autoimmune hepatitis preceded by acute hepatitis A infection. Am J Gastroenterol 2002;97:1568-9.
7. Vento S, Cainelli F, Renzini C, Concia E. Autoimmune hepatitis type 2 induced by HCV and persisting after viral clearance. Lancet 1997;350:1298-9.
8. Lewis JL, Zimmerman HJ. Drug-induced autoimmune liver disease. In: Krawitt EL, Wiesner RH, Nishioka M, eds. Autoimmune liver diseases. 2nd ed. Amsterdam: Elsevier, 1998:627-49.
9. Sterling MJ, Kane M, Grace ND. Pemoline-induced autoimmune hepatitis. Am J Gastroenterol 1996;91:2233-4.
10. Gough A, Chapman S, Wagstaff K, Emery P, Elias E. Minocycline induced autoimmune hepatitis and systemic lupus erythematosus-like syndrome. BMJ 1996; 312:169-72.
11. Nietsch HH, Libman BS, Pansze TW, Eicher JN, Reeves JR, Krawitt EL. Minocycline-induced hepatitis. Am J Gastroenterol 2000;95:2993-5.
12. Graziadei IW, Obermoser GE, Sepp NT, Erhart KH, Vogel W. Drug-induced lupus-like syndrome associated with severe autoimmune hepatitis. Lupus 2003;12: 409-12.
13. Donaldson PT, Albertini RJ, Krawitt EL. Immunogenetic studies of autoimmune hepatitis and primary sclerosing cholangitis. In: Krawitt EL, Wiesner RH, Nishioka M, eds. Autoimmune liver diseases. 2nd ed. Amsterdam: Elsevier, 1998:141-65.
14. Donaldson PT. Genetics in autoimmune hepatitis. Semin Liver Dis 2002;22:353-64.
15. Pando M, Larriba J, Fernandez GC, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. Hepatology 1999;30:1374-80.
16. Czaja AJ, Souto EO, Bittencourt PL, et al. Clinical distinctions and pathogenic implications of type 1 autoimmune hepatitis in Brazil and the United States. J Hepatol 2002;37:302-8.
17. Yoshizawa K, Ota M, Katsuyama Y, et al. Genetic analysis of HLA region of Japanese patients with type 1 autoimmune hepatitis. J Hepatol 2005;42:578-84.
18. Djilali-Saiah I, Renous R, Caillat-Zucman S, Debray D, Alvarez F. Linkage disequilibrium between HLA class II region and autoimmune hepatitis in pediatric patients. J Hepatol 2004;40:904-9.
19. Czaja AJ, Cookson S, Constantini PK, Clare M, Underhill JA, Donaldson PT. Cytokine polymorphisms associated with clinical features and treatment outcome in type 1 autoimmune hepatitis. Gastroenterology 1999;117:645-52.
20. Bittencourt PL, Palacios SA, Cancado EL, et al. Autoimmune hepatitis in Brazilian patients is not linked to tumor necrosis factor alpha polymorphisms at position –308. J Hepatol 2001;35:24-8.
21. Agarwal K, Czaja AJ, Jones DE, Donaldson PT. Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphisms and susceptibility to type 1 autoimmune hepatitis. Hepatology 2000;31:49-53.
22. Bittencourt PL, Palacios SA, Cancado ELR, et al. Cytotoxic T lymphocyte antigen-4 gene polymorphisms do not confer susceptibility to autoimmune hepatitis types 1 and 2 in Brazil. Am J Gastroenterol 2003;98:1616-20.
23. Fukagawa NK, Liang P, Li M, Ashikaga T, Reddy KR, Krawitt EL. Glutathione-S-transferase M1 null genotype in autoimmune hepatitis. Dig Dis Sci 2001;46: 2080-3.
24. Vogel A, Strassburg CP, Manns MP. Genetic association of vitamin D receptor polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. Hepatology 2002;35:126-31.
25. Wies I, Brunner S, Henninger J, et al. Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. Lancet 2000;355:1510-5.
26. Ma Y, Okamoto M, Thomas MG, et al. Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. Hepatology 2002;35:658-64.
27. Kerkar N, Choudhuri K, Ma Y, et al. Cytochrome P4502D6(193-212): a new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type 1-positive liver disease. J Immunol 2003;170:1481-9.
28. Hodgson HJ, Wands JR, Isselbacher KJ. Alteration in suppressor cell activity in chronic active hepatitis. Proc Natl Acad Sci U S A 1978;75:1549-53.
29. Nouri-Aria KT, Hegarty JE, Alexander GJM, Eddleston ALWF, Williams R. Effect of corticosteroids on suppressor-cell activity in "autoimmune" and viral chronic active hepatitis. N Engl J Med 1982;307: 1301-4.
30. Krawitt EL, Kilby AE, Albertini RJ, et al. An immunogenetic study of suppressor cell activity in autoimmune chronic active hepatitis. Clin Immunol Immunopathol 1988;46:249-57.
31. Lohse AW, Kögel M, Meyer zum Büschenfelde KH. Evidence for spontaneous immunosuppression in autoimmune hepatitis. Hepatology 1995;22:381-8.
32. Longhi MS, Ma Y, Bogdanos DP, Cheeseman P, Mieli-Vergani G, Vergani D. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. J Hepatol 2004;41:31-7.
33. Duchini A, McHutchison JG, Pockros PJ. LKM-positive autoimmune hepatitis in the western United States: a case series. Am J Gastroenterol 2000;95:3238-41.
34. Bridoux-Henno L, Maggiore G, Johanet C, et al. Features and outcome of autoimmune hepatitis type 2 presenting with isolated positivity for anti-liver cytosol antibody. Clin Gastroenterol Hepatol 2004;2: 825-30.
35. Schramm C, Kanzler S, zum Buschenfelde KH, Galle PR, Lohse AW. Autoimmune hepatitis in the elderly. Am J Gastroenterol 2001;96:1587-91.
36. Lim KN, Casanova RL, Boyer TD, Bruno CJ. Autoimmune hepatitis in African Americans: presenting features and response to therapy. Am J Gastroenterol 2001; 96:3390-4.
37. Hurlburt KJ, McMahon BJ, Deubner H, Hsu-Trawinski B, Williams JL, Kowdley KV. Prevalence of autoimmune liver disease in Alaska natives. Am J Gastroenterol 2002;97:2402-7.
38. Toda G, Zeniya M, Watanabe F, et al. Present status of autoimmune hepatitis in Japan — correlating the characteristics with international criteria in an area with a high rate of HCV infection. J Hepatol 1997;26:1207-12.
39. Boberg KM, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. Incidence and prevalence of primary biliary cirrho-

sis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. Scand J Gastroenterol 1998;33:99-103.
40. Nishioka M, Morshed SA, McFarlane IG, et al. Geographical variation in the frequency and characteristics of autoimmune liver disease. In: Krawitt EL, Wiesner RH, Nishioka M, eds. Autoimmune liver diseases. 2nd ed. Amsterdam: Elsevier, 1998:413-24.
41. Kosar Y, Kacar S, Sasmaz N, et al. Type 1 autoimmune hepatitis in Turkish patients: absence of association with HLA B8. J Clin Gastroenterol 2002;35:185-90.
42. Kessler WR, Cummings OW, Eckert G, Chalasani N, Lumeng L, Kwo PY. Fulminant hepatic failure as the initial presentation of acute autoimmune hepatitis. Clin Gastroenterol Hepatol 2004;2:625-31.
43. Heneghan MA, Norris SM, O'Grady JG, Harrison PM, McFarlane IG. Management and outcome of pregnancy in autoimmune hepatitis. Gut 2001;48:97-102.
44. Buchel E, Van Steenbergen W, Nevens F, Fevery J. Improvement of autoimmune hepatitis during pregnancy followed by flare-up after delivery. Am J Gastroenterol 2002;97:3160-5.
45. Samuel D, Riordan S, Strasser S, Kurtovic J, Singh-Grewel I, Koorey D. Severe autoimmune hepatitis first presenting in early post partum period. Clin Gastroenterol Hepatol 2004;2:622-4.
46. Abdo A, Meddings J, Swain M. Liver abnormalities in celiac disease. Clin Gastroenterol Hepatol 2004;2:107-12.
47. Obermayer-Straub P, Perheentupa J, Braun S, et al. Hepatic autoantigens in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Gastroenterology 2001;121:668-77.
48. Czaja AJ, Cassani F, Cataleta M, Valentini P, Bianchi FB. Frequency and significance of antibodies to actin in type 1 autoimmune hepatitis. Hepatology 1996; 24:1068-73.
49. Herkel J, Heidrich B, Nieraad N, Wies I, Rother M, Lohse AW. Fine specificity of autoantibodies to soluble liver antigen and liver/pancreas. Hepatology 2002;35: 403-8.
50. Roozendaal C, de Jong MA, van den Berg AP, van Wijk RT, Limburg PC, Kallenberg CGM. Clinical significance of antineutrophil cytoplasmic antibodies (ANCA) in autoimmune liver diseases. J Hepatol 2000;32:734-41.
51. Terjung B, Spengler U, Sauerbruch T, Worman HJ. "Atypical p-ANCA" in IBD and hepatobiliary disorders react with a 50-kilodalton nuclear envelope protein of neutrophils and myeloid cell lines. Gastroenterology 2000;119:310-22.
52. Krawitt EL. Sudden jaundice with isolated atypical perinuclear antineutrophil cytoplasmic antibodies. Ann Intern Med 1999;131:796.
53. Gregorio GV, Portmann B, Reid F, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology 1997;25: 541-7.
54. Lapierre P, Hajoui O, Homberg JC, Alvarez F. Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. Gastroenterology 1999; 116:643-9.
55. Batts KP, Ludwig J. Histopathology of autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis. In: Krawitt EL, Wiesner RH, Nishioka M, eds. Autoimmune liver diseases. 2nd ed. Amsterdam: Elsevier, 1998:115-40.
56. Goodman ZD, McNally PR, Davis DR, Ishak KG. Autoimmune cholangitis: a variant of primary biliary cirrhosis: clinicopathologic and serologic correlations in 200 cases. Dig Dis Sci 1995;40:1232-42.
57. Pratt DS, Fawaz KA, Rabson A, Dellelis R, Kaplan MM. A novel histological lesion in glucocorticoid-responsive chronic hepatitis. Gastroenterology 1997;113: 664-8.
58. Davis PA, Leung P, Manns MP, et al. M4 and M9 antibodies in the overlap syndrome of primary biliary cirrhosis and chronic active hepatitis: epitopes or epiphenomena. Hepatology 1992;16:1128-36.
59. Dufour J-F, DeLellis R, Kaplan MM. Reversibility of hepatic fibrosis in autoimmune hepatitis. Ann Intern Med 1997; 127:981-5.
60. Cotler SJ, Jakate S, Jensen DM. Resolution of cirrhosis in autoimmune hepatitis with corticosteroid therapy. J Clin Gastroenterol 2001;32:428-30.
61. Czaja AJ, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. J Hepatol 2004;40: 646-52.
62. Hennes EM, Zeniya M, Czaja AJ, et al. Simplified diagnostic criteria for autoimmune hepatitis. Hepatology 2005;42:295A. abstract.
63. Chazouilleres O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. Hepatology 1998;28:296-301.
64. Lohse AW, zum Buschenfelde KH, Franz B, Kanzler S, Gerken G, Dienes HP. Characterization of the overlap syndrome of primary biliary cirrhosis (PBC) and autoimmune hepatitis: evidence for it being a hepatitic form of PBC in genetically susceptible individuals. Hepatology 1999;29:1078-84.
65. Joshi S, Cauch-Dudek K, Wanless IR, et al. Primary biliary cirrhosis with additional features of autoimmune hepatitis: response to therapy with ursodeoxycholic acid. Hepatology 2002;35:409-13.
66. Romero-Gomez M, Wichmann I, Crespo J, et al. Serum immunological profile in patients with chronic autoimmune cholestasis. Am J Gastroenterol 2004;99: 2150-7.
67. Gregorio GV, Portmann B, Karani J, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology 2001;33:544-53.
68. Feldstein AE, Perrault J, El-Youssif M, Lindor KD, Freese DK, Angulo P. Primary sclerosing cholangitis in children: a long-term follow-up study. Hepatology 2003;38: 210-7.
69. Gohlke F, Lohse AW, Dienes HP, et al. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. J Hepatol 1996;24:699-705.
70. McNair AN, Moloney M, Portmann BC, Williams R, McFarlane IG. Autoimmune hepatitis overlapping with primary sclerosing cholangitis in five cases. Am J Gastroenterol 1998;93:777-84.
71. van Buuren HR, van Hoogstraten JF, Terkivatan T, Schalm SW, Vleggaar FP. High prevalence of autoimmune hepatitis among patients with primary sclerosing cholangitis. J Hepatol 2000;33:543-8.
72. Abdo AA, Bain VG, Kichian K, Lee SS. Evolution of autoimmune hepatitis to primary sclerosing cholangitis: a sequential syndrome. Hepatology 2002;36:1393-9.
73. Roberts SK, Therneau TM, Czaja AJ. Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. Gastroenterology 1996;110:848-57.
74. Johnson P, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. N Engl J Med 1995;333:958-63.
75. Czaja AJ, Freese DK. Diagnosis and treatment of autoimmune hepatitis. Hepatology 2002;36:479-97.
76. Krawitt EL, Bonis PAL. Treatment of autoimmune hepatitis. In: Rose BD, ed. UpToDate, version 13.1. Wellesley, Mass.: UpToDate, 2005.
77. Czaja AJ, Lindor KD. Failure of budesonide in a pilot study of treatment-dependent autoimmune hepatitis. Gastroenterology 2000;119:1312-6.
78. Alvarez F, Ciocca M, Canero-Velasco C, et al. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. J Hepatol 1999;30:222-7.
79. Rumbo C, Emerick KM, Emre S, Shneider BL. Azathioprine metabolite measurements in the treatment of autoimmune hepatitis in pediatric patients: a preliminary report. J Pediatr Gastroenterol Nutr 2002;35:391-8.
80. Langley PG, Underhill J, Tredger JM, Norris S, McFarlane IG. Thiopurine methyltransferase phenotype and genotype in relation to azathioprine therapy in autoimmune hepatitis. J Hepatol 2002;37:441-7.
81. Pratt DS, Flavin DP, Kaplan MM. The

successful treatment of autoimmune hepatitis with 6-mercaptopurine after failure with azathioprine. Gastroenterology 1996; 110:271-4.
82. Czaja AJ. Low-dose corticosteroid therapy after multiple relapses of severe HBsAg-negative chronic active hepatitis. Hepatology 1990;11:1044-9.
83. Fernandes NF, Redeker AG, Vierling JM, Villamil FG, Fong TL. Cyclosporine therapy in patients with steroid resistant autoimmune hepatitis. Am J Gastroenterol 1999;94:241-8.
84. Van Thiel DH, Wright H, Carroll P, et al. Tacrolimus: a potential new treatment for autoimmune chronic active hepatitis: results of an open-label preliminary trial. Am J Gastroenterol 1995;90:771-6.
85. Burak KW, Urbanski SJ, Swain MG. Successful treatment of refractory type 1 autoimmune hepatitis with methotrexate. J Hepatol 1998;29:990-3.
86. Venkataramani A, Jones MB, Sorrell MF. Methotrexate therapy for refractory chronic active autoimmune hepatitis. Am J Gastroenterol 2001;96:3432-4.
87. Kanzler S, Gerken G, Dienes HP, Meyer zum Buschenfelde KH, Lohse AW. Cyclophosphamide as alternative immunosuppressive therapy for autoimmune hepatitis — report of three cases. Z Gastroenterol 1997;35:571-8.
88. Czaja AJ, Carpenter HA, Lindor KD. Ursodeoxycholic acid as adjunctive therapy for problematic type 1 autoimmune hepatitis: a randomized placebo-controlled treatment trial. Hepatology 1999;30:1381-6.
89. Richardson PD, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. J Hepatol 2000;33:371-5.
90. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med 2005;353: 1261-73.
91. Sanchez-Urdazpal LS, Czaja AJ, van Hoek B, Krom RAF, Wiesner RH. Prognostic features and role of liver transplantation in severe corticosteroid-treated autoimmune chronic active hepatitis. Hepatology 1992;15:215-21.
92. Reich DJ, Fiel I, Guarrera JV, et al. Liver transplantation for autoimmune hepatitis. Hepatology 2000;32:693-700.
93. Ratziu V, Samuel D, Sebagh M, et al. Long-term follow-up after liver transplantation for autoimmune hepatitis: evidence of recurrence of primary disease. J Hepatol 1999;30:131-41.
94. Gonzalez-Koch A, Czaja AJ, Carpenter HA, et al. Recurrent autoimmune hepatitis after orthotopic liver transplantation. Liver Transpl 2001;7:302-10.
95. Duclos-Vallee JC, Sebagh M, Rifai K, et al. A 10 year follow up study of patients transplanted for autoimmune hepatitis: histological recurrence precedes clinical and biochemical recurrence. Gut 2003;52: 893-7.
96. Kerkar N, Hadzic N, Davies ET, et al. De-novo autoimmune hepatitis after liver transplantation. Lancet 1998;351:409-13.
97. Heneghan MA, Portmann BC, Norris SM, et al. Graft dysfunction mimicking autoimune hepatitis following liver transplantation in adults. Hepatology 2001;34: 464-70.
98. Salcedo M, Vaquero J, Banares R, et al. Response to steroids in de novo autoimmune hepatitis after liver transplantation. Hepatology 2002;35:349-56.
99. Mieli-Vergani G, Vergani D. De novo autoimmune hepatitis after liver transplantation. J Hepatol 2004;40:3-7.
100. Inui A, Sogo T, Komatsu H, Miyakawa H, Fujisawa T. Antibodies against cytokeratin 8/18 in a patient with de novo autoimmune hepatitis after living-donor liver transplantation. Liver Transpl 2005;11: 504-7.

IMAGES IN CLINICAL MEDICINE

Black Hairy Tongue



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An 85-year-old male cigar smoker with no notable medical history presented with black discoloration and hairy appearance of the tongue, which had lasted for several years. He said he did not use bismuth-containing compounds. Black hairy tongue, also known as lingua villosa nigra, is a painless, benign disorder caused by defective desquamation and reactive hypertrophy of the filiform papillae of the tongue. It is characterized clinically by an abnormal brownish-black coating of the dorsal surface of the tongue. The exact pathogenesis is unclear. A number of relevant etiologic factors have been assumed, including the use of topical or systemic antibiotics as well as psychotropic agents, dehydration, hyposalivation, trigeminal neuralgia, poor oral hygiene, smoking, ingestion of alcohol, and infections. Symptoms may include nausea, halitosis, dysgeusia, and unattractive appearance of the tongue. Therapeutic options of modest benefit include increasing hydration and salivation, discontinuing smoking, brushing the tongue with a soft toothbrush enhanced by previous application of 40 percent urea, applying topical retinoids or salicylic acid, or undergoing surgical excision.

CLINICAL PROBLEM-SOLVING

Needle in a Haystack

Krishna R. Polu, M.D., and Myles Wolf, M.D., M.M.Sc.

In this Journal *feature, information about a real patient is presented in stages (boldface type) to an expert clinician, who responds to the information, sharing his or her reasoning with the reader (regular type). The authors' commentary follows.*

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A 63-year-old man presented to the emergency department because of shortness of breath that had begun the evening before, after he had gone to bed, and worsened progressively during the night. He had had no fevers, chills, cough, hemoptysis, chest pain, or peripheral edema, and there was no history of congestive heart failure. Five months earlier, a pulmonary embolus was diagnosed; the patient received warfarin maintenance therapy; the results of prothrombin-time testing, expressed as an international normalized ratio (INR), were consistently above 2.0. The patient did not think that his current symptoms were similar to those he had experienced with the pulmonary embolism.

Many disorders can present with a sudden onset of shortness of breath, some of which are medical emergencies. Priority should be given to evaluating the possible diagnoses of an acute myocardial infarction or unstable angina. Congestive heart failure precipitated by malignant hypertension, supraventricular or ventricular arrhythmias, and acute valvular diseases such as endocarditis should also be ruled out. Noncardiac diseases that may be characterized by shortness of breath at presentation, such as pneumonia, pneumothorax, acute airway obstruction, sepsis, and renal disease, should also be considered. In this case, despite nearly six months of therapeutic warfarin, pulmonary embolism must remain in the differential diagnosis because of the patient's history and the potentially dire consequences of missing that diagnosis.

The patient's medical history included a horseshoe kidney (a structural renal malformation), mild hypertension that was not treated with medication, depression; and an anxiety disorder. The patient had been a heavy smoker for more than 50 years and continued to smoke one pack per day. He did not use recreational drugs or alcohol, lived alone, and worked as a custodian. His medications included mirtazapine and lorazepam. He also reported taking up to two 200-mg tablets of ibuprofen daily during the previous week for generalized malaise.

On physical examination, he appeared cachectic and chronically ill and was in distress, with a respiratory rate of 22 breaths per minute and an oxygen saturation of 90 percent while he was breathing ambient air. He was afebrile; his blood pressure was 189/115 mm Hg; and his heart rate was 128 beats per minute and regular. A cardiopulmonary examination revealed no jugular venous distention or peripheral edema; breath sounds were equal bilaterally with wet rales but no wheezes, and an S_4 heart sound was present, without a murmur or rub. The pulses were equal and symmetric; however, bruits were heard in both femoral arteries, which were rigid to palpation.

The patient's history of smoking and hypertension in conjunction with evidence of peripheral vascular disease on physical examination increases the likelihood of coronary artery disease complicated by congestive heart failure. Malignant hypertension could also precipitate acute pulmonary edema. Pericardial effusion with cardiac tamponade seems an unlikely diagnosis because of the elevated blood pressure, absence of jugular venous distention, and presence of rales. In addition, there is little evidence to support a diagnosis of pneumonia, pleural effusion, pneumothorax, or sepsis. The cachexia, which may or may not relate to the current acute presentation, will also require further evaluation.

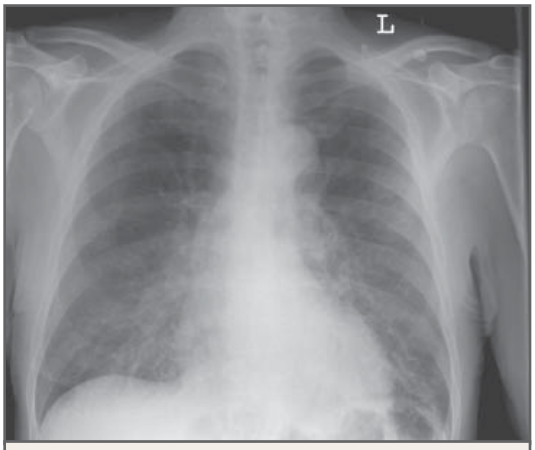

Figure 1. Anteroposterior Radiograph of the Chest, Demonstrating Interstitial Pulmonary Edema and Borderline Cardiomegaly.

A chest radiograph revealed bilateral perihilar alveolar infiltrates and borderline cardiomegaly, suggestive of cardiogenic pulmonary edema (Fig. 1). An electrocardiogram revealed sinus tachycardia at 120 beats per minute, left ventricular hypertrophy, and no evidence of ischemia or infarction. The white-cell count was 9600 cells per cubic millimeter, with 70 percent neutrophils, 20 percent lymphocytes, 6 percent monocytes, and 4 percent eosinophils; the hemoglobin level was 13.7 g per deciliter; and the platelet count was 178,000 cells per cubic millimeter. The creatine kinase level was 69 U per liter, and the troponin T level was 0.02 ng per milliliter. Levels of electrolytes, calcium, magnesium, phosphorus, and liver enzymes were normal. The INR was 2.2. The urea nitrogen level was 25 mg per deciliter (8.9 mmol per liter), and the serum creatinine level was 2.7 mg per deciliter (240 μmol per liter); five months earlier, the urea nitrogen level had been 12 mg per deciliter (4.3 mmol per liter), and the creatinine level had been 1.2 mg per deciliter (110 μmol per liter). The patient was treated with oxygen given nasally and nitroglycerin, furosemide, and morphine given intravenously. His symptoms resolved during the next 12 hours, after a diuresis of 1.5 liters. His blood pressure fell to 140/90 mm Hg, and his oxygen saturation rose to 98 percent. Oral metoprolol was added to the treatment regimen, and he was admitted to the medical service. During his hospitalization, no arrhythmias were noted on telemetry.

On the basis of the presentation, radiographic findings, and rapid response to therapy, pulmonary edema appears to be the most likely diagnosis. There is no evidence of acute myocardial infarction or arrhythmia, and a recurrent pulmonary embolism seems unlikely. An unexpected finding is the renal failure, which, on the basis of the previous tests, is subacute to acute. Although mild, rapidly reversible acute renal failure can accompany congestive heart failure, the triad of renal failure, hypertension, and alveolar infiltrates raises the possibility of a pulmonary–renal syndrome such as Goodpasture's syndrome or a vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA). The absence of hemoptysis and prompt resolution of the shortness of breath after the administration of diuretics argue against pulmonary hemorrhage as the cause of the alveolar infiltrates. However, patients with these and other vasculitides can present with isolated rapidly progressive glomerulonephritis. Evidence of dysmorphic red cells, red-cell casts, and proteinuria on the urinalysis would provide support for this diagnosis.

Other diagnoses to consider at this point are malignant hypertension, which can be characterized at presentation by both acute renal failure caused by renal microvascular ischemia and congestive heart failure caused by diastolic dysfunction and ischemia. We are not told whether the patient had retinal hemorrhages or papilledema, but the rapid and dramatic decrease in blood pressure in response to diuretics argues against the presence of malignant hypertension. Patients with myeloma can present with cardiac and renal involvement, and that diagnosis should also be con-

sidered. Bilateral renal-artery stenosis can also be characterized initially by pulmonary edema because of a combination of volume overload and diastolic dysfunction. In this patient's age group, atherosclerotic disease is the most common cause of renal-artery stenosis, and his lengthy smoking history, along with the presence of bilateral femoral bruits, makes this an important consideration. It would be unusual for the creatinine level to increase so quickly because of isolated renovascular disease, unless the patient was concomitantly using angiotensin-converting–enzyme inhibitors, angiotensin-receptor blockers, nonsteroidal antiinflammatory drugs (NSAIDs), or cyclooxygenase-2 inhibitors; this patient had been taking ibuprofen for a week. Finally, renal obstruction, interstitial nephritis, and acute tubular necrosis caused by medications or exposure to other potentially toxic agents are diagnostic considerations.

Renal ultrasonography revealed fused, low-lying kidneys, without hydronephrosis or increased echogenicity. Oliguria was not present, but the serum creatinine level rose from 2.5 to 3.0 mg per deciliter (220 to 270 μmol per liter) by the third hospital day. On further questioning, the patient reported constipation during the previous year, along with a diminished appetite and an unintentional weight loss of more than 30 kg (his weight decreased from 90.9 kg to 59.1 kg) during the previous six months. He had not had fevers, chills, night sweats, nausea, rash, arthralgias, abdominal pain, hematuria, or other urinary tract symptoms. Further physical examination revealed a lower midline abdominal mass, but there was no hepatosplenomegaly, ascites, lymphadenopathy, rash, edema, or arthritis. A urinalysis revealed a specific gravity of 1.020, a pH of 7.0, and the presence of leukocyte esterase (2+), protein (2+), and trace amounts of blood. Review of the sediment by a nephrologist revealed no casts or crystals; rare white cells and nondysmorphic red cells were seen. A 24-hour urine collection contained 308 mg of protein. A test for antinuclear antibodies (ANA) was positive at 1:640, with a speckled pattern. Tests for ANCA and antibodies against glomerular basement membrane and phospholipids were negative, as were serologic tests for hepatitis B and C viruses. Blood cultures were negative. The serum complement levels were normal, with no monoclonal spikes in the serum or urine electrophoresis.

The serologic workup is essentially negative, and the analysis of the urinary sediment effectively rules out rapidly progressive glomerulonephritis. The positive ANA test does not raise my level of suspicion of nephritis associated with systemic lupus erythematosus, because the complement levels are normal and should be low in active immune-complex diseases such as lupus. The weight loss, constipation, abdominal mass, and recent pulmonary embolism raise the possibility of an underlying cancer such as colorectal carcinoma, which can be associated with renal complications, including obstruction, tumor infiltration, and tumor-associated glomerular disease. The most common presentation of tumor-associated glomerular disease is the nephrotic syndrome, but this syndrome is ruled out by the absence of substantial proteinuria. Horseshoe kidneys can be associated with focal segmental glomerulosclerosis with proteinuria, but the progression of renal disease is typically much slower than in this case, and the patient has only minimal proteinuria. Acute renal failure associated with cancer is often due to obstruction by pelvic or retroperitoneal masses or by lymphadenopathy. Just as important is the fact that partial obstruction, even with preserved urinary output, can also cause renal failure. Therefore, the absence of hydronephrosis does not rule out obstruction, especially in patients with malignant tumors that encase the renal pelvis and ureters and thereby restrict their dilatation. Thus, the absence of oliguria and hydronephrosis on ultrasonography would not deter me from further pursuing a diagnosis of obstruction, especially since the unremarkable findings on the analysis of the urinary sediment are consistent with the presence of obstruction. The horseshoe kidney may also limit the reliability of the ultrasound examination to rule out obstruction.

The patient underwent technetium-99m–mercaptoacetyltriglycine nuclear scanning of the kidneys with furosemide administration. The study revealed fused kidneys with prompt excretion of isotope, indicating no evidence of obstruction. The left part of the kidney accounted for 39 percent of the split renal function, and the right accounted for 61 percent. On palpation, the abdominal mass was believed to be the ectopic horseshoe kidney.

Ureteral obstruction has been ruled out. Patients with tubulointerstitial diseases such as acute tu-

bular necrosis or acute interstitial nephritis caused by the ingestion of ibuprofen usually present with characteristic casts, along with leukocytes and red cells in the sediment; these were not seen. What else causes acute renal failure in the presence of a normal urinary sediment? Congestive heart failure is one cause, but the creatinine level generally improves when the acute episode is treated, just the opposite of the course in this case. Prerenal azotemia resulting from volume depletion is unlikely. The most likely explanation at this point is bilateral renovascular disease, presenting as acute renal failure precipitated by the use of NSAIDs. There is no history of recent exposure to angiotensin-converting–enzyme inhibitors or angiotensin-receptor blockers, which can precipitate a similar presentation.

Abdominal magnetic resonance angiography with gadolinium enhancement (Fig. 2) revealed the horseshoe kidney and marked atherosclerotic disease of the abdominal aorta. There were two right and two left renal arteries, all of which were well visualized and widely patent. A transthoracic echocardiogram showed moderate mitral regurgitation and a dilated left atrium and ventricle with segmental wall-motion abnormalities. The ejection fraction was 45 percent, and there was no pericardial effusion. The ascending aorta was dilated, and a mobile protruding atheroma was noted.

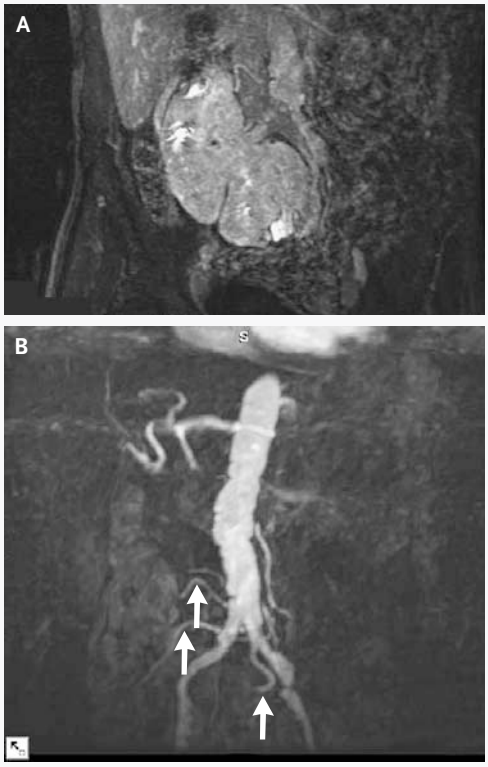

Figure 2. Magnetic Resonance Angiograms of the Renal Arteries.

Panel A shows crossed, fused ectopia, with the left kidney attached to the lower pole of the right kidney. In Panel B, multiple renal arteries (arrows) originate from the aorta and both common iliac arteries that supply the horseshoe kidney. There is marked atherosclerosis of the abdominal aorta.

Renal magnetic resonance angiography is highly sensitive (although not highly specific) for renal-artery stenosis. It is especially useful in cases such as this one; the renal arteries were well visualized and normal, ruling out bilateral renal-artery stenosis. Nonetheless, the aortic segments visualized on both the echocardiogram and the magnetic resonance angiogram suggest a substantial atherosclerotic burden, albeit with sparing of the renal arteries. I suspect that the patient's risk factors, visualized atherosclerosis, and wall-motion abnormalities point to undiagnosed coronary artery disease. Cardiac ischemia precipitated by volume overload induced by NSAIDs or an exacerbation of hypertension could explain the initial pulmonary edema.

The presence of extensive aortic atherosclerosis raises the possibility of another diagnosis not previously considered, atheroembolic kidney disease. We have not been told of any cutaneous signs of atheroembolism, such as livedo reticularis, or overt emboli with the so-called blue toe syndrome. Also, cholesterol embolism is often associated with eosinophilia, hypocomplementemia, and granular casts and leukocytes on the urinalysis, but these were not present in this patient. However, these manifestations can vary widely, and this patient did present with systemic symptoms that complicate atheroembolism, including anorexia and weight loss. With no other suitable working diagnosis, I must strongly consider atheroembolic kidney disease. I would perform a kidney biopsy, but only after carefully discussing the risks of the procedure with the patient, including the risk of recurrent pulmonary embolism related to discontinuing warfarin.

The patient's renal function stabilized, with a creatinine level of 3.1 mg per deciliter (270 μmol per

liter). Cardiac catheterization was deferred because of the renal failure. He left the hospital against medical advice but returned with recurrent pulmonary edema within two weeks. The serum creatinine level rose to 5.0 mg per deciliter (440 μmol per liter). There was no oliguria, and the results of a repeated review of the urinary sediment were unchanged. Subsequently, a percutaneous kidney biopsy was performed (Fig. 3). Pathological examination under light microscopy revealed global sclerosis in two of six glomeruli; the remaining glomeruli were unremarkable. Coarse vacuolization was found in the epithelium of tubules, with loss of brush borders, suggesting ischemic injury. The arteries showed intimal thickening and fibrosis consistent with the presence of hypertension. The immunofluorescence study was negative, ruling out immune-complex disease. Electron microscopy showed minimal segmental effacement of podocyte foot processes and no electron-dense deposits. Two needle-shaped cholesterol crystals were identified in separate afferent arterioles, consistent with the presence of atheroembolic kidney disease. During the subsequent two months, uremia developed and hemodialysis was initiated.

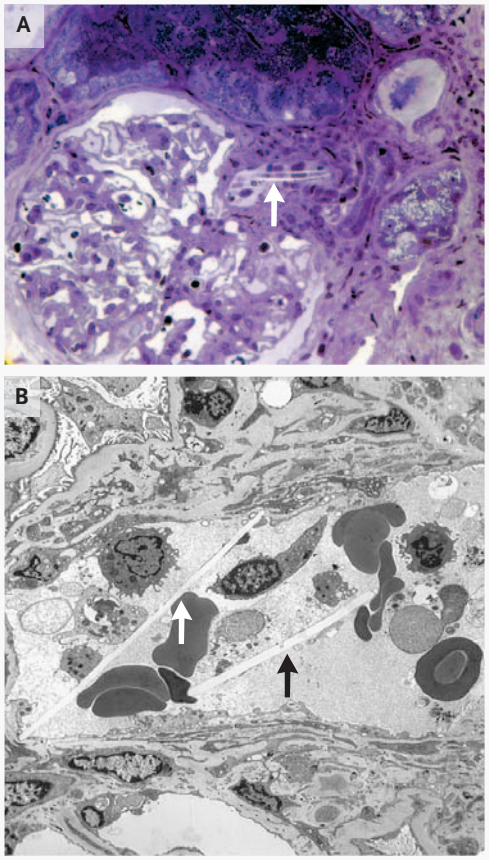

Figure 3. Percutaneous-Biopsy Specimen of the Kidney.
Both the light-microscopical view (Panel A, hematoxylin and eosin) and the electron-microscopical view (Panel B) show needle-like clefts from atheroemboli to afferent arterioles.

COMMENTARY

As this case illustrates, the difficulty in diagnosing renal atheroembolism is often related to the fact that the diagnosis is never even considered. In this instance, the diagnosis was especially difficult, because there were initially no obvious signs or symptoms. Furthermore, in trying to identify a single, unifying disease to connect the pulmonary embolism to the subsequent renal presentation, the discussant did not consider that the treatment of the initial process may have been the link between the thromboembolism and the renal disease. Indeed, although invasive aortic manipulation is the leading cause of atheroembolism, spontaneous atheroembolism is often precipitated by thrombolysis or anticoagulation, which was the likely cause in this case.[1,2] It was only after a detailed evaluation of other potential causes of renal failure proved fruitless and after magnetic resonance angiography and echocardiography, which were ordered for other reasons, showed evidence of diffuse atherosclerosis that the discussant considered the diagnosis of spontaneous atheroembolism and found the "needle in the haystack." In fairness, the discussant's approach highlighted the important principle of ruling out life-threatening diagnoses first and then evaluating patients for other treatable diagnoses. In this case, the delayed diagnosis had little clinical effect, since there are currently no effective treatments for atheroembolism.

Atheroembolic disease accounts for approximately 5 to 10 percent of cases of acute renal failure.[3] These rates may actually underestimate the true prevalence of the disease, because the nonspecific symptoms and indolent nature of the syndrome render the diagnosis elusive, as shown in this case. Typical signs, including eosinophilia, hypocomplementemia, livedo reticularis, and cu-

taneous emboli, are highly variable and may be absent.[4] Retinal emboli, known as Hollenhorst plaques, may provide clues to the diagnosis, but they occur only rarely.[5] As in this case, the results of urinalysis may be unrevealing or may misleadingly suggest other diagnoses, such as interstitial nephritis, acute tubular necrosis, or even glomerulonephritis.[6-8] Furthermore, most cases of atheroembolic kidney disease are triggered by angiography, in which case radiocontrast-induced nephropathy is easily invoked as an alternative diagnosis.[5,9]

Risk factors for atheroembolism include increased age, male sex, hypertension, a history of coronary or peripheral vascular disease, and a history of smoking.[4] In studies of patients with biopsy-proven atheroembolic kidney disease, anticoagulation was thought to be a precipitating factor in 33 to 55 percent of cases.[3-5] Anticoagulant agents are thought to destabilize atherosclerotic plaques by allowing cholesterol crystals that were previously covered by clot to be exposed to the circulation. The prognosis for patients with atheroembolic kidney disease is poor; in up to 25 percent, the condition progresses to end-stage renal disease, and in a recent prospective study, 38 percent died within five years.[5]

The identification of needle-shaped cholesterol clefts on examination of the renal-biopsy specimen is required to make the diagnosis (sites of atheroembolism dissolve during tissue fixation). However, even on renal biopsy, patchy involvement of the kidney can lead to a sampling error and a false negative finding. In addition, changes consistent with the presence of acute tubular necrosis, minimal change disease, and collapsing glomerulopathy, an aggressive subtype of focal segmental glomerulosclerosis, have been described in biopsy specimens from patients with atheroembolism.[10,11] Even when cholesterol clefts are found on biopsy, the finding may be incidental in a patient with another primary diagnosis. However, an incidental finding was unlikely in this case, since the course of the renal failure is consistent with this diagnosis, other potential diagnoses were reasonably ruled out, and the patient's diffuse atherosclerosis and recent anticoagulant therapy are recognized risk factors for atheroembolism.

Atheroembolic disease is an important yet underdiagnosed component of the spectrum of kidney disease associated with atherosclerosis. Physicians should consider this diagnosis in patients with underlying vascular disease who present with renal failure.

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REFERENCES

1. Moll S, Huffman J. Cholesterol emboli associated with warfarin treatment. Am J Hematol 2004;77:194-5.
2. Wong FK, Chan SK, Ing TS, Li CS. Acute renal failure after streptokinase therapy in a patient with acute myocardial infarction. Am J Kidney Dis 1995;26:508-10.
3. Mayo RR, Swartz RD. Redefining the incidence of clinically detectable atheroembolism. Am J Med 1996;100:524-9.
4. Thadhani RI, Camargo CA Jr, Xavier RJ, Fang LS, Bazari H. Atheroembolic renal failure after invasive procedures: natural history based on 52 histologically proven cases. Medicine 1995;74:350-8.
5. Scolari F, Ravani P, Pola A, et al. Predictors of renal and patient outcomes in atheroembolic renal disease: a prospective study. J Am Soc Nephrol 2003;14:1584-90.
6. Espejo B, Herrero JC, Torres A, et al. Immunoallergic interstitial nephritis vs. cholesterol atheroembolism: differentiating characteristics. Nefrologia 2003;23:125-30. (In Spanish.)
7. Scolari F, Tardanico R, Pola A, et al. Cholesterol crystal embolic disease in renal allografts. J Nephrol 2003;16:139-43.
8. Goldman M, Thoua Y, Dhaene M, Toussaint C. Necrotising glomerulonephritis associated with cholesterol microemboli. BMJ 1985;290:205-6.
9. Scolari F, Tardanico R, Zani R, et al. Cholesterol crystal embolism: a recognizable cause of renal disease. Am J Kidney Dis 2000;36:1089-109.
10. Liss KA, Gaughan WJ, McCue PA, Burke JF. Coexistence of atheroemboli and minimal-change disease. Clin Nephrol 1997;47:125-8.
11. Greenberg A, Bastacky SI, Iqbal A, Borochovitz D, Johnson JP. Focal segmental glomerulosclerosis associated with nephrotic syndrome in cholesterol atheroembolism: clinicopathological correlations. Am J Kidney Dis 1997;29:334-44.

EDITORIALS

The Promise of New Rotavirus Vaccines

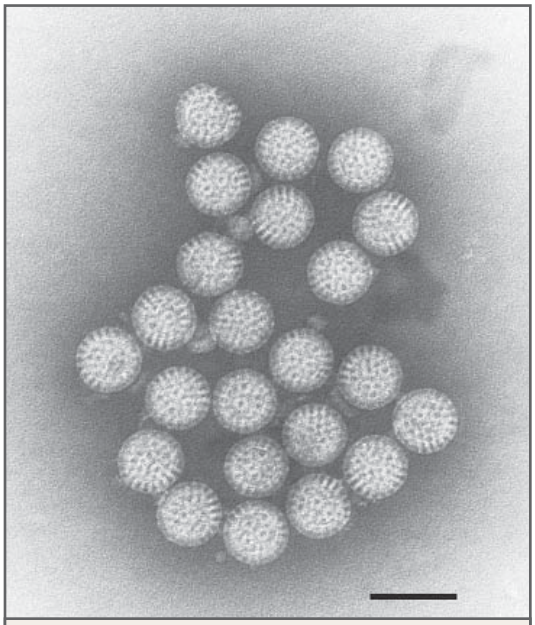
Roger I. Glass, M.D., Ph.D. and Umesh D. Parashar, M.B., B.S., M.P.H.

Rotavirus disease kills approximately half a million children annually in developing countries and accounts for one third of hospitalizations for diarrhea worldwide.[1] In 1999, global efforts to control the tremendous health burden of gastroenteritis suffered an abrupt and unanticipated setback. The first licensed rotavirus vaccine (RotaShield) was withdrawn from the U.S. market less than a year after its introduction because it was associated with an uncommon but potentially life-threatening adverse event, intussusception, at an estimated rate of 1 incident per 10,000 vaccine recipients.[2]

Debate ensued over the possible use of this vaccine in developing countries, where the health benefits, particularly a reduction in deaths from rotavirus, clearly exceeded the potential risks of the vaccine.[3,4] However, it soon became apparent that the introduction of a vaccine that had been withdrawn in the United States was untenable in developing countries and that new vaccines would be needed. The manufacture of the first licensed rotavirus vaccine was halted, and hope was lost for a vaccine that could have prevented much severe diarrhea in children around the world. Despite the prospect that other live oral rotavirus vaccines might also be associated with intussusception and despite the expense of conducting large-scale clinical trials for safety, two manufacturers accepted the challenge.

This issue of the *Journal* includes reports on the promising results of large clinical trials of two new rotavirus vaccines whose manufacturers moved ahead with trials despite the many challenges and risks.[5,6] The two new products, Rotateq from Merck and Rotarix from GlaxoSmithKline, are both live oral vaccines intended to be given to infants at the same time as their immunizations for diphtheria, pertussis, and tetanus, but they differ in their approaches, strains, and formulations. Rotarix is a monovalent vaccine derived from the most common human rotavirus strain, G1P[8], that has been attenuated by serial passage and is administered in two oral doses one to two months apart. The vaccine strain replicates well in the gut, is shed by more than 50 percent of patients receiving the vaccine after the first dose, and (like natural rotavirus infections) provides cross-protection against most other serotypes. By contrast, Rotateq is a pentavalent vaccine based on a bovine strain, WC3, that contains five human–bovine reassortant viruses. WC3 is naturally attenuated for humans but is not broadly cross-protective, so each reassortant virus contains a single gene encoding a major outer capsid protein from the most common human serotypes. The bovine virus grows less well in the human intestine, so the aggregate titer required to immunize a child is greater. In addition, the vaccine strains are infrequently shed in the stool, and three oral doses are required, with at least a month between doses.

Despite these differences, both vaccines demonstrate an impressive efficacy profile. The slight differences in observed efficacy against severe rotavirus disease (85 percent for Rotarix and 98 percent for Rotateq) might well be explained by differences in the classification of disease severity and the populations studied. GlaxoSmithKline conducted its trials primarily among infants of poor and middle-income families in Latin America, whereas the Merck vaccine was tested in the United States and Finland. A particularly exciting finding of great importance to public health (and to the economic burden of disease) was the magnitude of the reduction in hospitalizations



Courtesy of Charles D. Humphrey, Centers for Disease Control and Prevention, Atlanta.

Figure 1. Negative-Stain Electron Micrograph of Rotavirus A.

The virus is 70 to 75 nm in diameter and has three protein layers, two of which are visible, which is typical of rotavirus. This specimen was stained with 2 percent methylamine tungstate. The bar represents 100 nm.

for diarrhea of any cause, a decrease that was greater than expected given the number of diagnosed cases of rotavirus. In Latin America, Rotarix vaccination decreased hospitalizations for diarrhea among children under one year of age by 42 percent, and in the United States and Finland, Rotateq vaccination reduced hospitalizations by 63 percent during the first year of life. These studies, which identify the fraction of diarrhea attributable to rotavirus, indicate that more of the severe cases of diarrhea leading to hospitalization are probably caused by rotavirus than has been estimated from previous studies. In Latin America, the 42 percent reduction in hospitalizations may predict a similar reduction in mortality that could translate directly into improved child survival. In the United States, Rotateq reduced the number of lost workdays from rotavirus by nearly 87 percent, a welcome benefit with clear economic implications for families.[7,8]

Perhaps even more important, the two vaccines demonstrated a reassuring safety profile, particularly with respect to intussusception. Each of the two trials enrolled and monitored more than 60,000 infants, making them the largest prelicensure vaccine trials conducted to evaluate vaccine safety. Fortunately, neither trial identified a significant difference between vaccine and placebo in the risk of intussusception, suggesting that the problem of intussusception may have been a characteristic of RotaShield rather than a problem intrinsic to all live oral rotavirus vaccines. However, epidemiologic observations indicate that natural intussusception spares infants in the first three months of life, and infants immunized with RotaShield who were under three months of age had a substantially lower risk of intussusception (approximately 1 in 30,000) than did those who were older (<1 in 8000).[9] Since the first doses of both of the new vaccines were administered to infants who were under three months of age, the absence of an increased risk of intussusception might reflect the safer age at which these vaccines were tested. These trials leave open the question of whether either vaccine might cause intussusception if administered to older infants or to a larger number of infants. Given the troubling legacy of RotaShield and concern among the public and physicians over intussusception, an effective system of surveillance should be put in place after licensure to monitor this rare outcome. Hundreds of thousands of children will need to be immunized before a clean bill of health can be given to these vaccines.

As these vaccines become licensed and used in Europe, the United States, and many other countries, global interest will focus next on the effect these vaccines will have on reducing the number of clinic visits and hospitalizations, as well as economic costs, in industrialized countries and hospitalizations for diarrhea and deaths among children in the developing world.[10] However, a number of issues remain to be faced before rotavirus vaccines can realize their full potential. For the developed world, issues of price, acceptability, public awareness, and fear of intussusception will need to be addressed before the effect of this vaccine will be fully felt. The key question for the global community will be to determine whether these vaccines work equally well among the poorest children in the developing world. Live oral vaccines must replicate and be processed in the infant's gut in order to induce a good immune response and be protective. Replication is highly dependent on the dose of the vaccine administered and host factors

that might neutralize the virus, including maternal antibodies, breast-feeding, interfering bacterial or viral agents, and malnutrition. In addition, although both vaccines protected against the full range of serotypes in circulation in the trial population, Rotarix was less efficacious against the G2 strains, and it remains to be seen how the vaccines will perform in settings where nonvaccine serotypes are more prevalent. Both vaccines will need to demonstrate their efficacy in the difficult settings of developing countries if we are to achieve our goal of maximally decreasing global deaths from diarrhea. Fortunately, trials of the Rotarix vaccine have begun in South Africa and will start in Bangladesh and Malawi in the near future. In their report on Rotateq, the investigators indicate the need for Merck to conduct similar trials in the developing world, but no definite plans have been announced.

Anticipating the results of these trials, the Global Alliance for Vaccines and Immunization, the World Health Organization, and the Bill and Melinda Gates Foundation are encouraging and supporting the accelerated introduction of rotavirus vaccines in the poorest countries of the world, where rotavirus remains a fatal disease. Once the efficacy of these vaccines can be established in these populations, mechanisms to finance the introduction of vaccines, ensure a sustainable and affordable supply of vaccines, and expedite the introduction of these vaccines into routine immunization programs should become a global priority. The two reports in the *Journal* document these very large trials, conducted before licensure, to demonstrate both the safety and efficacy of these new vaccines against diarrhea, the second most common disease in children. As vaccines become licensed and used in the United States and Europe, we should expect to see a substantial reduction in winter hospitalizations, visits to doctors and clinics, and parents' workdays lost due to childhood diarrhea. With the successful introduction of rotavirus vaccines in industrialized countries, the global health community will be charged with expediting the availability of these lifesaving vaccines at an affordable price in the developing world. After a long period of waiting, the time for a rotavirus vaccine may have finally arrived.

The views expressed in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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From the Centers for Disease Control and Prevention, Atlanta.

1. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003;9:565-72.
2. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344:564-72. [Erratum, N Engl J Med 2001;344:1564.]
3. Peter G, Myers MG. Intussusception, rotavirus, and oral vaccines: summary of a workshop. Pediatrics 2002;110:e67.
4. Weijer C. The future of research into rotavirus vaccine. BMJ 2000;321:525-6.
5. Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006;354:23-33.
6. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006;354:11-22.
7. Tucker AW, Haddix AC, Bresee JS, Holman RC, Parashar UD, Glass RI. Cost-effectiveness analysis of a rotavirus immunization program for the United States. JAMA 1998;279:1371-6.
8. Lee BP, Azimi PH, Staat MA, et al. Nonmedical costs associated with rotavirus disease requiring hospitalization. Pediatr Infect Dis J 2005;24:984-8.
9. Simonsen L, Viboud C, Elixhauser A, Taylor RJ, Kapikian AZ. More on RotaShield and intussusception: the role of age at the time of vaccination. J Infect Dis 2005;192:Suppl 1:S36-S43.
10. Glass RI, Bresee JS, Turcios R, Fischer TK, Parashar UD, Steele AD. Rotavirus vaccines: targeting the developing world. J Infect Dis 2005;192:Suppl 1:S160-S166.

Intraperitoneal Chemotherapy Comes of Age

Stephen A. Cannistra, M.D.

Patients with advanced epithelial ovarian cancer typically receive intravenous taxane and platinum-based chemotherapy in an attempt to eradicate residual disease after surgical debulking. This treatment yields overall median survivals of approximately 37 months in patients with suboptimally debulked disease (residual tumor, >1.0 cm in diameter) and 49 months in those with optimally debulked disease (residual tumor, ≤1.0 cm in diameter).[1] Despite high response rates, in most patients relapse occurs, and efforts to improve treatment by escalating the doses of intravenous chemotherapy have been largely unsuccessful.[2] In contrast to intravenous drug administration,

the intraperitoneal route is capable of achieving high local concentrations of drugs such as cisplatin, with generally acceptable systemic side effects.[3] This strategy is particularly attractive in the treatment of a disease such as ovarian cancer, which remains largely restricted to the abdominal cavity for most of its natural history. The pharmacologic advantage of the intraperitoneal route for drugs such as cisplatin and paclitaxel is considerable, with intraperitoneal-to-plasma concentration ratios in the range of more than 20 and 1000, respectively.[4] This route allows the escalation of the dose of chemotherapy to a level that is not possible to achieve safely with intravenous drug administration.

In this issue of the *Journal*, Armstrong et al.[5] report compelling evidence in support of intraperitoneal chemotherapy for patients with newly diagnosed stage III, optimally debulked epithelial ovarian cancer. Patients in the Gynecologic Oncology Group (GOG) trial who had undergone optimal debulking were randomly assigned to a control group receiving intravenous paclitaxel and intravenous cisplatin or to an experimental group receiving intravenous paclitaxel on day 1, intraperitoneal cisplatin on day 2, and intraperitoneal paclitaxel on day 8. With a median follow-up of 50 months, there was a statistically significant prolongation of median progression-free survival and overall survival in the intraperitoneal group (a benefit of 5.5 and 15.9 months, respectively) associated with a reduction of 25 percent in the risk of death. A 15.9-month improvement in median overall survival is one of the largest benefits ever observed for a new therapy in gynecologic oncology.

Drugs delivered by the intraperitoneal route penetrate only to a depth of a few millimeters beneath the tumor surface.[6] Thus, patients with relatively small-volume residual disease (i.e., optimally debulked) are expected to benefit most from this approach. However, even small-volume tumor implants may extend well beneath the peritoneal surface, and patients with stage III disease frequently have metastases at other sites, such as retroperitoneal lymph nodes.[7] Eradication of disease in such sanctuaries requires chemotherapy that is delivered through the bloodstream. In this regard, a substantial fraction of cisplatin administered by the intraperitoneal route will eventually be absorbed systemically.[3] It follows that the administration of intraperitoneal cisplatin on day 2 in the GOG trial accomplishes two important goals: the achievement of high drug concentrations within the peritoneal cavity and the systemic delivery of the drug to hidden sites of disease outside the abdomen. In contrast to cisplatin, however, paclitaxel is poorly absorbed into the systemic circulation when administered by the intraperitoneal route. For this reason, the GOG trial was designed to include both intravenous paclitaxel on day 1 and intraperitoneal paclitaxel on day 8, to ensure adequate systemic delivery of the drug while at the same time achieving high drug concentrations within the peritoneal cavity.

The side effects of intraperitoneal chemotherapy included a high incidence of catheter-related complications, abdominal pain, metabolic abnormalities, and neuropathy. Almost half the patients received only three or fewer intraperitoneal courses because of toxic effects, often catheter-related (e.g., infection, blockage, or leak), and only 42 percent of the patients completed six cycles of the planned intraperitoneal therapy. Patients who underwent resection of the left side of the large bowel during the initial debulking surgery were less likely to begin intraperitoneal treatment.[8] Patients removed from the intraperitoneal group were generally able to complete a total of six cycles of first-line chemotherapy by switching to conventional intravenous administration for the remainder of the treatment. It is remarkable that such a clinically meaningful survival advantage was observed, despite the high attrition rate in the intraperitoneal group, suggesting that a substantial benefit from intraperitoneal chemotherapy may occur within the first several cycles of treatment. Although this hypothesis is provocative, the relationship between the number of intraperitoneal cycles received and the magnitude of the benefit can be assessed only in a randomized trial.

Many efforts to improve the tolerability of intraperitoneal therapy could be considered, including reduction of the dose of intraperitoneal cisplatin on day 2, administration of intravenous paclitaxel on day 1 over 3 hours instead of 24 hours, or omission of intraperitoneal paclitaxel on day 8 until tolerance of the first cycle of intraperitoneal cisplatin can be assessed. Although these measures are reasonable, it is unknown whether they will reduce the toxic effects and still preserve the benefits of the intraperitoneal

approach. The intraperitoneal placement of a single-lumen venous-access device attached to a subcutaneous port may also be preferable, as this device appears to have a lower tendency to fibrous-sheath formation or the development of a bowel obstruction, as compared with fenestrated catheters.[9] There is also interest in the use of intraperitoneal carboplatin instead of intraperitoneal cisplatin, in the hope of reducing toxic effects while preserving efficacy.[10] Although there is a pharmacologic advantage to intraperitoneal carboplatin, it is not known whether carboplatin is as effective as cisplatin when administered by the intraperitoneal route. This important question can be addressed only in a randomized trial.

The results of the GOG trial, taken together with data from two other randomized trials,[11,12] will influence clinical practice. It will now be appropriate for physicians to discuss intraperitoneal therapy with selected patients who have newly diagnosed, optimally debulked disease, making certain that these patients have a clear understanding of the benefits as well as the greater risk of side effects. Unlike the introduction of a new drug into patient care, however, the use of intraperitoneal therapy requires a new set of logistics for clinical practice. These include the need to schedule catheter placement (unless this was performed during the initial surgery) and multiple treatment visits as well as the need to provide intensive physician and nursing support for managing infusion-related abdominal pain and infections at the catheter site. With the assistance of a skilled oncology nursing staff, it should eventually be possible for many oncologists to administer the GOG regimen, or a modification of it, effectively. However, as is the case with any specialized technique, in the short term physicians unfamiliar with intraperitoneal therapy might consider referring appropriate patients to centers with expertise in this procedure.

As anticipated on the basis of the toxicity profile, the intraperitoneal regimen used in the GOG trial is associated with a reduced quality of life during the therapy and shortly after its completion, but there was a return to baseline one year after completion.[5] Given the survival advantage of the treatment, many patients will be willing to undergo intraperitoneal therapy, even after being informed of its short-term effects on the quality of life, and others will not be willing to do so. For these reasons, the decision to use intraperitoneal chemotherapy should be individualized. Despite increased toxic effects and the more complicated logistics of drug administration, the data from the GOG trial establish intraperitoneal chemotherapy as an important advance in the first-line treatment of patients with optimally debulked stage III disease.

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1. Cannistra SA. Cancer of the ovary. N Engl J Med 2004;351:2519-29.
2. McGuire WP III. High-dose chemotherapeutic approaches to ovarian cancer management. Semin Oncol 2000;27:Suppl 7:41-6.
3. Howell SB, Pfeifle CL, Wung WE, et al. Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 1982;97:845-51.
4. Rothenberg ML, Liu PY, Braly PS, et al. Combined intraperitoneal and intravenous chemotherapy for women with optimally debulked ovarian cancer: results from an intergroup phase II trial. J Clin Oncol 2003;21:1313-9.
5. Armstrong DK, Bundy B, Wenzel L, et al. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 2006;354:34-43.
6. Los G, Mutsaers PH, Lenglet WJ, Baldew GS, McVie JG. Platinum distribution in intraperitoneal tumors after intraperitoneal cisplatin treatment. Cancer Chemother Pharmacol 1990;25:389-94.
7. Benedetti-Panici P, Greggi S, Maneschi F, et al. Anatomical and pathological study of retroperitoneal nodes in epithelial ovarian cancer. Gynecol Oncol 1993;51:150-4.
8. Walker JL, Armstrong D, Huang HQ, et al. Intraperitoneal catheter outcomes in a phase III trial of intravenous versus intraperitoneal chemotherapy in optimal stage III ovarian and primary peritoneal cancer: a Gynecologic Oncology Group study. Gynecol Oncol 2006;100:27-32.
9. Alberts DS, Markman M, Armstrong D, Rothenberg ML, Muggia F, Howell SB. Intraperitoneal therapy for stage III ovarian cancer: a therapy whose time has come! J Clin Oncol 2002;20:3944-6.
10. Fujiwara K, Sakuragi N, Suzuki S, et al. First-line intraperitoneal carboplatin-based chemotherapy for 165 patients with epithelial ovarian carcinoma: results of long-term follow-up. Gynecol Oncol 2003;90:637-43. [Erratum, Gynecol Oncol 2003;91:662.]
11. Markman M, Bundy BN, Alberts DS, et al. Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 2001;19:1001-7.
12. Alberts DS, Liu PY, Hannigan EV, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 1996;335:1950-5.

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Inhibiting Inflammation in Rheumatoid Arthritis

Gary S. Firestein, M.D.

Targeting signal-transduction molecules, especially kinases, is a daunting task. First, these enzymes are often widely distributed in many tissues and contribute to a vast array of cellular processes essential for survival. Second, truly selective inhibitors can be difficult to synthesize because the target enzyme is often structurally similar to others and many such enzymes share substrates such as ATP. Two recent reports by a single group show how one can overcome these hurdles through a combination of serendipity and inventiveness: the researchers used a novel inhibitor of phosphatidylinositol-3′-kinase γ (PI3Kγ) to treat mouse models of the inflammatory diseases rheumatoid arthritis[1] and systemic lupus erythematosus.[2]

The PI3Ks have long been considered attractive candidates for therapeutic intervention. By catalyzing phosphorylation of the 3-hydroxy position of inositol in the phosphoinositide lipids, they regulate diverse cellular functions such as glucose transport, cell survival and proliferation, cytoskeletal rearrangement, and the production of oxygen radicals.[3] These heterodimers are divided into three groups on the basis of their subunit composition. The PI3Ks composed of α and β subunits are ubiquitous and are activated by many extracellular stimuli and cellular stresses.

Here is the serendipity: the expression of PI3Kγ is far more restricted; it is found primarily in hematopoietic cells. Even more fortuitously, the stimuli that activate PI3Kγ are comparatively few and include chemokines (cytokines that mobilize and activate leukocytes) and other chemoattractant proteins, such as the C5a fragment of complement, that signal through G-protein–coupled receptors. PI3Kγ is an attractive therapeutic target for immune and inflammatory diseases because it is expressed mainly in leukocytes and regulates the transport of cells into inflammatory sites after activation of chemokine receptors. Because many chemokine and chemoattractant receptors signal through PI3Kγ, one can potentially suppress numerous overlapping pathways at once.

The validity of this concept was supported by studies of a mouse model of rheumatoid arthritis. Mice lacking the *PI3Kγ* gene, as compared with wild-type mice, had significantly less synovial inflammation and joint destruction, on the basis of both the clinical extent of arthritis and a histologic evaluation of the joint. This particular model relies on the ability of systemically administered antibodies against collagen to bind cartilage and activate complement locally and is independent of the activity of T and B cells.[4] The release of chemotactic factors such as C5a in the joint after complement fixation and activation of Fc receptors on resident cells recruits circulating blood leukocytes to the joint and leads to synovitis (Fig. 1A).

Next comes inventiveness: using rational drug design, Camps and colleagues developed novel PI3Kγ inhibitors with a degree of selectivity, as determined by in vitro assays using monocytes and macrophages.[1] One compound completely arrested disease progression in the standard, collagen-induced model of arthritis (Fig. 1B). This model differs from the one used in the *PI3Kγ*-knockout mice because the mice were immunized with type II collagen rather than given premade antibodies against type II collagen. Therefore, it tests the effect of a therapeutic agent on the entire immune response, from initial antigen recognition to the late effects of autoantibody-induced damage to tissues. Because the drug was administered after the onset of clinical arthritis, the primary effect was probably on the ablation of signaling induced by chemokines, rather than the activation of T and B cells, which occurs very early in the model and is responsible for the production of autoantibody.

Although this study design does not permit one to differentiate the relative contributions of innate and adaptive immunity, it is closer to the real-world clinical situation in which patients

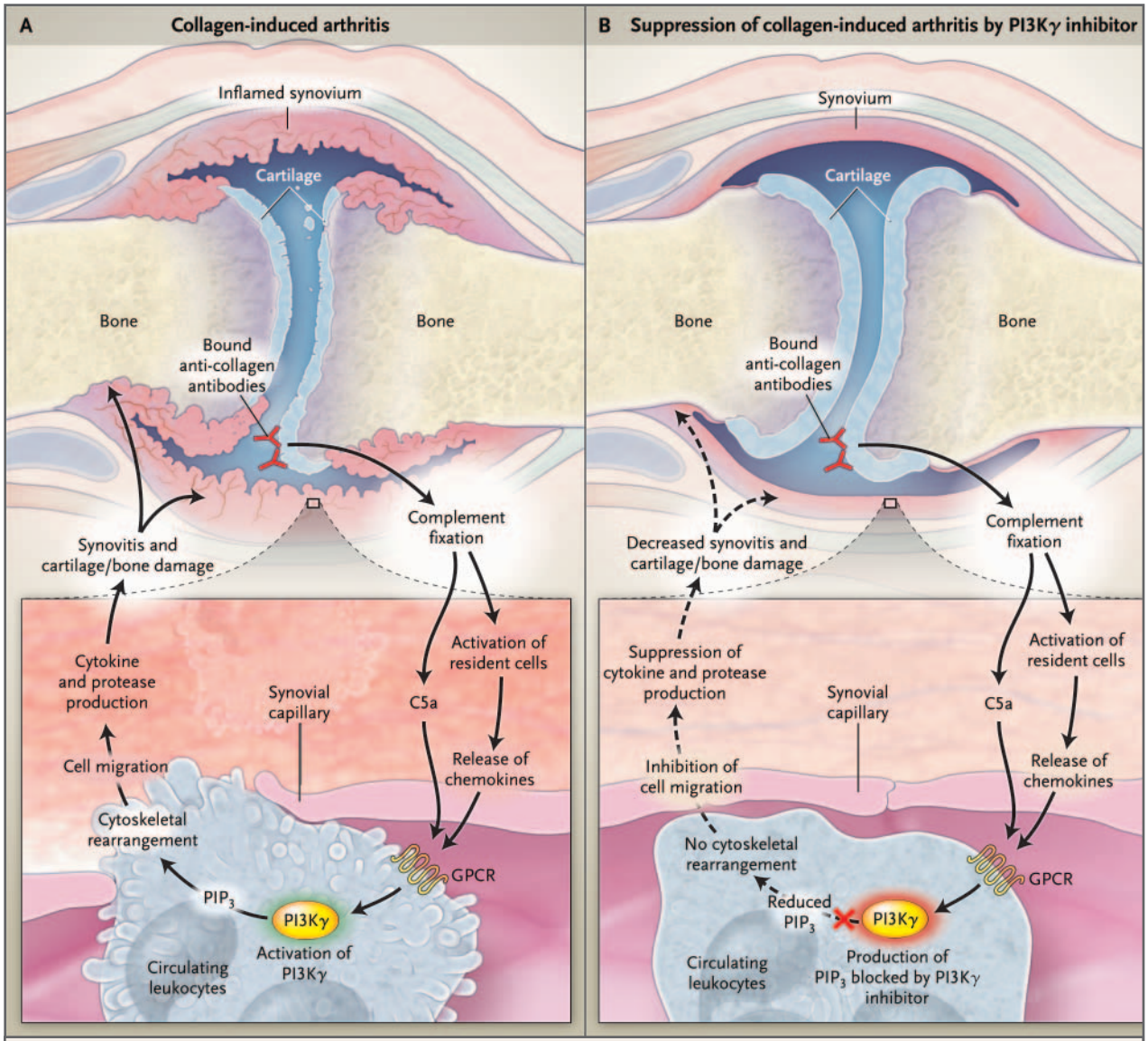

Figure 1. Ability of PI3Kγ Inhibitors to Block Inflammation and Joint Destruction in Arthritis.

Collagen-induced arthritis, a model of rheumatoid arthritis, is mediated by antibodies against type II collagen (anti-collagen antibodies) that bind to cartilage (Panel A). The bound antibodies can fix complement to produce C5a as well as activate resident cells by engaging Fc receptors, leading to the release of chemokines. The chemoattractants bind G-protein–coupled receptors (GPCR) on circulating leukocytes. The receptors activate PI3Kγ, which catalyzes the phosphorylation of phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-triphosphate (PIP_3), a second-messenger molecule that induces cytoskeletal rearrangement and cell migration into the inflamed synovium. The newly arrived cells produce inflammatory cytokines that further inflame the synovium and release proteases that damage cartilage and bone. Camps and colleagues[1] recently reported that a PI3Kγ inhibitor blocks both the production of PIP_3 and chemokine-induced migration of leukocytes into the joint (Panel B). By interfering with cell influx into the synovium, the inhibitor suppresses local production of cytokines and proteases, thereby protecting the joint from damage.

with established rheumatoid arthritis need to be treated. Administration of the PI3Kγ inhibitor also improved survival in the MRL–lpr strain, a mouse model of lupus, and longevity in the treated mice rivaled that among animals given a potent glucocorticoid. Although perhaps not predictive of results in patients,[5] these models provide preclinical proof-of-concept that selective inhibition of PI3Kγ may suppress inflammation while leaving the other, more widely distributed

isoforms untouched. This approach would theoretically have a reduced risk of adverse effects.

All these findings portend well for the eventual implementation of a strategy involving inhibitors of pivotal kinases in clinical practice. Several caveats must be kept in mind. The beneficial effect of the complete loss of PI3Kγ in the knockout-mouse model was relatively small, as compared with the effect of the small-molecule PI3Kγ inhibitor, in the standard collagen-induced model of arthritis. This could be due to an effect on T and B cells in the latter model. In addition, the compound has partial rather than complete selectivity over other PI3K isoforms and could potentially inhibit these enzymes at high doses. The long-term safety of this approach has yet to be established; the duration of treatment even in the mouse models of chronic disease was only a fraction of the time needed to assess the effect on host defenses and homeostasis. It is also uncertain whether PI3Kγ blockade will have unanticipated adverse effects if the protein is expressed at low levels in other nonhematopoietic tissues. Despite these questions, the studies represent a paradigm for modern drug development. Molecular biology and biochemistry established the hierarchy of signaling molecules; rational drug design produced an inhibitor of a pivotal molecule in the hierarchy. However, we must not lose sight of the fact that mouse models only imperfectly mimic the human condition. Clinical trials will be the ultimate test of the hypothesis that PI3Kγ is a therapeutic target for uniquely human diseases such as rheumatoid arthritis.

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

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1. Camps M, Ruckle T, Ji H, et al. Blockade of PI3Kγ suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. Nat Med 2005;11:936-43.
2. Barber DF, Bartolome A, Hernandez C, et al. PI3Kγ inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. Nat Med 2005;11:933-5.
3. Curnock AP, Logan MK, Ward SG. Chemokine signalling: pivoting around multiple phosphoinositide 3-kinases. Immunology 2002;105:125-36.
4. Nandakumar KS, Svensson L, Holmdahl R. Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. Am J Pathol 2003;163:1827-37.
5. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356-61.

CORRESPONDENCE

Normal Fasting Plasma Glucose Levels and Type 2 Diabetes

TO THE EDITOR: Tirosh et al. (Oct. 6 issue)[1] found higher fasting glucose levels within the normoglycemic range to be a risk factor for type 2 diabetes in young men. They collected blood in fluoride tubes, which were sent to the laboratory uncentrifuged. However, levels of glucose continue to decrease for up to four hours in fluoride tubes and can drop by variable amounts, sometimes exceeding 9 mg per deciliter.[2,3] Most of this decrease occurs in the two hours immediately after venipuncture.[2] A change of this magnitude could straddle two of the four top population quintiles described by Tirosh et al. Measurement bias, such as that introduced by failure to separate plasma from cells immediately or by the inherent variability of calibration, limits the use of simple cutoffs for plasma glucose levels in the identification of patients at risk for diabetes. We have suggested methods to minimize errors in venous glucose measurement.[4,5]

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1. Tirosh A, Shai I, Tekes-Manova D, et al. Normal fasting plasma glucose levels and type 2 diabetes in young men. N Engl J Med 2005;353:1454-62.
2. Chan AY, Swaninathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989;35:315-7.
3. Sidebottom RA, Williams PR, Kanarek KS. Glucose determinations in plasma and serum: potential error related to increased hematocrit. Clin Chem 1982;28:190-2.
4. Schwartz JG, Reichberg SB, Gambino RS. Glucose testing variability and the need for an expert oversight committee. CAP Today 2005;19:12-6.
5. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002;48:436-72.

THE AUTHORS REPLY: The technical point raised by Gambino and colleagues is well taken but is unlikely to have substantially affected our conclusions. Though potentially valuable for accurate blood glucose measurements in the future, new techniques such as those cited by Gambino and colleagues were not available to us nor to most investigators in studies that have provided cutoff definitions for blood glucose values. With a maximum of two hours between venipuncture and sample analysis, an expected rate of 14.1 percent impaired fasting-glucose levels was observed in our cohort of healthy young men.[1,2] The suggested possibility of an underestimation of 9 mg per deciliter in our blood glucose measurements would have raised this rate to more than 50 percent, a highly unlikely finding in such a population. Furthermore, the association between fasting plasma glucose and incident diabetes increased progressively, suggesting a linear trend from the lowest quintile. Finally, common clinical practice in primary care clinics relies on blood glucose measurements performed in a manner similar to

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the approach that we used. Therefore, we think that our results are probably relevant to the general practitioner in the assessment of the risk of diabetes in healthy young adults.

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1. DECODE Study Group. Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European cohorts. Diabetes Care 2003;26:61-9.
2. Qiao Q, Hu G, Tuomilehto J, et al. Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. Diabetes Care 2003;26:1770-80.

Genetic Modifiers in Cystic Fibrosis

TO THE EDITOR: Drumm and colleagues (Oct. 6 issue)[1] identified sequence variants in the *TGFβ1* gene as genetic modifiers of lung disease in cystic fibrosis. *TGFβ1* is encoded on chromosome 19q13, 4.5 Mbp from the *CFM1* locus that confers a risk of meconium ileus.[2] We scanned this region on 19q13 with use of single-nucleotide polymorphisms (SNPs) and microsatellites in a cohort of sibling pairs homozygous for the ΔF508 mutation with extreme clinical phenotypes[3] for a modulator of disease severity. Neither the two *TGFβ1* SNPs (–509 and codon 10)[1] nor the microsatellite D19S112 at *CFM1*[2] was associated with disease severity in our cohort.

Interrogation of the region between *TGFβ1* and *CFM1* by four microsatellites, however, revealed a significant transmission disequilibrium in clinically discordant sibling pairs (a peak at D19S197, P=0.003), suggesting a modulation of factors acting in trans[3] (Fig. 1). The region contains the *CEACAM* gene family encoding cell-adhesion molecules involved in the binding of pathogens and the regulation of differentiation, angiogenesis, and cancer. The *TGFβ1–CEACAM* region apparently contains at least one clinically relevant genetic modifier of cystic fibrosis.

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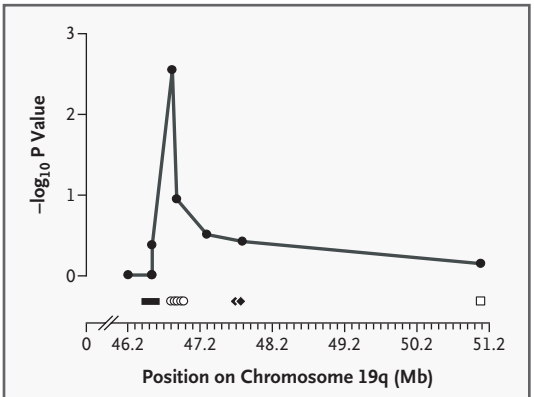

Figure 1. Transmission Disequilibrium among Clinically Discordant Sibling Pairs Homozygous for the ΔF508 Mutation.

The graph shows the results of a family-based test for linkage within families of ΔF508-homozygous sibling pairs with cystic fibrosis who are discordant for nutritional and pulmonary status.[3] Logarithmic P values[4] are displayed in a 5-Mbp segment of chromosome 19q13 for markers D19S400, single-nucleotide polymorphism (SNP) at codon 10 in *TGFβ1*, SNP at –509 of *TGFβ1* promoter, D19S197, three microsatellites near *CEACAM* genes (nucleotide 16143–16196 on AC005797, nucleotide 21541–21588 on AC024076, and nucleotide 21273–21314 on AC004558), and D19S112, whereby a transmission disequilibrium, P=0.003, was observed at D19S197. The positions of candidate genes are indicated by symbols below the physical map. The solid line denotes the *TGFβ1* locus; the open circles the *CEACAM* gene cluster consisting of *CEACAM4*, *CEACAM7*, *CEACAM5*, *CEACAM6*, and *CEACAM3*; the solid diamonds the *CEACAM* gene cluster consisting of *CEACAM1* and *CEACAM8*; and the open square *CFM1*. The length of the solid line indicates the region of high-density SNP mapping shown in Figure 1 of the article by Drumm et al.[1]

1. Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. N Engl J Med 2005;353:1443-53.
2. Zielenski J, Corey M, Rozmahel R, et al. Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. Nat Genet 1999;22:128-9.
3. Mekus F, Laabs U, Veeze H, Tummler B. Genes in the vicinity of CFTR modulate the cystic fibrosis phenotype in highly concordant or discordant F508del homozygous sib pairs. Hum Genet 2003;112:1-11.
4. Becker T, Knapp M. A powerful strategy to account for mul-

tiple testing in the context of haplotype analysis. Am J Hum Genet 2004;75:561-70.

TO THE EDITOR: Drumm et al. fail to include in their analysis the HLA class II genes and the neutrophil Fcγ receptor IIA (FcγRIIA) genes among those previously reported as potential modifiers in cystic fibrosis.[1,2] In patients with this disease, the HLA-DR7 allele and the FcγRIIA R allele have been associated with an increased risk of colonization by *Pseudomonas aeruginosa*. The finding related to the FcγRIIA R allele was obtained from a study of patients with cystic fibrosis who have the same *CFTR* genotype as the patients studied by Drumm et al.

Since the *TGFβ1* alleles have previously been shown to have a protective effect in smokers,[3] it may be reasonable to consider in the statistical analysis the covariate of the presence or absence of a history of smoking in order to avoid a potential confounding factor.

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2. De Rose V, Arduino C, Cappello N, et al. Fcgamma receptor IIA genotype and susceptibility to P. aeruginosa infection in patients with cystic fibrosis. Eur J Hum Genet 2005;13:96-101.
3. Celedon JC, Lange C, Raby BA, et al. The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). Hum Mol Genet 2004;13:1649-56.

THE AUTHORS REPLY: Rossi and Rossi call attention to two genetic variants associated with an increased risk of colonization by *P. aeruginosa* in cystic fibrosis. In a study of 98 adults with mixed *CFTR* genotypes, Aron et al.[1] reported that carriers of HLA-DR7 had a slight increase in colonization (100 percent, vs. 83 percent for noncarriers), but there was no difference in the age at which colonization occurred or in pulmonary function. HLA typing of our 808 patients who are homozygous for ΔF508 is ongoing. Last year, a study by De Rose et al.[2] reported that FcγRIIA variants were not associated with colonization by *P. aeruginosa* among 167 patients with mixed *CFTR* genotypes, but colonization was more prevalent among the 47 patients who were homozygous for the ΔF508 mutation who carried the R variant than among the 22 noncarriers when the results were analyzed by logistic regression ($P=0.04$). It will be interesting to see whether this variant is associated with the severity of lung disease in our larger population of ΔF508 homozygotes. A very small percentage of patients with cystic fibrosis have an appreciable smoking history, so this factor was not a potential confounder in our study.

Stanke (née Mekus) and colleagues[3] did not replicate the association we found of *TGFβ1* –509 and codon 10 variants with the severity of lung disease when they tested these two SNPs in sibling pairs with extreme clinical phenotypes. Since we obtained a robust association ($P=6\times10^{-8}$) with severity status for the *TGFβ1* codon 10 CC genotype in our total sample of 1306 subjects (808 in the initial study and 498 in the replication study), perhaps their study is underpowered. The study of patients with extreme clinical phenotypes cited by Stanke and colleagues involved a sample of 21 highly concordant sibling pairs (10 with severe disease and 11 with mild disease) and 13 discordant sibling pairs. In contrast to their negative findings with respect to *TGFβ1*, Stanke and colleagues report substantial transmission disequilibrium in their discordant sibling pairs as assessed by microsatellite testing, but the multiple testing they performed probably weakens this evidence. Even considering that only the eight loci reported in their letter were examined, the possibility of separate hypothesis tests for the concordant sibling pairs with mild or severe disease indicates that the most conservative Bonferroni method would generate an adjusted $P=0.07$. We look forward to examining studies reporting these new findings on chromosome 19q in the peer-reviewed literature, since the possibility of additional disease-severity loci or transacting modulators should be vigorously pursued.

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2. De Rose V, Arduino C, Cappello N, et al. Fcgamma receptor IIA genotype and susceptibility to P. aeruginosa infection in patients with cystic fibrosis. Eur J Hum Genet 2005;13:96-101.
3. Mekus F, Laabs U, Veeze H, Tummler B. Genes in the vicinity of CFTR modulate the cystic fibrosis phenotype in highly concordant or discordant F508del homozygous sib pairs. Hum Genet 2003;112:1-11.

Antibacterial Prophylaxis in Patients with Cancer and Neutropenia

TO THE EDITOR: The recent studies by Bucaneve et al.[1] and Cullen et al.[2] (Sept. 8 issue) suggest that many patients with cancer and neutropenia may benefit from levofloxacin prophylaxis. Although the reports were published in tandem, their clinical implications differ fundamentally. Bucaneve et al. targeted "high-risk" patients (stem-cell transplant recipients or patients with leukemia) with anticipated neutropenia lasting more than seven days, a previously accepted approach.[3]

In contrast, most patients studied by Cullen et al. had solid tumors. Because patients with such tumors are unlikely to have prolonged or profound neutropenia, they are at lower risk for infectious complications.[4] Although the authors do not report the average duration of neutropenia in the study patients, the outcomes for the 784 placebo recipients demonstrates their clinical stability. During neutropenia, 80 percent never had fever, and rates of severe infection or death were not significantly different between the two groups. Most levofloxacin recipients were therefore "treated" for problems that did not occur. These data do not support routine prophylaxis among "low-risk" patients, given drug costs and the hazards of antimicrobial resistance. Future efforts should define populations that will benefit from fluoroquinolone prophylaxis.

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1. Bucaneve G, Micozzi A, Menichetti F, et al. Levofloxacin to prevent bacterial infection in patients with cancer and neutropenia. N Engl J Med 2005;353:977-87.
2. Cullen M, Steven N, Billingham L, et al. Antibacterial prophylaxis after chemotherapy for solid tumors and lymphomas. N Engl J Med 2005;353:988-98.
3. Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. Ann Intern Med 2005;142:979-95.
4. Hughes WT, Armstrong D, Bodey GP, et al. 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis 2002;34:730-51.

TO THE EDITOR: Cullen et al. demonstrated that levofloxacin prophylaxis significantly reduced rates of neutropenic fever and hospitalization in patients with solid tumors or lymphoma who were receiving chemotherapy. Prophylaxis for 100 patients during each cycle of chemotherapy would be expected to prevent approximately six hospitalizations, without a significant effect on survival or the frequency of severe infections.

Quinolone-based regimens are the only orally administered, evidence-based option for outpatient management of neutropenic fever in lower-risk adults (adults at low risk for serious complications).[1] The lack of an alternative oral antibiotic that is active against *Pseudomonas aeruginosa* and the concern about a breakthrough infection in patients receiving quinolone prophylaxis are factors that might prompt physicians to use an intravenous antibiotic regimen and to have a lower threshold for hospitalization as initial management of neutropenic fever. In addition, concern about the emergence of resistant pathogens associated with the widespread use of quinolone prophylaxis in lower-risk patients with cancer cannot be overstated. Given the options of early use of a quinolone for many patients (prophylaxis) and later, targeted use (empirical therapy for neutropenic fever), later may be better.

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1. Freifeld AG, Brown AE, Elting L, et al. National Comprehensive Cancer Network practice guidelines in oncology: fever and neutropenia. (Accessed December 14, 2005, at http://www.nccn.org/physician_gls/f_guidelines.html.)

TO THE EDITOR: Cullen et al. reported that antibiotic prophylaxis with levofloxacin reduces the incidence of fever, probable infection, and hospitalization among patients whose solid tumors and lymphomas have been treated with standard-dose chemotherapy.

The study population included patients affected mainly by solid tumors; only 13 percent of patients had lymphoma. However, in our opinion, the number of patients with lymphoma is too limited to permit firm conclusions to be drawn regarding the benefit of levofloxacin prophylaxis in this subgroup. Furthermore, in the study by Cullen et al., patients who underwent randomization should be considered to have been at low risk for serious infective complications, according to the Multinational Association for Supportive Care in Cancer (MASCC) risk index,[1] and would have been expected to have a short duration of neutropenia. Consequently, these patients could have been safely managed as outpatients with oral quinolone,[2] reducing costs as well as the associated biologic consequences.[3]

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1. Klastersky J, Paesmans M, Rubenstein EB, et al. The Multinational Association for Supportive Care in Cancer risk index: a multinational scoring system for identifying low-risk febrile neutropenic cancer patients. J Clin Oncol 2000;18:3038-51.
2. Malik IA, Khan WA, Karim M, Aziz Z, Khan MA. Feasibility of outpatient management of fever in cancer patients with low-risk neutropenia: results of a prospective randomized trial. Am J Med 1995;98:224-31.
3. Baden LR. Prophylactic antimicrobial agents and the importance of fitness. N Engl J Med 2005;353:1052-4.

TO THE EDITOR: Fever was the main outcome analyzed in the two reports published in the September 8 issue.

Prophylaxis treatment given to between two and five patients prevented fever in one patient, with no effect on mortality or the severity of infection. This is a poor clinical outcome, and the problems associated with this strategy can be deleterious. Increasing resistance rates have been observed with increasing use of fluoroquinolones, a trend that was also shown by the authors. In addition, it is well known that prophylaxis might impair the isolation of organisms in culture, a problem common to both studies. At least three meta-analyses have shown a reduction in gram-negative bacteremia with quinolone-based regimens, but they have also shown that the reduction does not extend to infection-related mortality.[1-3] We agree with Cullen et al. that their results alone "cannot be used to determine whether a policy of antibacterial prophylaxis should be applied systematically." The emergence of multidrug-resistant organisms is a serious danger that no longer belongs to the future.

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1. Engels EA, Lau J, Barza M. Efficacy of quinolone prophylaxis in neutropenic cancer patients: a meta-analysis. J Clin Oncol 1998;16:1179-87.
2. van de Wetering MD, de Witte MA, Kremer LC, Offringa M, Scholten RJ, Caron HN. Efficacy of oral prophylactic antibiotics in neutropenic afebrile oncology patients: a systematic review of randomised controlled trials. Eur J Cancer 2005;41:1372-82.
3. Cruciani M, Rampazzo R, Malena M, et al. Prophylaxis with fluoroquinolones for bacterial infections in neutropenic patients: a meta-analysis. Clin Infect Dis 1996;23:795-805.

TO THE EDITOR: The prophetic editorial by Dr. Baden[1] warns of the emergence of resistant organisms with the use of levofloxacin prophylaxis in patients with cancer who have neutropenia. Although no study has shown a survival benefit, we began using levofloxacin prophylaxis in similar patients in 1998. These patients included those receiving treatment for hematologic cancers and solid tumors and those undergoing hematopoietic-cell transplantation. Initially, we observed a decrease in bloodstream infection. However, in August 2004, the frequency of bloodstream infection started to increase along with a concurrent increase in levofloxacin resistance among blood isolates (to 61 percent). In April 2005, several interventions were implemented, including the discontinuation of levofloxacin prophylaxis. Subsequently, we have observed a decrease in bloodstream infection to levels seen before the increase (Fig. 1). Levofloxacin resistance among blood isolates has also declined and is approaching the baseline rate (47 percent). Since it has been only eight months, we will continue to monitor the number and severity of cases of bloodstream

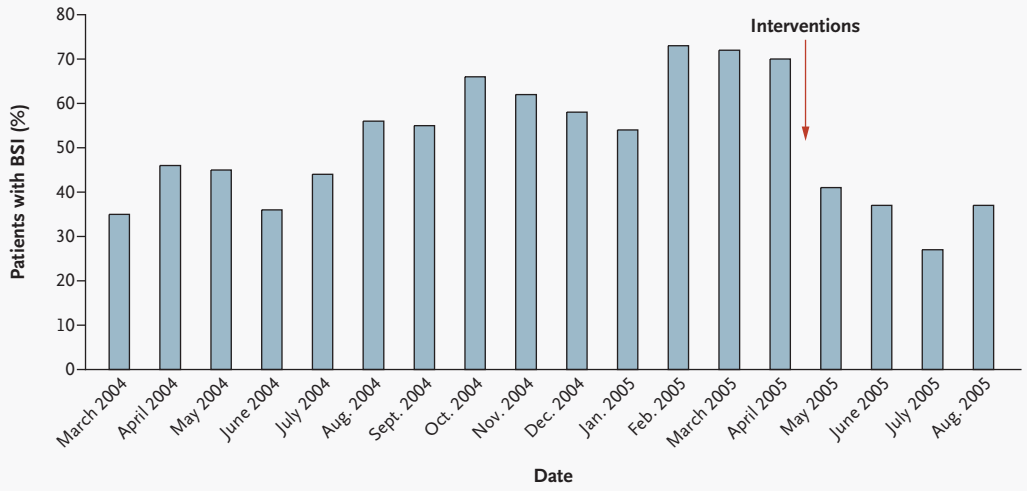

Figure 1. Patients with Bloodstream Infection (BSI).

infection. Although we cannot be certain, this may well be the very scenario that Dr. Baden feared.

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TO THE EDITOR: In their report on a randomized trial of prophylactic levofloxacin after chemotherapy, Cullen and coworkers report infection-related deaths. Overall mortality is not presented, in contrast to the companion article by Bucaneve et al. It would be helpful to know the rates for the Cullen et al. study, too.

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DR. BUCANEVE AND COLLEAGUES REPLY: Dr. Pasqualotto and colleagues raise three problems in the interpretation of the results of our study. The first point is that the occurrence of fever is considered a "poor" outcome to use in evaluating the efficacy of prophylaxis. We disagree. The occurrence of fever in patients with cancer who have neutropenia is a clinically relevant and frequent finding, and it is mandatory to treat these patients empirically to prevent early deaths, even in the absence of clinical signs or symptoms or microbiologic isolates documenting infection.[1]

The second problem mentioned is that our study was unable to document a significant effect of prophylaxis in reducing mortality. Our trial did not have enough power to show an effect of prophylaxis on mortality. However, a significant survival advantage from prophylaxis has been shown in a recently published meta-analysis.[2]

Finally, Pasqualotto et al. state that the emergence of multidrug-resistant organisms could be a serious and dangerous result of prophylaxis. The fear of emerging resistance is not a reason to avoid the prophylactic use of fluoroquinolones in high-risk patients with neutropenia. In fact, there is some evidence that resistance to fluoroquinolones is a multiclonal and reversible phenomenon[3,4] and does not adversely affect infection-related morbidity and mortality. Moreover,

the selective pressure exerted by fluoroquinolones may be due mainly to their wide use in the community.

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1. Reuter S, Kern WV, Sigge A, et al. Impact of fluoroquinolone prophylaxis on reduced infection-related mortality among patients with neutropenia and hematologic malignancies. Clin Infect Dis 2005;40:1087-93.
2. Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. Ann Intern Med 2005;142:979-95.
3. Hughes WT, Armstrong D, Bodey GP, et al. 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis 2002;34:730-51.
4. Tascini C, Menichetti F, Bozza S, et al. Molecular typing of fluoroquinolone-resistant and fluoroquinolone-susceptible Escherichia coli isolated from blood of neutropenic cancer patients in a single center. Clin Microbiol Infect 1999;5:457-61.

DR. CULLEN AND COLLEAGUES REPLY: The correspondents repeat concerns about antimicrobial resistance that we have expressed. Previous work has concentrated primarily on inpatients with hematologic cancers, and we set out to test the efficacy of prophylactic antibiotics in outpatients with solid cancers and lymphomas. We demonstrated significant reductions in febrile episodes and in hospitalizations for the treatment of infections. To translate these findings into recommendations, more information will be required on mortality, high-risk groups, adverse consequences of prophylaxis, and adverse consequences of neutropenic fever.

As mentioned, we did not report mortality from all causes during the study period. This was lower in the levofloxacin group but not significantly so. Our numbers can be combined with those published in the large, recent meta-analysis that did show a significant reduction in mortality from all causes with fluoroquinolone prophylaxis.[1]

It is important not to confuse the risk of neutropenic fever with the risk of serious consequences, according to MASCC criteria — which can only be applied strictly once infection has occurred. In response to the editorial and the correspondents, we are now examining our data for pretreatment patient characteristics, disease type, and cycle number to identify those at high risk for neutropenic fever, with the goal of increasing the effect and efficiency of prophylaxis in future studies. Physicians might prefer to avoid prophylaxis in those at low risk and to treat uncomplicated neutropenic fever with oral fluoroquinolones, although the safety of this strategy in an outpatient context has yet to be shown.

Antimicrobial resistance as a result of prophylaxis is a potential problem, but the clinical impact is not easily predictable. For instance, a recent study has shown that resistance resulting from prophylaxis does not necessarily reduce the long-term prophylactic efficacy of the antibacterial agent within an inpatient, hematologic-cancer unit.[2]

Prevention of death and the sepsis syndrome are not the only reasons for preventing neutropenic fever. Delays and dose reductions in subsequent cycles of chemotherapy may have serious long-term consequences for patients with cancer.

At least in part, resistance to the concept of routine antibacterial prophylaxis after chemotherapy assumes an acceptance of the ethical propriety of depriving current patients of a proven therapy in order to benefit future patients. This requires open debate based on clear evidence, which should result from more work in the areas discussed above.

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1. Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. Ann Intern Med 2005;142:979-95.
2. Kern WV, Klose K, Jellen-Ritter AS, et al. Fluoroquinolone resistance of Escherichia coli at a cancer center: epidemiologic evolution and effects of discontinuing prophylactic fluoroquinolone use in neutropenic patients with leukemia. Eur J Clin Microbiol Infect Dis 2005;24:111-8.

DR. BADEN REPLIES: Studies of novel antimicrobial prophylactic strategies are, by design, limited in their ability to detect the effects on the emergence of antimicrobial resistance. This limitation is due, in part, to the relatively short study duration and the delayed kinetics of this untoward

consequence. Further challenges include both limited funding for this type of research and reporting biases, as these types of observations are less novel and exciting than the results of the initial prophylactic study. The challenge in natural experiments involving actual clinical practice is to quantify precisely the effect of increased antimicrobial use on the emergence of resistance in the setting of complex medical care. I encourage Dr. Ito and colleagues to study in detail what has occurred at their institution, for an improved understanding of the risk-to-benefit ratio.

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Drotrecogin Alfa (Activated) in Severe Sepsis

TO THE EDITOR: In the article by Abraham et al. (Sept. 29 issue),[1] the ADDRESS (Administration of Drotrecogin Alfa [Activated] in Early Stage Severe Sepsis) trial showed no benefit of drotrecogin alfa (activated) (DrotAA) in patients with severe sepsis and a low risk of death (defined by single-organ failure or an Acute Physiology and Chronic Health Evaluation [APACHE II] score <25).[2] In contrast to the prematurely terminated PROWESS (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis) trial,[3] the ADDRESS study showed higher mortality in its subgroup of patients treated with DrotAA who had an APACHE II score of 25 or more. When the subgroups of the two trials that had an APACHE II score of 25 or more were combined with the use of standard meta-analysis software (Review Manager, version 4.2) and a random-effects model, there was substantial heterogeneity between the two results ($P=0.01$; $I^2=84$ percent, where I denotes quantification of the degree of heterogeneity), and no significant benefit in 28-day mortality was shown (relative risk, 0.90; 95 percent confidence interval, 0.54 to 1.49) (Fig. 1). Likewise, combining subgroups that had multiple-organ failure did not show a major benefit. The previous discrepant results in trials of treatment for sepsis that used early-stopping rules[4] or retro-

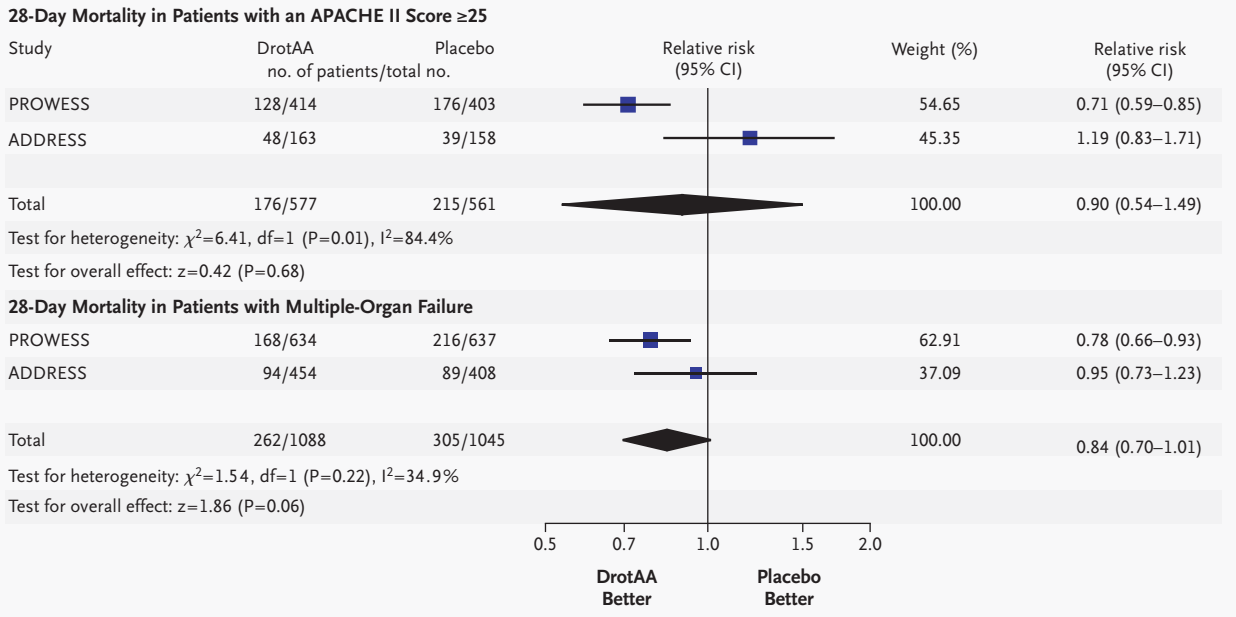

Figure 1. Effects of DrotAA on Combined 28-Day Mortality among Patients with Severe Sepsis in the PROWESS and ADDRESS Trials.

CI denotes confidence interval. A random-effects model was used to pool the relative risks. The size of each square is proportional to the relative weighting of the study's relative risk in the calculation of the overall pooled relative risk. The center of each diamond indicates the pooled relative risk, and the width of the diamonds reflects the size of the 95 percent confidence interval.

spective subgroups[5] and had apparently even higher risks of bleeding events, including intracranial hemorrhage (e.g., in the open-label DrotAA study Extended Evaluation of Recombinant Human Activated Protein C [ENHANCE][6]), suggest that another study in which a high risk of death is prospectively defined is urgently needed to determine whether DrotAA is beneficial even in this subgroup.

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1. Abraham E, Laterre P-F, Garg R, et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. N Engl J Med 2005;353:1332-41.
2. Siegel JP. Assessing the use of activated protein C in the treatment of severe sepsis. N Engl J Med 2002;347:1030-4.
3. Bernard GR, Vincent J-L, Laterre P-F, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001;344:699-709.
4. Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. JAMA 2003;290:238-47.
5. Angus DC, Birmingham MC, Balk RA, et al. E5 murine monoclonal antiendotoxin antibody in gram-negative sepsis: a randomized controlled trial. JAMA 2000;283:1723-30.
6. Vincent J-L, Bernard GR, Beale R, et al. Drotrecogin alfa (activated) treatment in severe sepsis from the global open-label trial ENHANCE: further evidence for survival and safety and implications for early treatment. Crit Care Med 2005;33:2266-77.

TO THE EDITOR: The lack of an observed treatment benefit with DrotAA in the subgroup of patients with APACHE II scores of 25 or more in the ADDRESS trial is worrisome, given that the agent is approved in the United States for this population. The authors attempted to explain this finding on the basis of statistical considerations, but questions remain. For this subgroup, baseline characteristics including each component of the APACHE II score, the site and type of infection, and types of organ dysfunction should be described according to treatment group to see if an imbalance existed. Similarly, the percentage of patients in each treatment group who received inadequate antibiotic therapy, had severe coexisting disorders, or were moribund should be presented.

Finally, a breakdown of the causes of death and types of adverse events, including bleeding events, in each treatment group may shed light on this result. If these investigations do not reveal additional potential explanations for the lack of observed efficacy in the subgroup of patients with high APACHE II scores, then a renewed call for a confirmatory, placebo-controlled trial of DrotAA is warranted.[1]

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1. Warren HS, Suffredini AF, Eichacker PQ, Munford RS. Risks and benefits of activated protein C treatment for severe sepsis. N Engl J Med 2002;347:1027-30.

TO THE EDITOR: The results of the ADDRESS trial of DrotAA in patients with severe sepsis are in startling contrast with those of the PROWESS trial of the same drug. Of particular interest is the high-risk group for whom the indication for DrotAA is currently widely accepted (APACHE II score ≥25). Within this group, there is an important discrepancy between the trials. The relative risk of death in the treatment group was 1.19 for the ADDRESS trial (95 percent confidence interval, 0.83 to 1.71) and 0.71 in the PROWESS trial (95 percent confidence interval, 0.59 to 0.84). The authors point out that the confidence intervals for the two studies overlap, and they imply that the results are therefore consistent with each other.

Our analysis of the data reveals that an alternative hypothesis is more likely. A z-test comparing the two relative risks shows that $P=0.01$, indicating a statistically significant difference between the relative risk observed in the high-risk subgroup in the ADDRESS trial and that observed in the high-risk subgroup in the PROWESS trial. We believe that these new data cast doubt on the evidence on which worldwide practice is currently based.

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THE AUTHORS REPLY: Drs. Baillie and Murray are correct that the relative risk (DrotAA vs. placebo) observed for patients with APACHE II scores of 25 or more in the ADDRESS trial is statistically different from that observed in the PROWESS trial. They are concerned that the outcomes observed are inconsistent between the trials. How-

ever, in neither trial was the APACHE II score used to stratify randomization. Therefore, subpopulations defined according to APACHE II scores cannot be assumed to be comparable. On the basis of PROWESS, DrotAA was approved for use in patients at high risk for death, but only after extensive analyses of important characteristics of the subgroups. In the ADDRESS trial, the subgroups defined by an APACHE II score of 25 or more differed statistically, in that more patients receiving DrotAA had multiple-organ dysfunction (46.7 percent vs. 31.4 percent, P=0.02) and respiratory dysfunction (64.2 percent vs. 50.3 percent, P=0.01) and more were 65 years of age or older (62.4 percent vs. 56.6 percent, P=0.02). Such imbalances in baseline characteristics limit the assessment of outcomes in this subpopulation.

Dr. Friedrich has similar concerns but also notes that combining the subgroups of patients with multiple-organ dysfunction in the ADDRESS and PROWESS trials does not indicate a major benefit of DrotAA (relative risk of death, 0.84; 95 percent confidence interval, 0.70 to 1.01). However, there seems to be an error in his estimate of the number of patients with multiple-organ dysfunction in the ADDRESS study. The number of patients with multiple-organ dysfunction included 455 who received DrotAA and 407 who received placebo, reflecting the higher number of patients with multiple-organ dysfunction who were randomly assigned to the active-treatment group. With the use of the correct data and a chi-square test, a combined analysis of all 2133 patients with multiple-organ dysfunction from both the PROWESS and ADDRESS trials yields a relative risk of 0.82 (P=0.007; 95 percent confidence interval, 0.71 to 0.95). Furthermore, sensitivity analysis with the use of logistic models to investigate a potential study effect reveals no significant interaction between the study and the assigned treatment, and the treatment-effect estimate (P=0.01) was unaltered.

Dr. LaRosa is correct to inquire about the baseline characteristics of the subgroups of patients in the ADDRESS trial with an APACHE II score of 25 or more. Imbalances in baseline characteristics coupled with the small sample size limit the interpretation of outcomes in this subgroup of patients.

The ADDRESS trial was designed to enroll patients who had severe sepsis and a low risk of death and for whom DrotAA was not indicated under the approved label applicable to the investigative site. The study was discontinued for reasons of futility, limiting any comparison between subpopulations in the ADDRESS trial and the high-risk populations in the PROWESS trial.

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γ-Hydroxybutyric Acid in Hair

TO THE EDITOR: The review (June 30 issue)[1] on γ-hydroxybutyric acid (GHB) generated correspondence (Oct. 13 issue),[2] which revealed that biochemical genetics laboratories can detect GHB.[2] There is an additional detection method worth noting.

When a medicolegal issue is present (e.g., a drug-facilitated crime), finding a hard-to-detect drug can be important.[3] GHB has amnesic properties[3] and is fully and rapidly metabolized to carbon dioxide and water. Even succinic acid, a product of GHB metabolism, becomes undetectable in urine within hours of ingestion.

However, the GHB in the body of a crime victim is still present, even after it has been totally removed from the circulation: GHB, like other substances, accumulates in hair, after a single exposure.[4] If the hair shaft is negative for GHB, the drug may still be detected in the root bulb, at hair concentrations measured in nanograms per milligram.[5]

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1. Snead OC III, Gibson KM. γ-Hydroxybutyric acid. N Engl J Med 2005;352:2721-32.
2. Sass JO, Superti-Furga A. γ-Hydroxybutyric acid. N Engl J Med 2005;353:1632-3.
3. McGinn CG. Close calls with club drugs. N Engl J Med 2005;352:2671-2.
4. Kintz P, Villain M, Ludes B. Testing for the undetectable in drug-facilitated sexual assault using hair analyzed by tandem mass spectrometry as evidence. Ther Drug Monit 2004;26:211-4.
5. Kalasinsky KS, Dixon MM, Schmunk GA, Kish SJ. Blood, brain, and hair GHB concentrations following fatal ingestion. J Forensic Sci 2001;46:728-30.

Ketoacidosis during a Low-Carbohydrate Diet

TO THE EDITOR: It is believed that low-carbohydrate diets work best in reducing weight when producing ketosis.[1] We report on a 51-year-old white woman who does not have diabetes but had ketoacidosis while consuming a "no-carbohydrate" diet. There was no family history of diabetes, and she was not currently taking any medications. While adhering to a regimen of carbohydrate restriction, she reached a stable weight of 59.1 kg, a decrease from 72.7 kg. After several months of stable weight, she was admitted to the hospital four times with vomiting but without abdominal pain. On each occasion, she reported no alcohol use. Her body-mass index (the weight in kilograms divided by the square of the height in meters) was 26.7 before the weight loss and 21.7 afterward. Laboratory evaluation showed anion-gap acidosis, ketonuria, and elevated plasma glucose concentrations on three of the four occasions (Table 1). She had normal concentrations of plasma lactate and glycosylated hemoglobin. Screening for drugs, including ethyl alcohol and ethylene glycol, was negative. Abdominal ultrasonography showed hepatic steatosis.

On each occasion, the patient recovered after administration of intravenous fluids and insulin, was prescribed insulin injections on discharge, and gradually reduced the use of insulin and then discontinued it while remaining euglycemic for six months or more between episodes. Testing for antibodies against glutamic acid decarboxylase and antinuclear antibodies was negative. Values on lipid studies were as follows: serum triglycerides, 102 mg per deciliter; high-density lipoprotein (HDL) cholesterol, 50 mg per deciliter; and calculated low-density lipoprotein (LDL) cholesterol, 189 mg per deciliter.

The patient strictly adhered to a low-carbohydrate diet for four years, with an estimated carbohydrate intake that was often less than 20 g per day. When she was put on a diet containing normal amounts of carbohydrates, her fasting plasma glucose concentration and the results of oral glucose-tolerance tests were normal. With a normal carbohydrate intake, she had no more episodes of ketoacidosis.

Citrate generated by glycolysis inhibits carnitine palmitoyltransferase complex I, limiting the beta-oxidation of fatty acids and thereby reducing ketogenesis. Lactate, which increases during starvation, can induce hepatic ketogenesis.[2] Low-carbohydrate, fat-rich meals can enhance alpha-cell secretion of glucagon and lower insulin concentrations.[3,4] Plasma fatty acid concentrations can be twice as high during low-carbohydrate diets as compared with the usual carbohydrate intake in the postabsorptive period.[5] Increased concentrations of free fatty acids in the absence of carbohydrate-induced inhibition of beta-oxidation of fatty acids and in the presence of an abnormally high ratio of glucagon to insulin and elevated concentrations of lactate may have caused ketoacidosis in our patient, who was trying to avoid all dietary carbohydrates. Low-carbohydrate, high-fat diets are generally associated with higher

Table 1. Laboratory Values during Four Episodes of Ketoacidosis.

Variable	Date			
	June 1999	May 2000	June 2002	Dec. 2002
Anion gap	33	26	35	31
pH	7.2	7.1	6.9	7.1
Urine ketones	Large	Large	Large	Large
Plasma glucose (mg/dl)	265	103	219	275
Glycosylated hemoglobin (%)	5.4	5.4	5.4	5.4
Lactate (mmol/liter)	—	0.8	0.8	1.2

concentrations of LDL and HDL cholesterol and lower serum concentrations of triglycerides than is the conventional intake of carbohydrates and fat.[1]

Benign dietary ketosis resulting from restricting carbohydrates could, theoretically, cause ketoacidosis in persons with a predisposition to the condition. Carbohydrate-restricted, high-fat diets may have adverse metabolic sequelae when followed for protracted periods.

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1. Bravata DM, Sanders L, Huang J, et al. Efficacy and safety of low-carbohydrate diets: a systematic review. JAMA 2003;289:1837-50.
2. Exton JH, Corbin JG, Harper SC. Control of gluconeogenesis in liver. V. Effects of fasting, diabetes, and glucagon on lactate and endogenous metabolism in the perfused rat liver. J Biol Chem 1972;247:4996-5003.
3. Gutniak M, Grill V, Efendic S. Effect of composition of mixed meals — low- versus high-carbohydrate content — on insulin, glucagon, and somatostatin release in healthy humans and in patients with NIDDM. Diabetes Care 1986;9:244-9.
4. Fukita Y, Gotto AM, Unger RH. Basal and postprotein insulin and glucagon levels during a high and low carbohydrate intake and their relationships to plasma triglycerides. Diabetes 1975;24:552-8.
5. Bisschop PH, De Sain-Van Der Velden MG, Stellaard F, et al. Dietary carbohydrate deprivation increases 24-hour nitrogen excretion without affecting postabsorptive hepatic or whole body protein metabolism in healthy men. J Clin Endocrinol Metab 2003;88:3801-5.

INSTRUCTIONS FOR LETTERS TO THE EDITOR

Letters to the Editor are considered for publication, subject to editing and abridgment, provided they do not contain material that has been submitted or published elsewhere. Please note the following: •Letters in reference to a *Journal* article must not exceed 175 words (excluding references) and must be received within three weeks after publication of the article. Letters not related to a *Journal* article must not exceed 400 words. All letters must be submitted over the Internet at http://authors.nejm.org. •A letter can have no more than five references and one figure or table. •A letter can be signed by no more than three authors. •Financial associations or other possible conflicts of interest must be disclosed. (Such disclosures will be published with the letters. For authors of *Journal* articles who are responding to letters, this information appears in the original articles.) •Include your full mailing address, telephone number, fax number, and e-mail address with your letter.

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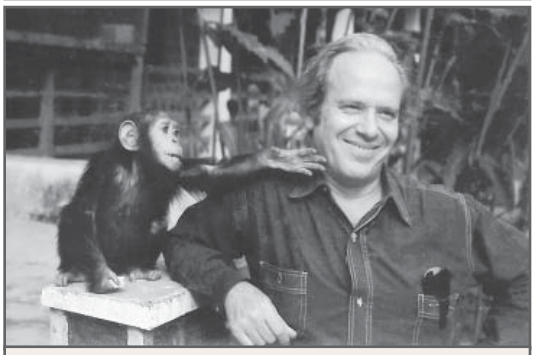
BOOK REVIEWS

A GENETIC AND CULTURAL ODYSSEY: THE LIFE AND WORK OF L. LUCA CAVALLI-SFORZA

By Linda Stone and Paul F. Lurquin. 227 pp., illustrated. New York, Columbia University Press, 2005. $47.50. ISBN 0-231-13396-0.



Courtesy of Linda Stone and Paul F. Lurquin/ Columbia University Press.

L. Luca Cavalli-Sforza in Pygmy Territory in the Central African Republic in the Late 1960s.

LUIGI LUCA CAVALLI-SFORZA IS ONE OF THE most influential population geneticists of our time. Now in his 80s, he is a principal investigator and professor emeritus in the Department of Genetics at Stanford University School of Medicine. I first encountered Cavalli-Sforza's name in 1992, when my Ph.D. thesis adviser, Dr. Iury Rychkov, well known in the field of anthropogenetics, showed me an issue of the journal *Gene Geography*, published by the University of Pavia, in Italy. The University of Pavia is Cavalli-Sforza's alma mater, where he trained as a physician and conducted his first experiments in measuring the virulence of anthrax and pneumonia-causing bacteria.

After spending some time in clinical practice at the end of World War II, Cavalli-Sforza decided to continue in basic research and turned to bacterial genetics. This was the beginning of his extraordinary scientific journey, as reflected in *A Genetic and Cultural Odyssey*, written by Linda Stone, an anthropologist, and Paul F. Lurquin, a geneticist. The book is an intelligent and serious scientific biography whose subject, according to Stone and Lurquin, "conveys a spirit of adventure coupled with a search for knowledge," which is the same reason, they say, that Cavalli-Sforza likes the tale of Ulysses's (Odysseus's) last adventure in Dante's *Divine Comedy* — and thus the word "odyssey" in the title of the book.

Cavalli-Sforza's early research in bacterial genetics had important implications for the understanding of bacterial conjugation and sexual reproduction. Unfortunately, this is not widely known, and like most geneticists, I was not aware of this work. The authors describe well this research period, which became a very important platform for future endeavors.

Cavalli-Sforza spent a few years in Cambridge, England, with Sir Ronald Fisher, a brilliant British statistician, and collaborated with Joshua Lederberg, who won the Nobel Prize in Physiology or Medicine in 1958. After Cambridge, Cavalli-Sforza returned to Italy and taught at the University of Parma, where he gradually developed a strong interest in human genetics, taking advantage of the parish registers of the Parma Valley. This shift from bacteria to humans was the turning point of his scientific career; his contributions to human population genetics have brought him international recognition.

Cavalli-Sforza studied the factors that make the frequencies of the human blood groups ABO, MN, and Rhesus so different from one population to another (e.g., from the Alaskan Eskimos to the Australian aborigines). He was among the first to suggest that studying many human populations would help us trace the origin of modern humans and learn how humans have populated the earth. Cavalli-Sforza's work on the Y chromosome supports the "out of Africa" theory of modern human origins. According to this theory, a small population of "Adam" and a population of "Eve" originated in East Africa about 100,000 years ago and then spread out of Africa to the rest of the world.

Stone and Lurquin were struck by "the way in which Cavalli-Sforza's work intersects science with society, a venture attempted by very few." His theories of prehistoric migrations of humans and the coevolution of genes, languages, and culture are exciting. Although by themselves cultural evo-

lution and transmission are purely theoretical and controversial, they do reflect the breadth of Cavalli-Sforza's research and its far-reaching implications. Cavalli-Sforza views cultural development as something similar to human biologic evolution; like genes, cultural traits are transmitted from generation to generation and migrate with people when they move from place to place.

A Genetic and Cultural Odyssey is a wonderful journey through the life to date of a giant who is nearly glued to science. Detailed descriptions of genetic methods and data are repeated in some chapters. One can take a detour around these paragraphs while not losing the excitement of the journey.

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GENETICS OF DEVELOPMENTAL DISABILITIES

(Pediatric Habilitation. Vol. 13.) Edited by Merlin G. Butler and F. John Meaney. 886 pp., illustrated. Boca Raton, Fla., Taylor & Francis, 2005. $199.95. ISBN 0-8247-5813-7.

The approach to a child with a developmental disability is frequently challenging for professionals and family members alike. *Genetics of Developmental Disabilities* does much to give the reader the practical information necessary to address this task. The editors have enlisted a strong group of contributors, many of whom have provided seminal work on the subject (Ann C.M. Smith on the Smith–Magenis syndrome, Charles J. Epstein on mouse models of Down's syndrome, John L. Hamerton on sex-chromosome abnormalities, Randi Hagerman on the fragile X syndrome, and Robert J. Shprintzen on the velocardiofacial syndrome, to name only a few).

The 25 chapters in the book are grouped into three sections. The first section consists of reviews of broad topics, including an excellent chapter on the history of mental retardation by Neri and Tiziano. A longer section on specific syndromes characterized by mental retardation follows. The final section takes a broader approach to the epidemiology of developmental disabilities such as attention deficit–hyperactivity disorder and even contains a chapter on alternative therapies.

The editors have targeted a wide audience, including medical geneticists, students, and health care professionals involved in the care of children with developmental disabilities. Overall, the book hits the mark by offering a great deal of information in a relatively short book. The chapter on the history of mental retardation is filled with facts that have gone unnoticed or been forgotten. How many families can boast of a pedigree that has produced both Sir Francis Galton, an early pioneer in the study of human heredity, and Charles Darwin? More pertinent to the subject is that two members of this pedigree had mental retardation, along with a relative with a seizure disorder and another with psychiatric disease. The chapters on specific conditions are generally well done, covering prevalent disorders that are frequent sources of questions from treating therapists.

Microdeletion, or contiguous gene syndromes, are now known to account for many common disorders producing developmental disabilities, and chapters in this book give specific attention to the Prader–Willi syndrome, Angelman's syndrome, the Williams syndrome, and the velocardiofacial syndrome. Each chapter covers the condition comprehensively and gives practical guidance. Yet in some respects, this combination is potentially among both the book's strengths and its weaknesses. The book is not necessarily useful as a quick reference source, given the depth of information presented. At the same time, readers looking for a comprehensive but rather brief chapter on a topic will find their needs met. Illustrations and tables are useful adjuncts to the textual information.

As I read the book, I looked forward with interest to the last section, although I equally enjoyed the first, with its historical focus. The effect of developmental disabilities on society as a whole is relatively profound, given current prevalence figures. The authors of the chapter on epidemiology note that 17 percent of children in the United States have been reported to have a disability, with 2 percent of school-age children having a serious developmental disability. Although one could quote a number of conflicting studies, the lifetime medical and nonmedical costs of this segment of the population, given these percentages, are staggering.

Increasing our understanding of the genetic basis of these developmental disabilities and of

the distant potential for effective treatment strategies is likely to have an equally large economic effect. The chapters on attention deficit–hyperactivity disorder and cerebral palsy reflect that we are still at an early point in the identification of genes that produce these conditions. With regard to the nonsyndromic child with an autism-spectrum disorder, learning disability, or mental retardation, we are still left with a small handful of clinical diagnostic tools that may fall short of answering questions such as how the condition occurs and what the risk of recurrence is within a family. This book, however, should provide the information that students, health care providers, and therapists need to address the questions they face on a daily basis.

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AN INTRODUCTION TO HUMAN MOLECULAR GENETICS: MECHANISMS OF INHERITED DISEASES

Second edition. By Jack J. Pasternak. 631 pp., illustrated. Hoboken, N.J., Wiley-Liss, 2005. $84.95. ISBN 0-471-47426-6.

The identification of the sequence of the human genome has unveiled the existence of 25,000 to 35,000 genes, but the functions of most of their products are still shrouded in mystery. Advances in genomics, proteomics, and the emerging field of lipidomics have raised hopes of having new ways of understanding the genetic basis of disease. Many intriguing findings about mendelian disease loci have surfaced over the past two decades, reinforcing the belief that simple mendelian inheritance is often not so simple. These recent developments are only a few of the reasons why the second edition of *An Introduction to Human Molecular Genetics* is an indispensable and timely textbook.

Discussion of the profound implications of the Human Genome Project is just one feature of this book. Like the previous edition, the book contains comprehensive discussions of mendelian inheritance, the molecular biology of the gene, and recombinant DNA technology and covers the genetic basis of mitochondrial diseases, muscle and neurologic disorders, ocular diseases, and cancer. The chapter on the mapping of the human genome is particularly thorough, up to date, and well written, which helps the reader crystallize intricate concepts.

This second edition has several new chapters. One, concerning bioinformatics, features the advent of genomics and proteomics and their contributions as new fields. Another engaging chapter emphasizes that about 75 percent of the inherited disorders that are evident at birth or soon thereafter, and most arising later in life, are multifactorial. This chapter offers insights into oligogenic and polygenic inheritance, as well as phenotypic variation within monogenic conditions — all of which are vivid testimony to the intricacy of genetic disorders. Other important additions to the second edition are the chapters on human population genetics and on genomic imprinting. The latter chapter illustrates how epigenetic processes can impart an additional level of complexity to regulatory systems.

The concluding chapter, another addition, concerns genetic testing and genetic counseling. It includes a discussion of the prevention of genetic discrimination by insurance providers and employers. In light of the plethora of information recently provided by the sequencing of the human genome, future editions of this book would benefit from a more comprehensive discussion of predictive genetic testing and its ethical, social, and legal ramifications. Genetic testing has profoundly affected society by increasing the gap between our ability to identify genetic risk and our ability to ameliorate it. Passionate discussions surround issues such as genetic testing in children, ownership of genetic material, and whether genetic information should be treated differently from routine medical information.

An Introduction to Human Molecular Genetics will be a useful resource for medical and dental students, as well as for advanced undergraduates and graduate students, research scientists, and physicians. The abundance of clearly presented information renders this textbook a chef d'œuvre.

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HUMAN EMBRYONIC STEM CELLS

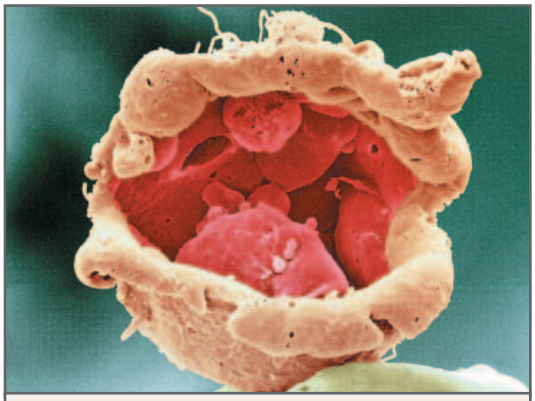
Edited by J. Odorico, S. Zhang, and R. Pedersen. 391 pp., illustrated. New York, Garland Science/BIOS Scientific, 2005. $165. ISBN 1-8599-6278-5.

THE SCIENTIFIC AND PUBLIC INTEREST IN stem-cell research has increased dramatically since human embryonic stem cells were first isolated in 1998. An increasing number of scientists and clinicians are joining stem-cell researchers to find ways of using knowledge of embryonic stem cells to understand the development of diseases and even to cure them. This book will be of tremendous help to those who have already started, or soon will start, a journey into the murky waters of stem-cell research.

The role of embryonic stem cells during development, the differentiation of these cells into derivatives of the three germ layers, the potential clinical and therapeutic uses of these cells, and the ethical and legal problems associated with their production and use are some of the topics that are summarized and discussed in an understandable and fluent way in this book. The editors have even included two solid and successful contributions about adult stem cells, a topic one would not immediately expect in a book titled *Human Embryonic Stem Cells.* These chapters will help readers to understand the potential and power of adult stem cells, as well as those of embryonic stem cells, and the ethical and legal issues associated with the production and use of both cell types.

Other authors focus on the differentiation of embryonic stem cells into hematopoietic, endothelial, and neural cells, as well as trophoblast, islet, and cardiomyocyte differentiation. Each of the chapters dealing with the various facets of embryonic stem-cell biology successfully reviews the basic aspects of the development of the organ system under discussion and introduces the role and importance of embryonic stem cells for these systems.

The chapters dealing with current and potential clinical and therapeutic applications of human embryonic stem cells will be of special interest to clinicians. The authors discuss recent progress and the promising results of therapeutic approaches employing these cells, including tissue engineering and somatic-cell nuclear transfer. Also reviewed are the requirements of and difficulties involved in therapy with embryonic stem cells.



Yorgos Nikas/Wellcome Photo Library.

Color-Enhanced Scanning Electron Micrograph of a Human Embryo at the Blastocyst Stage, Opened to Reveal the Inner Cell Mass.

A real strength of this book is that ethical, political, and legal aspects are discussed in detail, and the guidelines, regulations, and restrictions concerning embryonic stem-cell research are laid out systematically. This well-organized book is also an overview of the latest genomic and proteomic approaches and technological advances that can be used in stem-cell research, making it a useful compendium for basic scientists and clinicians working in the field.

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NOTICES

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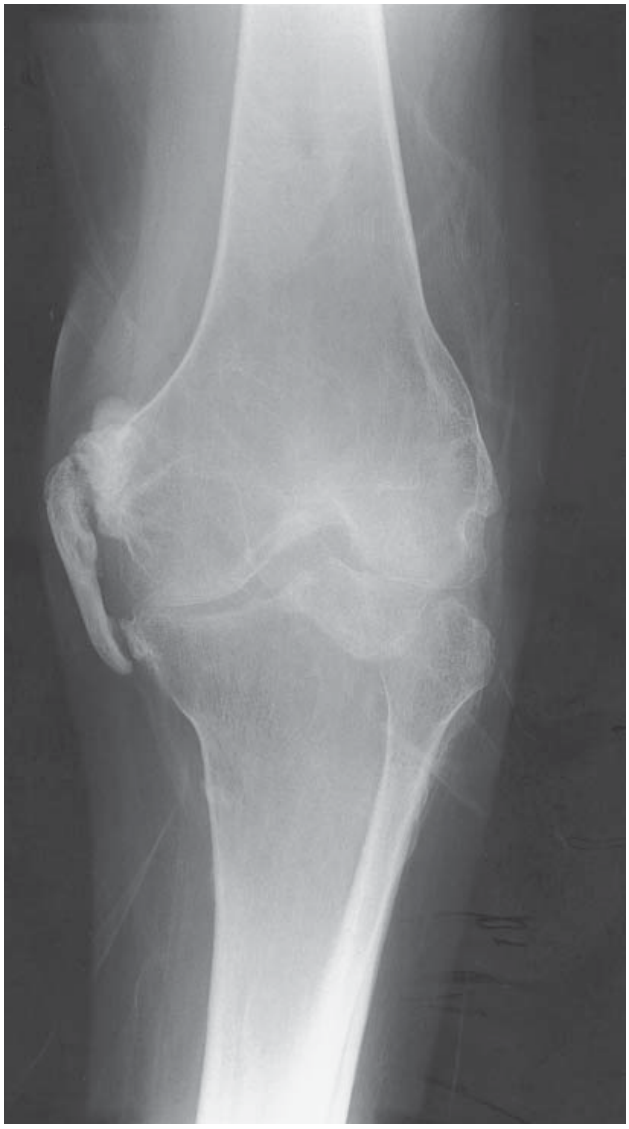
EUROPEAN SOCIETY OF CONTRACEPTION

The following meeting will be held: "9th Congress of the European Society of Contraception: Improving Life Quality through Contraception and Reproductive Health" (Istanbul, Turkey, May 3–6).

Contact ESC Central Office, c/o Orga-Med, Opalfeneweg 3, B-1740 Ternat, Belgium; or call (32) 2 582 08 52; or fax (32) 2 582 55 15; or e-mail esccentraloffice@contraception-esc.com; or see http://www.contraception-esc.com/.

IMAGES IN CLINICAL MEDICINE

Pellegrini–Stieda Syndrome



A 38-year-old man with an incomplete spinal cord injury secondary to a diving accident some years before, which caused tetraplegia but preserved some sensation below the neck, reported new pain in the left knee during the previous two months. He said there had been no recent trauma to the knee. An anteroposterior radiograph showed ossification corresponding to the medial collateral ligament, findings initially described by Pellegrini and Stieda in the early 1900s. Treatments for mild and moderate cases of the Pellegrini–Stieda syndrome include local corticosteroid injection and range-of-motion exercises. Surgical excision of calcifications and repair of the tear in the medial collateral ligament can be considered for refractory cases.

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