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Perspective

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Stem-Cell Research — Signposts and Roadblocks

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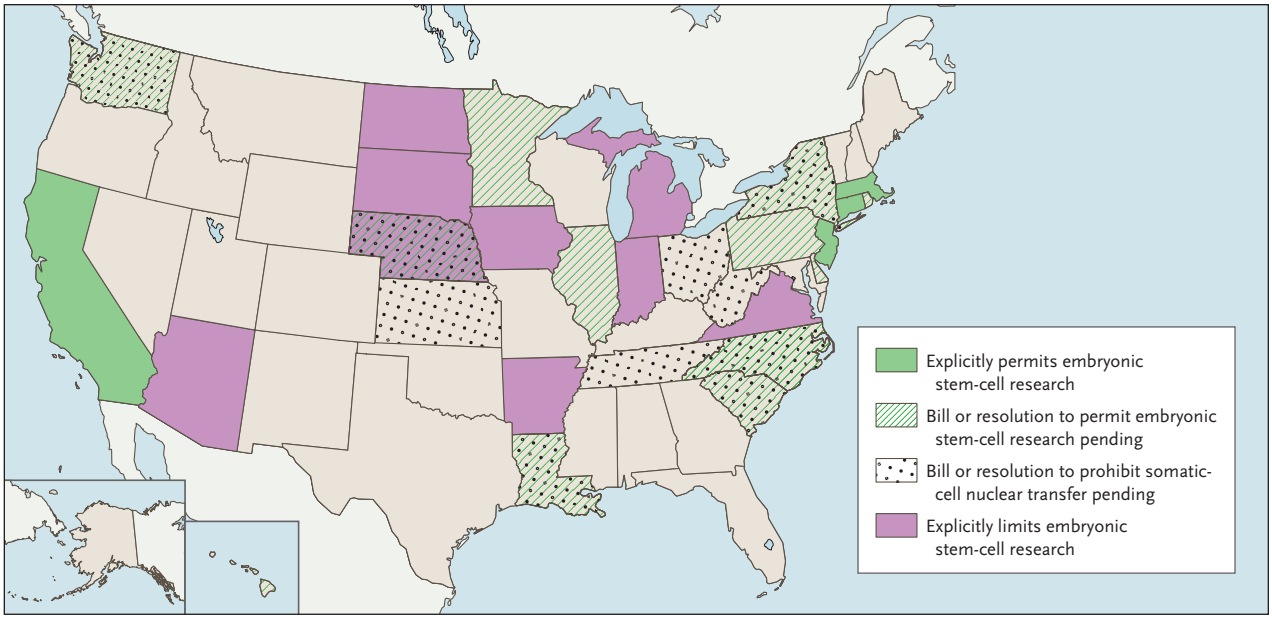
Embryonic stem-cell research, more than virtually any other scientific field, has been mired in political and ethical controversy. In recent weeks, however, important movement has occurred on ethical, scientific, and political fronts alike. First, at the end of April, the National Research Council and the Institute of Medicine (two branches of the National Academies, a nongovernmental scientific advisory body) issued new ethics guidelines for the conduct of such research, which were enthusiastically welcomed by scientists and policymakers.

Then, in mid-May, a Korean research team announced that they had derived lines of human embryonic stem cells carrying the genetic signatures of persons with disorders such as type 1 diabetes mellitus and spinal cord injury. This advance promises new opportunities for investigating the causes of disease and developing specific therapies — but also demonstrates that U.S. stem-cell researchers are falling behind their competitors.[1]

A few days later, the House of Representatives passed legislation supporting the expansion of federally funded human embryonic stem-cell research, setting the stage for a veto by President George W. Bush, who has ruled out providing funding for any such studies except those using certain cell lines that were derived before August 2001. Meanwhile, California and New Jersey are moving ahead with programs to sponsor the research, and similar initiatives are being considered by other states (see map).

Although the National Academies guidelines have no legal force, scientists predict that they will be widely adopted. The committee charged with drafting them started from the position that the cloning of humans for reproductive purposes should be banned but that research using human embryonic stem cells to study diseases and develop new treatments should proceed. In contrast, opponents of the research believe that it is immoral, since deriving new stem-cell lines requires the destruction of human embryos.

Human embryonic stem cells are derived from the inner cell



State Laws and Proposed Legislation Governing Embryonic Stem-Cell Research.

Most laws or proposed laws limiting embryonic stem-cell research explicitly prohibit only somatic-cell nuclear transfer (SCNT). Arizona's law prohibits the use of public funds for SCNT but does not prohibit the research. Nebraska has a law prohibiting the use of state funds for embryonic stem-cell research, but there are also proposed laws on both sides of the issue of SCNT. South Dakota is the only state with a law explicitly prohibiting all embryonic stem-cell research. Most laws or proposed laws permitting embryonic stem-cell research include SCNT. However, Delaware's proposed bill would permit research only on embryos from in vitro fertilization. North Carolina's proposed bill would permit research only on embryos from ectopic pregnancy, miscarriage, and in vitro fertilization. Another bill proposed in North Carolina would prohibit reproductive cloning and might prohibit SCNT for research; the language is unclear. Virginia's existing law on cloning is also unclear and might prohibit SCNT for research. All the laws and pending legislation shown on this map were enacted or proposed in the era of embryonic stem-cell research. Many states have laws governing research that were passed before 1998 that could be interpreted as prohibiting embryonic stem-cell research. The information is from the National Conference of State Legislatures and is accurate as of June 13, 2005.

mass of a blastocyst-stage embryo that has undergone cell division and development for about six days following fertilization. The new guidelines (see box) apply to research using stem cells obtained from human blastocysts, whether they have been created specifically for research or were originally made for reproductive purposes. They also apply to stem cells obtained by transferring the nucleus from a fully differentiated cell into a human oocyte whose own nucleus has been removed and inducing the resultant cell to develop into a blastocyst. This procedure, called somatic-cell nuclear transfer (SCNT), is the technique used by the Korean team. Although it holds great potential for yielding patient-specific therapies and cellular models of disease, SCNT requires that women donate fresh oocytes for research — a requirement that some find more ethically problematic than the donation of "leftover" frozen embryos by couples who have undergone in vitro fertilization (IVF) procedures. Unlike embryos, oocytes cannot be frozen for later use. So far, only a few U.S. researchers have sought permission to perform SCNT.

Korean veterinarian Woo Suk Hwang and his team "are now the world leaders" in SCNT, according to Douglas Melton, co-director of the Harvard Stem Cell Institute. Melton and his collaborators, who have used donated frozen embryos to derive new stem-cell lines, recently received permission from Harvard University, the Howard Hughes Medical Institute, and the District Attorney for Middlesex County, Massachusetts, to begin doing SCNT. "Had the [National Academies] guidelines been available two years ago, I think our process of getting permission would have been greatly facilitated," said Melton. (In May, the Massachusetts legislature passed a bill that would authorize expanded stem-cell re-

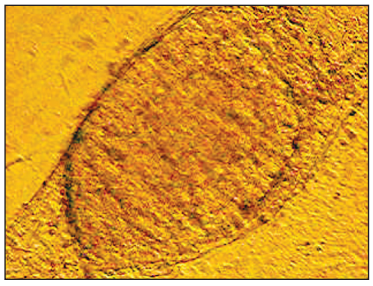
search, including SCNT, and subsequently overrode a veto by Republican Governor Mitt Romney.)

The ethics guidelines contain detailed recommendations with regard to donor recruitment and informed consent. They describe how stem cells should be characterized, handled, transferred, and stored. They recommend the establishment of an embryonic stem-cell research oversight committee at each institution, and they offer guidance on what kinds of experiments should be considered for approval and what kinds should be proscribed.

Adhering to a limit established by the British Warnock Commission in 1984, the guidelines stipulate that no blastocyst should be maintained in culture for longer than 14 days or past the time of appearance of the primitive streak, a band of cells that establishes the embryo's head–tail and right–left orientations. "It's also the point at which twinning is no longer possible — biological individuation," explained ethicist Jonathan D. Moreno of the University of Virginia, cochair of the guidelines committee. "Now, as a philosopher, I always want to say, anatomy is not ethics. But it's a signpost that says, 'Before this, you don't have structure; you don't have a biological individual.'"

The guidelines would also limit the creation of some kinds of chimeras, organisms containing both human and animal cells. Although chimeras have been made for years, the use of human embryonic stem cells creates the potential for much greater contribution of human cells to a chimeric animal. The committee recommended that chimeras made with the use of human embryonic stem cells not be allowed to breed and that such cells not be introduced into primate blastocysts. "I think the experiment one doesn't want to see done is to make some kind of human–primate chimera," Melton said. "That really causes us all to shiver and think about the essence of being human, about human dignity, and the rights of animals."

The guidelines urge caution on introducing human embryonic stem cells into the brains of nonhuman primates, an experiment



Human embryonic stem cells may differentiate into cardiomyocytes.

Nicolas Christoforou and John Gearhart, Institute for Cell Engineering, Johns Hopkins Medical Institutions.

that might be desirable, for example, during the development of cell-based therapies for disorders such as Parkinson's disease or stroke. Mice, points out John Gearhart, a stem-cell biologist at Johns Hopkins School of Medicine, do not live long enough to permit researchers to study the long-term fate of neural progenitor cells derived from embryonic stem cells and introduced into the mouse nervous system. "If you're concerned about your cells wandering off and doing something inappropriate, or turning into a tumor, how long does it take to reveal that?" asked Gearhart. The chances of creating a human-like brain in a monkey would be much smaller if human stem cells were introduced into an adult animal than if they were introduced into a primate embryo, fetus, or neonate, he added.

An estimated 400,000 frozen embryos are stored in the United States, primarily for use in IVF.[2] Some stem-cell researchers arrange with IVF clinics to obtain embryos donated by couples for research, from which they may derive stem-cell lines. The guidelines stipulate that donors and clinics may not be paid for embryos and that decisions to create embryos during infertility treatment should not be influenced by stem-cell scientists. At Boston IVF, where Melton has obtained embryos for research, physicians do not mention the possibility of donating embryos for this purpose until a couple indicates a wish to discard their remaining embryos, according to surgical director Alan Penzias. Penzias estimates that only about 10 percent of frozen embryos can be induced to develop into blastocysts.

Deriving new cell lines by means of SCNT will require recruiting women to donate oocytes, which have rarely been solicited for research purposes in this country. Currently, healthy, fertile women are sought as egg donors primarily for reproductive purposes — by IVF programs, infertile couples, or egg-donor agencies. According to the Centers for Disease Control and Prevention, egg donors are the patients in about 10 percent of the approximately 100,000 cycles of oocyte collection performed annually at U.S. IVF clinics. During a cycle, a woman injects a daily dose of recombinant human follicle-stimulating hormone subcu-

Key Elements of the National Academies' Guidelines for Human Embryonic Stem-Cell Research.*

Types of research requiring prior review and approval

Generation of new lines of human embryonic stem cells

Research in which human embryonic stem cells are introduced into animals

Research in which the identity of donors from whom human embryonic stem cells are derived is ascertainable

Studies that should not be permitted at this time

Research involving in vitro culture of an intact human embryo for longer than 14 days or until formation of the primitive streak begins

Research in which human embryonic stem cells are introduced into primate blastocysts

Research in which any embryonic stem cells are introduced into human blastocysts

Breeding of animals into which human embryonic stem cells have been introduced

Procurement of gametes, blastocysts, or cells

Institutional review boards should review any procurement of cells for the purpose of generating new human embryonic stem-cell lines

Consent from each donor should be obtained at the time that blastocysts are donated for research

Donors should be informed that they may withdraw consent until blastocysts are actually used in cell-line derivation

When donor gametes have been used in IVF, resulting blastocysts may not be used for research without the consent of all gamete donors

No payment may be provided for donating blastocysts for research

No payment should be provided for donating oocytes or sperm for research

Women who undergo hormonal induction to generate oocytes for research should be reimbursed only for direct expenses incurred as a result of the procedure

Decisions related to the creation of embryos for infertility treatment should be free of the influence of scientists seeking to derive or use human embryonic stem cells in research

Researchers may not ask members of an infertility-treatment team to generate more oocytes than necessary for the optimal chance of reproductive success

Derivation of new human embryonic stem-cell lines

Proposals for nuclear-transfer experiments involving human or nonhuman oocytes must have a strong scientific rationale, and studies to find alternatives to donated oocytes should be encouraged

Neither blastocysts made by means of nuclear transfer nor parthogenetic or androgenetic human embryos may be transferred to a uterus (human or animal) or cultured as intact embryos for longer than 14 days

Institutions should require documentation of the provenance of all human embryonic stem-cell lines

Research uses

Studies in which human embryonic stem cells are transplanted into animal fetuses need more careful consideration than those involving transplantation of such cells into adult animals

Consideration of any major functional contributions to the brain should be a main focus of review

Introduction of human embryonic stem cells into nonhuman mammalian blastocysts should be considered only when no other experiment can provide the information needed

* The National Academies have previously stated that no research aimed at reproductive cloning of a human being should be conducted. IVF denotes in vitro fertilization.

taneously for 10 to 14 days to trigger the development of multiple ovarian follicles. Oocytes are then collected transvaginally in a brief surgical procedure. There is some medical risk, primarily of ovarian hyperstimulation syndrome, which affects 2 to 5 percent of women undergoing hormonal stimulation. This syndrome can be painful, may necessitate hospitalization, and can cause hypotension, respiratory distress, renal failure, hemorrhage due to ovarian rupture, and very rarely even death.[3] The syndrome is related to the production of vascular endothelial growth factor, and severe cases can generally be prevented by administering the lowest doses of hormone necessary and carefully monitoring the ovarian response.

Women who donate oocytes for IVF are routinely paid, and some receive large sums. Penzias said the going rate in Boston is about $5,000 per cycle, and the American Society for Reproductive Medicine considers a fee of up to $10,000 acceptable under some circumstances. A recent advertisement in a college newspaper offered $25,000 for an egg donor who was an Ivy League student or alumna of northern or eastern European descent, 21 to 32 years of age, "healthy, athletic, very pretty, 5′7″–5′10.5″, outgoing, sense of humor preferred."[4] Expressing concern that financial incentives might unduly influence donors to accept unnecessary risk, the guidelines committee recommended that women who donate oocytes for research be reimbursed only for direct expenses and not receive payment for their

time, for lost wages, or as compensation.

That recommendation surprised some observers. Bernard Lo, director of the program in medical ethics at the University of California at San Francisco (UCSF), said that it ignores the reality that a competitive market already exists for egg donors, especially for those who are young, white, and well educated. "This apparently neutral rule that we're not going to pay anybody for research in fact means you are closing off financial opportunities for women of certain socioeconomic and ethnic backgrounds," he said. "We pay people to undergo risks in other types of research. It doesn't seem fair to have [oocyte donors] undergo clear medical risks and not offer them something for that." But Lo, who chairs UCSF's Campus Advisory Committee on the Ethics of Oocyte, Embryo, and Stem Cell Research, acknowledged that the issue is so troublesome that UCSF has not yet approved any protocols allowing retrieval of oocytes for research. "We thought about this a lot and really could not come up with a good answer," he said.

Lo and others predict that if egg donors are not paid, most will probably be people with relatives affected by one of the diseases often mentioned as targets of stem-cell research. "It's hard to imagine someone who isn't motivated for that kind of reason, but it's also important that people realize that this is a long-term scientific project," Lo said. "It's very unlikely that someone who donates now is going to have their materials used therapeutically for someone in their family."

The guidelines will probably be used in state and institutional policymaking, but they may have little impact in Washington. The Castle–DeGette bill, passed by the House of Representatives in May, would expand federal funding for the use of donated embryos in stem-cell research, but it would not allow such funding for SCNT, which many consider the approach most likely to yield new therapies. Nonetheless, President Bush has promised to veto the measure if it passes the Senate, declaring, "There is no such thing as a spare embryo."[5] Opponents of this research have lobbied for increased funding to study alternative sources of stem cells, but it is not yet clear whether a scientifically practical alternative that does not require the destruction of embryos can be identified.

Meanwhile, the funding of the $3 billion California stem-cell research initiative, passed by voters last year, has been delayed by lawsuits brought by its opponents. California universities and research institutions have been aggressively recruiting stem-cell scientists, and the new California Institute for Regenerative Medicine has assembled an impressive roster of outside advisers to set ethics standards and award grants. But some state legislators are seeking to amend the initiative, citing a lack of transparency, potential conflicts of interest among grant reviewers, and a desire to ensure that any therapies developed will be affordable. Zach Hall, the interim president of the institute, said that the proposed changes "would seriously impede our ability to do our science. . . . We haven't even gotten going, and already it's like they're crying, 'Foul!'"

According to Harvard's Melton, the only way to ensure steady progress in this emerging field is to have a consistent national policy. "In general, I'm not a big fan of state-led initiatives, because I think this is a federal issue," he said. "Either this research should go forward or it shouldn't. But if it should, our present policies make no sense at all, because their only consequence is to make the research go more slowly."

Dr. Okie is a contributing editor of the *Journal*.

An interview with stem-cell researcher John Gearhart can be heard at www.nejm.org

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2. Hoffman DI, Zellman GL, Fair CC, et al. Cryopreserved embryos in the United States and their availability for research. Fertil Steril 2003;79:1063-9.

3. Practice Committee of the American Society for Reproductive Medicine. Ovarian hyperstimulation syndrome. Fertil Steril 2003;80:1309-14.

4. Classified advertisement. Daily Pennsylvanian. May 27, 2005. (Also available at http://www.dailypennsylvanian.com.)

5. The president's stem cell theology. New York Times. May 26, 2005:A28.

CORRECTION

Stem-Cell Research — Signposts and Roadblocks

Stem-Cell Research — Signposts and Roadblocks . On page 2, the figure legend should have read: ``Nebraska has a law prohibiting the use of funds from tobacco-settlement dollars for embryonic stem-cell research. There are also proposed laws on both sides of the issue of SCNT,´´ rather than: ``Nebraska has a law prohibiting the use of state funds for embryonic stem-cell research, but there are also proposed laws on both sides of the issue of SCNT,´´ as printed.

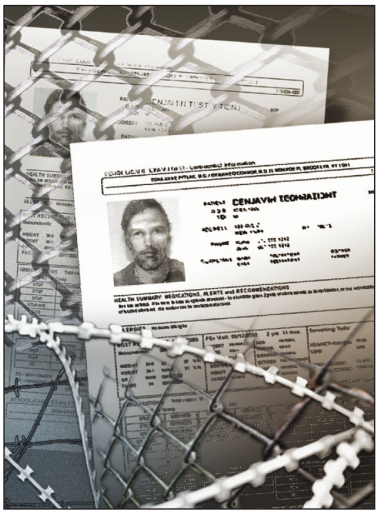
N Engl J Med 2005;353:2728-b

Doctors and Interrogators at Guantanamo Bay

M. Gregg Bloche, M.D., J.D., and Jonathan H. Marks, M.A., B.C.L.

Mounting evidence from many sources, including Pentagon documents, indicates that military interrogators at Guantanamo Bay have used aggressive counter-resistance measures in systematic fashion to pressure detainees to cooperate. These measures have reportedly included sleep deprivation, prolonged isolation, painful body positions, feigned suffocation, and beatings. Other stress-inducing tactics have allegedly included sexual provocation and displays of contempt for Islamic symbols.[1] The International Committee of the Red Cross (ICRC) and others charge that such tactics constitute cruel and inhuman treatment, even torture.

To what extent did interrogators draw on detainees' health information in designing and pursuing such approaches? The Pentagon has persistently denied this practice. After the ICRC charged last year that interrogators tapped clinical data to craft interrogation strategies, Defense Department officials issued a statement denying "the allegation that detainee medical files were used to harm detainees."[2] This spring, an inquiry led by Vice Admiral Albert T. Church, the inspector general of the U.S. Navy, concluded: "While access to medical information was carefully controlled at GTMO [Guantanamo Bay], we found in Afghanistan and Iraq that interrogators sometimes had easy access to such information."[3] The implication is that interrogators had no such access at Guantanamo and that medical confidentiality was shielded, albeit with exceptions. Other Pentagon officials have reinforced this message. In a memo made public last month, announcing "Principles . . . for the Protection and Treatment of Detainees," William Winkenwerder, the Assistant Secretary of Defense for Health Affairs, said that limits on detainees' medical privacy are "analogous to legal standards applicable to U.S. citizens."



But this claim, our inquiry has determined, is sharply at odds with orders given to military medical personnel — and with actual practice at Guantanamo. Health information has been routinely available to behavioral science consultants and others who are responsible for crafting and carrying out interrogation strategies. Through early 2003 (and possibly later), interrogators themselves had access to medical records. And since late 2002, psychiatrists and psychologists have been part of a strategy that employs extreme stress, combined with behavior-shaping rewards, to extract actionable intelligence from resistant captives.

A previously unreported U.S. Southern Command (SouthCom) policy statement, in effect since August 6, 2002, instructs health care providers that communications from "enemy persons under U.S. control" at Guantanamo "are not confidential and are not subject to the assertion of privileges" by detainees. The statement, from SouthCom's chief of staff, also instructs medical personnel to "convey any information concerning . . . the accomplishment of a military or national security mission . . . obtained from detainees in the course of treatment to non-medical military or other United States personnel who have an apparent need to know the information. Such information," it adds, "shall be communicated to other United States personnel with an apparent need to know, whether the exchange of information with the non-medical person is initiated by the provider or by the non-medical person." The only limit this policy imposes on caregivers' role in intelligence gathering is that they cannot act as interrogators.

The statement, embedded — along with policies on parking and alcohol — in the personnel section of the SouthCom Web site,[4] not only requires caregivers to provide clinical information to military and Central Intelligence Agency interrogation teams on request; it calls on them to volunteer information that they believe might be of value. It thereby makes them part of Guantanamo's surveil-

lance network, dissolving the Pentagon's purported separation between intelligence gathering and patient care.

Rather than being consistent with the presumption of confidentiality that applies to Americans even in prisons, the Guantanamo policy rejects this presumption. Within military prisons, personal health information cannot be given to correctional or law-enforcement officials unless they deem it necessary for health, safety, or security reasons. Confidentiality is also the starting point in federal and state prisons for civilians, albeit with similar exceptions for health, safety, and security. (Federal law permits disclosure of inmates' health information "to authorized federal officials for the conduct of lawful intelligence, counter-intelligence, and other national security activities.") There is debate over the scope of these exceptions, but there is consensus about the basic presumption of medical privacy.

Wholesale rejection of clinical confidentiality at Guantanamo also runs contrary to settled ethical precepts. Medical privacy is not an ethical absolute — caregivers in civilian and military settings have an obligation to report information to third parties when doing so can avert threats to the health or safety of identifiable persons — but confidentiality is the starting premise.

The laws of war defer to medical ethics. Additional Protocol I to the Geneva Conventions provides that medical personnel "shall not be compelled to perform acts or to carry out work contrary to the rules of medical ethics." Although the protocol has not been ratified by the United States, this principle has attained the status of customary international law. International human rights law (most important, the 1966 International Covenant on Civil and Political Rights) provides additional protection for privacy in general — in wartime and peacetime. Although this protection isn't absolute, exceptions must be justified by pressing public need, and they must represent the least restrictive way to meet this need. Wholesale abandonment of medical confidentiality hardly qualifies, especially when the "need" invoked is the crafting of counter-resistance measures that are prohibited by international law.

In what ways did military intelligence personnel draw on medical information for interrogation and counter-resistance purposes? Instructions to Guantanamo veterans not to discuss their service publicly have been an obstacle to answering this question. But available documents, an account of a fall 2004 briefing by the camp's commander (Brigadier General Jay Hood), and interviews with behavioral science professionals enable us to assemble parts of this picture.

During the camp's early months, interrogators could gain access to personal health information (and did so to set limits on practices that might put detainees' health at risk) but did not use psychological assessments of individual subjects. Conventional army intelligence doctrine has been unsympathetic to such input: it has relied instead on a mix of standard interrogation methods meant to appeal variously to subjects' insecurities, pride, and fears, within constraints set by the laws of war.[5] But by late 2002, growing frustration with the slow pace of intelligence production at Guantanamo led to calls from commanders for innovative tactics. Major General Geoffrey Miller, who took command of Guantanamo in late 2002, approved the creation of a "Behavioral Science Consultation Team" (BSCT, pronounced "Biscuit") in order to develop new strategies and assess intelligence production. A principal BSCT function was to engineer the camp experiences of "priority" detainees to make interrogation more productive.

A psychiatrist and a psychologist staffed the Guantanamo BSCT. Those initially assigned to this team both came from health care backgrounds; neither had much training in behavioral analysis of the sort that civilian psychologists perform for law-enforcement agencies. According to Hood's briefing, BSCT consultants prepared psychological profiles for use by interrogators; they also sat in on some interrogations, observed others from behind one-way mirrors, and offered feedback to interrogators. The first BSCT psychologist, Major John Leso, a specialist in assessing aviators' fitness to fly, attended part of the interrogation of Mohammed al-Qahtani, thought by many to be the "20th hijacker." (An extract from a log of this interrogation published in *Time* magazine last month refers to Leso as "Maj. L.")

There are strong indications that the Guantanamo BSCT has had access to personal health information. An internal, May 24, 2005, memo from the Army Medical Command, offering guidance to caregivers responsible for detainees, refers to the "interpretation of relevant excerpts from

medical records" for the purpose of "assistance with the interrogation process." The memo, provided to us by a military source, acknowledges this nontherapeutic role, urging health professionals who serve in this capacity to avoid involvement in detainee care, absent an emergency. This acknowledgment is consistent with other accounts of information flow from caregivers to behavioral science consultants to interrogators.

Competing behavioral science models have influenced the advice given to interrogators by BSCT members. One approach emphasizes fear and anxiety as counter-resistance tools; another favors rapport with detainees. The former approach, supported by some associated with the John F. Kennedy Special Warfare Center who have helped to formulate BSCT doctrine, builds on the premise that acute, uncontrollable stress erodes established behavior (e.g., resistance to questioning), creating opportunities to reshape behavior. Complex reward systems (e.g., the creation of multiple camp "levels" with different privileges) promote cooperation. Stressors tailored to the psychological and cultural vulnerabilities of individual detainees (e.g., phobias, personality features, and religious beliefs) are key to this approach and can be devised on the basis of detainee profiles.

Proponents of rapport-based interrogation counter that answers given under high stress are unreliable. Not only are people in acute distress inclined to say whatever they think might bring relief; the psychiatric sequelae of extreme stress — anxiety, depressed mood, and disordered thinking — impair the understanding of questions and produce incoherent answers. Rapport building, tailored to people's cognitive styles and cultural beliefs, takes time but yields better information, its defenders contend.

There is no scientific answer to the question of which interrogation strategy is more effective. For obvious ethical and legal reasons, there is unlikely to be one. At Guantanamo, the fear-and-anxiety approach was often favored. The cruel and degrading measures taken by some, in violation of international human rights law and the laws of war, have become a matter of national shame.

Clinical expertise has a limited place in the planning and oversight of lawful interrogation. Psychologists play such a role in criminal investigations, and medical monitoring of detainees is called for by international legal instruments. But proximity of health professionals to interrogation settings, even when they act as caregivers, carries risk. It may invite interrogators to be more aggressive, because they imagine that these professionals will set needed limits. The logic of caregiver involvement as a safeguard also risks pulling health professionals in ever more deeply. Once caregivers share information with interrogators, why should they refrain from giving advice about how to best use the data? Won't such advice better protect detainees, while furthering the intelligence-gathering mission? And if so, why not oversee isolation and sleep deprivation or monitor beatings to make sure nothing terrible happens?

Wholesale disregard for clinical confidentiality is a large leap across the threshold, since it makes every caregiver into an accessory to intelligence gathering. Not only does this undermine patient trust; it puts prisoners at greater risk for serious abuse. The global political fallout from such abuse may pose more of a threat to U.S. security than any secrets still closely held by shackled internees at Guantanamo Bay.

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An interview with Mr. Marks can be heard at www.nejm.org

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A Role for Oxidized Phospholipids in Atherosclerosis

Judith A. Berliner, Ph.D., and Andrew D. Watson, M.D., Ph.D.

Related Article, page 46

Atherosclerosis is a disease of the vessel wall involving lipid accumulation, chronic inflammation, cell death, and thrombosis that causes heart disease and stroke. Although elevated cholesterol levels are a recognized risk factor for atherosclerosis, a growing number of studies suggest that oxidized phospholipids may also play an important role in this condition.[1,2] Phospholipids, essential components of lipoproteins and cell membranes, are composed of fatty acids bound to a glycerol backbone containing a polar head group. They are susceptible to free-radical or enzymatic oxidation by myeloperoxidase, lipoxygenase, and other enzymes that are present in the vessel wall. The addition of oxygen to the polyunsaturated fatty acids produces prostaglandin-like molecules, some of which then decompose and fragment to form additional bioactive molecules.

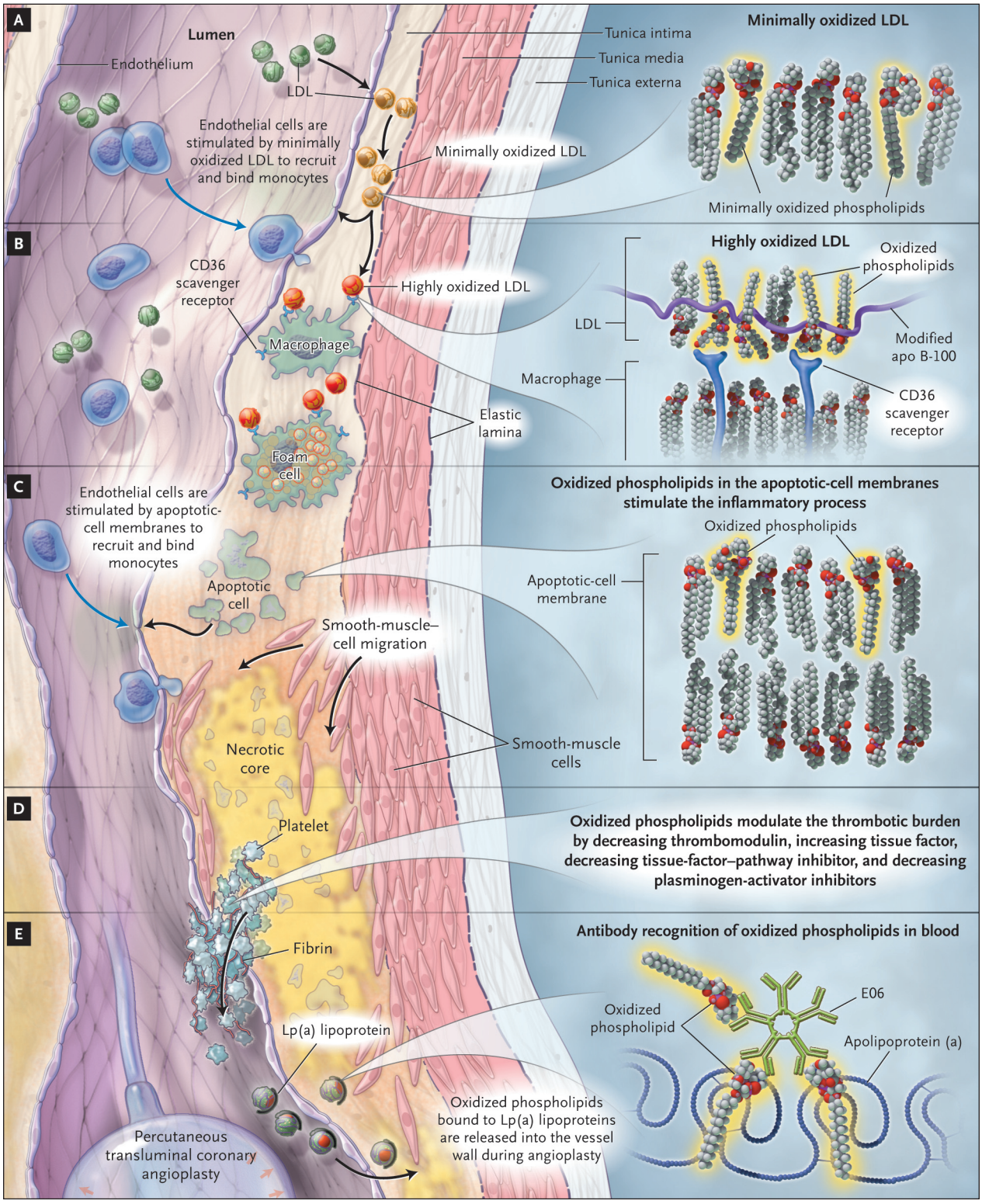
Oxidized phospholipids accumulate under conditions of oxidative stress during viral infections and in inflammatory conditions such as rheumatoid arthritis and atherosclerosis; they are also generated in apoptotic and necrotic cells.[1,2] Oxidized, but not native, phospholipids can interact with specific receptors that mediate atherogenesis. In addition, oxidized phospholipids contain reactive groups that can bind covalently to proteins, forming lipid–protein adducts. These modified proteins become dysfunctional, which can contribute to atherosclerosis. Phospholipid oxidation elicits an immune response by creating new epitopes that are recognized by antibodies of innate immunity, such as E06.[3] Thus, oxidized phospholipids are fundamentally distinct from unoxidized phospholipids in their ability to interact with cells, proteins, and the immune system in order to promote atherogenesis.

In vivo studies in human tissue have demonstrated the accumulation of oxidized phospholipids in the vessel wall at all stages of atherosclerosis, from early fatty streaks (in infants of mothers with hyperlipidemia) to advanced complex lesions, suggesting that these lipids may contribute to all stages of atherogenesis. In vitro studies and studies in which oxidized phospholipids were injected into animals have demonstrated that specific oxidized phospholipids can mediate many atherogenic processes — from the earliest entry of monocytes into the vessel wall to thrombus formation (see diagram). Oxidized phospholipids activate the endothelium to bind monocytes (but not neutrophils) and cause the endothelial cells and smooth-muscle cells to produce the potent monocyte chemoattractant protein 1 and the differentiation factor macrophage colony-stimulating factor. (Oxidized phospholipids can also induce responses that protect cells from oxidative stress and inhibit some acute, neutrophil-mediated inflammatory responses.[2])

In vivo, the presence of monocyte-binding molecules and chemotactic factors causes monocytes to migrate into the subendothelial space and to differentiate into macrophages. These macrophages can then release additional reactive oxygen species, further oxidizing low-density lipoprotein to a form that is recognized by scavenger receptors on macrophages and on smooth-muscle cells; this uptake results in the formation of foam cells. Oxidized phospholipids, either free or adducted to apolipoprotein B-100, are recognized by the CD36 scavenger receptor.[4] Furthermore, these phospholipids bind to C-reactive protein and could promote foam-cell formation through the Fcγ receptor.

As atherogenesis progresses (in response to cytokines produced by activated endothelial cells and macrophages), smooth-muscle cells proliferate, enter the intima, and form foam cells. Specific oxidized phospholipids at low concentrations stimulate the proliferation of smooth-muscle cells. Ultimately, the foam cells die by necrosis or apoptosis, and a necrotic core is formed. At higher concentrations, oxidized phospholipids have been shown to regulate smooth-muscle apoptosis by increasing the level of ceramide and facilitating the release of cytochrome *c* from mitochondria. All the while, the inflammation continues, with further entry of monocytes and lymphocytes into the vessel wall. This continuing entry may be facilitated by oxidized phospholipids that are present in the membranes of apoptotic and necrotic cells.

Ultimately, the plaque may rupture or erode, causing a thrombus to form. Key enzymes in the coagulation pathway are also targets of oxidized phospholipids, which increase the expression of



A Model of the Roles of Oxidized Phospholipids in the Development of Atherosclerosis.

Low-density lipoprotein (LDL) moves into the subendothelial space and becomes oxidized (Panel A). Inflammatory cells are recruited into the vessel wall, take up the oxidized LDL through scavenger receptors, and become foam cells (Panel B). The cell membranes of apoptotic cells continue to recruit inflammatory cells into the vessel wall (Panel C). Oxidized phospholipids also promote thrombosis through the modulation of thrombotic factors (Panel D). Modified Lp(a) lipoprotein, which accumulates in atherosclerotic lesions, can be detected at higher levels in the blood after angioplasty with the use of E06, an antibody that recognizes oxidized phospholipids (Panel E).

tissue factor in endothelial cells, while decreasing the expression of thrombomodulin and the activity of tissue-factor–pathway inhibitor. Platelet activation is also stimulated by oxidized phospholipids. Thus, oxidized phospholipids have proatherogenic effects on all vascular-wall cells.

Although many of the studies cited above were performed in vitro, there is growing evidence that oxidized phospholipids have a role in atherogenesis in vivo. Knocking out or inhibiting receptors that recognize oxidized phospholipids (including the platelet-activating–factor [PAF] receptor, CD36, and toll-like receptors 2 and 4) leads to a decrease in experimental atherosclerosis. Knocking out 12/15 lipoxygenase, an enzyme that oxidizes polyunsaturated fatty acids, also results in decreased atherosclerosis. Levels of myeloperoxidase, another oxidative enzyme, are correlated with the risk of coronary artery disease. High-density lipoprotein (HDL) has been shown to play a protective role in atherogenesis and alters the metabolism of oxidized phospholipids. HDL contains proteins (such as apolipoprotein A-I [apo A-I]) and enzymes (such as lecithin–cholesterol acyltransferase, paraoxonase, and PAF–acetylhydrolase) that can prevent the formation of oxidized phospholipids or destroy them once they have formed. Apo A-I transfers the phospholipids to HDL for destruction. Knocking out paraoxonase or PAF–acetylhydrolase increases atherosclerosis.

The same enzymes associated with HDL that destroy oxidized phospholipids are also inhibited by them, creating a balance so that in the absence of continued inflammation, HDL maintains enough functioning apo A-I and enzyme activity to be antiinflammatory. During an acute-phase response (e.g., after surgery) or during a chronic response (e.g., a chronic systemic inflammation such as atherosclerosis), the balance can shift, and HDL can become proinflammatory. In animal models of atherosclerosis, the balance has been shifted back by the transgenic or adenovirus-mediated expression of high concentrations of apo A-I or the exogenous administration of apo A-I or apo A-I–mimetic peptides.[5]

The study by Tsimikas et al., reported in this issue of the *Journal* (pages 46–57), demonstrated a correlation between the levels of oxidized phospholipids in the blood and levels of Lp(a) lipoprotein. The investigators also determined that increased levels of Lp(a) lipoprotein and oxidized phospholipids, in particles containing apolipoprotein B-100, correlated with the risk of coronary artery disease and that combined hypercholesterolemia plus increased levels of either oxidized phospholipids or Lp(a) lipoprotein greatly increased the odds of coronary artery disease. Thus, this study is the first to establish a causal connection between the levels of oxidized phospholipids and the risk of coronary artery disease.

In summary, phospholipids are ubiquitous molecules that are important to the structural integrity of cells and lipoproteins. When oxidized, however, they can promote inflammation, are taken up by scavenger receptors on macrophages, and are recognized by the innate immune system. Studies suggest that proteins and enzymes that remove or destroy oxidized phospholipids prevent atherosclerosis and that proteins and enzymes that produce or retain oxidized phospholipids promote atherosclerosis. Thus, oxidized phospholipids may be a diagnostic marker of coronary artery disease or may represent a potential target for therapeutic intervention.

Dr. Berliner is a professor of medicine and pathology and Dr. Watson is an assistant professor of medicine at the David Geffen School of Medicine, University of California, Los Angeles.

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2. Leitinger N. Oxidized phospholipids as modulators of inflammation in atherosclerosis. Curr Opin Lipidol 2003;14:421-30.

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CORRECTION

A Role for Oxidized Phospholipids in Atherosclerosis

A Role for Oxidized Phospholipids in Atherosclerosis . In the figure on page 10, the text in Panel D should have read, ``Oxidized phospholipids modulate the thrombotic burden by decreasing thrombomodulin, increasing tissue factor, decreasing tissue-factor–pathway inhibitor, and increasing plasminogen-activator inhibitor,´´ rather than `` . . . decreasing plasminogen-activator inhibitors,´´ as printed.

N Engl J Med 2005;353:1869

THIS WEEK in the JOURNAL

ORIGINAL ARTICLE

Inhaled Nitric Oxide for Critically Ill Preterm Infants

Premature infants with severe respiratory failure may be treated with inhaled nitric oxide, a controversial treatment that may reduce mortality or prevent bronchopulmonary dysplasia. In a randomized, placebo-controlled trial in neonates with respiratory failure after treatment with surfactant there was no difference in the rates of death or bronchopulmonary dysplasia.

This multicenter trial could not confirm the benefits of inhaled nitric oxide that were found in a smaller trial.

SEE P. 13; EDITORIAL, P. 82; CME, P. 110

ORIGINAL ARTICLE

Neurodevelopmental Outcomes among Premature Infants Given Inhaled Nitric Oxide

The authors previously reported the results of a single-center, randomized trial of inhaled nitric oxide in premature infants, showing reduced risks of death or chronic lung disease and of severe intraventricular hemorrhage or periventricular leukomalacia. Long-term follow-up revealed that children given nitric oxide had improved neurodevelopmental outcomes at two years of age.

The findings from this single-center trial of premature infants suggest benefits of inhaled nitric oxide on neurodevelopmental outcomes that warrant further study.

SEE P. 23; EDITORIAL, P. 82

ORIGINAL ARTICLE

Hydroxyurea Compared with Anagrelide in High-Risk Essential Thrombocythemia

The major risks in essential thrombocythemia are thrombosis and hemorrhage. In this large, randomized trial, the patients given anagrelide plus aspirin had higher rates of arterial thrombosis and serious hemorrhage, whereas the hydroxyurea group had a higher rate of venous thromboembolism. The rate of transformation to myelofibrosis was higher in the anagrelide group.

These results offer practical guidance for the treatment of patients with essential thrombocythemia and a high risk of thrombosis.

SEE P. 33; EDITORIAL, P. 85; CME, P. 111

ORIGINAL ARTICLE

Oxidized Phospholipids, Lp(a) Lipoprotein, and Coronary Artery Disease

Lp(a) lipoprotein is believed to be involved in the pathogenesis of coronary artery disease. This study provides evidence that its atherogenicity is related to proinflammatory oxidized phospholipids that are bound to it.

SEE P. 46; PERSPECTIVE, P. 9

BRIEF REPORT

Ovarian Transplantation between Monozygotic Twins

This report describes the successful transplantation of ovarian tissue from a fertile monozygotic twin to her 24-year-old twin sister, who had a 10-year history of ovarian failure. The recipient subsequently conceived and gave birth to a girl at 38 weeks' gestation.

SEE P. 58

CURRENT CONCEPTS

Preserving a Woman's Fertility

An increasing number of options are available for women who wish to preserve their fertility, particularly before cancer treatment: oophorectomy with cryopreservation, embryo cryopreservation, and aspiration of oocytes for cryopreservation immediately or after ovarian hyperstimulation. This article also describes the natural process of oocyte loss and methods of testing for decreased ovarian reserve.

SEE P. 64; CME, P. 109

CLINICAL PROBLEM-SOLVING

Double Jeopardy

A 36-year-old woman in her 34th week of pregnancy presented to the emergency department after the sudden onset of severe substernal chest pain that had awoken her in the early morning. She had diaphoresis and nausea but no dyspnea, dizziness, syncope, hemoptysis, cough, or fever.

SEE P. 75

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Progress in Human Somatic-Cell Nuclear Transfer

The efficiency of deriving blastocysts through the nuclear transfer of embryonic stem cells has been improved by a factor of more than 14.

SEE P. 87

The NEW ENGLAND JOURNAL of MEDICINE

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Inhaled Nitric Oxide for Premature Infants with Severe Respiratory Failure

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ABSTRACT

BACKGROUND

Inhaled nitric oxide is a controversial treatment for premature infants with severe respiratory failure. We conducted a multicenter, randomized, blinded, controlled trial to determine whether inhaled nitric oxide reduced the rate of death or bronchopulmonary dysplasia in such infants.

METHODS

We randomly assigned 420 neonates, born at less than 34 weeks of gestation, with a birth weight of 401 to 1500 g, and with respiratory failure more than four hours after treatment with surfactant to receive placebo (simulated flow) or inhaled nitric oxide (5 to 10 ppm). Infants with a response (an increase in the partial pressure of arterial oxygen of more than 10 mm Hg) were weaned according to protocol. Treatment with study gas was discontinued in infants who did not have a response.

RESULTS

The rate of death or bronchopulmonary dysplasia was 80 percent in the nitric oxide group, as compared with 82 percent in the placebo group (relative risk, 0.97; 95 percent confidence interval, 0.86 to 1.06; P=0.52), and the rate of bronchopulmonary dysplasia was 60 percent versus 68 percent (relative risk, 0.90; 95 percent confidence interval, 0.75 to 1.08; P=0.26). There were no significant differences in the rates of severe intracranial hemorrhage or periventricular leukomalacia. Post hoc analyses suggest that rates of death and bronchopulmonary dysplasia are reduced for infants with a birth weight greater than 1000 g, whereas infants weighing 1000 g or less who are treated with inhaled nitric oxide have higher mortality and increased rates of severe intracranial hemorrhage.

CONCLUSIONS

The use of inhaled nitric oxide in critically ill premature infants weighing less than 1500 g does not decrease the rates of death or bronchopulmonary dysplasia. Further trials are required to determine whether inhaled nitric oxide benefits infants with a birth weight of 1000 g or more.

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*Members of the Preemie Inhaled Nitric Oxide Study, which is part of the NICHD Neonatal Research Network, are listed in the Appendix.

N Engl J Med 2005;353:13-22.

Premature infants in respiratory failure can have dramatic improvements after treatment with exogenous surfactant. However, a subset of premature infants have suboptimal responses to surfactant[1] and may have pulmonary hypertension in association with severe respiratory failure.[2-6] Inhaled nitric oxide may benefit such infants by selectively dilating pulmonary vasculature, improving ventilation–perfusion matching, and decreasing the pulmonary inflammatory response.[7-9]

Inhaled nitric oxide had been shown to provide only short-term improvement in oxygenation in premature infants[10-14] until a recent single-center study reported an association between the administration of inhaled nitric oxide and a decrease in the incidence of bronchopulmonary dysplasia or death in a cohort of moderately ill infants.[15] We hypothesized that inhaled nitric oxide administered to premature infants with severe respiratory failure would reduce the incidence of death or bronchopulmonary dysplasia.

METHODS

HYPOTHESES AND OUTCOMES

The primary hypothesis was that administration of inhaled nitric oxide to neonates at less than 34 weeks of gestation, with a birth weight of 401 to 1500 g, and with severe respiratory failure would reduce the incidence of bronchopulmonary dysplasia or death (defined as death before discharge to home or within 365 days among hospitalized infants). Severe respiratory failure was defined as an oxygenation index of 10 or more on two consecutive measurements of arterial blood gases between 30 minutes and 12 hours apart. We used the conventional definition of bronchopulmonary dysplasia — treatment with oxygen at 36 weeks of gestation.[16] The oxygenation index was calculated as 100 × the fraction of inspired oxygen × the mean airway pressure (in centimeters of water) ÷ the partial pressure of arterial oxygen (PaO_2) (in millimeters of mercury).

The secondary hypotheses were that inhaled nitric oxide would not increase the incidence of grade 3 or 4 intraventricular hemorrhage or periventricular leukomalacia, and that it would decrease the number of days of assisted ventilation and oxygen use, the length of hospitalization, and the incidence of threshold retinopathy of prematurity.[17] In addition to the conventional definition of bronchopulmonary dysplasia, we assessed the "physiological requirement" for oxygen at 36 weeks of gestation in infants not on mechanical ventilation and receiving less than 30 percent oxygen by performing a stepwise reduction in oxygen delivery to the lowest oxygen concentration at which the oxygen saturation measured by pulse oximetry remained at least 90 percent.[18] Infants who were unable to maintain a saturation of 90 percent or more while breathing room air were classified as requiring supplemental oxygen and therefore having "physiological bronchopulmonary dysplasia."

CRITERIA FOR ELIGIBILITY

Neonates who had been born at less than 34 weeks of gestation, according to the best obstetric estimate, had a birth weight of 401 to 1500 g, required assisted ventilation, and had a diagnosis of respiratory distress syndrome, sepsis or pneumonia, aspiration syndrome, idiopathic persistent pulmonary hypertension, or suspected pulmonary hypoplasia were eligible. Eligible infants had received one dose of surfactant at least 4 hours before meeting the respiratory criteria for entry and had an oxygenation index of at least 10 on two consecutive measurements of arterial blood gas between 30 minutes and 12 hours apart.

On the basis of pilot data collected in National Institute of Child Health and Human Development (NICHD) Neonatal Research Network centers, we estimated that the rate of mortality or bronchopulmonary dysplasia in infants identified by the oxygenation-index criterion would be 75 percent. At the first interim analysis of the data safety and monitoring committee, the mortality rate was significantly higher than expected in both treatment groups. The committee requested that the entry criteria be modified to select a cohort whose severity of illness as measured by the oxygenation index was more similar to that of the targeted cohort. Data from the NICHD Neonatal Research Network were analyzed and used to revise the respiratory criteria for entry to an oxygenation index of at least 5 followed by an oxygenation index of at least 7.5, with the second determination made 30 minutes to 24 hours after the first. Hence, for purposes of analysis, the design was considered to have two strata based on the oxygenation-index entry criterion. Infants who required an indwelling arterial line were eligible from 4 to 120 hours after birth.

Infants were ineligible if they had congenital heart disease other than ventricular septal defect,

atrial-level shunt, or patent ductus arteriosus; any major congenital abnormality involving the respiratory system; thrombocytopenia (a platelet count ≤50,000 per cubic millimeter); or bleeding diathesis or if a decision had been made not to provide full treatment. The study was approved by the institutional review board of each study center, and written informed consent was obtained from the parents or guardians of all infants.

Clinical care was not mandated by the protocol, but each center agreed to its own management guidelines to define its approach to mean arterial pressure, partial pressure of carbon dioxide, pH, surfactant replacement therapy, high-frequency ventilation, targets for lung inflation, paralysis, and the use of indomethacin, corticosteroids, bronchodilators, sedation, anesthesia, and analgesia for the duration of the trial.

STUDY DESIGN AND RANDOMIZATION

A dedicated telephone system developed by the data center stratified infants according to center and birth weight (401 to 750 g, 751 to 1000 g, and 1001 to 1500 g). Infants were randomly assigned within each stratum, according to a permuted-block design, to receive inhaled nitric oxide or placebo. Randomization, administration of the study gas, and safety monitoring were performed by designated, nonblinded persons not involved in clinical care. To maintain blinding, they made mock adjustments in the control infants, used a proprietary delivery and monitoring unit (INOvent, Datex-Ohmeda) with a specially designed gauge cover secured with a numbered tether (to keep track of when and by whom the unit had been opened), used an oxygen analyzer upstream of the site of administration of the study gas, and covered the downstream oxygen analyzer. A shroud secured with tamper-resistant tape was used to cover the tank label, and a screen was used to ensure blinding when the gauge cover was opened. All other research and clinical personnel were blinded to the treatment assignment.

ADMINISTRATION OF STUDY GAS

The study protocol was based on previous trials of inhaled nitric oxide performed by the Neonatal Research Network.[19,20] When a study candidate had an initial measurement of arterial blood gas with a qualifying oxygenation index, parental consent was obtained, and an unblinded respiratory therapist set up the delivery system and analyzer (INOvent, Datex-Ohmeda) according to the manufacturer's guidelines. When a second qualifying measurement of arterial blood gas was obtained, infants were randomly assigned to either 5 ppm inhaled nitric oxide (INOmax, INO Therapeutics) or simulated flow. Primary-grade nitric oxide was supplied in a concentration of 800 ppm in nitrogen certified to be within ±1 percent of the stated nitric oxide content and to contain less than 5 ppm of nitrogen dioxide. If the study gas could not be initiated within 15 minutes, an additional sample of arterial blood gas was drawn as a baseline measurement and used to calculate the response to the study gas.

Response to the study gas was defined by the change in the PaO_2 between the baseline measurement and the measurement at 30 minutes without any alterations in ventilator or oxygen settings. A complete response was an increase of more than 20 mm Hg, a partial response an increase of 10 to 20 mm Hg, and no response an increase of less than 10 mm Hg. When a complete response occurred, administration of the same concentration of study gas was continued. For infants with less than a complete response, the study gas was increased to 10 ppm of inhaled nitric oxide or simulated flow, and arterial blood gas was measured again 30 minutes later. Infants who had a complete or partial response to 10 ppm of inhaled nitric oxide continued to be given that concentration; the study gas was discontinued in infants with no response at this flow level. If the condition of the infant deteriorated during administration of the initiation dose of the study gas, administration was discontinued and stabilization of the patient was attempted by such means as adjustment of the ventilator settings or inotropic infusions. If the patient was successfully stabilized, initiation of the study gas was tried again. If treatment with the study gas at the initiation dose was again accompanied by complications, the patient was classified as not having a response, and the study gas was withdrawn.

Weaning of the infants from the study gas followed a defined protocol and occurred 10 to 14 hours after the treatment had been initiated. Weaning was attempted only when the PaO_2 was more than 50 mm Hg and the oxygen saturation measured by pulse oximetry was greater than 90 percent. For weaning, the concentration of nitric oxide in the inhaled gas (or the simulated flow) was reduced as follows: 10.0, 5.0, 4.0, 3.0, 2.0, 1.0, 0.5, 0.0 ppm. If the oxygenation index was 5 or less, weaning was attempted every four to eight hours. Successful weaning was defined as a decrease in

the PaO_2 of less than 20 mm Hg and to a value no lower than 50 mm Hg and oxygen saturation greater than 90 percent in the 30 minutes after the weaning attempt.

The dose of the study gas could be increased if two consecutive oxygenation indexes measured 30 minutes apart were at least 7.5. The study gas could be reinitiated if the original entry criteria were met and if no more than 72 hours had passed since the study gas was discontinued. The maximal duration of the administration of the study gas was 336 hours, and the dose could not exceed 1 ppm after 240 hours.

SAFETY MONITORING

Blood methemoglobin concentrations were measured within the first 3 hours after administration of the study gas, and then after 12 and 24 hours. While the infants were receiving nitric oxide at a concentration of more than 5 ppm, the sampling interval was every 24 hours, and while they were receiving a concentration of less than 5 ppm, the interval was every 48 hours. Methemoglobin levels of 4 percent or more were managed by reducing the concentration of study gas by half until the level fell below 4 percent. The study gas was discontinued if the methemoglobin concentration exceeded 10 percent.

Continuous inhaled nitrogen dioxide concentrations were monitored, and if they exceeded 3 ppm, the delivery system was immediately checked and infants were weaned from the study gas in 50 percent increments until the concentration was below 3 ppm. If the concentration exceeded 5 ppm, the nitric oxide cylinder was changed; the study gas was discontinued if nitrogen dioxide concentrations remained greater than 5 ppm. Cranial ultrasound scans were performed on all infants at 28±3 days.

STATISTICAL ANALYSIS

Assuming an incidence of death or bronchopulmonary dysplasia of 75 percent, we determined that 220 infants would be required in each group to provide the study with 90 percent power to detect a reduction in death or bronchopulmonary dysplasia of 20 percent in the group given inhaled nitric oxide. All tests were two-tailed, with an alpha level of 0.05. We conducted the primary analysis according to the intention-to-treat principle.

Differences between the treatment groups in baseline characteristics, status at randomization, and response to study gas were tested with the use of t-tests for continuous variables and chi-square tests for categorical variables. Differences in the primary and secondary outcomes were tested with the use of Poisson regression models for categorical variables and linear regression models for continuous variables. The models included birth-weight category, oxygenation-index stratum, center, and treatment group and were used to calculate the adjusted relative risks and 95 percent confidence intervals.[21] The post hoc analysis used the same model (when appropriate) as the primary analysis, and the interactions were tested by adding the relevant variables to the model.

The interim analyses of the data safety and monitoring committee were performed after one third and two thirds of the study patients had reached an end point of the study. The efficacy stopping rule for the study was based on the O'Brien–Fleming boundary, with three analyses of the data for the primary outcome one third of the way through the study, two thirds of the way through, and at the conclusion of the trial. The nominal significance level was 0.05, and corresponding P values for the looks were 0.005, 0.01, and 0.04, respectively.[22]

INO Therapeutics provided the study gas, gas delivery systems, and site monitoring for all hospitals and capitation funding for the hospitals outside the NICHD Neonatal Research Network. The company was otherwise not involved in the study design, data analysis and interpretation, or preparation of the manuscript.

RESULTS

RECRUITMENT

At the recommendation of the data safety and monitoring committee, the trial was terminated after the second planned interim analysis, with 294 (67 percent) of the enrolled infants having reached a study end point (death, discharge to home, or 365 days of age). At that time, the incidence of grade 3 or 4 intraventricular hemorrhage or periventricular leukomalacia was significantly higher in the group being given inhaled nitric oxide than in the control group, and there was no apparent beneficial effect of treatment on the primary outcome. Recruitment ceased with enrollment of 420 patients instead of the planned enrollment of 440.

BASELINE CHARACTERISTICS AND STATUS AT RANDOMIZATION

From January 4, 2001, to September 26, 2003, 420 infants were enrolled in the trial. There were no sig-

nificant differences between treatment groups in the baseline characteristics (Table 1) or status at the time of randomization (Table 2). The distribution by birth weight did not differ significantly between the two treatment groups, with an overall distribution of 47 percent in the infants who weighed 401 to 750 g, 28 percent in those who weighed 751 to 1000 g, and 25 percent in those who weighed 1001 to 1500 g. The mean (±SD) oxygenation index at randomization was 24.6±16.3 for the first oxygenation-index stratum, and 20.4±17.4 for the second stratum.

The baseline characteristics for eligible infants who did not undergo randomization were similar to those for enrolled infants. The reasons for not enrolling were refusal of the parent (31 percent); unavailability of the parent (5 percent); or consent not being sought because of the recommendation of the attending physician (17 percent), unavailability of equipment (9 percent), use of high-frequency jet ventilation (8 percent), or other reasons (30 percent).

Table 1. Baseline Characteristics of the Infants.*

Characteristic	Inhaled Nitric Oxide (N=210)	Placebo (N=210)
Birth weight — g	840±264	837±260
Gestational age — wk	26±2	26±2
Male sex — no. (%)	133 (63)	127 (60)
Mother's race or ethnic group — no. (%)†		
White	95 (45)	96 (46)
Black	69 (33)	78 (37)
Hispanic	36 (17)	32 (15)
Other	10 (5)	4 (2)
Born at study hospital — no. (%)	165 (79)	159 (76)
Prenatal corticosteroids — no. (%)‡	119 (70)	114 (67)
Delivery by cesarean section — no. (%)	144 (69)	139 (66)
Apgar scores <4 at 1 min — no. (%)‡	92 (55)	87 (52)
Apgar scores <4 at 5 min — no. (%)‡	27 (16)	22 (13)
Cause of respiratory failure — no. (%)		
Respiratory distress syndrome	192 (91)	190 (90)
Sepsis or pneumonia	6 (3)	10 (5)
Aspiration syndromes	1 (<1)	0
Idiopathic persistent pulmonary hypertension of the newborn	6 (3)	5 (2)
Suspected pulmonary hypoplasia	5 (2)	5 (2)

* Plus–minus values are means ±SD.
† Race or ethnic group was self-reported.
‡ Data were not available for all infants.

Table 2. Status of Infants at Randomization.*

Status	Inhaled Nitric Oxide (N=210)	Placebo (N=210)
Age — hr	26±23	28±22
Oxygenation index†	23±17	22±17
Surfactant — no. of doses given	2±1	2±1
Type of ventilation — no. (%)		
High-frequency oscillatory ventilation	116 (55)	116 (55)
High-frequency flow interruption	9 (4)	8 (4)
Conventional mechanical ventilation	85 (40)	86 (41)
Inotropic support — no. (%)	127 (60)	126 (60)
Sedation or analgesia — no. (%)	155 (74)	150 (71)
Paralytic agents — no. (%)	31 (15)	25 (12)
Postnatal corticosteroids — no. (%)	20 (10)	22 (10)
Pulmonary air leaks — no. (%)	26 (12)	31 (15)
Pulmonary hemorrhage — no. (%)	22 (10)	15 (7)
Seizures — no. (%)	8 (4)	6 (3)

* Plus–minus values are means ±SD.
† The oxygenation index was calculated as 100 × the fraction of inspired oxygen × mean airway pressure (in centimeters of water) ÷ the partial pressure of arterial oxygen (in millimeters of mercury).

PRIMARY OUTCOME

There was no difference between the incidence of the primary outcome (bronchopulmonary dysplasia or death) between the group given inhaled nitric oxide and the placebo group (80 percent vs. 82 percent; relative risk, 0.97; 95 percent confidence interval, 0.86 to 1.06; P=0.52) (Table 3). The rate of bronchopulmonary dysplasia was 60 percent in the group given inhaled nitric oxide and 68 percent in the placebo group (relative risk, 0.90; 95 percent confidence interval, 0.75 to 1.08; P=0.26), and the rate of death was 52 percent in the group given inhaled nitric oxide and 44 percent in the placebo group (relative risk, 1.16; 95 percent confidence interval, 0.96 to 1.39; P=0.11). There were no discernible differences between the group given inhaled nitric oxide and the placebo group for the following variables: age at death (20 vs. 24 days, P=0.54) or cause of death (respiratory failure, 49 percent vs. 42 percent; neurologic insult, 4 percent vs. 1 percent; infection, 5 percent vs. 10 percent; necrotizing enterocolitis, 8 percent vs. 2 percent; support withdrawn, 19 percent vs. 26 percent; or other, 16 percent vs. 19 percent; P=0.13 for the equality of the distribution between the two treatment groups).

SECONDARY OUTCOMES

The frequency of severe intraventricular hemorrhage or periventricular leukomalacia was not sig-

Table 3. Primary and Secondary Outcomes.*

Outcome	Inhaled Nitric Oxide (N=210)	Placebo (N=210)	Relative Risk (95% CI)†	P Value
Primary — no. (%)				
Death or bronchopulmonary dysplasia‡	167 (80)	170 (82)	0.97 (0.86–1.06)	0.52
Death	109 (52)	93 (44)	1.16 (0.96–1.39)	0.11
Bronchopulmonary dysplasia§	65 (60)	86 (68)	0.90 (0.75–1.08)	0.26
Secondary				
Grade 3 or 4 IVH or PVL — no. (%)¶	69 (39)	50 (32)	1.25 (0.95–1.66)	0.11
Oxygen use — days‖	84±63	91±61		0.91
Physiological bronchopulmonary dysplasia — no. (%)**	50 (50)	69 (60)	0.87 (0.68–1.10)	0.17
Length of hospitalization — days‖	101±47	111±48		0.65
Duration of ventilation — days‖	39±45	47±53		0.56
Incidence of air leak — no. (%)‖	35 (35)	37 (32)	1.12 (0.78–1.61)	0.55
Threshold retinopathy of prematurity — no. (%)††	29 (30)	36 (32)	1.16 (0.81–1.64)	0.42

* Plus–minus values are means ±SD. CI denotes confidence interval, IVH intraventricular hemorrhage, and PVL periventricular leukomalacia.
† Values were adjusted for center, birth-weight group, and oxygenation-index entry stratum.
‡ The outcome of death or bronchopulmonary dysplasia is for 208 infants in the placebo group.
§ This outcome is for infants who were alive at 36 weeks (109 in the group receiving inhaled nitric oxide and 127 in the placebo group).
¶ Results of ultrasound examinations of the head were available for 179 infants in the group receiving inhaled nitric oxide and for 155 in the placebo group.
‖ This outcome is for infants who survived (101 in the group receiving inhaled nitric oxide and 117 in the placebo group).
** This outcome was defined according to the protocol of Walsh et al.,[18] for 100 infants in the group receiving inhaled nitric oxide and for 115 infants in the placebo group.
†† Examination for retinopathy of prematurity was performed in 98 infants in the group receiving inhaled nitric oxide and 112 infants in the placebo group.

nificantly different between the group given inhaled nitric oxide and the placebo group according to concurrent local radiology readings (39 percent vs. 32 percent, respectively; relative risk, 1.25; 95 percent confidence interval, 0.95 to 1.66; P=0.11) (Table 3) or by central reading performed after the trial was terminated (37 percent vs. 38 percent; relative risk, 0.97; 95 percent confidence interval, 0.74 to 1.27; P=0.81). The local reading was based on the worst results of evaluation among ultrasound examinations of the head performed during the administration of the study gas, at 28±3 days, and after 28 days of age. Ultrasound examinations of the head were not available for 86 infants, 93 percent of whom had died. Death occurred by 14 days in 91 percent and before 28 days in 98 percent. The central reading was based on the worst results of evaluation among all ultrasound examinations of the head performed during hospitalization. There were no significant differences in the two treatment groups with respect to the days on oxygen, the length of assisted ventilation, the length of hospitalization, the incidence of air leak, threshold retinopathy of prematurity, or "physiological bronchopulmonary dysplasia" for survivors (Table 3).

Thirty minutes after administration of the study gas, at a concentration of 5 ppm, the group given inhaled nitric oxide had a significant increase in the PaO_2 and a significant decrease in the oxygenation index as compared with the placebo group (Table 4). The PaO_2 and the oxygenation index showed no significant change in either group when the concentration of the study gas was increased to 10 ppm. More than 70 percent of the infants in the placebo group did not have a response to the study gas; these infants had a significantly shorter length of time on the study gas (39 vs. 76 hours).

There were 26 deviations from the protocol. Five ineligible infants were randomly assigned to a study group. One infant received the wrong study gas. Four incidents of unblinding occurred. Sixteen infants received open-label inhaled nitric oxide: sev-

en after undergoing randomization to inhaled nitric oxide and nine after undergoing randomization to placebo.

SAFETY AND TOXICITY

In the group given inhaled nitric oxide, two infants had a methemoglobin level of at least 4 percent, and one had a level of at least 8 percent. In the placebo group, two infants had a methemoglobin level of at least 4 percent; neither received open-label inhaled nitric oxide. In the group given inhaled nitric oxide, nitrogen dioxide concentrations were at least 3 ppm in four infants and at least 5 ppm in two infants. No infants in the placebo group had elevated nitrogen dioxide concentrations (Table 4).

POST HOC ANALYSES

Post hoc analyses evaluated the relationship among birth weight (≤1000 g or >1000 g), mode of ventilation (high-frequency ventilation or conventional mechanical ventilation), severity of illness (as measured by a median oxygenation index >17 vs. ≤17) in terms of the primary outcomes, and the incidence of grade 3 or 4 intraventricular hemorrhage or periventricular leukomalacia (Table 5). The interaction between treatment assignment and birth weight had a significant effect on death (P=0.02) as well as on death or bronchopulmonary dysplasia (P=0.02).

Infants with a birth weight above 1000 g who were treated with inhaled nitric oxide had a significantly lower rate of death or bronchopulmonary dysplasia than infants in the placebo group (50 percent vs. 69 percent; relative risk, 0.72; 95 percent confidence interval, 0.54 to 0.96; P=0.03). Infants with a weight of 1000 g or less who were treated with inhaled nitric oxide, as compared with those in the placebo group, had higher mortality (62 percent vs. 48 percent; relative risk, 1.28; 95 percent confidence interval, 1.06 to 1.54; P=0.01) and a higher rate of severe intraventricular hemorrhage (43 percent vs. 33 percent; relative risk, 1.40; 95 percent confidence interval, 1.03 to 1.88; P=0.03).

The interaction between treatment group and type of ventilation had a significant effect on mortality (P=0.03). Infants receiving inhaled nitric oxide by means of conventional mechanical ventilation had a significantly increased rate of death as compared with infants receiving placebo by means of conventional mechanical ventilation (62 percent vs. 40 percent; relative risk, 1.46; 95 percent confidence interval, 1.10 to 1.92; P=0.01). Infants given inhaled nitric oxide as compared with those given placebo by means of conventional mechanical ventilation had similar oxygenation indexes at randomization (22.6±19.2 vs. 17.6±14.1, P=0.06) and similar birth weights (814±255 g vs. 853±267 g, P=0.33). The interaction between the treatment group and the oxygenation index was not significant.

Table 4. Response to Study Gas.*

Variable	Inhaled Nitric Oxide (N=210)	Placebo (N=210)	P Value
Response to concentrations of 5 ppm — no.	208	204	
Increase in PaO_2 — no. (%)			<0.001
<10 mm Hg	60 (29)	151 (74)	
10–20 mm Hg	30 (14)	18 (9)	
>20 mm Hg	118 (57)	35 (17)	
Change in PaO_2 — mm Hg	57±88	8±53	<0.001
Change in oxygenation index	−8±13	1±17	<0.001
Response to concentrations of 10 ppm — no.	86	152	
Increase in PaO_2 — no. (%)			0.24
<10 mm Hg	53 (62)	109 (72)	
10–20 mm Hg	18 (21)	26 (17)	
>20 mm Hg	15 (17)	17 (11)	
Change in PaO_2 — mm Hg	8±35	10±37	0.68
Change in oxygenation index	−3±15	−1±11	0.29
Duration of administration of study gas — hr†	76±73	39±65	<0.001
Methemoglobin level ≥4% — no. (%)	2 (1)	2 (1)	0.99
Methemoglobin level ≥8% — no. (%)	1 (<1)	0	0.99
Nitrogen dioxide concentration ≥3.0 ppm — no. (%)	4 (2)	0	0.13
Nitrogen dioxide concentration ≥5.0 ppm — no. (%)	2 (1)	0	0.50

* Plus–minus values are means ±SD. PaO_2 denotes the partial pressure of arterial oxygen.
† The duration was calculated only in infants with a response.

DISCUSSION

We found that the administration of inhaled nitric oxide as used in this trial for premature infants with severe respiratory failure did not reduce the combined incidence of death or bronchopulmonary dysplasia. There were no significant differences in the secondary outcomes.

Previous randomized trials of the use of inhaled

Table 5. Post Hoc Analysis According to Birth Weight, Type of Ventilation, and Oxygenation Index.*

Variable	Inhaled Nitric Oxide	Placebo	Relative Risk (95% CI)†	P Value
	no. (%)			
Birth weight				
≤1000 g	158	158		
Death or bronchopulmonary dysplasia	141 (89)	133 (85)	1.04 (0.96–1.13)	0.29
Death	98 (62)	76 (48)	1.28 (1.06–1.54)	0.01
Bronchopulmonary dysplasia	49 (73)	65 (73)	1.02 (0.85–1.23)	0.84
Grade 3 or 4 IVH or PVL	55 (43)	39 (33)	1.40 (1.03–1.88)	0.03
>1000 g	52	52		
Death or bronchopulmonary dysplasia	26 (50)	35 (69)	0.72 (0.54–0.96)	0.03
Death	11 (21)	17 (33)	0.65 (0.36–1.18)	0.16
Bronchopulmonary dysplasia	16 (38)	21 (57)	0.68 (0.45–1.05)	0.08
Grade 3 or 4 IVH or PVL	14 (27)	11 (30)	0.95 (0.53–1.69)	0.86
Type of ventilation				
Conventional mechanical ventilation	85	86		
Death or bronchopulmonary dysplasia	69 (81)	63 (74)	1.04 (0.91–1.19)	0.55
Death	53 (62)	34 (40)	1.46 (1.10–1.92)	0.01
Bronchopulmonary dysplasia	17 (52)	33 (60)	0.90 (0.65–1.24)	0.53
Grade 3 or 4 IVH or PVL	29 (43)	24 (36)	1.20 (0.80–1.78)	0.37
High-frequency ventilation	125	124		
Death or bronchopulmonary dysplasia	98 (78)	105 (85)	0.93 (0.84–1.04)	0.21
Death	56 (45)	59 (48)	0.96 (0.75–1.24)	0.75
Bronchopulmonary dysplasia	48 (63)	53 (75)	0.89 (0.72–1.10)	0.29
Grade 3 or 4 IVH or PVL	40 (36)	26 (30)	1.41 (0.96–2.08)	0.08
Oxygenation index				
≤17	100	110		
Death or bronchopulmonary dysplasia	71 (71)	83 (75)	0.93 (0.81–1.08)	0.37
Death	45 (45)	40 (36)	1.27 (0.96–1.68)	0.09
Bronchopulmonary dysplasia	30 (51)	50 (66)	0.80 (0.61–1.06)	0.12
Grade 3 or 4 IVH or PVL	30 (33)	27 (30)	1.18 (0.79–1.76)	0.42
>17	110	100		
Death or bronchopulmonary dysplasia	96 (87)	85 (86)	1.02 (0.92–1.12)	0.75
Death	64 (58)	53 (53)	1.11 (0.88–1.40)	0.39
Bronchopulmonary dysplasia	35 (70)	36 (72)	0.98 (0.77–1.24)	0.85
Grade 3 or 4 IVH or PVL	39 (45)	23 (35)	1.38 (0.97–1.96)	0.07

* Data were not available for all infants in the categories of bronchopulmonary dysplasia and grade 3 or 4 intraventricular hemorrhage (IVH) or periventricular leukomalacia (PVL). Some infants had bronchopulmonary dysplasia and died. CI denotes confidence interval.

† Values were adjusted for center, birth-weight group, and oxygenation-index stratum.

nitric oxide in premature infants have shown varied results. In a trial of 80 premature infants with severe hypoxemic respiratory failure, Kinsella et al. reported a decrease in the number of days on a ventilator and a trend toward a decreased incidence of bronchopulmonary dysplasia.[10] The Franco–Belgian randomized trial of inhaled nitric oxide showed no significant decrease in bronchopulmonary dysplasia or death in a cohort of premature infants with a median oxygenation index of approximately 20.[11,12] A recent trial by Schreiber et al. studied a less critically ill cohort with a median oxy-

genation index of 6.94 on mechanical ventilation after the use of surfactant.[15] A significant decrease in bronchopulmonary dysplasia or death and severe intracranial hemorrhage was reported, but the benefit was confined to the cohort with an oxygenation index below 6.94.

The results of our trial may seem to be inconsistent with those of the trial of Schreiber et al. However, the infants enrolled in our trial were smaller and sicker than those in the trial of Schreiber et al. Benefit in the Schreiber trial was limited to infants with an oxygenation index below 6.94, but only 17 infants in our trial had an oxygenation index in this range. Benefit (i.e., decreased bronchopulmonary dysplasia or death) in our trial was evident only in infants with a birth weight above 1000 g (Table 5), and the patients in the trial of Schreiber et al. had significantly higher birth weights than those in our trial. Their trial also had a larger proportion of black infants, but our analysis did not reveal a significant effect of race on responses to inhaled nitric oxide.

The rate of intraventricular hemorrhage has been a concern in trials of preterm infants given inhaled nitric oxide, because nitric oxide is known to inhibit platelet aggregation and increase bleeding time.[23-28] Two small pilot studies of the use of inhaled nitric oxide showed high rates of intraventricular hemorrhage,[29,30] but larger randomized controlled trials have not confirmed those findings.[10-15] Review of our data on intraventricular hemorrhage at the second planned interim analysis showed an increased rate of severe intraventricular hemorrhage or periventricular leukomalacia in the group given inhaled nitric oxide as compared with the placebo group (39 percent vs. 27 percent, P=0.02), but this difference was not significant at the conclusion of the trial. Severe intraventricular hemorrhage or periventricular leukomalacia as the cause of death was not significantly higher in the group given inhaled nitric oxide, although our trial was not adequately powered to address this question.

Post hoc analyses suggested hypotheses that deserve further study. Infants with a birth weight above 1000 g seemed to benefit from inhaled nitric oxide therapy, with a decrease in the incidence of death or bronchopulmonary dysplasia without any increase in the rate of intraventricular hemorrhage. In contrast, infants with a birth weight of 1000 g or less who were treated with inhaled nitric oxide had an apparent increase in mortality and a higher rate of intraventricular hemorrhage. Infants receiving inhaled nitric oxide by means of conventional mechanical ventilation also seemed to have higher rates of death than those receiving inhaled nitric oxide by means of high-frequency ventilation. The article by Mestan et al. in this issue of the *Journal*[31] documents improved neurodevelopmental outcome at 2 years of age in infants who received inhaled nitric oxide; neurodevelopmental follow-up at 18 to 22 months of age for the infants enrolled in our trial is in progress.

In conclusion, the use of inhaled nitric oxide in premature infants who had a birth weight of less than 1500 g and severe respiratory failure did not result in a decrease in the rate of death or bronchopulmonary dysplasia. The use of inhaled nitric oxide in premature infants born at less than 34 weeks of gestation should be confined to clinical trials until those who benefit can be identified.[32]

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APPENDIX

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ORIGINAL ARTICLE

Neurodevelopmental Outcomes of Premature Infants Treated with Inhaled Nitric Oxide

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ABSTRACT

BACKGROUND

Chronic lung disease and severe intraventricular hemorrhage or periventricular leukomalacia in premature infants are associated with abnormal neurodevelopmental outcomes. In a previous randomized, controlled, single-center trial of premature infants with the respiratory distress syndrome, inhaled nitric oxide decreased the risk of death or chronic lung disease as well as severe intraventricular hemorrhage and periventricular leukomalacia. We hypothesized that infants treated with inhaled nitric oxide would also have improved neurodevelopmental outcomes.

METHODS

We conducted a prospective, longitudinal follow-up study of premature infants who had received inhaled nitric oxide or placebo to investigate neurodevelopmental outcomes at two years of corrected age. Neurologic examination, neurodevelopmental assessment, and anthropometric measurements were made by examiners who were unaware of the children's original treatment assignment.

RESULTS

A total of 138 children (82 percent of survivors) were evaluated. In the group given inhaled nitric oxide, 17 of 70 children (24 percent) had abnormal neurodevelopmental outcomes, defined as either disability (cerebral palsy, bilateral blindness, or bilateral hearing loss) or delay (no disability, but one score of less than 70 on the Bayley Scales of Infant Development II), as compared with 31 of 68 children (46 percent) in the placebo group (relative risk, 0.53; 95 percent confidence interval, 0.33 to 0.87; P=0.01). This effect persisted after adjustment for birth weight and sex, as well as for the presence or absence of chronic lung disease and severe intraventricular hemorrhage or periventricular leukomalacia. The improvement in neurodevelopmental outcome in the group given inhaled nitric oxide was primarily due to a 47 percent decrease in the risk of cognitive impairment (defined by a score of less than 70 on the Bayley Mental Developmental Index) (P=0.03).

CONCLUSIONS

Premature infants treated with inhaled nitric oxide have improved neurodevelopmental outcomes at two years of age.

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Over the past decade, advances in neonatal–perinatal medicine have resulted in increased survival rates among premature infants.[1] Despite therapies such as antenatal corticosteroids and surfactant replacement, however, the rates of birth-weight–specific abnormal neurodevelopmental outcomes have remained constant.[2,3] Other therapies, such as postnatal corticosteroids,[4] have been associated with abnormal neurodevelopment. The disabling neurologic conditions — cerebral palsy, hearing loss, and blindness — adversely affect the long-term neurodevelopmental outcomes among premature infants. However, even premature infants who have relatively uncomplicated neonatal courses are at substantial risk for developmental delays in cognition and motor skills.[5]

In a double-blind, randomized, placebo-controlled, single-center trial, we demonstrated that inhaled nitric oxide significantly decreased the incidence of chronic lung disease and death by 24 percent in premature infants with the respiratory distress syndrome.[6] Inhaled nitric oxide also decreased the incidence of severe intraventricular hemorrhage and periventricular leukomalacia by 47 percent. Because these pulmonary and neurologic conditions are associated with abnormal neurodevelopmental outcomes, we hypothesized that premature infants who had received inhaled nitric oxide would have improved neurodevelopmental outcomes. Accordingly, we prospectively studied the neurodevelopmental outcomes among this cohort of infants at two years of age.

METHODS

PRIMARY OUTCOME

This study was a prospective, longitudinal follow-up of the neurodevelopmental outcomes among premature infants who were randomly assigned to receive inhaled nitric oxide or placebo. The primary outcome, as specified in the protocol for this follow-up study, was an abnormal neurodevelopmental outcome at two years of age. We used three neurodevelopmental outcomes[7]: disability, defined as cerebral palsy, bilateral blindness, or bilateral hearing loss; delay, defined by a score on the Mental or Psychomotor Developmental Index of the Bayley Scales of Infant Development II that was at least 2 SD below the mean (i.e., less than 70) without disability; and normal, defined by the absence of both disability and delay. An abnormal neurodevelopmental outcome was defined as either disability or delay. The study was approved by the institutional review board of the University of Chicago. Written informed consent was obtained from the children's parents.

INITIAL STUDY

The initial study, conducted at the University of Chicago from October 1998 to October 2001, was a single-center, randomized, placebo-controlled trial of 207 premature infants requiring mechanical ventilation and surfactant-replacement therapy (mean [±SD] gestational age, 27.2±2.7 weeks). Treatment with inhaled nitric oxide (INO Therapeutics) was initiated at a dose of 10 ppm for the first day, followed by a dose of 5 ppm for six days or until extubation. A total of 105 patients received inhaled nitric oxide, and 102 patients received placebo. Infants were also randomly assigned to receive intermittent mandatory ventilation or high-frequency oscillatory ventilation.

FOLLOW-UP

Close contact with the original cohort was maintained, and children were scheduled for evaluation at one and two years of age, corrected for prematurity. Data for the present study were collected at two years of corrected age. Patients were evaluated at the University of Chicago Neonatal High-Risk Follow-up Clinic. Medical records, including visual and hearing examinations, were reviewed. Maternal race or ethnic group was self-reported, as recorded in the maternal medical record. Socioeconomic status was determined by interviewing the mothers at follow-up. The children's height, weight, and head circumference were measured. A physical examination was performed by a pediatrician, a neurologic examination was performed by a pediatric neurologist, and infant development was assessed by a certified neonatal occupational therapist, dedicated to the High-Risk Clinic. Infant development was assessed with the use of the Bayley Scales of Infant Development II Mental and Psychomotor Developmental Indexes,[8] adjusted for prematurity. Examiners and parents were unaware of the patients' treatment assignments in the initial study.

Patients were screened for cerebral palsy with the use of the following criteria[9]: abnormalities in neuromotor tone, deep tendon reflexes, and either coordination or movement; delay in motor milestones; and abnormalities in primitive reflexes and postural reactions. Patients with spastic hemiple-

gia, diplegia, hemidiplegia, or quadriplegia received a diagnosis of cerebral palsy.[10]

Patients were screened for visual impairment by means of a parental questionnaire, and the diagnosis was confirmed by a pediatric ophthalmologist. Blindness was defined as a corrected visual acuity worse than 20/200. Hearing loss was defined as impairment requiring a hearing aid.

The proportions of children in the two groups who had cognitive impairment (defined by a score of less than 70 on the Bayley Mental Developmental Index), performance impairment (defined by a score of less than 70 on the Bayley Psychomotor Developmental Index), or both were compared. However, since the Bayley Scales of Infant Development II system does not assign numerical values for scores of less than 50, the proportion of children in each group with scores of less than 50 was also calculated.

STATISTICAL ANALYSIS

Clinical and demographic characteristics of the study groups were compared by means of Fisher's exact tests for categorical variables and two-sample t-tests or Wilcoxon rank-sum tests for continuous variables. To control for sex and corrected age in the analysis of anthropometric measures, z scores were generated with the use of the Centers for Disease Control and Prevention (CDC) growth charts.[11]

The primary outcome was analyzed with the use of a generalized linear model with a logarithmic link (log-binomial model) to obtain relative risks and corresponding 95 percent confidence intervals. Using this model, we calculated adjusted relative risks, controlling individually for potential confounders: birth weight, sex, and (as measures of socioeconomic status) whether the mother had graduated from high school and the presence or absence of any employed person in the household. Adjustments were also made for the presence or absence of prolonged (more than seven days) postnatal exposure to corticosteroids, the type of ventilation used (high-frequency oscillatory or intermittent mechanical), and the initial oxygenation index, calculated by means of the following equation: 100 × the fraction of inspired oxygen × mean airway pressure (in centimeters of water) ÷ partial pressure of arterial oxygen (in millimeters of mercury). Because of the relatively few events in some subgroups, it was impossible to adjust for all potential confounders simultaneously in the multivariate analysis. Therefore, we controlled only for the confounders found to be significant predictors of neurodevelopmental outcome: birth weight and sex.

To better characterize factors mediating the primary outcome, we adjusted the primary outcome separately and simultaneously for the potential intermediate variables of the presence or absence of chronic lung disease and intraventricular hemorrhage or periventricular leukomalacia. In cases in which the log-binomial model did not converge, a modified Poisson regression was fitted.[12] All analyses were prespecified, unless noted as post hoc. All P values are two-sided.

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RESULTS

PATIENT DISPOSITION

Of the 207 infants enrolled in the initial study, 168 were alive at two years of age (89 in the group given inhaled nitric oxide and 79 in the placebo group). A total of 29 infants were lost to follow-up: 13 families could not be reached, and 16 families were contacted but did not come to the clinic. The 29 infants lost to follow-up had greater birth weights (1255±453 g vs. 994±360 g, P<0.001) and gestational ages (28.6±3.0 weeks vs. 27.4±2.5 weeks, P=0.03) than those who were included in the follow-up study.

From January 2000 to February 2004, 139 patients from the initial study were evaluated. Three patients underwent neurologic examinations but not Bayley testing. Two of the three patients were identified as having cerebral palsy, categorized as disabled, and included in the analysis. The third patient (who had received inhaled nitric oxide) was identified as not having cerebral palsy but was excluded from the analysis, since it could not be determined whether the child had normal or delayed neurodevelopment. Accordingly, 138 patients (70 who had received inhaled nitric oxide and 68 who had received placebo; 82 percent of survivors) were analyzed.

CHARACTERISTICS OF THE FOLLOW-UP COHORT

In the follow-up cohort, 89 of the 168 patients who were alive at two years of age (53 percent) had received inhaled nitric oxide, similar to the proportion of patients enrolled in the original study (51

percent). The baseline characteristics of the infants at the time of enrollment in the initial study did not differ significantly between study groups (Table 1). In contrast, the group given inhaled nitric oxide had a decreased incidence of severe intraventricular hemorrhage or periventricular leukomalacia (P=0.02) (Table 1). There was no significant difference in the mean corrected age at follow-up (24.9±7.9 months in the group given inhaled nitric oxide and 25.2±8.4 months in the placebo group, P=0.84). Maternal characteristics did not differ significantly in terms of important factors

Table 1. Characteristics of the Follow-up Cohort and Their Mothers.*

Characteristic	Inhaled Nitric Oxide (N=70)	Placebo (N=68)	P Value
Infants			
Birth weight — g	1026±366	958±356	0.27
Gestational age — wk	27.5±2.4	27.2±2.6	0.50
Corrected age at follow-up — mo	24.9±7.9	25.2±8.4	0.84
Male sex — no. (%)	39 (56)	33 (49)	0.50
Initial oxygenation index†			
Median	6.6	7.2	0.37
Interquartile range	4.0–11.5	4.5–14.3	
1-Minute Apgar score			
Median	5	5	0.65
Interquartile range	3–6	3–6	
5-Minute Apgar score			
Median	7	7	0.54
Interquartile range	7–8	6–8	
Antenatal corticosteroids — no./total no.(%)	40/69 (58)	38/67 (57)	1.0
Surfactant — no. of doses	2.2±0.9	2.3±1.0	0.44
Prolonged postnatal exposure to corticosteroids — no. (%)‡	6 (9)	6 (9)	1.0
At end of original study — no. (%)‡			
Chronic lung disease§	27 (39)	37 (54)	0.09
Severe intraventricular hemorrhage or periventricular leukomalacia¶	6 (9)	16 (24)	0.02
Household			
Mother has less than high-school education — no./total no. (%)	11/66 (17)	9/62 (15)	0.81
Single parent — no./total no. (%)	35/66 (53)	26/65 (40)	0.16
Foster care — no./total no. (%)	6/70 (9)	6/68 (9)	1.0
Household without an employed person — no./total no. (%)	18/67 (27)	12/68 (18)	0.30
Maternal race or ethnic group — no./total no. (%)‖			
Black	44/70 (63)	52/68 (76)	0.23
White	14/70 (20)	8/68 (12)	
Other	12/70 (17)	8/68 (12)	

* Plus–minus values are means ±SD.
† The initial oxygenation index was calculated by means of the following equation: 100 × the fractional inspiratory oxygen concentration × mean airway pressure (in centimeters of water) ÷ partial pressure of arterial oxygen in millimeters of mercury).
‡ Prolonged exposure was defined as more than seven days.
§ Chronic lung disease was defined as the need for supplemental oxygen at 36 weeks' postmenstrual age plus abnormal findings on chest radiography.
¶ Severe intraventricular hemorrhage or periventricular leukomalacia was defined by a Papile grade of III or IV.[13]
‖ Race or ethnic group was self-reported.

known to influence neurodevelopmental outcomes, including the level of education, marital status, employment status, and race or ethnic group.[5]

NEURODEVELOPMENTAL OUTCOMES

Patients treated with inhaled nitric oxide had approximately half the risk of having an abnormal neurodevelopmental outcome as those in the placebo group (relative risk, 0.53; 95 percent confidence interval, 0.33 to 0.87; P=0.01). Eight patients (12 percent) in the placebo group were identified as disabled, as compared with six patients (9 percent) in the group given inhaled nitric oxide. Of the 14 disabled patients, 1 (in the placebo group) had isolated hearing loss. The remainder had cerebral palsy, and two of these (in the placebo group) were also blind. All six of the patients with cerebral palsy in the group given inhaled nitric oxide were diplegic. In the placebo group, two were diplegic, four hemiplegic, and one quadriplegic. The percentage of patients categorized as having developmental delays was higher in the placebo group than in the group given inhaled nitric oxide (34 percent vs. 16 percent).

We also assessed associations between the neurodevelopmental outcome and other factors, including birth weight, sex, whether the mother had graduated from high school, and the presence or absence of any employed person in the household. Lower birth weight and male sex were associated with an increased risk of an abnormal neurodevelopmental outcome, but low socioeconomic status was not (Table 2). We also considered relationships between the primary outcome and the type of ven-

Table 2. Risk Factors for the Primary Outcome of Abnormal Neurodevelopment at Two Years of Age.

Risk Factor	Neurodevelopmental Outcome		Relative Risk (95% CI)*	P Value
	Normal (N=90)	Abnormal (N=48)		
Birth weight				
Mean — g	1047±374	891±316		
Per 100-g increment			0.91 (0.85–0.99)	0.02
Sex — no./total no. (%)				
Male	39/72 (54)	33/72 (46)	2.02 (1.21–3.36)	0.007
Female	51/66 (77)	15/66 (23)	1.00	
Maternal education — no./total no. (%)				
Less than high school	15/20 (75)	5/20 (25)	0.73 (0.33–1.63)	0.61
High school or higher	71/108 (66)	37/108 (34)	1.00	
Household without an employed person — no./total no. (%)				
Yes	19/30 (63)	11/30 (37)	1.17 (0.50–2.74)	0.83
No	69/103 (67)	34/103 (33)	1.00	
Type of ventilation — no./total no. (%)				
High-frequency oscillatory	44/66 (67)	22/66 (33)	0.92 (0.58–1.46)	0.86
Intermittent mechanical	46/74 (62)	26/74 (35)	1.00	
Prolonged postnatal exposure to corticosteroids — no./total no. (%)				
Yes	8/12 (67)	4/12 (33)	0.94 (0.41–2.16)	1.0
No	80/124 (65)	44/124 (35)	1.00	
Chronic lung disease — no./total no. (%)				
Yes	33/64 (52)	31/64 (48)	2.11 (1.29–3.43)	0.002
No	57/74 (77)	17/74 (23)	1.00	
Severe intraventricular hemorrhage or periventricular leukomalacia — no./total no. (%)				
Yes	10/22 (45)	12/22 (55)	1.76 (1.10–2.81)	0.05
No	80/116 (69)	36/116 (31)	1.00	

* CI denotes confidence interval.

tilation used and the presence of prolonged postnatal exposure to corticosteroids (more than seven days), chronic lung disease, and severe intraventricular hemorrhage or periventricular leukomalacia. Of these factors, only chronic lung disease and severe intraventricular hemorrhage and periventricular leukomalacia were associated with an increased risk of an abnormal neurodevelopmental outcome (Table 2).

Because other factors contributed to an abnormal neurodevelopmental outcome, we assessed the extent to which these factors accounted for the significant effect of inhaled nitric oxide. We first adjusted the relative risk of the primary outcome for the confounding factors of birth weight, sex, whether the mother had graduated from high school, the presence or absence of any employed person in the household, type of ventilation, and whether there was prolonged postnatal exposure to corticosteroids. After adjustment for each of these factors separately, the risk of an abnormal neurodevelopmental outcome remained essentially unchanged among patients treated with inhaled nitric oxide as compared with those in the placebo group (Table 3).

Table 3. Primary Outcome Adjusted for Potential Confounders and Intermediate Variables.

Potential Confounders and Intermediate Variables	Relative Risk of Abnormal Neurodevelopmental Outcome (95% CI)*	P Value
None	0.53 (0.33–0.87)	0.01
Potential confounders		
Birth weight	0.57 (0.35–0.93)	0.02
Sex	0.52 (0.32–0.82)	0.006
Mother's graduation from high school	0.48 (0.28–0.82)	0.007
Household without an employed person	0.49 (0.29–0.82)	0.006
Type of ventilation	0.53 (0.33–0.87)	0.01
Prolonged postnatal exposure to corticosteroids	0.53 (0.33–0.87)	0.01
Simultaneous adjustment for birth weight and sex	0.55 (0.35–0.88)	0.01
Potential intermediate variables		
Severe intraventricular hemorrhage or periventricular leukomalacia	0.55 (0.34–0.89)	0.01
Chronic lung disease	0.59 (0.36–0.95)	0.03
Simultaneous adjustment for chronic lung disease and severe intraventricular hemorrhage or periventricular leukomalacia	0.60 (0.38–0.96)	0.03

* CI denotes confidence interval.

Furthermore, in a post hoc analysis simultaneously adjusting for both birth weight and sex, the risk of an abnormal neurodevelopmental outcome remained essentially unchanged (Table 3).

Inhaled nitric oxide may have decreased the incidence of an abnormal neurodevelopmental outcome through its effects on the incidences of chronic lung disease and severe intraventricular hemorrhage or periventricular leukomalacia. However, the decreased risk of an abnormal neurodevelopmental outcome persisted after separate adjustment for these intermediate variables (Table 3). In addition, simultaneous, post hoc adjustment for the presence of chronic lung disease and severe intraventricular hemorrhage or periventricular leukomalacia minimally attenuated the relative risk in the group given inhaled nitric oxide, as compared with the placebo group (0.60 vs. 0.53), indicating that a reduced risk of chronic lung disease, severe intraventricular hemorrhage or periventricular leukomalacia, or both outcomes does not fully explain the effect of inhaled nitric oxide on neurodevelopmental outcome. Because of the relatively small size of our cohort and small number of events, simultaneous adjustment for all four significant confounding and intermediate variables was not possible.

In the original study, inhaled nitric oxide preferentially decreased the incidence of chronic lung disease and death among premature infants having oxygenation indexes below the median of 6.94. The median oxygenation index in the follow-up cohort was 6.8. For the comparison of the group given inhaled nitric oxide with the placebo group, the relative risk of an abnormal neurodevelopmental outcome was 0.52 (95 percent confidence interval, 0.26 to 1.01) in the group with an initial oxygenation index of less than 6.94 and 0.38 (95 percent confidence interval, 0.16 to 0.93) for those with an index of 6.94 or greater. However, the interaction between the initial oxygenation index and the primary outcome in this post hoc analysis was not significant (P=0.59).

BAYLEY SCORES

Because we observed few cases of disability and the distribution of these cases did not differ significantly between groups, inhaled nitric oxide may have improved neurodevelopmental outcome solely through a decrease in neurodevelopmental delay. However, in this study, a diagnosis of neurodevelopmental delay excluded children with cerebral palsy. Thus, we performed a post hoc analysis to

compare Bayley scores of all children regardless of disability diagnosis (Table 4). As compared with the placebo group, the group given inhaled nitric oxide had approximately half the risk of either cognitive impairment (defined by a score of less than 70 on the Mental Developmental Index) or performance impairment (defined by a score of less than 70 on the Psychomotor Developmental Index), or both (relative risk, 0.55; 95 percent confidence interval, 0.33 to 0.93; P=0.03). This difference was the result of a decreased incidence of cognitive impairment in the group given inhaled nitric oxide, as compared with the placebo group (relative risk, 0.53; 95 percent confidence interval, 0.29 to 0.94; P=0.03). The risk of performance impairment did not differ significantly between groups (relative risk, 0.73; 95 percent confidence interval, 0.33 to 1.61; P=0.48). The distributions of scores for the Mental and Psychomotor Developmental Indexes in the group given inhaled nitric oxide and the placebo group are shown in Figure 1.

ANTHROPOMETRIC MEASUREMENTS

Median height and head circumference were similar in the two groups, as were their respective CDC-referenced, median z scores (Table 4). In contrast, the group given inhaled nitric oxide had a significantly higher median weight than the placebo group

Table 4. Neurodevelopmental and Anthropometric Outcomes.

Outcome	Inhaled Nitric Oxide (N=70)	Placebo (N=68)	P Value
Abnormal neurodevelopmental outcome	17 (24)	31 (46)	0.01
Disability — no. (%)*	6 (9)	8 (12)	
Cerebral palsy	6 (9)	7 (10)	0.78
Blindness	0	2 (3)	0.24
Hearing loss	0	1 (1)	0.49
Delay without disability — no. (%)	11 (16)	23 (34)	
Bayley scores†			
Mental Developmental Index score <70 — no. (%)	13 (19)	24 (36)	0.03
Psychomotor Developmental Index score <70 — no. (%)	9 (13)	12 (18)	0.48
Either score <70 — no. (%)	16 (23)	28 (42)	0.03
Both scores <70 — no. (%)	6 (9)	8 (12)	0.58
Anthropometric measures			
Height			
Median — cm	84.5	83.9	0.55
Interquartile range — cm	81.2 to 88.5	81 to 88.3	
z Score for height for age‡	−0.23	−0.59	0.32
Interquartile range for z score	−0.83 to 0.36	−1.25 to 0.41	
Weight			
Median — kg	11.7	10.8	0.04
Interquartile range — kg	10.5 to 13.5	9.5 to 12.2	
z Score for weight for age‡	−0.49	−1.07	0.02
Interquartile range for z score	−1.51 to 0.61	−2.25 to −0.38	
Head circumference			
Median — cm	47.4	47.5	1.0
Interquartile range — cm	46 to 49	46 to 49	
z Score for head circumference for age‡	−0.33	−0.48	0.73
Interquartile range for z score	−1.20 to 0.64	−1.45 to 0.65	

* Some patients had more than one disability.
† Two patients (one in each group) did not undergo Bayley testing.
‡ A z score is the deviation from the mean value of the sex-specific and age-specific reference population, divided by the standard deviation for the reference population. It was generated with the use of the Centers for Disease Control and Prevention growth charts.

(P=0.04). Furthermore, the group given inhaled nitric oxide had significantly higher age- and sex-adjusted z scores for weight (P=0.02) (Table 4).

DISCUSSION

In this follow-up study of premature infants who were randomly assigned at birth to receive inhaled nitric oxide or placebo for seven days, patients treated with inhaled nitric oxide had a significantly lower risk of an abnormal neurodevelopmental outcome at two years of age. Increased birth weight and female sex also reduced the risk of an abnormal neurodevelopmental outcome. However, the beneficial effect of inhaled nitric oxide persisted after simultaneous adjustment for these confounding variables.

We have previously shown that inhaled nitric oxide decreases the risk of chronic lung disease and death, as well as of severe intraventricular hemorrhage or periventricular leukomalacia.[6] These conditions were also risk factors for an abnormal neurodevelopmental outcome. However, the effect of inhaled nitric oxide persisted after simultaneous adjustment for these potential intermediate variables. This observation indicates that the beneficial effect of inhaled nitric oxide on neurodevelopment is not simply explained by effects on chronic lung disease and severe intraventricular hemorrhage or periventricular leukomalacia.

The population for which we report the incidence of abnormal neurodevelopmental outcomes and cerebral palsy includes only infants who required mechanical ventilation and surfactant-replacement therapy during the newborn period. Consequently, comparison of these incidences with those in other reports is difficult, since these incidences are generally reported for all premature newborn infants, and only about 50 percent of infants weighing less than 1500 g at birth require mechanical ventilation and surfactant replacement[14]; the rates of these neurologic sequelae are considerably lower in premature infants who do not require such treatments.[15,16] Nonetheless, the rate of an abnormal neurodevelopmental outcome in our control group is similar to disability rates previously reported among children with very low birth weights and extremely low birth weights.[17] The incidence of cerebral palsy in our control group is also similar to previously reported rates.[5,10,18]

In our original study, the effect of inhaled nitric oxide on the combined incidence of chronic lung disease and death was confined to infants with initial oxygenation indexes that were less than the median. In contrast, our present data did not show a significant effect of the initial oxygenation index on the relationship between inhaled nitric oxide and neurodevelopmental outcome.

We observed that patients treated with inhaled nitric oxide had a significantly lower risk of having Bayley scores of less than 70. This difference was due to the significantly lower percentage of children in the inhaled nitric oxide group who had cognitive impairment. These findings suggest that inhaled nitric oxide primarily improves cognitive outcome. Developmental testing of premature infants at two years of age with the use of the Bayley Scales of Infant Development II provides an early opportunity to estimate future developmental capabilities.[19,20] However, the correlation of developmental assessments with ultimate developmental achievement is greater for assessments at five

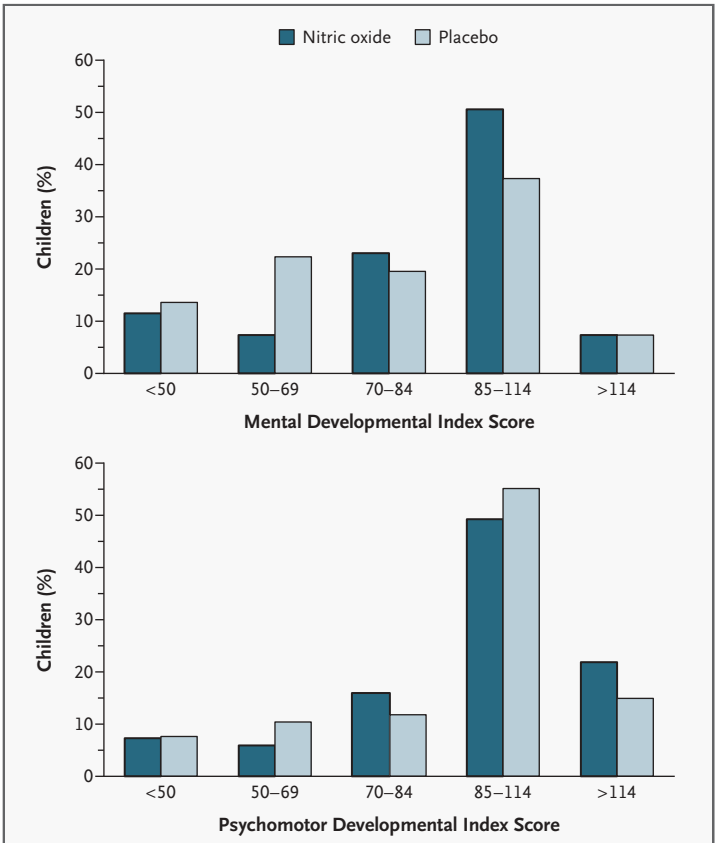

Figure 1. Distribution of Scores for the Bayley Scales of Infant Development II.
A score of less than 70 on either index indicated impairment.

and eight years of age.[21] Consequently, continued follow-up of this cohort will provide data on the robustness of the effect of inhaled nitric oxide on neurodevelopment.

How might inhaled nitric oxide given to premature infants at birth result in improved neurodevelopmental status at two years of age? Although the head circumferences of both groups of infants were similar and in the normal range, infants treated with nitric oxide had improved somatic growth and may have been healthier overall than infants given placebo, permitting better neurodevelopment. Alternatively, studies have suggested that inhaled nitric oxide may be delivered to sites outside the lung.[22-24] Thus, treatment with nitric oxide may have directly affected the brain through mechanisms involving the cerebral vasculature[25] or neuronal maturation.[26-28] Nonetheless, the mechanisms by which inhaled nitric oxide improves neurodevelopmental outcome remain unclear. Comparison of data from this and out previous study[6] with the results reported by Van Meurs et al. in this issue of the *Journal*,[29] suggests that further research is needed to define the optimal dose of inhaled nitric oxide and the duration of treatment in premature infants. It will be of interest to see whether a difference emerges between the groups in neurodevelopmental outcomes.

In conclusion, premature infants treated with inhaled nitric oxide throughout the first week of life, as described in our study, had better neurodevelopmental status at two years of age than their placebo-treated counterparts. By increasing the likelihood of a premature infant's survival without neurodevelopmental delay, inhaled nitric oxide may significantly improve the quality of life of both the child and his or her family and may decrease the societal burden of caring for these high-risk children.

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ORIGINAL ARTICLE

Hydroxyurea Compared with Anagrelide in High-Risk Essential Thrombocythemia

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ABSTRACT

BACKGROUND

We conducted a randomized comparison of hydroxyurea with anagrelide in the treatment of essential thrombocythemia.

METHODS

A total of 809 patients with essential thrombocythemia who were at high risk for vascular events received low-dose aspirin plus either anagrelide or hydroxyurea. The composite primary end point was the actuarial risk of arterial thrombosis (myocardial infarction, unstable angina, cerebrovascular accident, transient ischemic attack, or peripheral arterial thrombosis), venous thrombosis (deep-vein thrombosis, splanchnic-vein thrombosis, or pulmonary embolism), serious hemorrhage, or death from thrombotic or hemorrhagic causes.

RESULTS

After a median follow-up of 39 months, patients in the anagrelide group were significantly more likely than those in the hydroxyurea group to have reached the primary end point (odds ratio, 1.57; 95 percent confidence interval, 1.04 to 2.37; P=0.03). As compared with hydroxyurea plus aspirin, anagrelide plus aspirin was associated with increased rates of arterial thrombosis (P=0.004), serious hemorrhage (P=0.008), and transformation to myelofibrosis (P=0.01) but with a decreased rate of venous thromboembolism (P=0.006). Patients receiving anagrelide were more likely to withdraw from their assigned treatment (P<0.001). Equivalent long-term control of the platelet count was achieved in both groups.

CONCLUSIONS

Hydroxyurea plus low-dose aspirin is superior to anagrelide plus low-dose aspirin for patients with essential thrombocythemia at high risk for vascular events.

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THE PRINCIPAL FEATURE OF ESSENTIAL thrombocythemia, a clonal hematologic stem-cell disorder,[1-4] is thrombosis, with arterial events being more common than venous events. Hemorrhage also occurs, particularly if the platelet count is very high. In the long term, some cases transform to myelofibrosis, myelodysplasia, or acute myeloid leukemia. Factors that increase the risk of thrombosis are an age of more than 60 years, prior thrombosis, and, to a lesser extent, cardiovascular risk factors.[5-7] The importance of the platelet count as a risk factor is unclear, but a reduction in platelet count reduces the frequency of thrombosis, and aspirin relieves the microvascular symptoms of essential thrombocythemia.

Hydroxyurea is widely used as first-line therapy for high-risk patients, often in combination with low-dose aspirin. A previous randomized study demonstrated that hydroxyurea controlled the platelet count and reduced the incidence of thrombotic events in patients with a high risk of thrombosis.[8] Patients treated with hydroxyurea alone have a low incidence of leukemic transformation (3 to 4 percent),[9,10] whereas those given more than one cytotoxic agent are at increased risk for acute myeloid leukemia. It is not clear whether this increased risk is an effect of the treatment or a consequence of aggressive disease.

Anagrelide was developed as an inhibitor of platelet aggregation but was later found to reduce the platelet count at doses lower than the amount required to inhibit platelet aggregation.[11,12] The drug blocks megakaryocyte differentiation[13,14] and proliferation[15] and inhibits the action of cyclic AMP phosphodiesterase.[16] Despite the lack of evidence of efficacy reported in a randomized trial, anagrelide is commonly used as first-line therapy for high-risk patients with essential thrombocythemia, even though it is substantially more expensive than hydroxyurea. Here we report the results of the United Kingdom Medical Research Council Primary Thrombocythemia 1 study, which compared hydroxyurea plus aspirin with anagrelide plus aspirin in patients with essential thrombocythemia at high risk for thrombosis.

METHODS

STUDY POPULATION

We conducted an open-label, randomized trial comparing hydroxyurea plus aspirin with anagrelide plus aspirin in patients with essential thrombocythemia at high risk for vascular events. Patients were eligible if they met diagnostic criteria for essential thrombocythemia and were at high risk for thrombotic or hemorrhagic events. Both patients with newly diagnosed disease and previously treated patients who were at least 18 years old were eligible.

We used the diagnostic criteria of the Polycythemia Vera Study Group for essential thrombocythemia.[17] The Supplementary Appendix (available with the full text of this article at www.nejm.org) lists reasons for exclusion of patients from the study. Patients were classified as at high risk if they met one or more of the following criteria: an age of at least 60 years; current or previous platelet counts of 1 million per cubic millimeter or more; a history of ischemia, thrombosis, or embolism; hemorrhage caused by essential thrombocythemia; hypertension requiring therapy; and diabetes requiring the administration of a hypoglycemic agent.

In the United Kingdom, Ireland, and Australia, 815 patients were randomly assigned to receive one of the study drugs in 138 centers between August 20, 1997, and August 15, 2002. Six patients, three in each group, were misdiagnosed as having essential thrombocythemia (two patients with chronic myeloid leukemia, two with reactive thrombocytosis, one with idiopathic myelofibrosis, and one with polycythemia vera); these patients were excluded from the analysis. Of the remaining 809 patients, 404 were randomly assigned to receive hydroxyurea plus aspirin and 405 to receive anagrelide plus aspirin; patients in both groups were followed for a median of 39 months (range, 12 to 72). Only six patients were lost to follow-up, including four as a result of emigration. Complete follow-up information was available until the last visit of these six patients. The institutional research ethics committees in each center approved the study protocol, and written informed consent was obtained from all patients.

Patients who were assigned to receive hydroxyurea were started on 0.5 to 1 g daily; those assigned to receive anagrelide were started on 0.5 mg twice daily. Doses were subsequently adjusted to maintain the platelet count at less than 400,000 per cubic millimeter. Treatment was considered to have failed in patients whose platelet count was not less than 600,000 per cubic millimeter after having received the assigned therapy for at least three months; these patients left the study. All patients received aspirin at a daily dose of 75 mg (100 mg in

Australia). If aspirin was contraindicated, alternative agents were used: dipyridamole in 13 patients and clopidogrel in 4 patients. The protocol recommended delaying the introduction of aspirin in patients with very high platelet counts.

Information that was recorded at each visit included thrombotic or hemorrhagic events or transformation to neoplasm, other adverse events, a full blood count, measurement of the spleen, and a list of all other medications the patient was receiving. Follow-up forms requesting details of principal endpoint diagnoses were completed annually.

END POINTS

The composite primary end point was the time from randomization until the patient died from thrombosis or hemorrhage or had an arterial or a venous thrombotic event or a serious hemorrhage (see the Supplementary Appendix). Secondary end points were the time to the first arterial or venous thrombotic event or to the first serious hemorrhage; the time to death; the incidence of transformation to myelofibrosis, acute myeloid leukemia, myelodysplasia, or polycythemia vera; and control of the platelet count.

In the Supplementary Appendix, we list definitions of myocardial infarction, stroke, transient ischemic attack, deep-vein thrombosis, pulmonary embolism, serious hemorrhage, and transformation to acute myeloid leukemia, myelodysplasia, and polycythemia vera; also described is how transformation to myelofibrosis was determined.[18-24] All primary and secondary end points that were reported before July 31, 2004, were validated by a committee of clinicians who were blinded to the patients' treatment assignments. Two clinicians evaluated each event independently, and the study chairman resolved any disagreements. A committee of three hematopathologists who were blinded to the treatment assignments reviewed the results of the bone marrow biopsies of all patients with myelofibrotic or other transformations. Two hematologists who were blinded to treatment assignments independently reviewed all bone marrow aspirates and peripheral-blood smears from patients with transformations, and the study chairman resolved any disagreements.

STATISTICAL ANALYSIS

To detect a doubling in the rate of the primary end point (from 2 percent to 4 percent per year)[6,8] in either group over a median of four years of follow-up, with 80 percent power and a significance level of 0.05, we estimated that the trial would require that 560 patients be randomly assigned to a study group. Randomizations were undertaken and conveyed by telephone or fax to the Clinical Trial Service Unit in Oxford, United Kingdom. Minimization[25] was used to ensure that equal numbers of patients were assigned to each group, both overall and within subgroups defined by previous treatment (no treatment vs. aspirin or cytoreductive therapy or both) and previous treatment assignment (for patients who were initially in groups at low and intermediate risk).

Annual interim analyses were assessed by the data monitoring committee of the Medical Research Council, which uses the Haybittle–Peto stopping guideline, a difference of at least 3 SE between the two groups.[26] In 2002, this committee noted an excess of vascular events and deaths close to the boundary of 3 SE, together with an excess of myelofibrosis, other adverse events, and withdrawal from treatment. In 2003, the difference in vascular events and deaths exceeded the boundary of 3 SE, and the differences in myelofibrosis, other adverse events, and withdrawal from treatment were maintained. On September 1, 2003, the trial was closed and a letter sent to investigators recommending that they consider changing the treatment of participating patients from anagrelide to hydroxyurea. Because the Haybittle–Peto guideline is a conservative stopping rule, P values do not require adjustment for the interim analyses.[27]

Differences in baseline characteristics according to treatment assignment were assessed with the use of the chi-square test (two-by-two tables), the Mantel–Haenszel test for trend (with a grouped timing of trial entry), or the Mann–Whitney U test (for continuous data). Wilcoxon rank-sum tests were used to compare platelet counts between the two groups at three monthly time points for the first two years after randomization. Kaplan–Meier analysis and the log-rank test[28] were used to compare time to event from randomization on an intention-to-treat basis, with data of surviving patients censored on August 31, 2003, or (for those lost to follow-up) on the date of the last follow-up. The observed number of events (O) minus the expected number of events (E) in the anagrelide group and its variance (V) were calculated from the log-rank survival analysis and used to calculate the odds ratio[28] (as the exponent of $[(O-E) \div V]$). Tests for interaction were used to assess whether the

Table 1. Baseline Characteristics of the Patients.*

Feature	Hydroxyurea plus Aspirin (N=404)	Anagrelide plus Aspirin (N=405)
Demographic characteristics		
Male sex — no. (%)	180 (45)	162 (40)
Median age at entry — yr (range)	62 (21–88)	61 (23–88)
Laboratory and clinical features at diagnosis		
Platelet count — $\times 10^{-3}$/mm^3		
Mean	1011±320	1004 ±344
Median (range)	947 (425–2250)	930 (208–2320)
Hemoglobin — g/liter		
Mean†	139±15	140±14
Median (range)	140 (82–174)	140 (99–174)
White-cell count — $\times 10^{-3}$/mm^3		
Mean	10.4±3.3	10.0±3.3
Median (range)	9.9 (4.2–27.4)	9.4 (3.1–32.2)
Neutrophil count — $\times 10^{-3}$/mm^3		
Mean	7.1±2.8	7.0±2.9
Median (range)	6.6 (1.9–18.7)	6.4 (1.4–27.2)
Splenomegaly — no./total no. (%)	27/362 (7)	36/351 (10)
Laboratory and clinical features at trial entry		
Platelet count — $\times 10^{-3}$/mm^3		
Mean	853±383	839±359
Median (range)	837 (212–2860)	812 (211–2372)
Hemoglobin — g/liter		
Mean	136±15	138±14
Median (range)	136 (86–181)	138 (93–174)
White-cell count — $\times 10^{-3}$/mm^3		
Mean	9.1±3.5	8.8±3.2
Median (range)	8.8 (2.0–24.0)	8.6 (1.7–25.6)
Neutrophil count — $\times 10^{-3}$/mm^3		
Mean	6.0±2.9	5.9±2.7
Median (range)	5.7 (0.6–19.4)	5.6 (0.9–19.8)
Splenomegaly — no./total no. (%)	22/345 (6)	26/335 (8)

treatment effect differed among subgroups of patients.[29]

In the Supplementary Appendix, we describe how the trial was conceived, conducted, and analyzed. Shire, the manufacturer of anagrelide, provided the drug at a reduced price but was otherwise not involved in the trial.

RESULTS

BASELINE CHARACTERISTICS

There were no significant differences between the two groups with respect to laboratory and clinical features at diagnosis or trial entry (Table 1). The groups were well matched with respect to risk fac-

Table 1. (Continued.)

Feature	Hydroxyurea plus Aspirin (N=404)	Anagrelide plus Aspirin (N=405)
Time between diagnosis and enrollment — no. (%)		
<3 mo	250 (62)	240 (59)
3 mo to 5 yr	109 (27)	114 (28)
>5 yr	45 (11)	51 (13)
Prior treatment — no. (%)		
None	77 (19)	68 (17)
Aspirin or other antiplatelet agent alone	194 (48)	199 (49)
Any cytoreductive agent	133 (33)	138 (34)
Hydroxyurea	118 (29)	123 (30)
Anagrelide	0 (0)	1 (<1)
Interferon alfa	7 (2)	7 (2)
Busulfan	13 (3)	23 (6)
Other (phosphorus-32, mitobronitol, or unknown agent)	12 (3)	10 (2)
Thrombotic and hemorrhagic risk factors — no./total no. (%)		
Previous arterial thrombosis	72/400 (18)	74/402 (18)
Previous venous thromboembolism	29/400 (7)	20/402 (5)
Previous peripheral vascular disease	152/400 (38)	131/402 (33)
Previous angina	22/400 (6)	21/402 (5)
Regular daily cigarette smoking at enrollment	62/331 (19)	48/342 (14)
Diabetes	17/400 (4)	12/402 (3)
Hypertension	94/400 (24)	90/402 (22)
Previous hemorrhage	26/400 (6)	23/402 (6)

* Plus–minus values are means ±SD.
† No male patient had a hemoglobin level of more than 175 g per liter, and one female patient had a hemoglobin level of more than 165 g per liter.

tors for thrombosis and hemorrhage and to hematologic transformation. Approximately one third of patients in each group had previously received hydroxyurea.

CONTROL OF THE PLATELET COUNT

Control of the platelet count was similar in the two groups by nine months after trial entry and subsequently (Fig. 1). At three and six months after trial entry, platelet counts in the anagrelide group were significantly higher than those in the hydroxyurea group (P<0.001 for both time points). The difference remained significant when the analysis was restricted to patients with newly diagnosed disease at trial entry (data not shown) and therefore did not reflect a need for patients who had previously received a diagnosis of essential thrombocythemia to change from hydroxyurea to anagrelide after randomization to the anagrelide group. The median white-cell count in the hydroxyurea group was significantly and persistently lower than that in the anagrelide group (P<0.001), starting at three months after trial entry (data not shown).

VASCULAR END POINTS

As compared with the hydroxyurea group, the anagrelide group had a significantly higher rate of the composite primary end point of arterial or venous

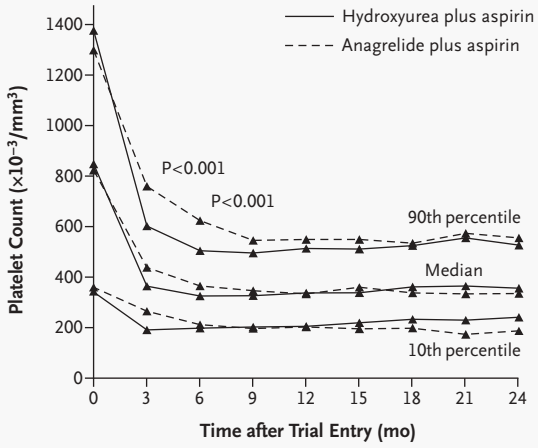


Figure 1. The Median and 10th and 90th Percentiles of the Platelet Count, According to Month after Trial Entry.

Data points are based on patients with a platelet count recorded within one month of each time point and remaining on their assigned treatment. Platelet counts were significantly different between the two groups at three and six months after trial entry but not at other time points.

thrombosis, serious hemorrhage, or death from vascular causes (odds ratio, 1.57; 95 percent confidence interval, 1.04 to 2.37; P=0.03) (Table 2). The estimated risk of the primary end point at five years was 16 percent in the anagrelide group (95 percent confidence interval, 12 to 21) and 11 percent in the hydroxyurea group (95 percent confidence interval, 7 to 14), with a median follow-up of 39 months (Fig. 2). The rates of the primary end point were also compared in prespecified subgroups of patients (newly diagnosed vs. previously diagnosed disease; previous cytoreductive therapy vs. no previous cytoreductive therapy; and previous hydroxyurea therapy vs. no previous hydroxyurea therapy). There was no evidence of heterogeneity of treatment effect between these subgroups.

Analyses of prespecified secondary vascular end points revealed statistically significant differences between the two groups (Table 2 and Fig. 3). Arterial thrombosis developed in more than twice as many patients in the anagrelide group as in the hydroxyurea group (odds ratio, 2.16; 95 percent confidence interval, 1.27 to 3.69; P=0.004). There were significantly more transient ischemic attacks in the anagrelide group than in the hydroxyurea group (14 vs. 1; odds ratio, 5.72; 95 percent confidence interval, 2.08 to 15.73; P<0.001). The rates of myocardial infarction, unstable angina, and thrombotic stroke were higher in the anagrelide group but not significantly different from the rates in the hydroxyurea group. There was also a significant increase in the rate of serious hemorrhage in the anagrelide group (odds ratio, 2.61; 95 percent confidence interval, 1.27 to 5.33; P=0.008), with gastrointestinal hemorrhage being particularly common (odds ratio, 3.54; 95 percent confidence interval, 1.33 to 9.44; P=0.01).

By contrast, the rate of venous thromboembolism in the anagrelide group was approximately one fourth that in the hydroxyurea group (odds ratio, 0.27; 95 percent confidence interval, 0.11 to 0.71; P=0.006), and there was a significantly lower rate of deep-vein thrombosis in the anagrelide group (odds ratio, 0.20; 95 percent confidence interval, 0.06 to 0.71; P=0.009). Pulmonary emboli developed in only seven patients, but five of the seven were in the hydroxyurea group. The rates of death from any cause and death from thrombotic or hemorrhagic causes were not significantly different between the two groups, although the study was not powered to detect any difference in mortality.

Since patients who received anagrelide were more likely to withdraw from their assigned treatment than were patients who received hydroxyurea (Table 3), survival analyses were repeated with data that were censored on the date of the patients' withdrawal from treatment. The various rates of arterial or venous thrombosis, serious hemorrhage, and reaching the composite primary end point all remained statistically significant, with minimal changes in the P values (data not shown).

The rates of the primary end point in the two groups were compared for various periods after trial entry. There was no significant heterogeneity between the odds ratio for events in the first nine months and the odds ratio for subsequent events (data not shown). The differences in platelet count in the first nine months are therefore unlikely to be of clinical significance.

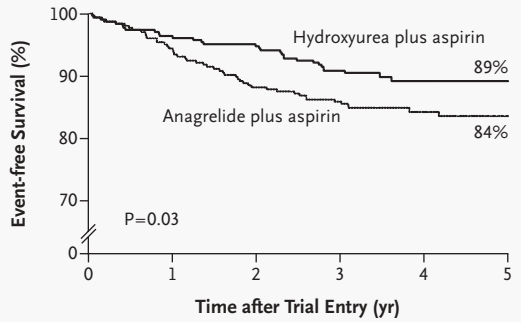
DISEASE TRANSFORMATION

As compared with the hydroxyurea group, the anagrelide group had a significantly increased rate

Table 2. Number of Patients Reaching Principal Study End Points.*

Feature	Hydroxyurea plus Aspirin (N=404)	Anagrelide plus Aspirin (N=405)	Odds Ratio (95% CI)	P Value†
	no. of patients			
Primary end point				
Arterial or venous thrombosis, serious hemorrhage, or death from thrombosis or hemorrhage	36	55	1.57 (1.04–2.37)	0.03
Secondary end point				
Arterial thrombosis	17	37	2.16 (1.27–3.69)	0.004
Myocardial infarction	7	13	1.84 (0.76–4.41)	NS
Unstable angina	2	4	1.94 (0.39–9.63)	NS
Stroke	7	9	1.30 (0.49–3.47)	NS
Transient ischemic attack	1	14	5.72 (2.08–15.73)	<0.001
Other‡	2	0		NE
Venous thromboembolism	14	3	0.27 (0.11–0.71)	0.006
Deep-vein thrombosis	9	1	0.20 (0.06–0.71)	0.009
Pulmonary embolism	5	2	0.43 (0.01–1.87)	NS
Hepatic-vein thrombosis	1	0		NE
Serious hemorrhage	8	22	2.61 (1.27–5.33)	0.008
Gastrointestinal bleeding	3	13	3.54 (1.33–9.44)	0.01
Intracranial bleeding	4	1	0.30 (0.05–1.75)	NS
Nasal bleeding	1	4	3.34 (0.58–19.25)	NS
Other bleeding§	0	4		NE
Death	27	31	1.15 (0.69–1.93)	NS
Thrombotic cause¶	9	11	1.23 (0.51–2.94)	NS
Hemorrhagic cause	4	4	1.01 (0.25–4.02)	NS
Hematologic cause (transformation)	4	3	0.77 (0.18–3.39)	NS
Other cause	12	14	1.17 (0.54–2.53)	NS
Hematologic transformation				
Myelofibrosis	5	16	2.92 (1.24–6.86)	0.01
Mo after trial entry — median (range)	30 (7–54)	28 (10–52)		
Mo after diagnosis — median (range)‖	34 (27–107)	45 (12–182)		
Acute myeloid leukemia or myelodysplasia	6	4	0.67 (0.20–2.33)	NS
Mo after trial entry — median (range)	26 (7–46)	43 (8–55)		
Mo after diagnosis — median (range)¶	36 (26–58)	83 (9–150)		
Polycythemia vera	1	1	1.00 (0.06–1.60)	NS

* CI denotes confidence interval, NS not significant, and NE not able to be evaluated (since one group had no events).
† P values were obtained with the use of log-rank analysis.
‡ This category includes arterial embolus of either the lower or upper limbs.
§ This category includes retroperitoneal, pericardial, urinary, and obstetrical hemorrhage.
¶ This category includes three patients who died suddenly of presumed cardiac causes (one in the hydroxyurea group and two in the anagrelide group).
‖ There was no significant difference in the duration of disease before transformation with use of the Wilcoxon rank-sum test.



No. at Risk						
Hydroxyurea plus aspirin	404	388	298	204	129	57
Anagrelide plus aspirin	405	379	272	190	119	52

Figure 2. Kaplan–Meier Estimates of Survival Free of the Primary End Point of Arterial or Venous Thrombosis, Serious Hemorrhage, or Death from Any of These Causes.

of transformation to myelofibrosis (odds ratio, 2.92; 95 percent confidence interval, 1.24 to 6.86; P=0.01) (Table 2). The estimated actuarial risk of myelofibrosis five years after trial entry was 2 percent for the hydroxyurea group (95 percent confidence interval, 0 to 5) and 7 percent for the anagrelide group (95 percent confidence interval, 3 to 10) (Fig. 3D). Of the 21 patients with myelofibrotic transformation, 3 had died by September 2003, all in the anagrelide group.

The increased rate of myelofibrosis in the anagrelide group remained evident after patients who had previously received busulfan were excluded from the analysis (P=0.04). None of the 21 patients had anemia, leukoerythroblastic findings on peripheral-blood smears, or systemic symptoms at trial entry, and only 1 (5 percent) had splenomegaly (as compared with 7 percent for the trial as a whole). Furthermore, the higher rate of myelofibrotic transformation was not an artifact of the precise definition that was used, since making the diagnostic criteria more stringent by requiring the inclusion of three clinical or laboratory features instead of two, or by the exclusion of any one of the five criteria from the set, did not affect the statistical significance.

Myelodysplasia or acute myeloid leukemia developed in 10 patients, 4 in the anagrelide group and 6 in the hydroxyurea group (Table 2). Median survival was 14 months from transformation, and seven patients had died by September 2003. Polycythemia vera developed in two patients (one in each group), at 3 and 49 months after trial entry.

SAFETY AND SIDE EFFECTS

The number of patients who withdrew from the assigned treatment before closure of the trial was higher in the anagrelide group than in the hydroxyurea group (148 vs. 79, P<0.001) (Table 3). Significantly more patients withdrew from the anagrelide group because of side effects (88 vs. 43, P<0.001) or because either an end point (particularly myocardial infarction or hematologic transformation) or a serious adverse event had developed (most commonly, cardiac failure, serious arrhythmia, or pancytopenia). The lower rate of withdrawal from treatment with hydroxyurea was still evident even when analysis was restricted to patients who had not previously received hydroxyurea (P<0.001).

The rates of nonthrombotic cardiovascular events (particularly palpitations), gastrointestinal events (especially diarrhea and abdominal pain), noncardiac edema, headache, and constitutional symptoms were all significantly higher in the anagrelide group (Table 3). The rate of dermatologic side effects, including mouth ulcers, was significantly increased in the hydroxyurea group.

DISCUSSION

This study of more than 800 patients with essential thrombocythemia who were at high risk for thrombosis shows that, as compared with hydroxyurea plus aspirin, anagrelide plus aspirin was associated with higher rates of arterial thrombosis, serious hemorrhage, transformation to myelofibrosis, and treatment withdrawal but a lower rate of venous thromboembolism. The participation of many secondary and tertiary hematology centers and the involvement of three countries suggest that these conclusions can be generalized.

The rates of major arterial and venous thrombosis in the hydroxyurea group in this trial were similar to those in the hydroxyurea group in the study of Cortelazzo and colleagues[8] (actuarial rate of first thrombosis, 4 percent at two years in both trials), which suggests that the study populations in the two trials were broadly similar. However, the rate of major arterial and venous thrombosis in the anagrelide group in our trial was less than that ob-

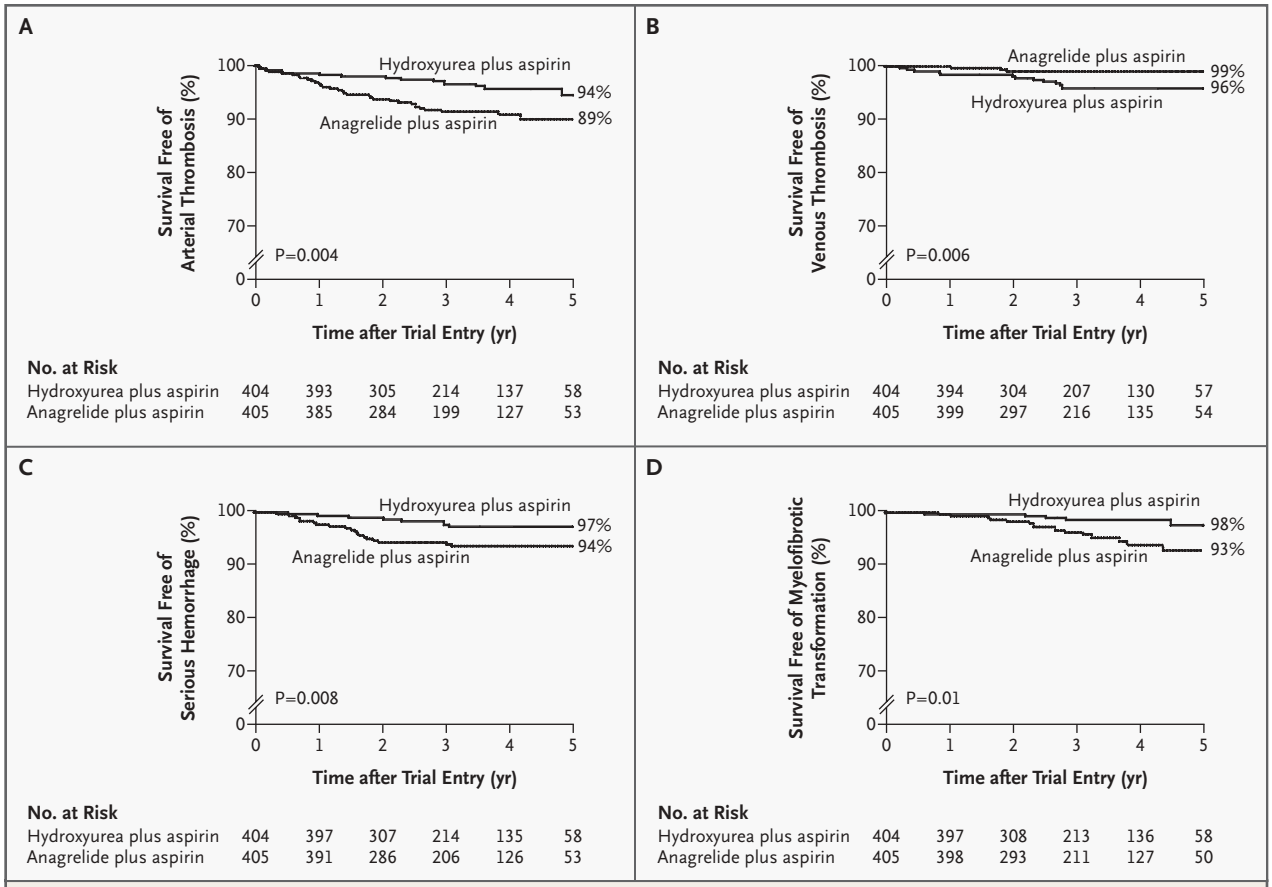


Figure 3. Kaplan–Meier Estimates of Survival Free of the Secondary End Points of Arterial Thrombosis (Panel A), Venous Thrombosis (Panel B), Serious Hemorrhage (Panel C), and Myelofibrotic Transformation (Panel D).

served in the control group (which did not receive hydroxyurea) in the Italian study (actuarial rate of first thrombosis, 8 percent vs. 26 percent at two years, respectively). Since more than 80 percent of the thrombotic events in the Italian trial were arterial, these comparisons suggest that anagrelide partially protects against arterial thrombosis. It is interesting to note that both trials reported a marked effect of hydroxyurea on rates of transient ischemic attack, which suggests a particular role for hydroxyurea in the prevention of this complication.

In contrast to the rate of arterial thrombosis, the rate of venous thrombosis was significantly lower in the anagrelide group. Since the incidence of venous thrombosis in untreated patients with high-risk essential thrombocythemia is unknown, it is unclear whether this rate is increased by hydroxyurea or decreased by anagrelide. The optimal treatment of a patient with prior venous thrombosis will depend not only on individual circumstances but also on the fact that arterial thrombosis is more than three times more common than venous thrombosis in essential thrombocythemia.

The equivalent long-term control of the platelet count in both groups implies that, in addition to lowering the platelet count, either hydroxyurea or anagrelide may modulate thrombosis by other mechanisms. The lower white-cell count in patients receiving hydroxyurea may be relevant, since white cells contribute to the procoagulant response at sites of vascular injury.[30] Moreover, neutrophil activation occurs in essential thrombocythemia and correlates with activation of both endothelial cells and the coagulation cascade.[31] Hydroxyurea also has direct effects on endothelial function[32] and acts as a nitric oxide donor.[33]

Table 3. Treatment Withdrawal and Adverse Events.

Feature	Hydroxyurea plus Aspirin (N=404)	Anagrelide plus Aspirin (N=405)	P Value*
Withdrawal from treatment			
No. of patients who withdrew from assigned treatment	79	148	<0.001
Reason for withdrawal			
Side effect	43	88	<0.001
Serious adverse or end-point event	4	22	<0.001
Lack of platelet control	15	19	NS
Pregnancy or other contraindication	2	8	0.03
Choice of patient	10	5	NS
Other reason	5	6	NS
Adverse events			
Nonthrombotic cardiovascular events	27	92	<0.001
Cardiac failure (including acute ventricular failure)	7	14	NS
Arrhythmia (atrial flutter, atrial fibrillation, need for pacemaker)	4	8	NS
Palpitations (including irregular pulse)	7	63	<0.001
Other nonthrombotic cardiovascular event†	12	22	NS
Gastroenterologic events	36	59	0.01
Diarrhea	6	18	0.01
Nausea and vomiting	12	16	NS
Peptic ulcer, esophagitis, and gastritis	18	18	NS
Abdominal pain	1	9	0.008
Irritable-bowel symptoms	0	5	NE
Inflammatory bowel disease	2	2	NS
Other gastroenterologic event‡	8	18	0.04

The increased risk of serious hemorrhage in the anagrelide plus aspirin group may reflect interference of anagrelide with platelet function in a way that synergizes with low-dose aspirin. Anagrelide blocks platelet phosphodiesterase activity[16] and at high doses (0.5 to 10.0 mg per kilogram of body weight) inhibits thrombus formation in animal models.[34] Although the results of most assays of platelet function are normal in patients with essential thrombocythemia who receive anagrelide, some subtle effects on platelet function have been reported.[35,36] The results presented here suggest that if anagrelide is used, the decision whether to use concurrent aspirin therapy should depend on the relative risk of arterial thrombosis and hemorrhage in each patient.

The incidence of transformation to myelofibrosis was higher in the anagrelide group than in the hydroxyurea group. The reason for this difference is unknown. Hydroxyurea reduces reticulin fibrosis in a variety of myeloproliferative disorders, including essential thrombocythemia.[37,38] By contrast, the many immature forms that arise when anagrelide blocks differentiation of megakaryo-

Table 3. (Continued.)

Feature	Hydroxyurea plus Aspirin (N=404)	Anagrelide plus Aspirin (N=405)	P Value*
Dermatologic event	45	29	0.05
Rash	10	15	NS
Leg ulcer	20	9	0.04
Mouth ulcers	8	1	0.02
Other dermatologic event	16	7	NS
Hematologic event (excluding transformation)	24	35	NS
Iron-deficiency anemia	4	10	NS
Other anemia	13	22	NS
Thrombocytopenia, neutropenia, or both	8	5	NS
Other hematologic event	1	4	NS
Event involving other systems			
Noncardiac edema	5	25	<0.001
Headache	8	51	<0.001
Constitutional symptoms§	12	41	<0.001
Diabetes	10	3	0.05
Peripheral vascular disease	11	11	NS
Minor hemorrhage	42	50	NS
Nonhematologic cancer	14	11	NS

* P values were obtained with the use of log-rank analysis. NS denotes not significant, and NE not able to be evaluated (since one group had no events).
† Other nonthrombotic cardiovascular events include hypertension, 5 patients in the hydroxyurea group and 9 patients in the anagrelide group (NS); chest pain, 4 patients and 8 patients, respectively (NS); aortic aneurysm, 2 patients and 2 patients, respectively (NS); and multiorgan failure, 1 patient and 3 patients, respectively (NS).
‡ Other gastroenterologic events included gallstones, no patients in the hydroxyurea group and 1 patient in the anagrelide group (NE); abnormal liver-function tests, no patients and 4 patients, respectively (NE); diverticular disease, 1 patient and 4 patients, respectively (NS); ascites, no patients and 1 patient, respectively (NE); celiac disease, no patients and 1 patient, respectively (NE); colonic polyp, no patients and 1 patient, respectively (NE); volvulus, 1 patient and no patients, respectively (NE); constipation, 2 patients and 5 patients, respectively (NS); and hemorrhoids, 4 patients and 3 patients, respectively (NS).
§ Constitutional symptoms included fatigue, weight change, fevers, flushing, sleep disturbance, and loss of appetite.

cytes may produce relatively high levels of profibrotic cytokines.

In summary, the results of this trial suggest that hydroxyurea plus aspirin should remain first-line therapy for patients with essential thrombocythemia at high risk for vascular events.

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ORIGINAL ARTICLE

Oxidized Phospholipids, Lp(a) Lipoprotein, and Coronary Artery Disease

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ABSTRACT

BACKGROUND

Lp(a) lipoprotein binds proinflammatory oxidized phospholipids. We investigated whether levels of oxidized low-density lipoprotein (LDL) measured with use of monoclonal antibody E06 reflect the presence and extent of obstructive coronary artery disease, defined as a stenosis of more than 50 percent of the luminal diameter.

METHODS

Levels of oxidized LDL and Lp(a) lipoprotein were measured in a total of 504 patients immediately before coronary angiography. Levels of oxidized LDL are reported as the oxidized phospholipid content per particle of apolipoprotein B-100 (oxidized phospholipid:apo B-100 ratio).

RESULTS

Measurements of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels were skewed toward lower values, and the values for the oxidized phospholipid:apo B-100 ratio correlated strongly with those for Lp(a) lipoprotein ($r=0.83$, $P<0.001$). In the entire cohort, the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels showed a strong and graded association with the presence and extent of coronary artery disease (i.e., the number of vessels with a stenosis of more than 50 percent of the luminal diameter) ($P<0.001$). Among patients 60 years of age or younger, those in the highest quartiles for the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels had odds ratios for coronary artery disease of 3.12 ($P<0.001$) and 3.64 ($P<0.001$), respectively, as compared with patients in the lowest quartile. The combined effect of hypercholesterolemia and being in the highest quartiles of the oxidized phospholipid:apo B-100 ratio (odds ratio, 16.8; $P<0.001$) and Lp(a) lipoprotein levels (odds ratio, 14.2; $P<0.001$) significantly increased the probability of coronary artery disease among patients 60 years of age or younger. In the entire study group, the association of the oxidized phospholipid:apo B-100 ratio with obstructive coronary artery disease was independent of all clinical and lipid measures except one, Lp(a) lipoprotein. However, among patients 60 years of age or younger, the oxidized phospholipid:apo B-100 ratio remained an independent predictor of coronary artery disease.

CONCLUSIONS

Circulating levels of oxidized LDL are strongly associated with angiographically documented coronary artery disease, particularly in patients 60 years of age or younger. These data suggest that the atherogenicity of Lp(a) lipoprotein may be mediated in part by associated proinflammatory oxidized phospholipids.

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Human coronary atherosclerosis is a chronic inflammatory disease that is superimposed on a background of lipid abnormalities. Proinflammatory oxidized low-density lipoprotein (LDL) may be a unifying link between lipid accumulation and inflammation in the vessel wall. In humans, oxidized LDL in plasma and within atherosclerotic lesions is strongly associated with coronary artery disease, acute coronary syndromes, and vulnerable plaques.[1-7]

Lp(a) lipoprotein is a lipoprotein of unknown physiologic function that is composed of apolipoprotein B-100 (apo B-100) to which apolipoprotein(a) is covalently bound. Increased plasma levels of Lp(a) lipoprotein are independent predictors of the presence of angiographically documented and clinical coronary artery disease, particularly in patients with hypercholesterolemia.[8] However, the underlying mechanisms by which Lp(a) lipoprotein contributes to the pathogenesis of atherosclerosis are not well understood. We recently showed that proinflammatory oxidized phospholipids are strongly associated with Lp(a) lipoprotein in human plasma.[5-7,9] Therefore, we hypothesized that the presence of oxidized phospholipids on apo B-100–containing lipoproteins may explain some of the atherogenic properties of Lp(a) lipoprotein, and we designed this study to evaluate the relationship between circulating oxidized LDL, Lp(a) lipoprotein, and angiographically documented coronary artery disease.

METHODS

STUDY DESIGN

We designed the current study on the basis of a previous study in which we had enrolled a total of 504 consecutive patients (97.2 percent of whom were white), 18 to 75 years of age, who were undergoing clinically indicated coronary angiography at the Mayo Clinic between June 1998 and December 1998.[10] Race was self-reported. The exclusion criteria, which have been described previously, included prior coronary revascularization and the presence of diabetes mellitus.[10] Arterial plasma samples were obtained from the femoral sheath before angiography and were placed in tubes containing EDTA and frozen at −70°C until the analyses were performed. Hypercholesterolemia was defined as a total cholesterol level of at least 250 mg per deciliter (6.5 mmol per liter), an LDL level of at least 150 mg per deciliter (3.9 mmol per liter), or ongoing treatment with lipid-lowering agents. The study was approved by the Mayo Clinic institutional review board, and all patients gave written informed consent.

ANGIOGRAPHIC ANALYSIS

The maximal stenosis in each of 27 coronary-artery segments was assessed by a cardiologist, who was unaware of risk factors, with the use of handheld calipers or in visual analysis according to the segmental classification system of the Coronary Artery Surgery Study. The extent of angiographically documented coronary artery disease was quantified as follows: normal coronary arteries (smooth, with either no stenosis or a stenosis of <10 percent of the luminal diameter), mild disease (a stenosis of 10 to 50 percent of the luminal diameter in one or more coronary arteries or their major branches), or one-vessel, two-vessel, or three-vessel disease, defined as a stenosis of more than 50 percent of the luminal diameter in one, two, or three coronary arteries or their major branches.[10]

LABORATORY ANALYSES

Analyses of apo B-100, Lp(a) lipoprotein, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were performed with the use of commercially available kits. LDL cholesterol was estimated with the use of the Friedewald formula. High-sensitivity C-reactive protein (CRP) (lower limit of detection, 0.15 mg per liter) was measured as described elsewhere.[11]

Our assay of oxidized LDL determines the content of oxidized phospholipids per particle of apo B-100 (oxidized phospholipid:apo B-100 ratio) and is performed with the use of the murine monoclonal antibody E06, which specifically binds to the phosphorylcholine moiety of oxidized but not native phospholipids.[6,7] We have previously used the term OxLDL-E06 to describe the name of this assay. In brief, a dilution of plasma at 1:50 in phosphate-buffered saline was added to microtiter wells coated with monoclonal antibody MB47, which specifically binds apo B-100 particles. Under these conditions, a saturating amount of apo B-100 was added to each well, and consequently, equal numbers of apo B-100 particles were captured in each well for all assays. The oxidized phospholipid:apo B-100 ratio was measured by chemiluminescent enzyme-linked immunosorbent assay with the use of biotinylated E06, as described elsewhere.[6,7]

STATISTICAL ANALYSIS

Discrete data are presented as frequencies and percentages, and continuous variables as means and standard deviations or as medians and interquartile ranges if the distributions were skewed. Spearman's correlation coefficient was used to measure the linear associations between the rank values of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels as well as lipid levels and other clinical risk factors. The association of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels with the extent of coronary artery disease was tested by one-way analysis of variance of the log-transformed values followed by a one-degree-of-freedom test for trend. The percentages of patients with obstructive coronary artery disease and the odds ratios were calculated for quartiles of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels for all patients, according to age (≤60 years or >60 years), and according to the presence or absence of hypercholesterolemia.

Logistic-regression models were used to estimate the associations between patients' characteristics and lipid measurements and obstructive coronary artery disease. Multiple logistic-regression analysis was used to estimate the partial associations between the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels and obstructive coronary artery disease, with adjustment for age, sex, smoking status, the presence or absence of hypertension, and levels of LDL cholesterol, HDL cholesterol, triglycerides, and CRP. The base-2 logarithms ($\log_2$) of the oxidized phospholipid:apo B-100 ratio and the levels of Lp(a) lipoprotein, triglycerides, and CRP were used in all the logistic-regression models to account for skewness in the distributions. Thus, odds ratios for these variables reflect the change in odds for an increase of 1 $\log_2$ (the equivalent of a doubling of the value) in the measure.

RESULTS

The baseline clinical characteristics of the patients, indications for coronary angiography, lipid measurements, and CRP levels are shown in Table 1. The distributions of both the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels were skewed toward lower values, with 85 percent of the patients having levels lower than 0.4 and 45 mg per deciliter, respectively (Fig. 1). In the entire population, a strong correlation (r=0.83, P<0.001) was noted between the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels.

ASSOCIATION WITH THE EXTENT OF ANGIOGRAPHICALLY DOCUMENTED DISEASE

In the entire study group, the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels were strongly associated with a graded increase in the extent of coronary artery disease (P<0.001 for both analyses) (data not shown). These relationships were markedly stronger for patients 60 years of age or younger than for patients older than 60 years (Fig. 2).

ASSOCIATION WITH OBSTRUCTIVE CORONARY ARTERY DISEASE

The proportion of patients with obstructive coronary artery disease increased consistently with increases in the oxidized phospholipid:apo B-100 ratio and in Lp(a) lipoprotein levels (Table 2). This association was particularly evident among patients 60 years of age or younger, among whom the highest quartiles of the oxidized phospholipid:apo B-100 ratio (odds ratio, 3.12; P<0.001) and Lp(a) lipoprotein levels (odds ratio, 3.64, P<0.001) were associated with a significantly higher risk, as compared with the lowest quartiles. This association was not present among patients older than 60 years.

The combined effects of hypercholesterolemia plus either the oxidized phospholipid:apo B-100 ratio or Lp(a) lipoprotein levels greatly increased the probability of obstructive coronary artery disease. When compared with patients in the lowest quartile who did not have hypercholesterolemia, patients in the highest quartile of the oxidized phospholipid:apo B-100 ratio or Lp(a) lipoprotein levels who had hypercholesterolemia were significantly more likely to have obstructive coronary artery disease (Table 3). These relationships were markedly accentuated among patients 60 years of age or younger (for the oxidized phospholipid:apo B-100 ratio, odds ratio, 16.8 [P<0.001]; for Lp(a) lipoprotein levels, odds ratio, 14.2 [P<0.001]), as compared with those older than 60 years (for the oxidized phospholipid:apo B-100 ratio, odds ratio, 4.95 [P=0.003]; for Lp(a) lipoprotein levels, odds ratio, 4.92 [P=0.007]).

The relationship of the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels to coronary artery disease remained fundamentally similar after the exclusion from analysis of 41 patients with acute myocardial infarction within six weeks

before enrollment. Also, there was a stronger association between the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein levels and coronary artery disease in patients with hypercholesterolemia who were taking statins than among such patients who were not taking statins, but differences in the odds ratios were not statistically significant (data not shown).

PREDICTORS OF OBSTRUCTIVE CORONARY ARTERY DISEASE

Without adjustment for other risk factors, the oxidized phospholipid:apo B-100 ratio was predictive of obstructive coronary artery disease (odds ratio per doubling, 1.19; 95 percent confidence interval, 1.05 to 1.34; P=0.005) as was the Lp(a) lipoprotein level (odds ratio per doubling, 1.22; 95 percent confidence interval, 1.07 to 1.40; P=0.003). Similarly, male sex (odds ratio, 4.33; 95 percent confidence interval, 2.95 to 6.35; P<0.001), age (odds ratio per decade, 1.48; 95 percent confidence interval, 1.25 to 1.75; P<0.001), current smoking (odds ratio, 1.65; 95 percent confidence interval, 1.16 to 2.35; P=0.006), hypertension (odds ratio, 1.81; 95 percent confidence interval, 1.27 to 2.58; P=0.001), LDL cholesterol (odds ratio per increase of 25 mg per deciliter [0.65 mmol per liter], odds ratio, 1.28; 95 percent confidence interval, 1.12 to 1.45; P=0.003), and triglyceride levels (odds ratio per doubling, 1.27; 95 percent confidence interval, 1.00 to 1.61; P=0.05) were also predictive, whereas HDL cholesterol (odds ratio per increase of 10 mg per deciliter [2.3 mmol per liter], 0.64; 95 percent confidence interval, 0.56 to 0.74; P<0.001) was a negative predictor. CRP (odds ratio per doubling, 1.08; 95 percent confidence interval, 0.98 to 1.19; P=0.12) was not a predictor of obstructive coronary artery disease.

Among patients 60 years of age or younger, the odds ratios per doubling for the oxidized phospholipid:apo B-100 ratio (1.43; 95 percent confidence interval, 1.20 to 1.71; P<0.001) and Lp(a) lipoprotein level (1.41; 95 percent confidence interval, 1.16 to 1.73; P<0.001) were significant, whereas among those older than 60 years they were no longer significant (for the oxidized phospholipid:apo B-100 ratio: odds ratio per doubling, 1.05; 95 percent confidence interval, 0.89 to 1.25; P=0.58; and for Lp[a] lipoprotein levels: odds ratio per doubling, 1.09; 95 percent confidence interval, 0.90 to 1.32; P=0.37).

Multivariable analysis with the use of logistic-regression models to derive adjusted odds ratios for coronary artery disease showed that an increase in the oxidized phospholipid:apo B-100 ratio (odds ratio per doubling, 1.21; 95 percent confidence interval, 1.05 to 1.39; P=0.007) was an independent predictor of obstructive coronary artery disease, as were male sex (odds ratio, 4.27; 95 percent confidence interval, 2.59 to 7.03; P<0.001), age (odds ratio per decade, 1.72; 95 percent confidence interval, 1.41 to 2.10; P<0.001), an increase in LDL cholesterol (odds ratio per 25 mg per deciliter, 1.28; 95 percent confidence interval, 1.11 to 1.48; P<

Table 1. Baseline Characteristics and Lipid Levels in the Study Group.*

Variable	Value
Age — yr	60.1±10.9
Female sex — no. (%)	193 (38)
White race — no. (%)†	490 (97)
Hypertension — no. (%)	232 (46)
Current smoker — no. (%)	40 (8)
Previous myocardial infarction — no. (%)	77 (15)
Congestive heart failure — no. (%)	59 (12)
Family history of coronary artery disease — no. (%)	128 (25)
Hypercholesterolemia — no. (%)	286 (57)
Statin therapy — no. (%)	142 (28)
Serum creatinine level — mg/dl	
Median	1.1
Interquartile range	1.0–1.3
Indications for angiography — no. (%)‡	
Myocardial infarction within 6 wk before enrollment	41 (8)
Unstable angina	147 (29)
Dyspnea on exertion	137 (27)
Ischemia on nuclear stress test	125 (25)
Other	166 (33)
Lipid levels — mg/dl	
Total cholesterol	207±45
LDL cholesterol	124±37
HDL cholesterol	48±15
Triglycerides	
Median	153
Interquartile range	112–207
Apolipoprotein B-100	98±21
Lp(a) lipoprotein	
Median	21.1
Interquartile range	8.8–39.6
C-reactive protein — mg/liter	
Median	2.9
Interquartile range	1.2–6.7

* The study group was made up of 504 patients. Plus–minus values are means ±SD. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein.
† Race was self-reported.
‡ Patients could have more than one indication for angiography.

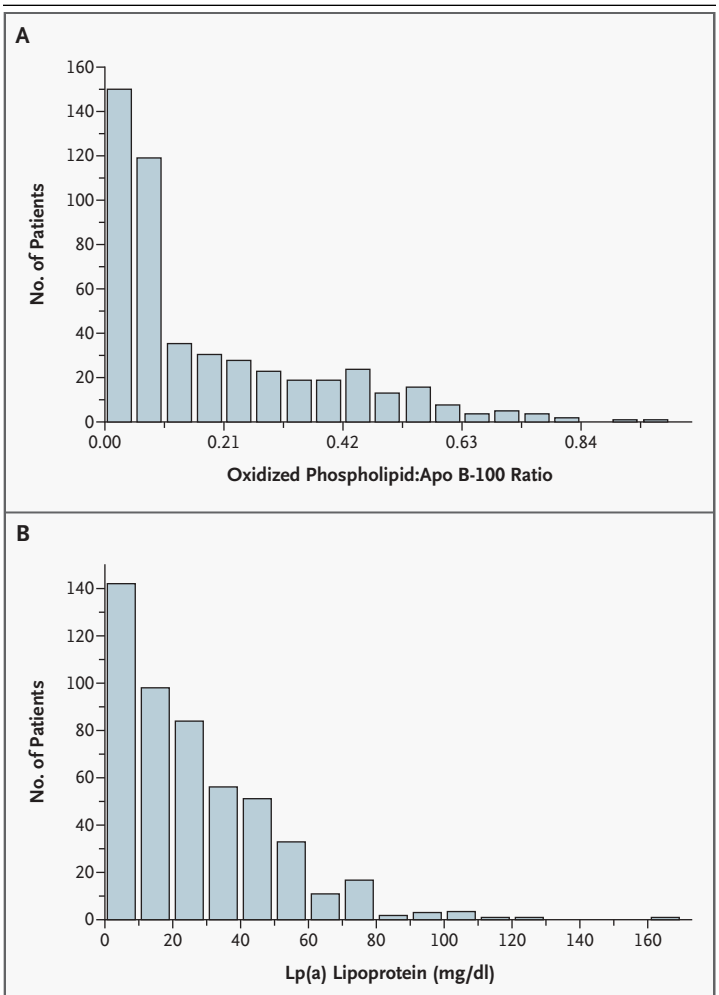

Figure 1. Frequency Distribution of the Oxidized Phospholipid:Apo B-100 Ratio (Panel A) and Lp(a) Lipoprotein Levels (Panel B).

Oxidized phospholipid:apo B-100 ratio denotes the oxidized phospholipid content per particle of apolipoprotein B-100.

0.001), and hypertension (odds ratio, 1.67; 95 percent confidence interval, 1.10 to 2.52; P=0.016), whereas an increase in HDL cholesterol levels (odds ratio per 10 mg per deciliter, 0.75; 95 percent confidence interval, 0.63 to 0.90; P=0.002) was a negative predictor. An increase in CRP (odds ratio per doubling, 1.09; 95 percent confidence interval, 0.97 to 1.22; P=0.16) was not a predictor of obstructive coronary artery disease. When Lp(a) lipoprotein was added to the model and the oxidized phospholipid:apo B-100 ratio was removed, Lp(a) lipoprotein was also an independent predictor (odds ratio per doubling, 1.20; 95 percent confidence interval, 1.02 to 1.40; P=0.02). As in the unadjusted data, the odds ratios per doubling for the oxidized phospholipid:apo B-100 ratio (1.49; 95 percent confidence interval, 1.20 to 1.84; P<0.001) and for Lp(a) lipoprotein (1.42; 95 percent confidence interval, 1.12 to 1.81; P=0.004) among patients 60 years of age or younger were significantly accentuated, whereas among those older than 60 years they were no longer significant (for the oxidized phospholipid: apo B-100 ratio: odds ratio per doubling, 1.00; 95 percent confidence interval, 0.82 to 1.22; P=0.96; for Lp(a) lipoprotein: odds ratio per doubling, 1.05; 95 percent confidence interval, 0.84 to 1.31; P=0.69).

Interestingly, in the entire study group, when Lp(a) lipoprotein was forced into the model with the oxidized phospholipid:apo B-100 ratio, there was a trend toward significance of the oxidized phospholipid:apo B-100 ratio (odds ratio per doubling, 1.21; 95 percent confidence interval, 0.95 to 1.54; P=0.12), whereas Lp(a) lipoprotein levels no longer remained an independent predictor of coronary artery disease (odds ratio per doubling, 1.00; 95 percent confidence interval, 0.76 to 1.32; P=0.99). However, when patients were analyzed according to age, the oxidized phospholipid:apo B-100 ratio, but not Lp(a) lipoprotein levels, was an independent predictor of obstructive coronary artery disease among those 60 years of age or younger, but not among those older than 60 years (Fig. 3). CRP was also a predictor of obstructive coronary artery disease among patients 60 years of age or younger, but not among those older than 60 years. When the 41 patients with acute myocardial infarction, who also had the highest levels of CRP, were removed from the analysis, CRP was no longer a predictor of obstructive coronary artery disease (odds ratio per doubling, 1.06; 95 percent confidence interval, 0.85 to 1.33; P=0.58), but the oxidized phospholipid:apo B-100 ratio (odds ratio per doubling, 1.55; 95 percent confidence interval, 1.05 to 2.27; P=0.03) remained a significant predictor. When the data were evaluated according to the absence of coronary artery disease, as compared with the presence of any coronary artery disease, the odds ratios were slightly smaller, but in general, the trends described were maintained, so that younger patients had higher odds ratios than older patients.

CORRELATIONS BETWEEN OXIDIZED LDL LEVELS AND OTHER BIOMARKERS

Levels of LDL cholesterol were weakly associated with levels of Lp(a) lipoprotein (r=0.17, P<0.001),

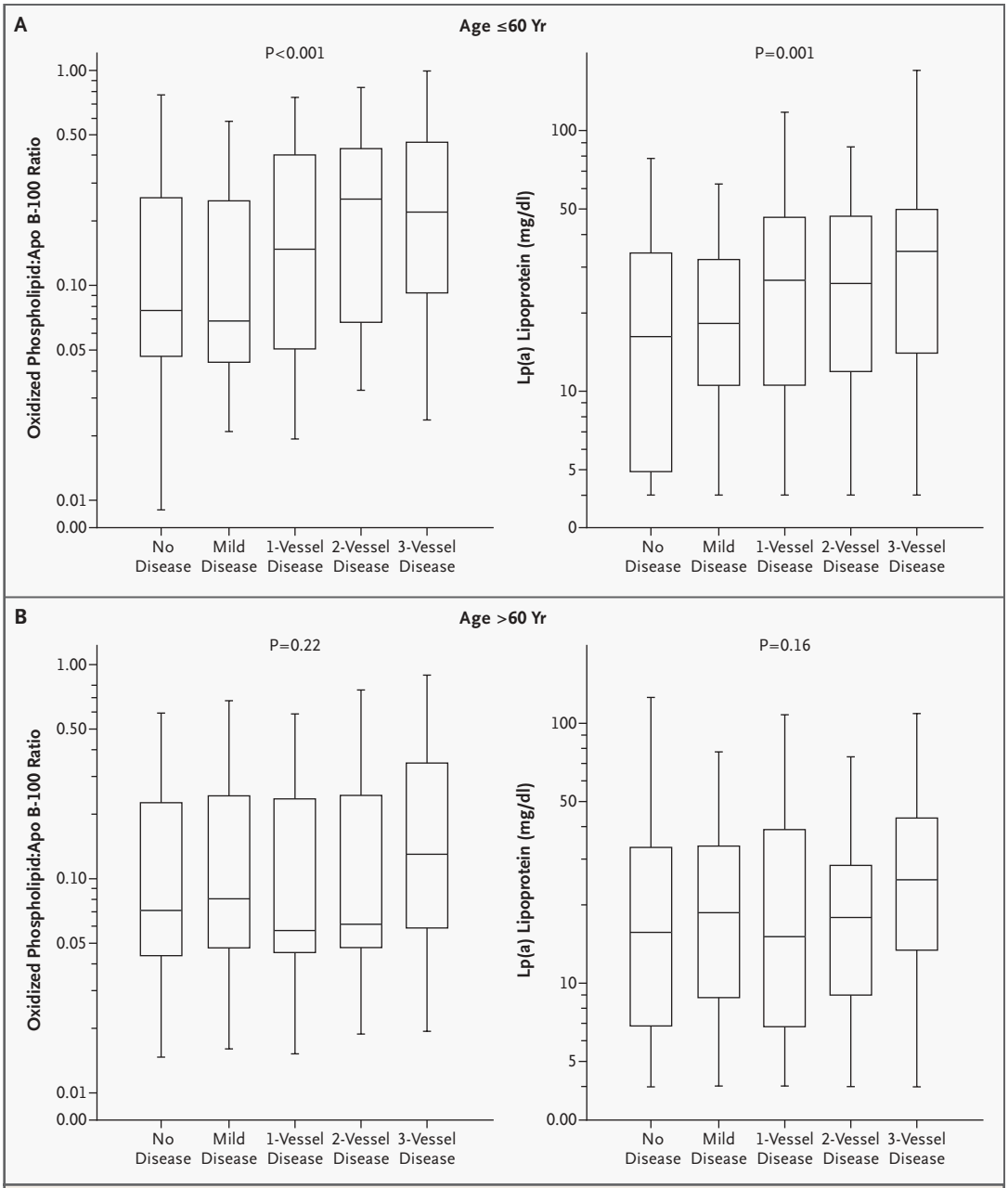

Figure 2. Association of the Oxidized Phospholipid:Apo B-100 Ratio and Lp(a) Lipoprotein Levels to the Extent of Coronary Artery Disease (CAD) in 239 Patients 60 Years of Age or Younger and 265 Patients Older Than 60 Years.

The extent of coronary artery disease is categorized as no disease (stenosis of less than 10 percent of the luminal diameter), mild disease (stenosis of 10 to 50 percent of the luminal diameter), or one-vessel, two-vessel, or three-vessel disease (all patients with a stenosis of more than 50 percent of the luminal diameter). Each box represents the median and interquartile range of values, with the I bars (whiskers) extended to the minimum and maximum. Oxidized phospholipid:apo B-100 ratio denotes the oxidized phospholipid content per particle of apolipoprotein B-100. Values for the oxidized phospholipid:apo B-100 ratio and Lp(a) lipoprotein are shown on a logarithmic scale.

Table 2. Odds Ratios for Obstructive Coronary Artery Disease (CAD) According to Quartiles for the Ratio of Oxidized Phospholipids to Apolipoprotein B-100 and Levels of Lp(a) Lipoprotein.*

Patient Group	Oxidized Phospholipid:Apo B-100 Ratio			Lp(a) Lipoprotein		
	Total No.	No. with CAD (%)	OR (95% CI)	Total No.	No. with CAD (%)	OR (95% CI)
All patients						
Quartile I	126	59 (47)	1.00	126	56 (44)	1.00
Quartile II	125	64 (51)	1.19 (0.73–1.96)	126	62 (49)	1.21 (0.74–1.99)
Quartile III	126	68 (54)	1.33 (0.81–2.18)	127	70 (55)	1.54 (0.94–2.52)
Quartile IV	125	79 (63)	1.95 (1.18–3.23)	125	83 (66)	2.47 (1.48–4.12)
P for trend			0.009			<0.001
Age ≤60 yr						
Quartile I	58	19 (33)	1.00	60	18 (30)	1.00
Quartile II	53	17 (32)	0.97 (0.44–2.15)	57	19 (33)	1.17 (0.53–2.54)
Quartile III	60	27 (45)	1.68 (0.79–3.55)	58	28 (48)	2.18 (1.02–4.63)
Quartile IV	68	41 (60)	3.12 (1.50–6.48)	64	39 (61)	3.64 (1.73–7.68)
P for trend			<0.001			<0.001
Age >60 yr						
Quartile I	68	40 (59)	1.00	66	38 (58)	1.00
Quartile II	72	47 (65)	1.32 (0.66–2.61)	69	43 (62)	1.22 (0.61–2.43)
Quartile III	66	41 (62)	1.15 (0.57–2.30)	69	42 (61)	1.15 (0.58–2.28)
Quartile IV	57	38 (67)	1.40 (0.67–2.83)	61	44 (72)	1.91 (0.91–4.01)
P for trend			0.46			0.13

* OR denotes odds ratio, and CI confidence interval. For the oxidized phospholipid:apo B-100 ratio, quartiles I through IV correspond to the following values: <0.047, 0.047 to 0.089, >0.089 to 0.294, and >0.294, respectively. For Lp(a) lipoprotein, quartiles I through IV correspond to the following values: <8.8, 8.8 to 21.1, >21.1 to 39.7, and >39.7 mg per deciliter, respectively. Samples for the oxidized phospholipid:apo B-100 ratio were unavailable for two patients.

and with the oxidized phospholipid:apo B-100 ratio ($r=0.09$, $P=0.05$). CRP levels correlated weakly with LDL cholesterol levels ($r=0.10$, $P=0.02$) and triglyceride levels ($r=0.11$, $P=0.01$). There were no significant correlations between the oxidized phospholipid:apo B-100 ratio or Lp(a) lipoprotein levels and CRP levels, age, body-mass index, blood pressure, and serum creatinine level.

DISCUSSION

This study shows an association between the oxidized phospholipid:apo B-100 ratio in plasma and the presence and extent of angiographically documented coronary artery disease. The association is independent of all clinical and lipid-related risk factors, except one, Lp(a) lipoprotein, which also has a strong association with angiographically documented coronary artery disease. The odds ratios for angiographically documented coronary artery disease associated with the Lp(a) lipoprotein level were nearly identical with those associated with the oxidized phospholipid:apo B-100 ratio. However, among patients younger than 60 years of age, the oxidized phospholipid:apo B-100 ratio remained an independent predictor of obstructive coronary artery disease. There was a strong correlation between levels of Lp(a) lipoprotein and the oxidized phospholipid:apo B-100 ratio. These observations, in conjunction with previous studies from our laboratory showing that in plasma such oxidized phospholipids are predominantly physically present on Lp(a) lipoprotein,[5-7,9] as opposed to other lipoproteins, lend strong support to the hypothesis that, in the setting of enhanced oxidative stress, proinflammatory oxidized phospholipids may, in part, mediate the atherogenicity of Lp(a) lipoprotein.

The natural murine monoclonal IgM autoantibody E06, cloned from apolipoprotein E-receptor–deficient mice,[12] is functionally identical with classic natural T15 murine antibodies that bind phosphorylcholine on the cell-wall polysaccharide of patho-

Table 3. Odds Ratios for Obstructive Coronary Artery Disease (CAD) According to Quartiles of the Ratio of Oxidized Phospholipids to Apolipoprotein B-100 and Levels of Lp(a) Lipoprotein in Patients without and with Hypercholesterolemia (HC).*

Patient Group	Oxidized Phospholipid:Apo B-100 ratio					LP(a) Lipoprotein				
	No HC		HC		P Value	No HC		HC		P Value
	% *with CAD*	*OR (95% CI)*	% *with CAD*	*OR (95% CI)*		% *with CAD*	*OR (95% CI)*	% *with CAD*	*OR (95% CI)*	
All patients										
Quartile I	29	1.00	67	4.93 (2.31–10.5)		31	1.00	60	3.41 (1.63–7.11)	
Quartile II	44	1.92 (0.91–4.06)	56	3.10 (1.54–6.25)		35	1.18 (0.55–2.56)	59	3.28 (1.64–6.56)	
Quartile III	38	1.47 (0.68–3.19)	64	4.36 (2.16–8.79)		39	1.42 (0.67–3.02)	67	4.57 (2.25–9.29)	
Quartile IV	39	1.54 (0.70–3.40)	77	8.13 (3.88–17.1)	<0.001	48	2.04 (0.93–4.48)	77	7.30 (3.53–15.1)	<0.001
Age ≤60 yr										
Quartile I	14	1.00	61	9.33 (2.64–33.0)		15	1.00	50	5.80 (1.71–19.7)	
Quartile II	27	2.21 (0.61–7.97)	37	3.53 (1.03–12.0)		15	1.05 (0.25–4.39)	48	5.44 (1.67–17.7)	
Quartile III	28	2.33 (0.64–8.45)	57	8.00 (2.51–25.5)		37	3.36 (1.01–11.2)	61	8.96 (2.66–30.2)	
Quartile IV	43	4.59 (1.39–15.1)	74	16.8 (5.11–55.2)	<0.001	46	4.97 (1.46–16.9)	71	14.2 (4.37–46.3)	<0.001
Age >60 yr										
Quartile I	45	1.00	71	3.00 (1.10–8.18)		47	1.00	69	2.47 (0.90–6.77)	
Quartile II	61	1.85 (0.67–5.15)	68	2.57 (1.01–6.54)		54	1.31 (0.47–3.65)	67	2.33 (0.92–5.89)	
Quartile III	48	1.11 (0.39–3.14)	71	2.90 (1.11–7.58)		42	0.80 (0.28–2.31)	71	2.77 (1.09–7.03)	
Quartile IV	31	0.55 (0.15–1.92)	80	4.95 (1.76–13.9)	0.003	50	1.12 (0.36–3.53)	81	4.92 (1.77–13.7)	0.007

* OR denotes odds ratio, and CI confidence interval. For the oxidized phospholipid:apo B-100 ratio, quartiles I through IV correspond to the following values: <0.047, 0.047 to 0.089, >0.089 to 0.294, and >0.294, respectively. For Lp(a) lipoprotein, quartiles I through IV correspond to the following values: <8.8, 8.8 to 21.1, >21.1 to 39.7, and >39.7 mg per deciliter, respectively. The P values indicate whether any two of the eight groups (defined by quartile and hypercholesterolemia status) have significantly different proportions of subjects with CAD.

gens such as pneumococcus and provide optimal protection from pneumococcal infections.[13] In vitro, E06 binds to and prevents the uptake of oxidized LDL and apoptotic cells by scavenger receptors of macrophages. Binder et al. have also shown that the immunization of mice with *Streptococcus pneumoniae* results in increased titers of IgM oxidized LDL autoantibodies and reduction in the progression of atherosclerosis.[14,15] These observations suggest that seemingly unrelated proatherogenic processes, such as oxidation, apoptosis, and infection, share molecular mimicry of the phosphorylcholine epitopes found on proinflammatory oxidized phospholipids.[16]

Although previous studies have shown that plasma oxidized LDL levels are elevated in patients with clinically manifest stable coronary artery disease[17,18] and acute coronary syndromes,[2,4,5,19] our study shows that oxidized phospholipids present on particles of apo B-100 and primarily on Lp(a) lipoprotein correlate with both the presence and extent of angiographically documented coronary artery disease. Although most of the oxidized LDL is present within the vessel wall,[20] this study suggests that the small amounts of minimally modified LDL (e.g., particles of apo B-100 that contain oxidized phospholipids) are present in the circulation. This finding is also consistent with previous studies from our laboratory showing that the oxidized phospholipid:apo B-100 ratio (with oxidized LDL measured with use of antibody E06) rises abruptly after acute coronary events[5] and immediately after percutaneous coronary intervention[6] — situations in which the release of oxidized phospholipids (or oxidized LDL, or both) from the vessel wall might be postulated.

A potential pathophysiological relationship between levels of oxidized phospholipids and Lp(a) lipoprotein is strongly supported by this study and by data from earlier studies from our laboratory showing that oxidized phospholipids are physically associated with Lp(a) lipoprotein[5-7] bound to

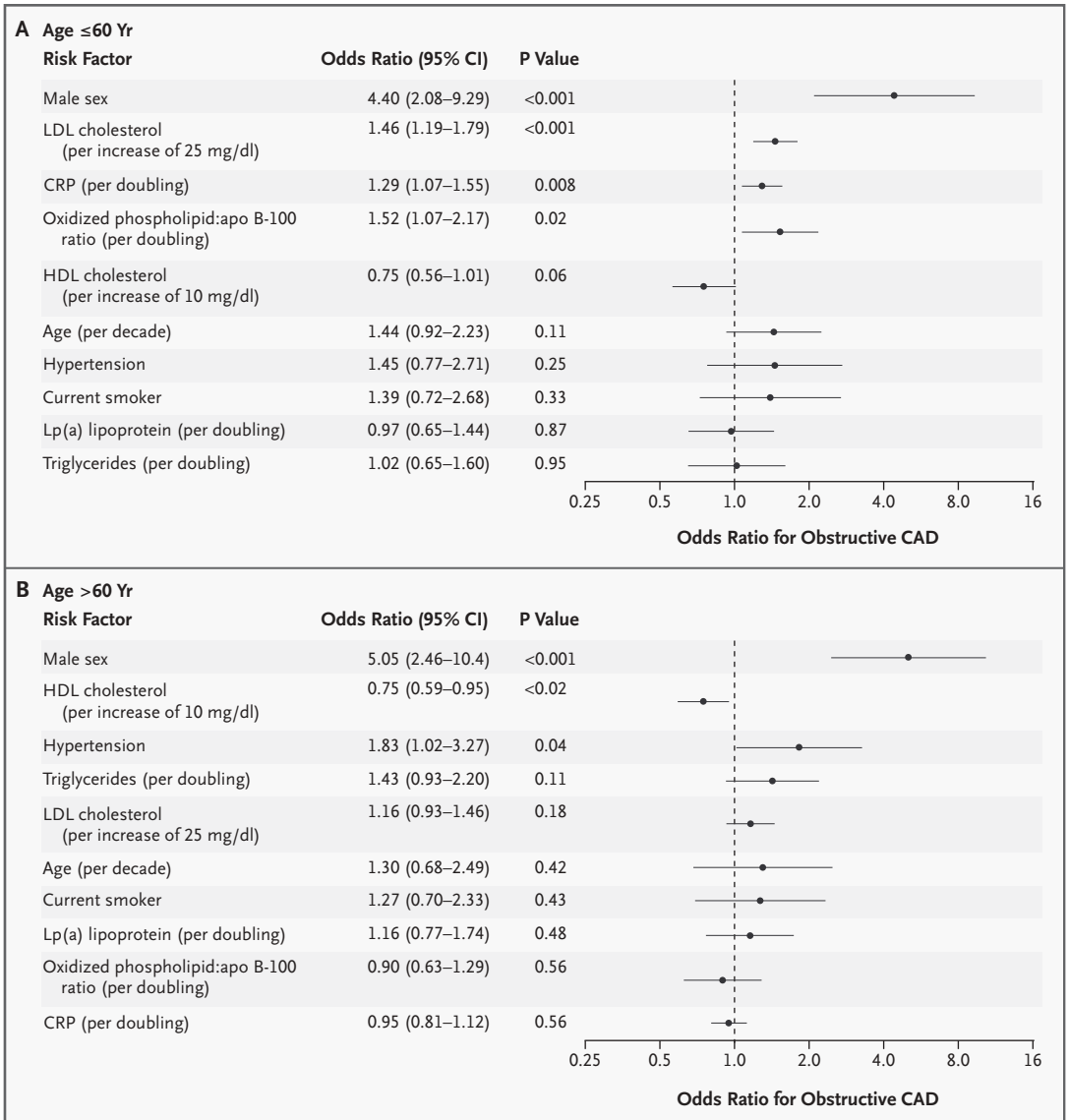

Figure 3. Odds Ratios for Obstructive Coronary Artery Disease (CAD) Associated with Selected Risk Factors among Patients 60 Years of Age or Younger and Those Older Than 60 Years, from the Multivariable Analysis.

CI denotes confidence interval, LDL low-density lipoprotein, CRP C-reactive protein, oxidized phospholipid:apo B-100 ratio the ratio of oxidized phospholipid content per particle of apolipoprotein B-100, and HDL high-density lipoprotein. Risk factors are shown in descending order of significance. In this analysis, Lp(a) lipoprotein was forced into the model with the oxidized phospholipid:apo B-100 ratio.

lysine residues on isolated fragments of kringle V of apolipoprotein(a)[9] and also in the lipid phase of Lp(a) lipoprotein (unpublished data). In addition, the kringle V fragments containing such oxidized phospholipids induce inflammatory responses by up-regulating secretion of interleukin-8 by cultured human macrophages.[9,21]

In this study, we have shown that the predictive abilities of levels of oxidized LDL and Lp(a) lipoprotein for obstructive coronary artery disease are highly interdependent. In the entire study group, when Lp(a) lipoprotein was excluded from the multivariable analysis, the odds ratios for the oxidized phospholipid:apo B-100 ratio were similar to those for traditional risk factors such as age, hypertension, and LDL cholesterol. Similarly, without the ox-

idized phospholipid:apo B-100 ratio in the analysis, Lp(a) lipoprotein levels stood as an independent predictor, as has been shown in a recent meta-analysis.[8] In the entire study group, with the oxidized phospholipid:apo B-100 ratio in the model, there was no added ability of Lp(a) lipoprotein levels to explain the risk of obstructive coronary artery disease, suggesting that measures of oxidized LDL and Lp(a) lipoprotein represent a common path of biologic influence on the risk for coronary artery disease. However, in patients 60 years of age or younger, the oxidized phospholipid:apo B-100 ratio maintained its independent predictive power even with Lp(a) lipoprotein in the model. This observation supports the hypothesis that much of the risk attributable to Lp(a) lipoprotein levels can be explained by the binding of oxidized phospholipids by Lp(a) lipoprotein, but that in younger patients, an additional risk associated with oxidized phospholipids may be present, perhaps through proinflammatory pathways independent of Lp(a) lipoprotein.

The physiologic role of Lp(a) lipoprotein is unknown. We and others have suggested that a potential physiologic role of Lp(a) lipoprotein may be to bind and detoxify proinflammatory oxidized phospholipids.[5-7,22] Lp(a) lipoprotein, which is present only in humans and Old World primates (although a partially related gene arose separately in hedgehogs), may have evolved to provide protection against various oxidative stressors. For example, Lp(a) lipoprotein has been shown to be involved in wound healing[23] and possibly in preventing angiogenesis in tumor models,[24] and elevated levels have been noted in centenarians in a manner consistent with human longevity.[25]

Similarly, oxidized phospholipids are generated not only during atherogenesis but also in inflammation and apoptosis,[13,26] which suggests that housekeeping functions involving the clearance of such oxidized phospholipids may have evolved for maintaining general health as well as vascular health. In this regard, Lp(a) lipoprotein may act in a way similar to CRP, which Chang et al. have shown also binds specifically to the phosphorylcholine moiety of oxidized phospholipids and apoptotic cells.[27] Indeed, we and others have shown that Lp(a) lipoprotein acts as an acute-phase reactant in patients with acute coronary syndromes.[5,6,28] It has also been reported to be highly enriched (higher by a factor of 7 than LDL) in platelet-activating factor acetyl hydrolase,[29,30] an enzyme that potentially could detoxify such oxidized phospholipids by removing the oxidized fatty acid.

Thus, when present at low levels, Lp(a) lipoprotein may serve a protective function by binding and participating in the transfer and possible degradation of oxidized phospholipids formed during normal homeostasis or in acutely stressful situations. However, when Lp(a) lipoprotein levels are chronically elevated (as determined genetically), especially in a milieu of chronically increased oxidative stress, Lp(a) lipoprotein, with its content of oxidized phospholipids, may be proatherogenic, particularly since it has enhanced binding to the extracellular matrix of the artery wall.[31-33]

The association between the oxidized phospholipid:apo B-100 ratio and angiographically documented coronary artery disease in our study was much stronger for patients 60 years of age or younger than for older patients. The reasons for this association are not entirely clear, but many previous studies have documented a strikingly similar relationship between Lp(a) lipoprotein levels and angiographically documented disease among younger patients only.[34-40] By excluding patients with diabetes and previous coronary revascularization from our study, we may have preferentially enriched the study group with younger patients with fewer traditional risk factors. In addition, increasing age, which is a surrogate for known and unknown risk factors, is itself one of the strongest risk factors for coronary artery disease. Thus, the independent effects of oxidized LDL and Lp(a) lipoprotein levels appear to diminish with age, presumably because of the cumulative contributions of additional risk factors that affect the clinical expression of atherosclerosis.

The limitations of this study include the fact that angiography is not a precise method for quantifying atherosclerosis. In addition, we have not yet defined the exact oxidized phospholipids, their physical location within Lp(a) lipoprotein, or the rates of flux, binding, and removal of oxidized phospholipids that are on Lp(a) lipoprotein.

In conclusion, we have documented that plasma levels of oxidized phospholipids present on apo B-100–containing lipoproteins and predominantly on Lp(a) lipoprotein reflect the presence and extent of angiographically documented coronary artery disease. We propose that in settings of enhanced oxidative stress and elevated Lp(a) lipoprotein levels, a proinflammatory milieu may predominate that contributes to the clinical expression of cardiovas-

cular disease. Further studies are needed to explore these mechanisms and to determine whether these measures of oxidation predict clinical events.

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Dr. Tsimikas reports having served as a consultant to and on the speakers' bureau of Pfizer and General Electric and having received investigator-initiated grants from these companies. Dr. Witztum reports having served as a consultant to AtheroGenics and on the speakers' bureau of Merck. Dr. Berger reports having received research funding and honoraria from Aventis, Bristol-Myers Squib, and Sanofi and having served on scientific advisory boards for Genentech and Johnson & Johnson. Dr. Kornman is an employee of Interleukin Genetics and reports holding equity in the company. Dr. McConnell reports having received grant support from diaDexus. A patent for the potential use of the E06 antibody has been awarded to the University of California in the names of Dr. Witztum and colleagues and has been licensed by the University of California to AtheroGenics.

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***JOURNAL* INDEX**

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BRIEF REPORT

Ovarian Transplantation between Monozygotic Twins Discordant for Premature Ovarian Failure

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SUMMARY

Monozygotic 24-year-old twins presented with discordant ovarian function. One had had premature ovarian failure at the age of 14 years, whereas her sister had normal ovaries and three naturally conceived children. After unsuccessful egg-donation therapy, the sterile twin received a transplant of ovarian cortical tissue from her sister by means of a minilaparotomy. Within three months after transplantation, the recipient's cycles resumed and serum gonadotropin levels fell to the normal range. During the second cycle, she conceived, and her pregnancy progressed uneventfully. At 38 weeks' gestation, she delivered a healthy-appearing female infant.

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NUMEROUS STUDIES HAVE CONFIRMED THAT OVARIAN GRAFTS CAN RE-store ovarian function and fertility in sterilized animals.[1-6] With the rise in the number of young survivors of neoplastic and other diseases who are rendered sterile by gonadotoxic drugs or radiation, the possibility of ovarian transplantation in humans is receiving increased attention. Some centers offer to bank ovarian tissue of young patients with cancer, with the aim of restoring fertility by transplanting thawed ovarian tissue after they are cured or in long-term remission. If ovarian transplantation is proven to be safe and effective in humans, fertility preservation might become readily available for young women who need to delay childbearing for medical or social reasons. We report a case of ovarian transplantation between healthy monozygotic twins by means of a simple cortical-graft technique. One twin had premature menopause, whereas the other twin was fertile and volunteered to donate one of her ovaries.

CASE REPORT

The patients were 24-year-old twin sisters who were confirmed to be monozygotic by means of genetic fingerprinting at 15 loci. One of the sisters (the donor) had three naturally conceived children and had been using oral contraceptives during the year preceding transplantation. Secondary amenorrhea had developed in the other twin (the recipient) at the age of 14 years, after three years of cycles with scanty menses. She had had no menses for 10 years before the ovarian transplantation. Her serum follicle-stimulating hormone (FSH) level at the age of 15 years ranged from 49 to 104 mIU per milliliter. When she was 20 years old, a laparoscopic examination and ovarian biopsy revealed atrophic, elongated ("streak") gonads with no follicles and a small uterus with an otherwise normal reproductive tract. At the age of 23 years, the sisters underwent two un-

successful cycles of in vitro fertilization (IVF) at another center. The donor sister received controlled ovarian stimulation with standard doses of hormones for egg donation. A total of 14 oocytes (9 of which were mature) were recovered from 16 preovulatory follicles, and 7 zygotes were generated by IVF. Pregnancy was not achieved after the transfer of two fresh embryos or after the transfer of two cryopreserved embryos in a subsequent cycle of IVF.

The twins declined any further attempts at egg donation. Informed that an attempt could be made to transplant ovarian tissue, they requested that this approach be tried instead to restore fertility to the menopausal sister. The women were in excellent general health, and the donor's ovarian function was considered to be normal on the basis of her reproductive history, peripheral hormone levels, ovarian ultrasonographic findings, and previous ovarian stimulation for IVF. Peripheral hormones measured in the infertile twin one day before the transplantation remained at menopausal levels: 75 mIU of FSH per milliliter, 32 mIU of luteinizing hormone (LH) per milliliter, and 4 pg of estradiol per milliliter.

METHODS

SURGERY

The surgical procedure was approved by the ethics review committee of St. Luke's Hospital, and both sisters provided written informed consent. They were screened for human immunodeficiency virus type 1 and hepatitis B and C viruses, and the recipient stopped taking oral contraceptives as hormone-replacement therapy two weeks before surgery. The donor underwent laparoscopic removal of her left ovary under general anesthesia. The excised ovary was then dissected ex vivo. The ovarian cortical tissue was trimmed to a thickness of 1 to 2 mm by excising medullary tissue and bursting small antral follicles. This dissection was performed in Leibovitz (L15) medium on ice in less than one hour (Fig. 1A).

Meanwhile, the recipient underwent a minilaparotomy through a 3.5-cm incision above the pubis. The cortex of each streak ovary was resected under magnification of 16× to 25×, exposing the entire raw surface of the medulla. No follicles were observed. Hemostasis was meticulously controlled in the medulla with the use of pinpoint microbipolar forceps and continuous irrigation with heparin-treated saline in order to prevent the formation of a hematoma under the graft, but at the same time care was taken to avoid impairing revascularization by minimizing the amount of cautery. A section of approximately one third of the donor ovarian cortex was laid over the raw medulla of each ovary in the recipient and sutured onto the medulla with the use of 9-0 nylon interrupted stitches (Fig. 1B, 1C, and 2). The remaining third of the donor ovarian cortex was cryopreserved after equilibration in 1.5 M 1,2-propanediol and 0.1 M sucrose and slow cooling in an

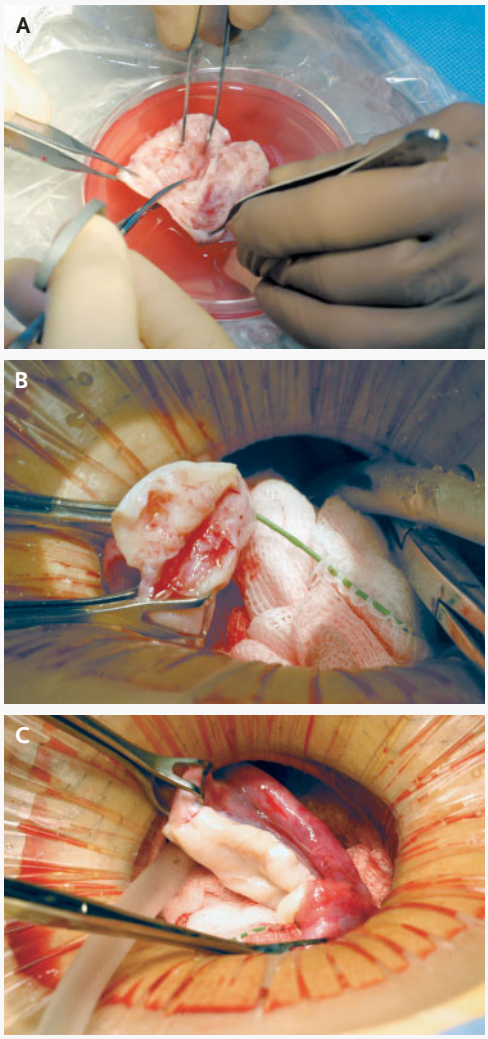

Figure 1. Ovarian Transplantation.
Panel A shows the ex vivo dissection of the donor ovarian cortex. Panel B shows the donor cortex being sutured to the medulla of the recipient after the empty recipient cortex has been removed. Panel C shows the transplant after the completion of the procedure.

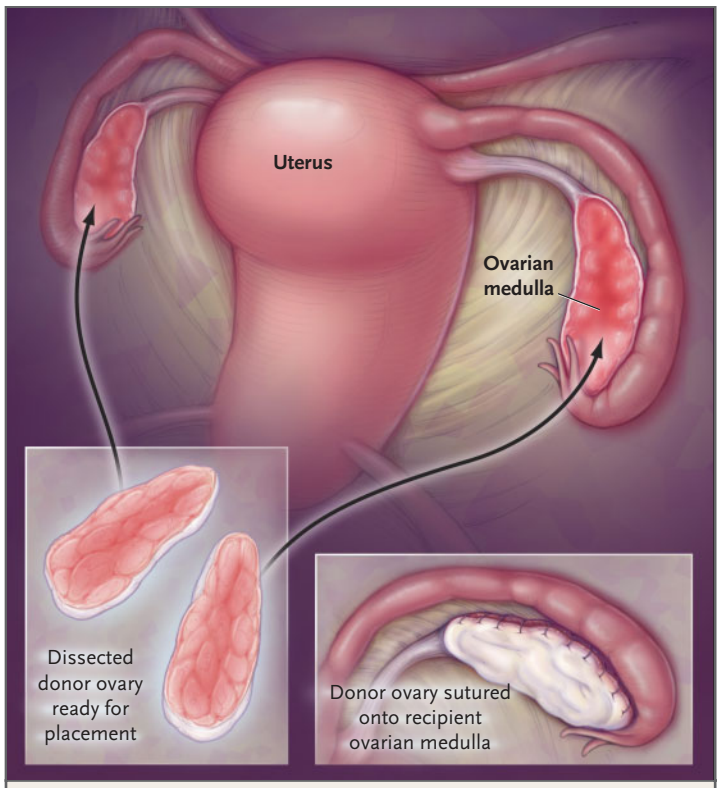

Figure 2. Schematic Illustration of Ovarian Transplantation.

automated freezer.[7,8] Tissue from the ovaries of both women were fixed in Bouin's fluid for histologic examination.

LABORATORY INVESTIGATIONS

Spare cortical and medullary tissues were cultured for cytogenetic analyses according to standard procedures, including G-banding. In addition, enzymatically digested ovarian tissue obtained by direct harvest was examined by fluorescence in situ hybridization. Both women were also evaluated for skewed X-chromosome inactivation at the androgen receptor locus[9] and for an increase in the number of CGG repeats in the *FMR1* gene (indicative of fragile X syndrome) by means of Southern analysis or the polymerase chain reaction, since these mutations have been associated with premature ovarian failure.[10,11] Serum samples were analyzed for FSH, LH, and estradiol with the use of standard immunoassays.

RESULTS

Both patients recovered rapidly and returned to their home state one day after surgery. Seventy-one days after transplantation, a 14-mm follicle had developed in the recipient; the serum estradiol level at this time was 154 pg per milliliter (Table 1) and the uterine-lining thickness was 8 mm. At 80 days, she had her first postoperative menses, although it lasted only a single day. The endocrine profile indicated that her ovaries then remained quiescent until 48 days later (128 days after transplantation), when a 14-mm follicle was again noted sonographically. The thickness of the uterine lining was 10 mm, the estradiol level was 193 pg per milliliter, and the LH level was 123.4 mIU per milliliter. Her basal body temperature then rose from 36.4° to 36.9°C and remained elevated for 13 days. At 141 days after transplantation, her serum FSH and LH had decreased to 9.1 and 4.7 mIU per milliliter, respectively. The following day, a heavy menstrual period began, and her basal body temperature dropped.

On day 3 of the second menstrual cycle (145 days after transplantation), the recipient's FSH level was 7.1 mIU per milliliter. On day 26 of the same cycle, her β human chorionic gonadotropin level was 828 mIU per milliliter. Ultrasonographic examination five weeks after her second menstrual period (day 176 after transplantation) revealed a normal intrauterine pregnancy with a well-defined secondary yolk sac; the β human chorionic gonadotropin level was then 30,289 mIU per milliliter. She had conceived naturally. At seven weeks, ultrasonography verified that she had a normally developing intrauterine pregnancy associated with a fetal heart rate of 148 beats per minute. The 22-week ultrasonogram was normal. She gave birth to a healthy-appearing girl at 38 weeks' gestation by vaginal delivery. The infant's birth weight was 3600 g, and the Apgar scores were 9 at one minute and 10 at five minutes.

All specimens (peripheral lymphocytes, ovarian cortex, and ovarian medulla) from both patients had a normal 46,XX karyotype, and G-banding did not reveal any structural chromosomal aberrations. Moreover, dual-colored fluorescence in situ hybridization of 250 cells from each sample of uncultured tissue with the use of probes for two genes on the sex chromosomes (*DXZ1* and *SRY*, Vysis) did not reveal any XX–XY mosaicism. The degree of inactivation of the X chromosome was within normal limits in both patients (50:50 for the donor and 65:35 for the recipient), and neither patient had an increase in the number of CGG repeats in the *FMR1* gene that was in the premutation or mutation range.

Tissue sections from each sister were compared.

Table 1. Serum Gonadotropin and Estradiol Levels before and after Isologous Ovarian Grafting.*

Days	Follicle-Stimulating Hormone	Luteinizing Hormone	Estradiol
	mIU/ml		*pg/ml*
Before transplantation			
1	74.6	32.1	4
After transplantation			
44	90.3	33.7	—
71	45.6	36.9	154
80		First menses	
84	34.9	—	—
100	68.5	26.2	54
128	50.2	123.4	193
141	9.1	4.7	196
142		Second menses	
145	7.1	—	—
167†	—	—	—

* Dashes indicate that a measurement was not obtained.
† On day 167 after transplantation (day 26 of the second menstrual cycle), the β human chorionic gonadotropin level was 828 mIU per milliliter.

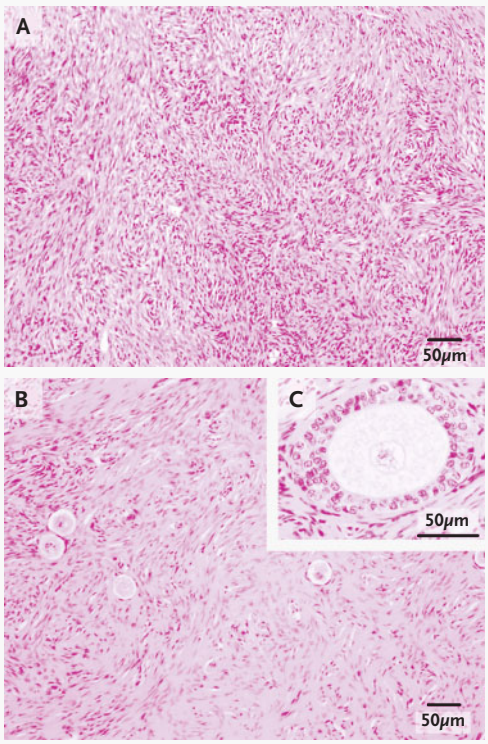

Figure 3. Histologic Findings in the Streak Ovary of the Recipient (Panel A) and the Ovary of the Donor (Panels B and C) (Hematoxylin and Eosin).
Follicles were completely absent in the recipient's ovary (Panel A). In contrast, her twin sister's ovary had four primordial follicles (Panel B) and a developing follicle (Panel C) in this section.

The streak ovaries of the recipient had no detectable follicles (Fig. 3). There was extensive fibrosis, including fibrotic remnants of old corpora albicantia presumably dating from the sporadic ovulatory cycles that had last occurred more than a decade earlier. The smaller fragments of tissue from the donor ovary appeared histopathologically normal and contained follicles of various sizes. Tissue from the contralateral ovary of the donor was not available for study, but its size and appearance suggested that this ovary was also normal.

DISCUSSION

We describe a successful pregnancy in a prematurely menopausal monozygotic twin who received an ovary from her healthy, fertile twin. The pregnancy was established spontaneously, without ancillary medical assistance. It is extremely unlikely that the restoration of ovarian function in this patient after transplantation was due to residual follicles in the streak ovary of the recipient; she had a decade-long history of amenorrhea with elevated gonadotropin levels on all occasions on which they were measured and no detectable follicles on pathological examination.

Studies in animals have indicated that only primordial follicles survive transplantation-related ischemia,[12] but the rapid onset of cyclicity in our patient suggested that at least one growing follicle had survived and grown to maturity. However, follicle recruitment may not have normalized until the second cycle, about three months after transplantation, when serum levels of gonadotropin and estrogen returned to levels characteristic of those in young, fertile women. Endometrial thickness also reached preovulatory dimensions at this time, and an LH surge and elevated basal body temperature indicated that ovulation had occurred. The patient conceived naturally during this cycle.

Grafting of ovarian cortex was chosen because it is minimally invasive and involves substantially less

risk and recovery time than a vascular graft (which one of us described in a report of testis transplantation between male homozygous twins discordant for anorchia[13]). Moreover, cortical grafting (in contrast to vascular anastomosis) affords the opportunity to cryopreserve additional ovarian tissue for a future transplantation, should it be required. To date, whole-ovary cryopreservation has been successful only in rats and would be unlikely to be effective with the much larger human ovary.[14] Although heterotopic grafts of human (or monkey) cortical tissue can generate mature oocytes for IVF,[15-17] we did not pursue that strategy in the present case because the twins had declined further attempts at IVF.

An important technical limitation of cortical grafting, whether orthotopic or heterotopic, is the potential for follicle atresia during the period of ischemia, before the tissue becomes revascularized[12,18]; such an event might compromise long-term ovarian function. Yet experimental studies suggest that long-term ovarian function may not be compromised by grafting.[6] Studies in animals have indicated that a short interval before conception is related to adequate follicular reserves and that the use of grafted ovarian cortex can result in prolonged resumption of fertility.[6] In the current case, the recipient's low levels of serum FSH on day 3 of the second cycle (after she had received only two thirds of one ovary from her sister) and the rapid return of normal ovarian function suggest that normal menstrual cycles and fertility may resume in this patient after pregnancy. If, however, ovarian function were not maintained over the long term, the recipient could receive a second graft of remaining cryopreserved tissue.

The procedure we used is based on a simple method that was first described in sheep.[19-21] In the first reported clinical cases of ovarian autografting, ovarian function was transitory, perhaps because of a paucity of follicles in the grafted tissue as a result of prior chemotherapy.[22,23] Recently, a viable pregnancy was reported after ovarian-tissue banking and transplantation in a patient who was thought to be sterile after treatment for Hodgkin's disease.[24] However, doubts have been expressed as to whether ovulation in that conception cycle occurred from the grafted tissue or from tissue left in situ and in close proximity to the graft.[25] It is impossible to verify the origin of the fertilized egg in that case retrospectively, but the present report provides a compelling demonstration that cortical grafts can restore fertility. Furthermore, our technique involved a single, simple procedure rather than repeated laparoscopic procedures to attach multiple slices of tissue to the pelvic wall. Cryopreservation is not required for this procedure, but if a second transplantation is required, frozen–thawed tissue can be used; experimental evidence suggests that cryopreservation should have only a minor effect on follicular wastage.[7] Routine genetic testing revealed no obvious cause for the discordance between these monozygotic twin sisters, nor any obvious cause for concern about the health of future offspring.

Although ovarian transplantation between monozygotic twins will be rare, the demonstration that ovarian function can be restored and that natural conception and successful pregnancy can be achieved after transplantation of ovarian tissue may have broader implications for preserving fertility in young women, such as those who require potentially sterilizing treatment for cancer.

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CLINICAL TRIAL REGISTRATION

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REVIEW ARTICLE

CURRENT CONCEPTS

Potential Options for Preservation of Fertility in Women

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THE CHOICE OF DELAYING PREGNANCY HAS BECOME THE NORM FOR many women in developed countries. Among some women, however, achieving pregnancy may be difficult or impossible at a later time. The ability to preserve fertility with various methods has become a key issue for some women. Although the need is most pressing among women with cancer, the same therapeutic options may be available for many other women who are reaching an advanced reproductive age. However, in this group, the use of the available techniques is controversial and should be considered experimental.

THE NATURAL PROCESS OF OOCYTE LOSS

The progressive loss of oocytes from fetal life through menopause is a normal process. Female infants have 6 to 7 million oocytes at 20 weeks of gestation, when progressive atresia occurs, resulting in 1 to 2 million oocytes at birth, approximately 25,000 at the age of 37 years, and 1000 at the age of 51 years, the average age of natural menopause in the United States.[1,2] The mechanism underlying this process is poorly understood and involves multiple factors encoded by genes on the X chromosome, as well as on autosomes.[3] Although ovarian aging in mice has a hypothalamic component, this has not been found to be the case in humans. Also, recent data point to the presence of ovarian stem cells, which presumably could lead to replenishment, in mice[4]; however, no such convincing data exist for humans.

It has been hypothesized that in gonadal dysgenesis various deletions on the X chromosome cause such rapid atresia of oocytes[5] that few girls with this condition reach puberty with functioning ovaries. However, it is also possible that such genetic defects result in a smaller complement of oocytes during gonadal development. Although environmental factors may be important, genetic factors predict 44 to 87 percent of the variance in the age at menopause.[6,7]

In normal women, at approximately 37.5 years of age, an accelerated atresia of the oocytes begins[1,2] (Fig. 1). This accelerated loss is poorly understood and is often associated with a small monotropic rise in the level of follicle-stimulating hormone (FSH) and decreased fecundity,[9,10] as well as an increased risk of aneuploidy. The subtle increase in the level of FSH is thought to increase atresia, which is coupled with an accelerated loss of follicles and a further increase in FSH, thus resulting in a positive-feedback loop.[8]

In a classic study of normal women undergoing donor insemination, pregnancy rates at one year declined from 74.0 percent among 20-year-olds to 61.5 percent among women between the ages of 31 and 35 years and to 55.8 percent among women between the ages of 36 and 40 years.[11] Among women undergoing in vitro fertilization–embryo transfer in the United States, deliveries per oocyte retrieved decreased from

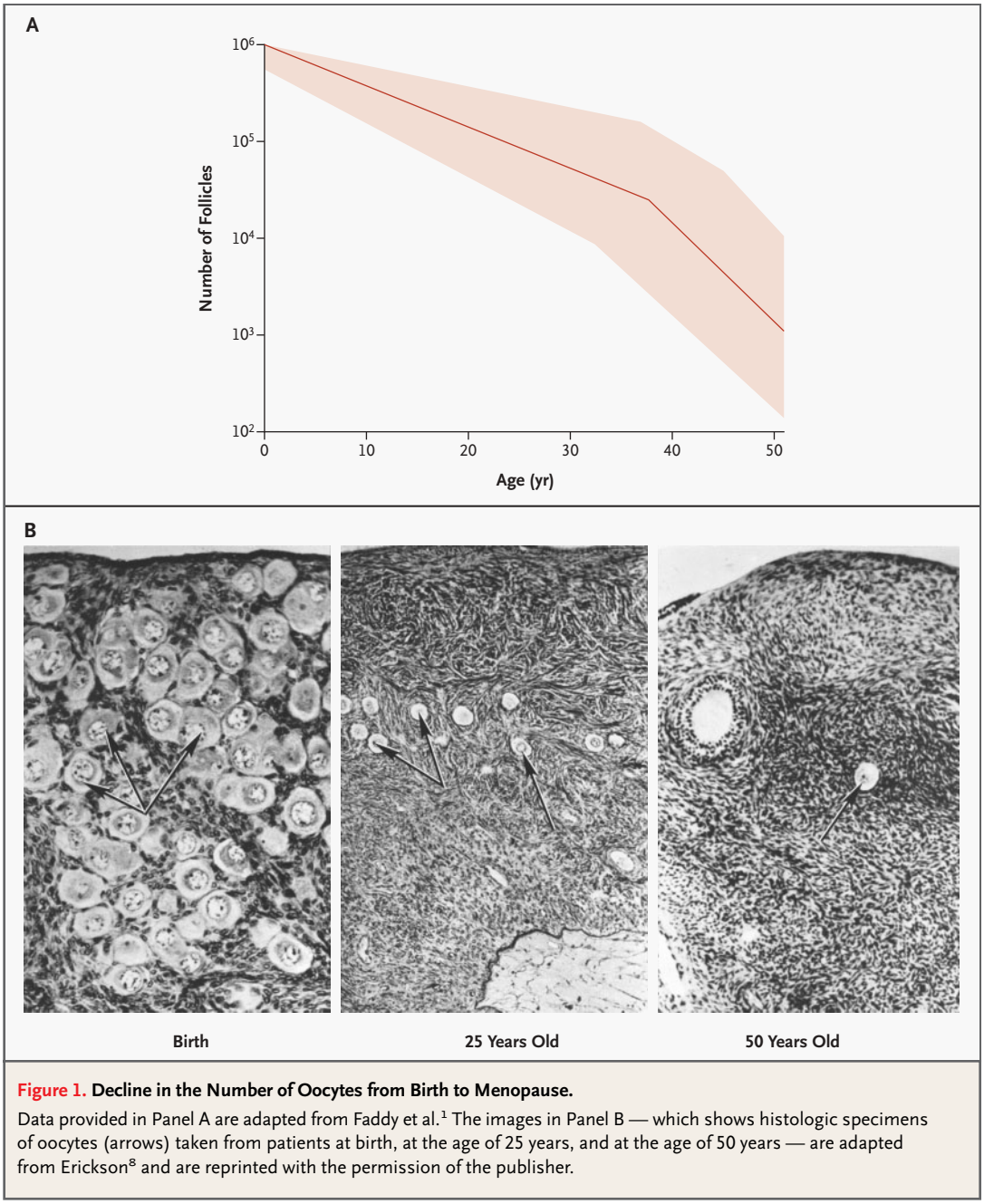

Figure 1. Decline in the Number of Oocytes from Birth to Menopause.

Data provided in Panel A are adapted from Faddy et al.[1] The images in Panel B — which shows histologic specimens of oocytes (arrows) taken from patients at birth, at the age of 25 years, and at the age of 50 years — are adapted from Erickson[8] and are reprinted with the permission of the publisher.

36.9 percent among women under the age of 35 years to 20.5 percent among women between the ages of 38 and 40 years and to 10.7 percent among women between the ages of 41 and 42 years.[12] This decline is largely due to implantation failure and an increased rate of aneuploidy.

It has been hypothesized that there is a fixed window of some 13 years before menopause, during which accelerated ovarian atresia takes place. Accordingly, women who are destined to go through menopause at the age of 45 years (10 percent of the population) might be expected to have accelerated atresia and reduced fecundity beginning at the age of 32 years.[13] However, this hypothesis has not been proved,[14] and it is not known how many women in their early 30s have accelerated atresia. Nevertheless, although it is clear that women with a strong family history of early menopause would be

at risk for reduced fecundity at an earlier age, no data suggest at what age fecundity decreases.

As atresia continues, both the number and quality of oocytes fall below a critical level, and the rate of aneuploidy increases — a finding that is related at least in part to problems of the meiotic spindle[15,16] resulting in nondisjunction. This process leads to a greater risk of spontaneous abortion once pregnancy occurs.

DEFINING THE POPULATION AT RISK FOR REPRODUCTIVE FAILURE

Aging is the most significant factor influencing the ability to conceive. As stated earlier, in normal women, fecundity begins to decline at a more rapid pace after the age of 37.5 years. Women who do not plan to conceive until after this age may wish to consider options to preserve fertility, although some of these approaches remain experimental.

Premature ovarian failure, which is defined as menopause before the age of 40 years or hypergonadotropic amenorrhea, occurs in up to 0.9 percent of women in the general population and has multiple causes, including the involvement of several genes.[17] Once premature ovarian failure has been established, fertility is usually lost, although spontaneous pregnancies may occur in approximately 5 to 6 percent of patients after the diagnosis.[18,19] Familial premature ovarian failure and environmental factors that may deplete ovarian follicles define this risk category. Various environmental factors and toxic exposures may also affect the age at menopause. The most well-documented of these factors is smoking, which can delay menopause by one to two years.[20]

Pelvic diseases — such as endometriosis, neoplasms, and infection — may require surgery, which by removing and destroying cortical tissue depletes the follicular or oocyte reservoir and may lead to early menopause. In addition, pelvic surgery may lead to the formation of adhesions, which may affect the ability to conceive naturally.

Among the woman at greatest risk for the inability to reproduce are those undergoing treatment for cancer. Table 1 shows summary data on the effects of multidrug chemotherapy with or without radiotherapy (predominantly involving nonpelvic organs) in women, stratified according to age. Both the age of the patient and the type and dose of the chemotherapeutic agent influence the progression to ovarian failure, with alkylating agents increasing the risk of premature ovarian failure by a factor of 9.[21] Among teenagers who are being treated for cancer, the risk of premature ovarian failure increases by a factor of 4; among women between the ages of 21 and 25 years, the risk increases by a factor of 27.[22]

Several follow-up studies have shown that among women under the age of 20 years, the rate of amenorrhea after treatment is in the range of 20 to 50 percent, and early menopause is also a risk. Among women who were treated for cancer, premature ovarian failure occurred in 17 percent at a mean age of 26 years[23] and in 42 percent in the third decade,[21] with an overall rate of premature ovarian failure of approximately 60 percent. Spontaneous pregnancies have been found to occur in

Table 1. Ovarian Function and Fertility Potential after Cancer Treatment.

Treatment	Amenorrhea	Premature Ovarian Failure	Ovarian Reserve	Chance of Spontaneous Pregnancy
	% of patients			*% of patients*
Multidrug chemotherapy with or without irradiation				
Age, <20 yr	20–50	17	Decreased	28
Age, >25 yr	80–90	90–100*	Decreased	5
Bone marrow transplantation (age, <20 yr)	>90	80–100	Decreased	0
Pelvic irradiation (without ovariopexy)	6–20 Gy	Increased (dependent on dose and age of patient)	Decreased	Variable uterine compromise

* Premature ovarian failure occurs in 42 percent of women in their 30s and in 60 percent of all patients treated.

28 percent of young women treated for cancer,[23] although decreased ovarian function has been documented in these women.[23,24] In women more than 25 years of age, the rate of amenorrhea rises to 80 to 90 percent, with virtually all women having premature ovarian failure and a reported spontaneous pregnancy rate of only 5 percent.[25,26] Bone marrow transplantation (with its associated treatments) carries the worst prognosis, even when it is performed in children. Only 19 percent of children so treated have normal ovarian function,[27] and in older persons, ovarian failure is virtually universal.[28]

Pelvic irradiation by itself has significant consequences. Complete ovarian failure occurs with a dose of 20 Gy in women under 40 years of age and with only 6 Gy in older women.[29] A dose of 4 Gy may result in the loss of half the ovarian follicles.[30] Ovariopexy, or moving the ovary away from the irradiation field (which is usually performed laparoscopically[31]), results in the preservation of ovarian function in 60 to 100 percent of patients.[32] However, the uterus is extremely vulnerable to irradiation and decreases in volume by 40 percent.[33] It has been suggested that if pregnancy occurs, problems may ensue that are related to abnormalities in uterine function.

TESTING FOR DECREASED OVARIAN RESERVE

Decreased ovarian reserve is defined by a poor ovarian follicular response to stimulation, which by implication signifies a decreased number of oocytes. Are there tests that may help to identify women who have decreased ovarian reserve? Even with normal ovulatory cycles, FSH levels may be elevated early in the menstrual cycle, signaling a decreased ovarian reserve. On day 3 of the menstrual cycle, serum FSH levels are usually less than 10 mIU per milliliter in most assays. FSH levels that are more than 15 mIU per milliliter on day 3 suggest a decreased ovarian reserve and a reduced probability of pregnancy; if values exceed 20 mIU per milliliter, the probability of pregnancy is close to nil. Although these levels vary from one cycle to the next, it has been suggested that any elevation signals a poor prognosis.[34] Measures of estradiol that are obtained concurrently are useful, since values that are more than 80 pg per milliliter signify disrupted folliculogenesis, which does not allow for an accurate interpretation of FSH measurements. Abnormal tests of ovarian reserve indicate that the probability of pregnancy is approximately 5 percent.[34,35]

Other tests of ovarian reserve include the clomiphene citrate challenge test (with a higher FSH level after the administration of clomiphene indicating decreased ovarian reserve),[36-38] the gonadotropin-releasing hormone agonist test,[39] and measurements of levels of inhibin B[40,41] or müllerian inhibiting substance,[42,43] which reflect the health of granulosa cells. Baseline assessment of the number of antral follicles by vaginal ultrasonography also has been shown to be reasonably predictive of ovarian reserve.[44] Although an antral follicle count of less than 5 usually signifies a poorer prognosis,[45] no reliable cutoff points are available for levels of inhibin B or müllerian inhibiting substance, and although these measurements have not been recommended for routine use,[46] measurements of müllerian inhibiting substance may be most useful. Recent data suggest that the positive predictive value is the same for day 3 levels of FSH and the clomiphene challenge test, at approximately 90 percent.[47] Both tests, however, have low sensitivity (range, 7 to 26 percent) but high specificity (range, 98 to 99 percent).[47]

Testing women who are at risk is useful but limited in its success. An abnormal result of FSH testing suggests a very poor prognosis yet may not be predictive of the absolute possibility to conceive; a normal result, although reassuring, has been reported in the setting of decreased ovarian reserve.[24] To obtain greater sensitivity, the results of several tests may be considered together,[13] but such an approach has not been studied to date. Given the current shortcomings, if a test result is abnormal, it should prompt a more aggressive approach to the testing of fertility.

OPTIONS FOR PRESERVING FERTILITY

For women who wish to defer pregnancy, what are the realistic options? Various cryopreservation strategies have been used, with variable success rates.

For patients with cancer, Figure 2 provides an algorithm of various possibilities. In children, it is preferable to cryopreserve ovarian tissue (described below) before treatment begins. In older patients with cancer, all cryopreservation methods are options, but freezing embryos or oocytes is preferred. Among these options, ovarian stimulation and oocyte retrieval are more reliable than the aspiration

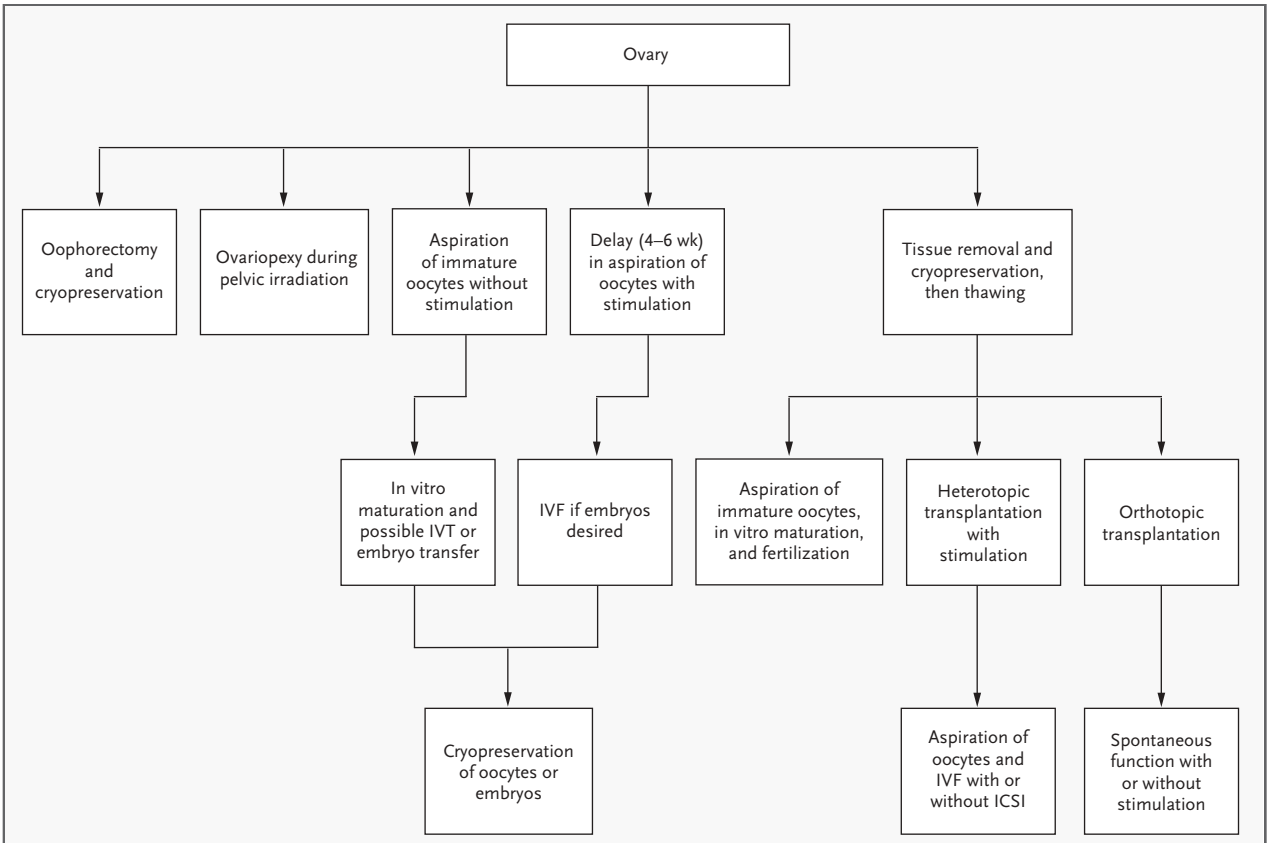

Figure 2. Options for the Preservation of Fertility in Patients with Cancer.

Options include the removal of tissue and the aspiration of oocytes with or without stimulation with ovulation-inducing agents. After aspiration, immature oocytes may undergo in vitro maturation. Various cryopreservation techniques are also available. Ovarian transposition (ovariopexy) is used when pelvic irradiation is necessary. With the exception of ovariopexy and embryo cryopreservation performed in centers that are experienced in the techniques, the other possibilities — orthotopic transplantation and heterotopic transplantation with subsequent in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) — are considered experimental.

of immature oocytes for in vitro maturation; however, ovarian stimulation necessitates a delay in treatment of four to six weeks.

In all scenarios, the most successful approach is embryo cryopreservation. This technique affords a pregnancy rate of 20 to 30 percent per transfer of two to three embryos.[12] However, this approach requires in vitro fertilization and a participating male partner, although frozen sperm from a donor may also be used. If many mature oocytes are retrieved, there is an opportunity to carry out several attempts at embryo transfer from a single cycle. Although this method is appropriate for women at great risk for ovarian failure or to extend the fertility potential of women between the ages of 38 and 40 years, it may be problematic for some women without a partner and is not applicable to children.

Oocyte cryopreservation is another potential option. Because of the fragility of the meiotic spindle and the formation of ice crystals, the success of this approach has been limited but is improving. Newer cryopreservation methods, particularly the use of vitrification (Fig. 3)[48,49] and intracytoplasmic sperm injection for fertilizing oocytes,[48-52] have resulted in viable pregnancies. For this option, ovarian stimulation is used as described above in order to retrieve mature oocytes. The obstacle to the success of this approach is oocyte survival during the thawing process, which is generally about 37 percent.[51] Although the pregnancy rate per cycle (after intracytoplasmic sperm injection and embryo transfer) has been reported to be as high as 22 to 25 percent,[51,52] this rate is not routine, and on the basis of each embryo generated, the realistic success of

this approach is only 2.2 percent,[52] or a 3 percent pregnancy rate per thawed oocyte.[53] Fewer than 100 births have been reported to date from oocyte cryopreservation. Because of the limited success of this technique and a lack of long-term experience with it (including data on birth outcomes), the American Society for Reproductive Medicine has recently recommended that this method not be routinely attempted and that it be carried out only under the aegis of research.[54] Nevertheless, commercialization of this approach has already begun.

In specialized centers, aspiration of immature oocytes from fresh tissue or follicular aspirates may be attempted so that oocytes can then undergo in vitro maturation. The matured oocytes may be vitrified or fertilized by intracytoplasmic sperm injection and cryopreserved.[55,56] This technique may also be used with cryopreserved tissue. These techniques have been used in patients with cancer but mostly are applicable to women with polycystic ovaries who are infertile.

In instances in which a large yield of harvested oocytes is desired for optimal results in cryopreservation, the use of agents to stimulate ovulation is a potential concern among patients with cancer. The aspiration of immature follicles for in vitro maturation can mitigate this concern, although this technique is not applicable to all patients or a realistic option in all centers. In addition, ovarian aspiration carries some risk of hemorrhage and infection (<1 percent). Stimulation agents should be a concern only in patients with estrogen-sensitive cancers such as breast cancer. In such cases, alternative stimulation regimens may be considered, such as the use of tamoxifen or aromatase inhibitors,[57] although these regimens are less effective without added gonadotropins.

Ovarian cryopreservation is an attractive approach to the preservation of fertility and has proved successful in several animal models.[58-60] The rationale for this approach is that primordial follicles in excised ovarian tissue may be more resistant to freezing and thawing than are mature oocytes and may be preserved without a delay in treatment. The principal obstacle to the success of this technique is poor oocyte viability. This rate is affected by the cryopreservation methods and ischemic damage, which occurs after thawing and transfer either orthotopically (to the ovarian site) or heterotopically (to another site, such as to the forearm) because of the lack of an adequate blood supply. Ischemic damage is the greater of these challenges,[59,61,62] and for this reason it has been proposed to excise an intact ovary with its vascular pedicle for cryopreservation, with later vascular reanastomosis.[62-64] However, this procedure is more surgically invasive than other approaches and has not been recommended because of the chance of spontaneous ovarian function after treatment.

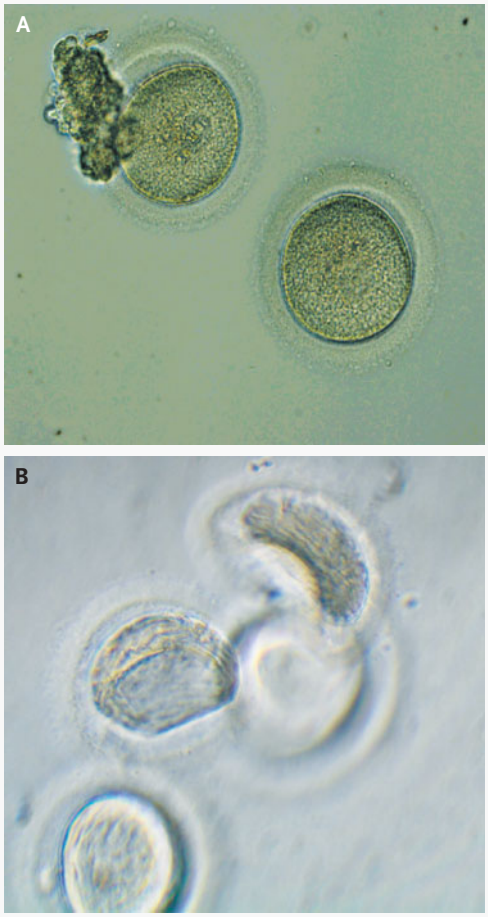

Figure 3. Transformation of Oocytes during Vitrification. Panel A shows mature fresh oocytes. Panel B shows vitrified oocytes that have been frozen for two years and then thawed. Vitrification refers to a rapid freezing process that gives the oocytes a glass-like appearance. Slides are courtesy of Cha Research Institute, Seoul, South Korea.

Some of the debate about ovarian cryopreservation relates to its efficacy, although hundreds of samples all over the world have been frozen.[65] A recent report by Donnez et al. describes the sole pregnancy occurring after orthotopic transplantation and gives encouragement to the field.[66] However,

in this case, the pregnancy may not have resulted from the cryopreserved tissue, since the patient had intact ovaries. This approach also remains fairly invasive, requiring two to four operations, and is somewhat inefficient.

A major concern is the short-term viability of the transplant. For an allograft to be successful, there is a small window of opportunity before the viability of the transplanted tissue is lost. Oktay et al.[67-69] have shown that there may be only nine months to three years of endocrine function after tissue replacement. With heterotopic transplantation of autologous ovarian tissue to the forearm, Oktay and colleagues[69] were able to produce a single four-cell embryo but reported no pregnancy after multiple attempts at oocyte harvesting — a finding suggesting that the site of replacement may also be an important variable. Because of the small window of opportunity, it would seem ideal to stimulate transplanted tissue in order to obtain multiple oocytes for fertilization by intracytoplasmic sperm injection. However, the viability of the tissue is such that a poor yield of oocytes is to be anticipated.[70] Although intermittent ovarian failure occurs and the prognosis for spontaneous pregnancy is low in this population of patients who are at risk, Bath et al.[71] recently reported a spontaneous pregnancy in a woman whose ovarian tissue had been frozen but not transplanted.

In patients with cancer, there is a risk of transmission of cancer cells from the excised ovarian tissue, although this risk appears to be low.[65] Genetic problems are also possible with cryopreservation, but the risk is probably very low on the basis of several studies with oocytes.[72,73] The cryopreservation of ovarian tissue should still be considered experimental[54] and is not the best option for women, with or without cancer, in cases in which other options are available. Elsewhere in this issue of the *Journal*, Silber et al. describe a case of ovarian transplantation between monozygotic twins in which the recipient twin had unexplained premature ovarian failure.[74] Although this rare case may not be directly relevant for patients with cancer, the feasibility of the microsurgical technique, in which large pieces of fresh ovarian cortex from the donor are transplanted to an atrophic ovary with documented viability, is a valuable contribution.

Table 2 lists several possible approaches to preserving fertility in women with cancer, along with evidence-based success rates. If pregnancy is desired immediately on remission, attempts may be begun soon after treatment. If this approach is considered, a more aggressive method involving an assisted reproductive technique should be used because the rate of spontaneous pregnancy is low (about 5 percent)[34,35] and early menopause is to be anticipated.

Another potential approach is to protect primordial follicles from chemotherapy, irradiation, or both. Pretreatment with a gonadotropin-releasing hormone agonist or antagonist to keep the ovaries quiescent during chemotherapy has been suggested[75] but without convincing data in humans.[76,77] A novel approach, which has been shown to be effective in mice, is to suppress ovarian apoptosis by disruption of the sphingomyelinase gene or by treatment with sphingosine-1-phosphate.[78] This latter approach is untested in humans and has unknown consequences with regard to the efficacy of cancer treatment.

Although the above techniques are all options for the treatment of patients with cancer (Fig. 2), the woman who does not have cancer but wishes merely to preserve fertility presents the greatest challenge. The cryopreservation of ovarian tissue in this setting is not warranted, and the cryopreservation of oocytes has to be considered experimental.[54] Because embryo cryopreservation has a reasonable success rate and has been carried out for many years, it may be a reasonable approach for appropriate candidates.

For all women, a final option to consider is oocyte donation. This technique affords the highest pregnancy rates, in the range of 40 to 50 percent per cycle.[12] With this technique, age is not a factor, with pregnancy possible in women in the mid-50 years, as long as the uterus remains healthy; the lat-

Table 2. Options for Preserving Fertility in Women with Cancer.

Option	Percentage of Patients Who Become Pregnant
Spontaneous cycles (acceptance of risk)	5–28 in 3–6 yr after treatment (age-related)
Pretreatment with GnRH agonist or antagonist or with sphingosine-1-phosphate*	Experimental
Cryopreservation	
Embryo	20–30 per transfer (for 3 embryos)
Oocyte	3 per thawed oocyte
Ovarian tissue	Unknown; 1 pregnancy reported
Oocyte donation	40–50 per transfer (for 2–3 embryos)

* GnRH denotes gonadotropin-releasing hormone.

ter is a potential concern in women who have undergone pelvic irradiation. Concern about a genetic risk of cancer (although rare) would be eliminated by this approach. In the future, nuclear or cytoplasmic transfer and similar techniques may be availabe for women who want to have their own genetic progeny.[79] Clearly, these experimental techniques are not approved for use at the present time. Other research that may play a role in the future includes the derivation of oocytes from embryonic stem cells[80] and developments in nuclear reprogramming in which germ cells can be turned into pluripotent cells.[81]

CONCLUSIONS

Because oocytes that are lost cannot be replaced, identifying women who are at risk for early ovarian failure, particularly among those with cancer, is important. Counseling about the various options should take into account the age of the woman and when she may wish to conceive. If pregnancy is to be delayed indefinitely, various cryopreservation options are available. Finally, if a woman is unclear about her wishes, she should be told that oocyte donation at a later time may still be an option.

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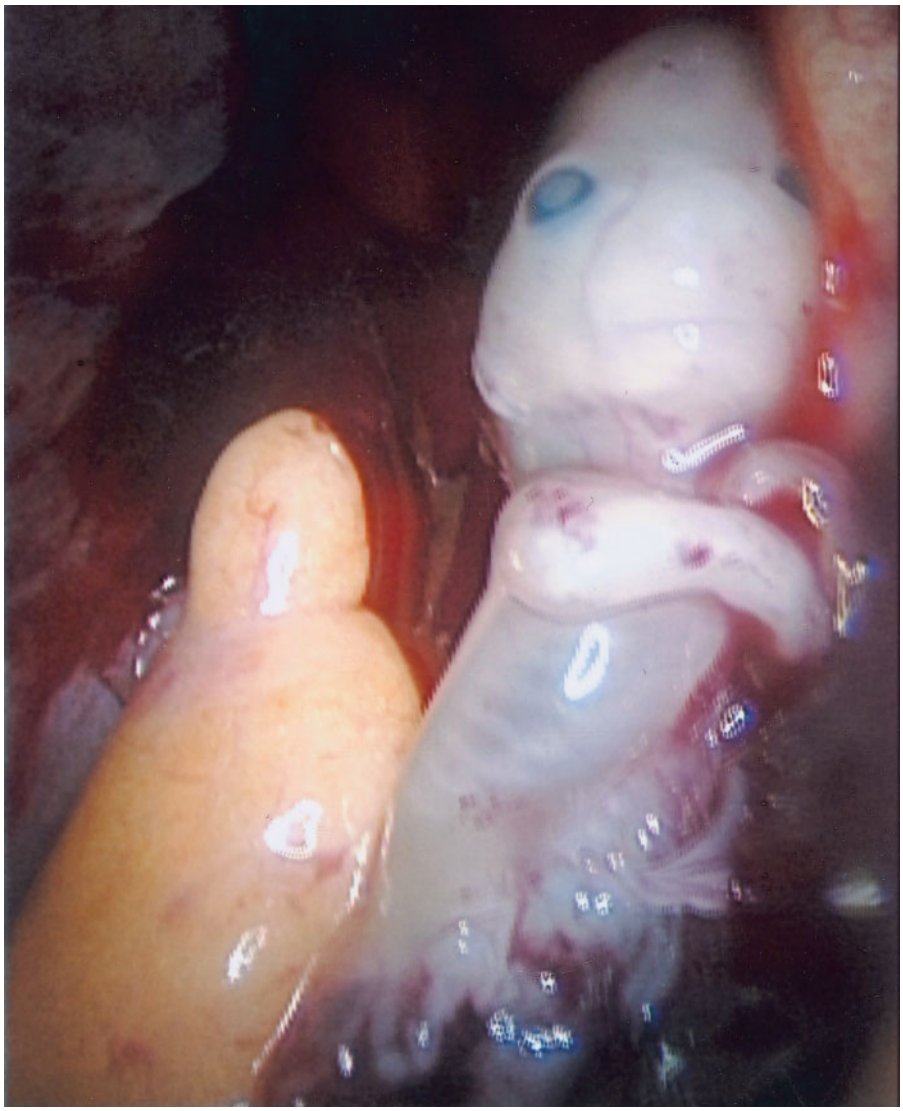
IMAGES IN CLINICAL MEDICINE

Ruptured Ectopic Pregnancy



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A 33-YEAR-OLD WOMAN, PARA 2, PRESENTED TO THE EMERGENCY DEPARTMENT WITH A REPORT OF VAGINAL bleeding and abdominal pain of several days' duration. She had no history of pelvic inflammatory disease. Right adnexal tenderness was noted on physical examination. On screening, the serum β human chorionic gonadotropin level was 47,281 IU per liter, corresponding to an embryonic gestational age of six weeks. Ultrasonography demonstrated free fluid in the peritoneum, an empty uterus, and a right adnexal mass containing a gestational sac and live embryo. During emergency laparoscopy, the surgeon viewed the right ovary, which contained an intact chorion, and a 2 cm embryo extruding from the right fallopian tube. A large hemoperitoneum was present, with active bleeding from the implantation site. The fallopian tube could not be salvaged. The surgery was performed without complication, and the patient's recovery was uneventful. Tests for gonorrhea and chlamydia were negative. The final pathological findings were consistent with a ruptured first-trimester ectopic pregnancy secondary to chronic follicular salpingitis.

CLINICAL PROBLEM-SOLVING

Double Jeopardy

Brahmajee K. Nallamothu, M.D., M.P.H., Mona Saint, M.D., M.P.H., Sanjay Saint, M.D., M.P.H., and Debabrata Mukherjee, M.D.

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.*

A 36-year-old woman in her 34th week of pregnancy presented to the emergency department after the onset of severe substernal chest pain. The chest pain was sudden in onset and had awoken her from sleep in the early morning. She also noted diaphoresis and nausea. She did not have dyspnea, dizziness, syncope, hemoptysis, cough, or fever.

Chest pain in a pregnant woman may be the result of various conditions, ranging from benign to life-threatening diseases. Cardiovascular causes include a hypertensive crisis, an acute coronary syndrome, pericarditis, myocarditis, and aortic dissection. Possible pulmonary disorders are pulmonary thromboembolism, spontaneous pneumothorax, and pneumonia. Peptic ulcer disease, gastroesophageal reflux disease, esophageal spasm, costochondritis, and even herpes zoster should also be considered.

The patient's previous pregnancies had been complicated by preterm labor and a miscarriage. She had had an ectopic pregnancy two years earlier. Her current pregnancy was complicated by hyperemesis gravidarum, and she had required total parenteral nutrition support through a peripherally inserted central catheter for the past 12 weeks. Her only medication was famotidine. She did not smoke cigarettes or use alcohol. She said she did not use illicit drugs.

This woman's history of preterm labor and spontaneous abortion suggests the possibility of the antiphospholipid-antibody syndrome. The antiphospholipid-antibody syndrome results in a hypercoaguable state and has been associated with myocardial infarction and pulmonary thromboembolism. It may also be associated with systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome — all of which may cause pericarditis or pleuritis. An additional concern is the peripherally inserted central catheter, which might cause thromboembolism, although this is a rare occurrence. My differential diagnosis remains broad and includes pulmonary thromboembolism, an acute coronary syndrome, aortic dissection, and pericarditis.

In the emergency department, the patient was alert and in distress from her chest pain. She was afebrile, with a blood pressure of 88/60 mm Hg, a pulse of 108 beats per minute, and a respiratory rate of 20 breaths per minute. Oxygen saturation as determined by pulse oximetry was 98 percent, with the patient breathing room air. An examination of the neck showed no jugular venous distention or carotid bruits, and auscultation of the chest revealed no wheezes or crackles. The heart sounds were normal, and there was no murmur, rub, or gallop. An abdominal examination revealed a gravid abdomen without tenderness. There was no cyanosis, clubbing, or edema of the arms or legs. A peripherally inserted central catheter in her left arm was functioning well.

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The fetal heart rate was approximately 150 beats per minute.

Hypotension and tachycardia may suggest a cardiovascular condition as the cause of her chest pain, although these findings may also occur during normal pregnancy. Obtaining symmetric blood pressures from both arms would be important, since aortic dissection is possible. The absence of a rub during the examination of the heart sounds makes pericarditis less likely than other possible causes, but it cannot be completely ruled out. The normal lung examination and oxygen saturation make pneumothorax and pneumonia improbable choices. Pulmonary thromboembolism remains a possibility, despite the normal oxygen saturation.

Electrocardiography showed sinus tachycardia with ST-segment elevation of 1 mm in leads II, III, and aVF (Fig. 1). Chest radiography revealed a normal cardiac silhouette with no evidence of pneumothorax, consolidation, or effusion. Initial laboratory studies revealed the following: hemoglobin, 9.0 g per deciliter; hematocrit, 27 percent; platelet count, 126,000 per cubic millimeter; white-cell count, 7000 per cubic millimeter; sodium, 134 mmol per liter; potassium, 3.6 mmol per liter; chloride, 104 mmol per liter; bicarbonate, 17 mmol per liter; blood urea nitrogen, 5 mg per deciliter (1.8 mmol per liter); and serum creatinine, 0.4 mg per deciliter (35.4 μmol per liter). The coagulation studies, including an activated partial-thromboplastin time (aPTT), were normal.

The results of electrocardiography are consistent with acute myocardial injury in the inferior wall, probably due to occlusion of the right coronary artery. Lateral changes and the ST-segment elevation of 1 mm in lead V_1 suggest that the infarction involves the inferolateral wall and right ventricle, respectively. A right-sided electrocardiogram should be obtained. Although rare, myocardial infarction is a well-described complication of pregnancy and is estimated to occur in 1 in 10,000 women during the peripartum period. Coronary atherosclerosis is a frequent cause, but this patient has no risk factors for atherosclerosis. Other causes include thromboembolism, a spontaneous coronary-artery dissection, and severe coronary-artery vasospasm.

The normal chest radiograph essentially rules out pneumothorax and pneumonia. Pulmonary thromboembolism also appears less likely than other possibilities, given the clinical presentation and the electrocardiogram, but it is not completely ruled out. Although a widened mediastinum would suggest aortic dissection, chest radiography is insensitive for this condition. For a dissection to explain the electrocardiographic changes, the dissection would have to extend into the right coronary artery. Initial routine laboratory studies show anemia and a mild decrease in the platelet count, and coagulation studies were normal. These findings suggest that a diagnosis of the antiphospholipid-antibody syndrome is not as likely as some other choices.

A transthoracic echocardiogram may be helpful at this point, because it can be obtained rapidly with no harm to the fetus and would confirm wall-motion abnormalities due to myocardial infarction. In addition, it might detect dilatation of the pulmonary artery, right ventricle, and right atrium or hypokinesis of the right ventricle, as may be seen with pulmonary thromboembolism. Although such an echocardiogram cannot be used to definitively rule out acute aortic dissection, it may detect an intimal flap in the proximal aorta. However, because "time is muscle," additional studies should not markedly delay decisions regarding immediate reperfusion therapy.

Owing to the lack of an on-site cardiac catheterization laboratory, the patient was transferred on an emergency basis to a nearby facility for cardiac catheterization with possible percutaneous coronary intervention. Before she was transported, an aspirin was given, intravenous heparin and nitroglycerin were started, and a single 5-mg dose of intravenous metoprolol was given. A transient decrease in blood pressure during transport responded to intravenous fluids and discontinuation of nitroglycerin.

Although there is some debate about the relative benefits of transfer for emergency cardiac catheterization and percutaneous coronary intervention in patients presenting with myocardial infarction with ST-segment elevation, in this case the decision is easier. The cause of this patient's condition has not been established, and both the mother and fetus remain in jeopardy. Cardiac catheterization would help establish the diagnosis as well as offer the possibility of definitive treatment. The risk to a third-trimester fetus associated with radiation from the procedure is considered negligible if proper shielding of the abdomen is used. Intravenous

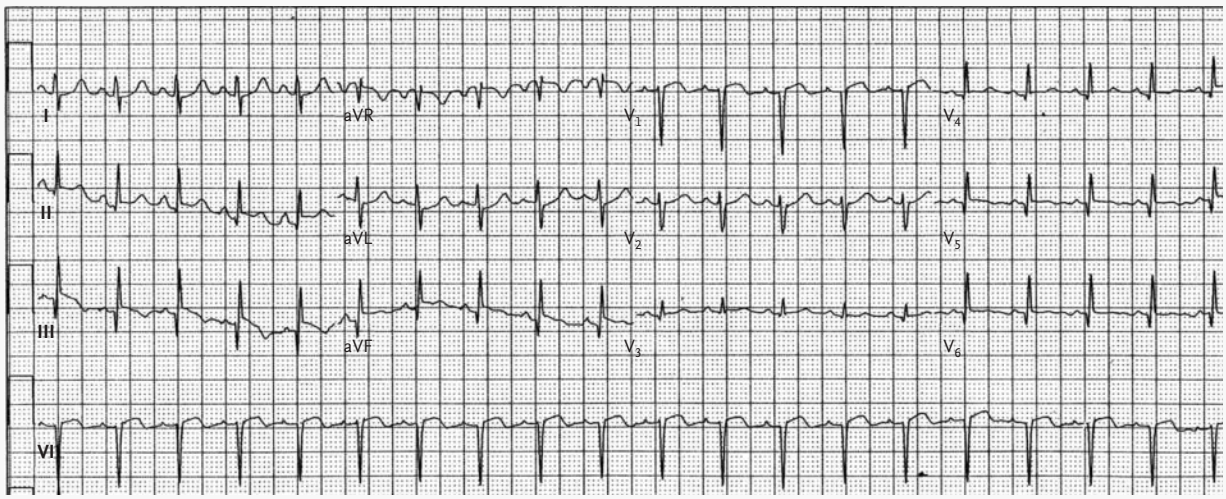

Figure 1. Electrocardiogram.
The electrocardiogram shows a pattern of myocardial injury that involves the inferior and inferolateral walls with ST-segment elevation and Q waves present. There is also mild ST-segment elevation in V1, suggesting right ventricular involvement.

fibrinolysis is an alternative for reperfusion and could be given immediately. However, it is rarely used in pregnancy because of limited data and the potential for bleeding.

The precipitous drop in blood pressure with intravenous nitroglycerin suggests right ventricular involvement, and further use of nitroglycerin should be avoided. Aggressive resuscitation with intravenous fluids is appropriate. Intravenous heparin probably has limited value in this setting and may be detrimental, because acute aortic dissection with right coronary involvement has not been ruled out.

Transthoracic echocardiography, performed at the second hospital, revealed hypokinesis of the inferior wall and posterior wall, with an overall ejection fraction estimated at 50 percent. The right ventricle appeared to be normal in size and function. There were no other valvular abnormalities noted.

Cardiac catheterization on an emergency basis is needed. At this point, the potential benefits of the procedure in guiding management and allowing definitive therapy outweigh its risks, which include bleeding complications, cardiac arrest, and prolonged arrhythmias.

Cardiac catheterization was performed after shielding the patient's abdomen with a lead apron. Coronary angiography showed a normal left main coronary artery, left anterior descending coronary artery, and left circumflex coronary artery. An injection of contrast medium into the right coronary artery revealed a total occlusion of the proximal vessel, with contrast-medium staining suggestive of a dissection flap (Fig. 2A and Video Clip 1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org).

The cause of a spontaneous dissection of a coronary artery during pregnancy is not well understood. The hormonal and hemodynamic alterations that take place during pregnancy may lead to morphologic changes in the collagen of coronary arteries and weaken the media layer. Spontaneous coronary-artery dissection may also occur in patients with underlying atherosclerotic plaque, connective-tissue diseases, immunological diseases or Kawasaki's disease. Regardless, the goal now should be immediate restoration of flow with percutaneous coronary intervention.

A temporary pacemaker was placed in the right ventricle. Eptifibatide, an intravenous glycoprotein IIb/IIIa receptor blocker, and clopidogrel were

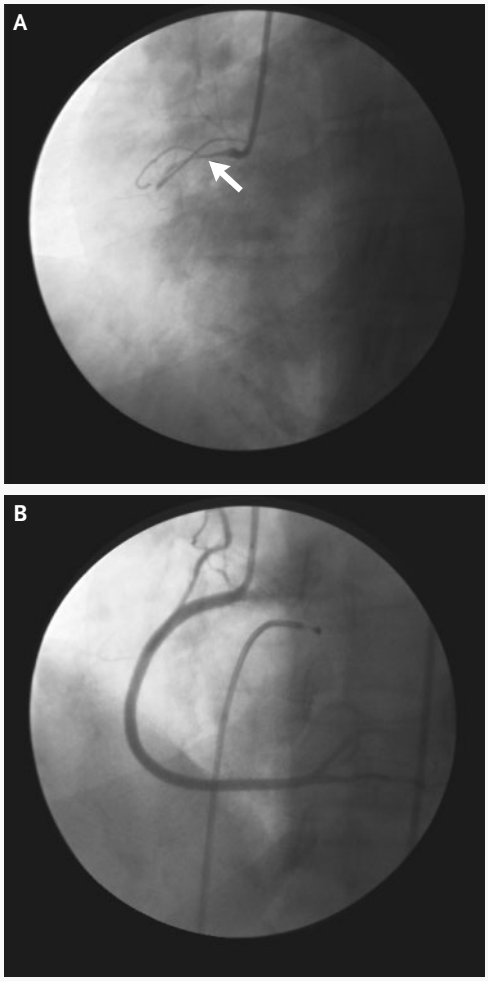

Figure 2. Coronary Angiography.
The right coronary artery shows a proximal occlusion (arrow), presumably secondary to a dissection flap (Panel A). The right coronary-artery lumen and flow are restored to normal after percutaneous coronary balloon angioplasty and stenting (Panel B).

given. The right coronary-artery occlusion was crossed and then dilated with balloon angioplasty several times. Multiple intracoronary paclitaxel-coated stents were deployed to tack up the dissection flap. The final angiography revealed no stenosis and normal intracoronary blood flow (Fig. 2B and Video Clip 2 in the Supplementary Appendix). The patient's chest pain resolved, and repeated electrocardiography showed improvements in ST-segment elevations. The fetal heart rate remained in the normal range throughout the procedure.

It would be ideal to have a full obstetrical team available if any hemodynamic compromise occurs in such a case. The interventions performed were reasonable on the basis of clinical-trial evidence from patients who were not pregnant, although data are lacking in pregnancy. Adjunctive therapy with a glycoprotein IIb/IIIa receptor blocker has been associated with improved clinical outcomes after percutaneous coronary intervention, but it may increase risk of bleeding as a result of inhibition of platelet aggregation; this is a particular concern if an emergency delivery is required. Dual antiplatelet therapy with aspirin and clopidogrel is recommended to prevent subacute stent thrombosis. Stenting — as opposed to traditional balloon angioplasty — is theoretically preferable when coronary-artery dissection occurs, in order to adequately tack down the dissection flap. The role of drug-eluting stents has not been established in this setting.

The patient's hospital course was complicated by an episode of ventricular fibrillation one day after the percutaneous coronary intervention. She was treated with immediate cardioversion and did not require cardiopulmonary resuscitation. Three days after this episode, still in her 34th week of gestation, labor was induced, and she delivered a healthy baby girl. After delivery, treatment with an angiotensin-converting–enzyme inhibitor was begun, in addition to the aspirin, clopidogrel, and metoprolol she was taking already. An implantable cardioverter–defibrillator was also placed before discharge.

An angiotensin-converting–enzyme inhibitor may be beneficial for ventricular remodeling during the early postinfarction period, but it may not be needed in the long term in the absence of coronary atherosclerosis. Early use of statins is valuable in most patients with myocardial infarction, but their role here is uncertain. The use of an implantable cardioverter–defibrillator is highly controversial in this patient, especially as the episode occurred within 24 hours of her myocardial infarction and her ejection fraction remained relatively preserved.

The optimal timing for delivery of the fetus after the mother has had a myocardial infarction is unknown. If possible, waiting for up to two to three weeks has been recommended in order to allow for adequate myocardial healing. Earlier delivery may have been reasonable in this case, given the successful revascularization and the episode of ven-

tricular fibrillation. Data are limited to guide the mode of delivery (vaginal or cesarean). Although an episode of spontaneous coronary-artery dissection is not considered an absolute contraindication to future pregnancies, it may be best for the patient to avoid them. Of course, this decision requires an individualized approach, because data on the potential for recurrence are limited.

COMMENTARY

Evaluating chest pain in pregnant women can be challenging. Physicians need not only to distinguish between life-threatening and benign conditions, but also to quickly determine the best and safest treatment options for both the patient and the fetus.[1] With the advent of fetal heart-rate monitoring and improved antenatal testing, potential jeopardy to the fetus has emerged as both a medical and a legal consideration.[2] However, it is important that maternal health remains the primary determining factor in obstetrical treatment decisions.

During emergency medical conditions, the tendency often is to "order first and think later." This is especially true when it comes to diagnostic imaging studies. Even though there is considered to be little risk to the fetus from radiation during the final trimester,[3,4] the desire to avoid unnecessary exposure underscores the importance of the history, physical examination, and simple tests such as electrocardiography in assessing chest pain in pregnant women. Fetal heart tones also should be quickly determined in any viable pregnancy, because a fetal status that was not reassuring might alter how the situation is managed.

During normal pregnancy, left-axis deviation, ST-segment and T-wave abnormalities, and nonsignificant Q waves in leads III and avF may occur, but they are readily distinguishable in most cases from the more pronounced changes observed with acute coronary syndromes.[5] Whereas other conditions that were considered in this case (such as pulmonary thromboembolism) may produce ST-segment changes, the findings were highly suggestive of myocardial infarction with ST-segment elevation. Furthermore, the echocardiogram confirmed the suspicion of myocardial infarction and justified the emergency cardiac catheterization.

Spontaneous coronary-artery dissection is a rare but well-described cause of acute coronary syndromes.[6,7] Its true incidence is unknown, since cases are often diagnosed post mortem. Most reported cases have been in young women during the peripartum period or in patients with coronary atherosclerosis. The clinical presentation of a patient with this condition is highly variable and depends primarily on the vessel involved and the rate and magnitude of the dissection. Patients may present with chronic stable angina, acute coronary syndromes, cardiogenic shock, or sudden death from cardiac causes.[7] Although the exact cause of spontaneous coronary dissection in pregnancy is unknown, it is believed to relate to structural changes within the blood-vessel wall in response to hormonal changes associated with pregnancy, as well as to hemodynamic stress caused by increased coronary blood flow during pregnancy. Autoimmune conditions, such as systemic lupus erythematosus and the antiphospholipid-antibody syndrome, have also been linked to coronary-artery dissections.

Treatment includes medical therapies to reduce ischemia and revascularization performed on an emergency basis when indicated. Aspirin, anticoagulant agents, and beta-blockers are safe and immediate options in most patients.[8] In this case, a decision was made to transfer the patient quickly to a specialized center with the capability of performing emergency cardiac catheterization and percutaneous coronary intervention for reperfusion. As the discussant stated, fibrinolytic agents in this setting are relatively contraindicated because of the potential risk of maternal hemorrhage. Propagation of spontaneous coronary-artery dissection has also been described with fibrinolysis.[9]

Although surgical revascularization has been used, it is typically reserved for cases that involve multiple vessels or the left main coronary artery. Percutaneous coronary intervention — and in particular, coronary stenting — has otherwise been successful at reestablishing flow and tacking down the dissection flap.[10] The role of drug-eluting stents is unknown in this setting. Drug-eluting stents dramatically reduce the rate of restenosis after deployment in patients with coronary-artery disease. However, they have not been evaluated in patients with coronary-artery dissection or pregnancy. Paclitaxel has been used only rarely in pregnant women with advanced cancer as a systemic agent.[11,12] In this limited instance, the agent caused no apparent side effects in the fetus. The early delivery that occurred three days after treatment with this agent also minimized exposure of the fetus.

Ventricular tachyarrrhythmias that occur soon after myocardial infarction are thought to be due

primarily to electrical instability and increased sympathetic tone.[13] As mentioned by the discussant, the use of an implantable cardioverter–defibrillator is controversial in this setting, because in-hospital ventricular tachyarrhythmias within 48 hours after myocardial infarction have not been consistently associated with long-term survival after discharge.[14,15] Lidocaine can be safely used during pregnancy to prevent recurrences of ventricular tachyarrhythmias; however, amiodarone should not be given because of the potential association with neonatal thyroid dysfunction.

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EDITORIALS

Inhaled Nitric Oxide for Preterm Infants — Who Benefits?

Richard J. Martin, M.B., F.R.A.C.P., and Michele C. Walsh, M.D.

Endogenously released nitric oxide is widely recognized to play a key role in multiple vertebrate organ systems, and its deficiency disrupts pulmonary parenchymal and vascular development. These observations have suggested that exogenously inhaled nitric oxide might protect the respiratory and the central nervous systems during a critical phase of development and thus improve outcomes in preterm infants.

In this issue of the *Journal*, Mestan et al.[1] and Van Meurs et al.[2] assess the utility of inhaled nitric oxide in preterm neonates with respiratory failure, with contrasting results. Mestan and colleagues report improved cognitive neurodevelopmental outcome in two-year-olds who had been treated with inhaled nitric oxide as neonates.[1] This is a very reassuring follow-up to their previous report in the *Journal* that showed a reduced rate of death or bronchopulmonary dysplasia in premature infants treated with inhaled nitric oxide (relative risk, 0.76; 95 percent confidence interval, 0.60 to 0.97).[3] In contrast, Van Meurs and colleagues report that treatment with inhaled nitric oxide as compared with placebo resulted in no overall improvement in survival to hospital discharge and suggests worse outcomes in a subgroup of neonates with birth weights of 1000 g or less.[2]

How can these discrepant results be explained? Although the studies investigate the same drug, few other aspects of the studies are similar. Mestan and colleagues studied premature infants with an average birth weight of 992 g and a gestational age of 27.4 weeks. In contrast, the infants in the study by Van Meurs et al. were considerably smaller (mean weight, 839 g) and less mature (average gestational age, 26 weeks); 47 percent had a birth weight of less than 750 g.

In addition, the infants enrolled in the study by Mestan et al. had a median oxygenation index (a measure of the severity of respiratory distress, calculated as 100 × the fraction of inspired oxygen × the mean airway pressure [in centimeters of water] ÷ the partial pressure of arterial oxygen [in millimeters of mercury]) of approximately 7, indicating that they were substantially less ill than the infants in the study by Van Meurs et al., who had a mean oxygenation index in excess of 20. (Oxygenation indexes that are less than 10 reflect mild disease, 10 to 20 moderate disease, and greater than 20 critical respiratory distress.) The mortality in the placebo group studied by Van Meurs et al. as compared with that in the placebo group studied by Mestan et al. (44 percent vs. 22.5 percent) is additional evidence that the participants in the study by Van Meurs et al. were more ill.

Plausible explanations for the discrepant results are that effects of inhaled nitric oxide may differ depending on the severity of illness and that administration of inhaled nitric oxide to critically ill preterm infants with respiratory failure does not improve outcome. In the study by Van Meurs et al., a post hoc analysis of the subgroup of infants with birth weights of 1000 g or less revealed a higher incidence of severe hemorrhagic or ischemic brain injury in the group treated with nitric oxide. However, it is uncertain whether these very ill infants had an intraventricular hemorrhage before their exposure to inhaled nitric oxide; platelet inactivation induced by inhaled nitric oxide may have exacerbated a preexisting complication. Post hoc analysis of the subgroup with birth weights greater than 1000 g actually revealed a significantly reduced rate of the combined outcome of death or bronchopulmonary dysplasia ($P=0.03$) with inhaled nitric oxide therapy.

There is also a striking difference in the racial composition of the two studies. Seventy percent of

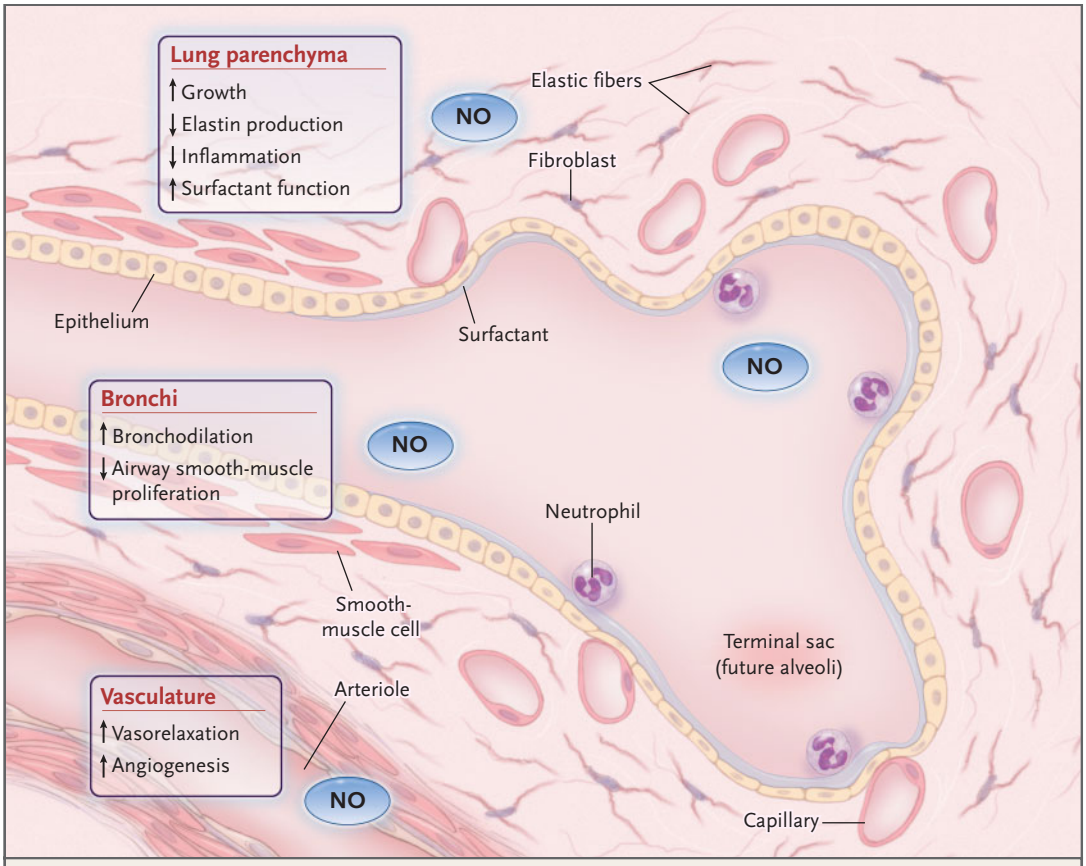

Figure 1. Proposed Effects of Nitric Oxide on the Development of the Respiratory System.

Endogenously released and exogenously inhaled nitric oxide (NO) may influence many facets of perinatal lung development, including lung parenchyma, bronchi, and vascular structures. This schematic figure depicts a fetal or preterm lung during the transition from a saccular to an alveolar stage, corresponding to 25 to 28 weeks of gestation. Endogenous nitric oxide is released primarily from epithelial and endothelial cells that contain nitric oxide synthase, and it is implicated in the structural and functional aspects of the development of pulmonary vasculature and airway smooth muscle. Nitric oxide also contributes to growth of the lung parenchyma and to extracellular matrix deposition and may modulate surfactant and inflammation in the developing lung. Animal models of bronchopulmonary dysplasia that have deficient levels of nitric oxide synthase show that inhaled nitric oxide can preserve lung growth. The implications of supplementation with inhaled nitric oxide in preterm infants are currently uncertain. The upward arrows indicate increased and the downward arrows decreased.

the infants in the trial by Mestan et al. and 35 percent in the trial by Van Meurs et al. were black. Race or ethnic group is a very crude marker of potential biologic differences in drug response, known as pharmacogenomics.[4] However, there are no previous data in infants to suggest differential responsiveness to nitric oxide between blacks and whites.

Whereas the trial by Mestan et al. was a single-center study, the study by Van Meurs et al. was larger and involved many centers. Drug delivery was performed by blinded personnel, and outcomes were evaluated by observers blinded to therapy, minimizing the chance of bias in the outcome assessments. Even with these precautions, however, small trials have been shown to overestimate the effects of treatment, as compared with multicenter studies.[5] It is likely that this is caused in part by the variations in concomitant processes of care that occur among centers as opposed to the processes within a single center.

In the study by Mestan et al., the finding of a decrease in the rate of neurodevelopmental impairment at two years of age in patients who had been treated with inhaled nitric oxide as infants, as compared with those who had been treated with placebo (24 percent vs. 46 percent, respectively), is impres-

sive. The mechanism for this beneficial result is not immediately obvious. The improved outcome was associated with significantly less severe hemorrhagic and ischemic brain injury and a trend toward less bronchopulmonary dysplasia, although other factors, such as better growth, may also contribute. Infants with bronchopulmonary dysplasia have a number of related coexisting conditions, including poor growth, congestive heart failure, reactive airway disease, intermittent hypoxemic episodes, and exposure to postnatal steroids.[6] It is therefore not surprising that numerous studies have shown a persistent link between bronchopulmonary dysplasia and adverse neurodevelopmental outcomes. Endogenous nitric oxide directly influences neural function, and inhaled nitric oxide may have more potent protective systemic effects than previously believed.[7] However, there is currently no clear evidence that inhaled nitric oxide or active circulating nitrosylated proteins directly affect the developing brain.[8]

Inhaled nitric oxide was introduced to the therapeutic armamentarium as a means of rapidly improving oxygenation in term infants with primary pulmonary hypertension. Oxygenation improves in many preterm infants with respiratory failure within minutes after exposure to inhaled nitric oxide, presumably as a consequence of improved ventilation–perfusion matching. However, as shown by Van Meurs et al. as well as others,[9,10] the administration of inhaled nitric oxide for one to three days does not necessarily translate into lower mortality or morbidity. Meanwhile, there is increasing interest in the effect of inhaled nitric oxide given for several weeks at low doses to modulate development of the respiratory system (Fig. 1), including effects on angiogenesis and the maturation of lung parenchyma and airway smooth muscle.[11-13]

Meanwhile, what should the clinician conclude from these two studies? The sobering finding of increased mortality and increased hemorrhagic or ischemic brain injury with inhaled nitric oxide therapy in the subgroup of severely ill infants with birth weights of 1000 g or less, although a post hoc finding, must not be ignored. It may reflect the production of biologically toxic byproducts, such as peroxynitrite, from the combination of nitric oxide with the high oxygen concentration to which these very sick infants are exposed. Therefore, short-term use of inhaled nitric oxide cannot be considered an effective rescue therapy for very preterm infants with profound respiratory failure.[2,9,10] In contrast, less ill preterm infants may benefit from this therapy, both in the short term and over the long term, as suggested by the study by Mestan et al.

Another recent report suggests that in less ill infants, inhaled nitric oxide may permit aggressive weaning from inspired oxygen and improve biomarkers of oxidative stress.[14] Currently, at least two large, multicenter, randomized trials of prolonged inhaled nitric oxide exposure beginning shortly after birth are completing enrollment; we are participating in one of them. Pending the results, it is prudent to avoid the use of inhaled nitric oxide in preterm infants in the first week of life. The benefits and risks of inhaled nitric oxide need further scrutiny before its use becomes widespread.

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When and How to Treat Essential Thrombocythemia

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Essential thrombocythemia is one of the chronic myeloproliferative disorders, a heterogeneous group of diseases involving clonal hematopoietic stem cells that also includes polycythemia vera, idiopathic myelofibrosis, and chronic myelogenous leukemia.[1] Among these disorders, essential thrombocythemia has the most favorable outcome: patients with this disease have a life span that nearly rivals that of a healthy population matched by age and sex.[2] The principal causes of death in patients with essential thrombocythemia are thrombosis, hemorrhage, and progression to myelofibrosis or acute myelogenous leukemia. The myelosuppressive therapy that prevents vascular events in essential thrombocythemia may itself increase the risk of transformation to myelofibrosis or acute myelogenous leukemia. The challenge in treating essential thrombocythemia is to prevent bleeding and thrombosis without increasing this risk.

A risk-adapted treatment strategy can help physicians to meet this challenge. The important risk factors for thrombotic events in patients with essential thrombocythemia are an age of 60 years or more and previous vascular episodes,[3] whereas hemorrhagic complications are paradoxically associated with extreme thrombocythemia. Young, asymptomatic patients with a platelet count of less than 1.5 million per cubic millimeter should be considered at low risk for thrombosis and hemorrhagic events, and do not require myelosuppressive treatment. By contrast, patients who are at least 60 years of age or who have a history of serious bleeding or thrombosis or a platelet count of 1.5 million per cubic millimeter or more should receive cytoreductive treatment. Hypertension, dyslipidemia, diabetes, and smoking can also increase the risk of thrombosis; patients with these coexisting conditions may constitute an "intermediate risk" category, but the assignment of patients to this subgroup is not universally accepted.

The myelosuppressive agent of choice in patients who have essential thrombocythemia and a high risk of thrombosis is hydroxyurea. This drug was found to be effective in reducing the incidence of thrombosis in a trial that randomly assigned 114 high-risk patients to receive either hydroxyurea or no cytoreductive therapy. After a median follow-up of 27 months, thrombosis developed in 3.6 percent of the treated patients, whereas 24 percent of the untreated patients had one or more thrombotic events.[4]

The major concern regarding hydroxyurea is whether it is leukemogenic. However, to date, no randomized studies with sufficient statistical power have been conducted to determine whether the risk of leukemia in patients treated with hydroxyurea is higher than the inherent risk that essential thrombocythemia will evolve into acute myelogenous leukemia. Nevertheless, the putative risk of leukemia associated with hydroxyurea prompted investigators to test new drugs that lack this potential, such as anagrelide and interferon alfa.

Anagrelide is an imidazoquinazoline derivative that reduces the platelet count in essential thrombocythemia and other myeloproliferative diseases.[5] It selectively inhibits maturation of megakaryocytes, with either minimal or no effect on the other blood-cell precursors. There is extensive experience with anagrelide, which is licensed in the United States as a first-line agent for the control of thrombocytosis associated with any myeloproliferative disorder. Clinical studies of anagrelide, however, have not been randomized, have lacked control groups, have enrolled relatively few patients, and have had limited follow-up.

In this issue of the *Journal*, Harrison and colleagues[6] report the results of a randomized trial that compares hydroxyurea with anagrelide (plus low-dose aspirin in both groups) in 809 high-risk patients with essential thrombocythemia. After a median follow-up of 39 months, the trial was terminated early by the data-monitoring committee because of excess rates of vascular events and transformation to myelofibrosis in the anagrelide group. Control of the platelet count and the incidence of leukemia were similar in the two groups. The investigators concluded that hydroxyurea plus aspirin was superior to anagrelide plus aspirin for patients with essential thrombocythemia who are at high risk for vascular events.

Besides the outcome, this well-designed and well-conducted trial has important features. First, it shows that control of the platelet count alone should not be taken as an appropriate surrogate end point to judge the efficacy of a treatment for essential thrombocythemia, because there was an excess of vascular events in the anagrelide group despite a reduction in the platelet count that was similar to

the reduction in the hydroxyurea group. A likely explanation for this finding is the broader myelosuppressive activity of hydroxyurea, which also affects leukocytes and red cells. There is growing evidence that these cells play an important role in the pathogenesis of thrombosis in essential thrombocythemia.[7]

Second, anagrelide and low-dose aspirin appear to act synergistically to increase the risk of bleeding complications. This effect was unexpected and may result from the inhibition of platelet function by the two drugs given simultaneously. In contrast, the combination of hydroxyurea and aspirin provided protection against thrombosis with only a small risk of bleeding. This finding is in agreement with recent data from a randomized trial involving patients with polycythemia vera (about 50 percent of whom received hydroxyurea), in which aspirin reduced the risk of serious thrombosis and overall mortality without increasing the risk of serious bleeding.[8]

Third, the study by Harrison et al. showed that progression to myelofibrosis was about three times as frequent in the anagrelide group as it was in the hydroxyurea group. Evolution into myelofibrosis is part of the natural history of essential thrombocythemia and occurs in about 3 percent of patients after 5 years, 8 percent after 10 years, and 15 percent after 15 years.[9] However, the risk of myelofibrosis differs according to the baseline characteristics of the bone marrow[10]: it is very low in so-called true essential thrombocythemia, in which the marrow has none of the histopathological features of myelofibrosis, and is higher when the marrow shows prefibrotic stages of myelofibrosis. For this reason, a limitation of the trial by Harrison et al. is the lack of a baseline examination of bone marrow. A trial comparing hydroxyurea with anagrelide in true essential thrombocythemia alone is ongoing in Europe.

Last but not least, transformation into acute myelogenous leukemia was found in similar numbers of patients in the hydroxyurea and anagrelide groups. This finding reassures us about the low leukemogenic potential of hydroxyurea and confirms the recently reported results of a large prospective study of polycythemia vera, in which there was no increase in acute myelogenous leukemia among patients treated with hydroxyurea, as compared with patients treated with phlebotomy alone.[11]

In summary, for now, hydroxyurea plus aspirin should be the standard of treatment for patients with essential thrombocythemia who are at high risk for thrombosis. A new avenue for the treatment of essential thrombocythemia and other myeloproliferative diseases was opened by the recent identification of an acquired mutation of the *JAK2* gene in most patients with polycythemia vera and about one half the patients with essential thrombocythemia or myelofibrosis.[12-15] The predicted consequence of this mutation is constitutive tyrosine kinase activity of Janus kinase 2 (JAK2), resulting in proliferative and survival advantages of hematopoietic progenitor cells. New tyrosine kinase inhibitors are being developed with the aim of discovering targeted therapy for these diseases. We foresee clinical trials comparing tyrosine kinase inhibitors with standard therapy in the near future.

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CORRESPONDENCE

Intensive Lipid Lowering with Atorvastatin in Coronary Disease

TO THE EDITOR: LaRosa and colleagues (April 7 issue)[1] report that among patients with stable coronary heart disease (CHD), an intensive statin regimen provides a 22 percent reduction in the risk of cardiovascular events when compared with a standard regimen. However, despite the 20 percent reduction in the risk of death from CHD in the group that received 80 mg of atorvastatin per day, there was no significant difference between the groups in overall mortality. This finding reflects an increase in the risk of death from noncardiovascular causes, which is undoubtedly a matter of concern.

In patients with an acute coronary syndrome, high-dose atorvastatin, as compared with standard-dose pravastatin, was associated with reductions of 28 and 30 percent in overall mortality and mortality related to CHD, respectively, by two years.[2] The benefit of high-dose atorvastatin emerged as early as 30 days after the start of treatment with the statin.[2] A lower cardiovascular risk among patients with stable CHD than among patients with an acute coronary syndrome, together with longer exposure to the potential toxicity of high-dose atorvastatin (2.0 vs. 4.9 years), may explain the difference in the effect on overall mortality in the Treating to New Targets (TNT) study[1] and the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE IT–TIMI 22) trial.[2] Thus, when treatment with very high statin doses is considered, the selection of patients whose risk of cardiovascular events is high enough to outweigh the potential risk of toxic drug-related effects is of utmost importance.

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1. LaRosa JC, Grundy SM, Waters DD, et al. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 2005;352:1425-35.
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TO THE EDITOR: In his editorial on the study by LaRosa and colleagues, Pitt[1] expresses concern regarding the nonsignificant difference in mortality from noncardiovascular causes between high-dose and low-dose statin treatment. This difference, however, may be due to the play of chance. To evaluate all available data, we performed a meta-analysis of the three trials comparing intensive and standard lipid lowering to date with respect to mortality from cardiovascular and noncardiovascular causes. As for mortality from noncardiovascular causes, the trial reported by LaRosa et al. (TNT, in which 10 mg of atorvastatin per day was compared with 80 mg of atorvastatin per day in a population of 10,001 patients) showed a nonsignificantly higher rate with high-dose statin therapy; the second trial (PROVE IT–TIMI 22, in which 40 mg of pravastatin per day was compared with 80 mg of atorvastatin per day in a population of 4162 patients) showed a lower rate with high-dose therapy[2]; and in the third (Aggrastat-to-Zocor

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[A-to-Z], in which 20 mg of simvastatin per day was compared with 80 mg of simvastatin in a population of 4497 patients), the difference in dose had no effect.[3] On the other hand, mortality from cardiovascular causes in the three trials was significantly reduced, by 24 percent (P=0.004), adding further support to the trend toward reduced mortality from cardiovascular causes seen with intensive statin therapy in the individual trials. This analysis should provide reassurance that intensive lipid lowering does not appear to have any adverse effect on mortality from noncardiovascular causes and in fact that it is associated with a substantial benefit in preventing morbidity and mortality from cardiovascular causes.

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Dr. Cannon reports having received research grant support from AstraZeneca, Bristol-Myers Squibb, Merck, Merck/Schering-Plough, and Sanofi-Aventis and having served on paid advisory boards for AstraZeneca, Bristol-Myers Squibb, GlaxoSmithKline, Merck/Schering-Plough Partnership, Merck, Pfizer, and Sanofi-Aventis. Dr. Braunwald reports having received research grant support from Sanofi-Aventis, Bristol-Myers Squibb, and Merck/Schering Plough.

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TO THE EDITOR: When a drug designed to prevent cardiovascular disease fails to lower overall mortality rates, as in the TNT study, it is mandatory to report meticulously the number, nature, and severity of adverse effects, because the benefits from reductions in the incidence of coronary events may be outweighed by such complications.

A nonfatal heart attack or stroke may have few sequelae; the consequences of aggressive statin treatment, including rhabdomyolysis, polyneuropathy,[1] aggressive or suicidal behavior,[2] amnesia,[3] or serious congenital defects[4] could be much more catastrophic for patients. Although such adverse effects of statins have been rare, there can be little doubt that their incidence would rise with an increase in dosage by a factor of eight. We are therefore concerned about the cursory manner in which the authors dealt with this important issue; in particular, we object to the arbitrary exclusion of adverse effects that they considered unrelated to treatment (e.g., five cases of rhabdomyolysis). What other untoward effects not reported did they consider unrelated to treatment?

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TO THE EDITOR: The study by LaRosa et al. adds to the growing literature indicating that the lower the low-density lipoprotein (LDL) cholesterol level, the better the outcome among patients with known coronary artery disease. It would be useful clinically to have two further analyses of their data. First, what were the clinical outcomes of the patients stratified according to their achieved LDL cholesterol levels? Statins have many pleiotropic effects in addition to their direct lipid-lowering effect.[1] However, if the actual LDL cholesterol level achieved were in fact a predictor of future events, as found in the PROVE IT trial,[2] then clinicians could use the lowest dose of atorvastatin necessary to achieve that goal, thereby minimizing medication-related side effects and costs.

Second, what role does high-density lipoprotein (HDL) cholesterol play? For example, a target LDL cholesterol level of 100 mg per deciliter in a patient with an HDL cholesterol level of 55 mg per deciliter might be clinically superior to an LDL cholesterol target of 70 mg per deciliter in a patient with an HDL cholesterol level of 25 mg per deciliter. There are abundant examples in the literature suggesting that the ratio of LDL (or total) cholesterol to HDL choles-

terol is a better predictor of clinical outcomes than the LDL cholesterol level itself.[3,4]

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TO THE EDITOR: LaRosa et al. report a 22 percent relative reduction in the risk of major cardiovascular events with 80 mg of atorvastatin per day as compared with a dose of 10 mg per day. The cohort assigned to the higher dose also had a lower mean LDL cholesterol level (77 mg per deciliter vs. 101 mg per deciliter). According to the data presented, changing the target of statin therapy from an LDL cholesterol level of 100 mg per deciliter to a level of 70 mg per deciliter is not warranted. In PROVE IT–TIMI 22, the benefit of intensive lipid-lowering therapy was driven primarily by the subgroup (27 percent of the population) with an LDL cholesterol level greater than 125 mg per deciliter at randomization.[1] It would be interesting to see a similar subgroup analysis in the current study, given that substantial numbers of patients had an LDL cholesterol level above 100 mg per deciliter at randomization. It is entirely possible that the benefit of the higher dose of atorvastatin is attributable to the subgroup of patients whose baseline LDL cholesterol levels were greater than 100 mg per deciliter. If that is the case, then no change in existing guidelines is warranted.

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THE AUTHORS REPLY: Dr. Southern and Dr. Modest both suggest the examination of clinical benefits in groups stratified according to LDL cholesterol levels during treatment. In addition, Dr. Modest asks about the relationship between HDL cholesterol levels during treatment and cardiovascular risk. We have not yet performed these analyses.

Dr. Ravnskov and colleagues attribute conditions to statins in what are, at best, associations that have not been demonstrated in this or previous statin trials. The increased incidence they speculate would be present with the higher dose was not confirmed in this large study, which was conducted over a five-year period (with almost 25,000 patient-years of atorvastatin therapy at a dose of 80 mg per day). With regard to the cases of rhabdomyolysis, none of the on-site investigators believed they could be attributed to statin use, and none met American College of Cardiology–American Heart Association–National Heart, Lung, and Blood Institute criteria for rhabdomyolysis. The decision as to whether an adverse event was considered unrelated to treatment was made by the investigator with direct responsibility for the patient, and not by the authors. Even if Dr. Ravnskov and colleagues disagree, they cannot deny that the incidence is extremely low and unrelated to the dose of atorvastatin used.

The comparison of data from the TNT, PROVE IT–TIMI 22, and A-to-Z trials by Dr. Cannon and colleagues is reassuring, although we acknowledge, as do Dr. Auer and colleagues, that the mechanisms underlying the benefit from high-dose statin therapy in acute coronary syndromes may not be exactly the same as they are in stable CHD.

We concur with Dr. Cannon and colleagues that the play of chance could be driving the observed results in the TNT study. TNT was not powered to detect a treatment effect on death from any cause. Previous large-scale secondary-prevention studies in which death from any cause was not the primary end point have also failed to find a significant effect on overall mortality. We cannot draw definitive conclusions about mortality from noncardiovascular causes on the basis of a study substantially underpowered to study it; we can only repeat that no single cause of death explains what is most likely a chance observation.

We will face a similar challenge in designing future LDL-lowering studies focusing on clinical outcomes. Since placebo-controlled trials involving patients with stable disease are no longer ethical, we will be challenged to power controlled studies around events with a low incidence, such as death from any cause. We estimated in TNT that a sample size of roughly 35,000 patients (17,500 in each

group) would have been necessary to detect a treatment effect on overall mortality.

Previous studies, adequately powered to detect differences in mortality, have clearly demonstrated the mortality benefits associated with the use of statins. We are now in an era in which randomized, comparative clinical studies are likely only to demonstrate benefits in terms of reductions in morbidity. In TNT, mortality from any cause in both treatment groups was lower than that in the treatment group in all previous trials of statins in secondary prevention. In this new era, TNT found significant reductions in morbidity from cardiovascular causes (including a highly significant reduction in the risk of stroke) with the use of 80 mg of atorvastatin per day to lower LDL cholesterol levels substantially below the current LDL cholesterol goals.

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A Proposal for Universal Coverage

TO THE EDITOR: Four thoughtful pieces in the March 24 issue[1-4] call attention to the need for universal health insurance and to the financial problems that must be resolved. The two Sounding Board articles, by Emanuel and Fuchs[1] and Mongan and Lee,[2] state that more federal taxes would be required, but neither suggests a unified insurance plan to replace the present costly mix of government entitlements and multiple for-profit plans and neither considers the possibility of changing the organization of practice or the system for compensating physicians in order to reduce costs and improve the quality of care. Such major reforms may not be politically feasible right now, but what will happen if the whole system begins to implode is anybody's guess. Mongan and Lee are therefore correct in urging physicians to become involved with these issues now, before the financial crisis gets much worse.

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1. Emanuel EJ, Fuchs VR. Health care vouchers — a proposal for universal coverage. N Engl J Med 2005;352:1255-60.
2. Mongan JJ, Lee TH. Do we really want broad access to health care? N Engl J Med 2005;352:1260-3.
3. Weissman JS. The trouble with uncompensated hospital care. N Engl J Med 2005;352:1171-3.
4. Kronick R. Financing health care — finding the money is hard and spending it well is even harder. N Engl J Med 2005;352:1252-4.

TO THE EDITOR: The health care voucher plan presented by Emanuel and Fuchs is an inventive alternative to our current method of health care financing, but one might argue that it falls into the authors' self-identified trap of "incremental reform," rather than "major surgery." Vouchers would shift insurance costs from employers to consumers without curbing the excessive administrative costs created by our private insurance system. The establishment of the proposed Federal Health Board, Institute for Technology and Outcomes Assessment, and bodies to undertake risk adjustment and administration of a valued-added tax (VAT) would add further expense. Administrative costs for private insurance systems are more than twice those of public programs[1]; drastically reducing these costs through regulation of the insurance industry would slow the upward pressure on health care costs overall. Reining in administrative spending would free up finances for patient care and development of system infrastructure, allowing us to move toward universal coverage. We should rightly concentrate on cost control as the first step of reform. Otherwise, we could open ourselves up to even more explosive growth in health care spending.

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DRS. EMANUEL AND FUCHS REPLY: We agree with Dr. Relman about the need to reform the health care delivery system. The universal voucher system we propose would do precisely that, not by regula-

tion or exhortation but by changing the incentives for physicians and hospitals. The experience of the past 40 years has shown that finance reform is a necessary prerequisite for widespread changes in the organization and delivery of care. Incentives need to be made more coherent, and they need to provide a financial as well as professional rationale for the implementation of electronic medical records, practice guidelines, physician extenders (e.g., nurse practitioners and physician assistants), drug formularies, and other mechanisms to improve quality and reduce costs. This is why we believe reform of financing must precede reform of the delivery system.

We agree with Ms. Hinkel that administrative costs should be reduced. The voucher system would do that by eliminating billions of dollars devoted to administering Medicaid and other means-tested insurance, especially by eliminating costly eligibility determinations. Additional billions would be saved by eliminating employer-based insurance. The voucher system would usher in the consolidation of the more than 1000 health insurance companies seeking business from millions of employers with costly annual negotiations of contracts — another savings of administrative costs.

However, not all administrative costs are wasteful. Systematic technology and outcomes assessment involve administrative costs that are essential for a well-functioning system that delivers cost-effective, high-quality care. One of the big defects of the current health care system is that we do not monitor the quality of the care actually delivered to most patients. Administrative costs are what we must pay for the information necessary to determine what works and what interventions are worth the money. Risk adjustment also entails administrative costs, but it furthers the important goal of preventing "cherry picking and lemon dropping." Such expenditures are worthwhile, as are administrative ones that reduce the hidden costs of fraud and abuse.

The voucher proposal is not a panacea, but it furthers the widely agreed-on goals of universal coverage, improvements in the organization and delivery of care, and reduced administrative costs in a manner congruent with basic American values.

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One Surprise after Another

TO THE EDITOR: In the report by Leeper et al. (April 7 issue),[1] the endomyocardial-biopsy specimen in their Figures 2 and 3 shows chronic changes, including extensive fibrosis, as stated by the authors, but it does not support a diagnosis of myocarditis.

The most widely accepted histologic criteria for the diagnosis of active myocarditis[2] require the presence of an inflammatory infiltrate and associated myocyte damage (Fig. 1). Myocardial changes, including myocyte hypertrophy, degeneration, and varying degrees of myocardial fibrosis, are typical of a chronic process, such as idiopathic dilated cardiomyopathy.[3] The occasional presence of lymphocytes may be associated with areas of fibrosis.[4] Cocaine use may have contributed to the multifocal fibrosis.[5] Patients with dilated cardiomyopathy may undergo acute clinical decompensation without a secondary or superimposed pathologic process.

It has become increasingly apparent that histologic descriptors alone may not be sufficient to characterize myocarditis fully. However, despite limited sensitivity and specificity, the endomyocardial biopsy is an important diagnostic tool,

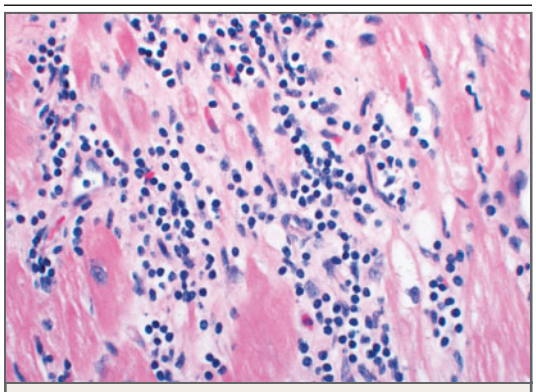

Figure 1. Active Myocarditis Consisting of an Interstitial Lymphocytic Infiltrate with Associated Myocyte Damage.

particularly when the findings are positive. Those of the biopsy discussed by Leeper and colleagues are not.

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TO THE EDITOR: Leeper et al. do not mention polymerase-chain-reaction (PCR) testing for viral genomes in the endomyocardial-biopsy material. Such testing could have provided valuable additional information in the differential diagnosis of dilated cardiomyopathy. Where resources permit, it is important for practitioners who are performing catheterization and endomyocardial biopsy in suspected myocarditis to consider special handling of tissue in order to complete the PCR viral study, since the Dallas criteria[1] for the diagnosis of active or borderline myocarditis are insufficiently sensitive to confirm a diagnosis of lymphocytic myocarditis, inflammatory cardiomyopathy, or both in many cases.

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1. Aretz HT, Billingham ME, Edwards WD, et al. Myocarditis: a histopathologic definition and classification. Am J Cardiovasc Pathol 1987;1:3-14.

THE AUTHORS AND A COLLEAGUE REPLY: The Dallas criteria for the microscopical diagnosis of myocarditis, to which Winters and McManus refer, have been criticized for both low sensitivity and poor interobserver concordance.[1] Part of the problem is the wide spectrum of inflammatory activity seen in myocarditis. Winters and McManus's Figure 1, which clearly shows florid inflammation, is on one end of the spectrum. On the other end, we consider our patient's sparse lymphocytic infiltrate and rare myocyte damage to be more typical.[2] In light of this, we believe that the biopsy findings, when viewed in the context of the patient's profound acute deterioration (with dynamic electrocardiographic abnormalities and rapidly escalating levels of troponins) and clinical rebound, are consistent with the presence of active myocarditis, perhaps superimposed on a chronic process.

Given the shortcomings of histology-based strategies, we appreciate Fett's comments regarding PCR. Though initially plagued by technical problems and variable sensitivities, nested PCR is becoming an important and reliable tool in the identification of causative viral agents in acute myocarditis. Recent advances now allow detection of ongoing viral replication, which may help direct antiviral therapy, and the identification of enterovirus in myocardium, which has been associated with a poor prognosis.[3,4] As test standardization and test characteristics improve and the significance of viral presence in myocardium is better defined, the use of PCR is likely to become a standard approach to the diagnosis of acute myocarditis.

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Osteonecrosis of the Jaw and Bisphosphonates

TO THE EDITOR: Cases of osteonecrosis of the jaw in connection with the use of bisphosphonates were reported in 2003.[1,2] In 2004, the International Myeloma Foundation conducted a Web-based survey to assess the risk factors for osteonecrosis of the jaw. Of 1203 respondents, 904 had myeloma and 299 breast cancer. Both osteonecrosis and suspicious findings, including bone erosions and spurs plus exposed bone, were assessed. Sixty-two patients with myeloma had osteonecrosis of the jaw and 54 had suspicious findings; 13 patients with breast cancer had osteonecrosis and 23 had suspicious findings — a total of 152 patients with either osteonecrosis or suspicious findings. Of the patients with myeloma, 71 percent had received zoledronic acid and 29 percent had received only pamidronate.

Figure 1 displays the cumulative incidence of osteonecrosis of the jaw among patients receiving either zoledronic acid alone or pamidronate alone who responded to the survey. With censoring at 36 months, osteonecrosis of the jaw developed in 10 percent of 211 patients receiving zoledronic acid, as compared with 4 percent of 413 patients receiving pamidronate (P=0.002 by the log-rank test). The earlier onset of osteonecrosis of the jaw among patients receiving zoledronic acid during the first 36 months reflects remarkably well the reported increase in the occurrence of osteonecrosis in the first 36 months after the Food and Drug Administration approved the drug in 2001.

The censored 36-month estimates of osteonecrosis, suspicious findings, or both did not differ between patients with myeloma and those with breast cancer (P>0.50). No other therapies, including corticosteroids and thalidomide, conferred an added risk over time (P>0.50). However, a history of underlying dental problems, such as infection or dental extraction, was present in 81 percent of patients with myeloma and in 69 percent of patients with breast cancer who had osteonecrosis of the jaw, as compared with 33 percent of those without osteonecrosis (P<0.001 and P=0.01, respectively, in a two-sided test). Maxillofacial surgery was a particular problem for patients with osteonecrosis of the jaw, since the surgery resulted in areas of nonhealing bone and soft tissue that were larger than those in patients who did not undergo surgery.

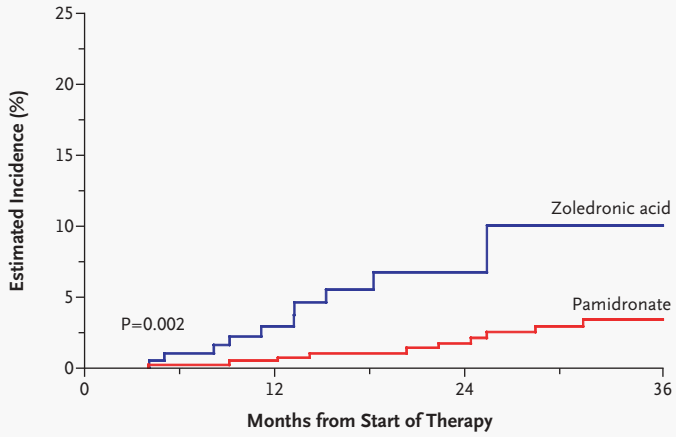

Figure 1. Time to the Onset of Osteonecrosis of the Jaw in Patients with Myeloma Receiving Zoledronic Acid or Pamidronate.

Among patients receiving zoledronic acid, the occurrence of osteonecrosis of the jaw is particularly notable at months 4, 8, 9, 11, 13, 15, and 18. With data censored at 36 months, the estimated incidence among patients receiving zoledronic acid was 10 percent and that among those receiving pamidronate was 4 percent. Without censoring, the mean time to the onset of osteonecrosis among patients receiving zoledronic acid was 18 months, as compared with 6 years for patients receiving pamidronate (P=0.002).

In September 2004, Novartis, the manufacturer of pamidronate (Aredia) and zoledronic acid (Zometa), issued post-marketing guidelines[3] for both drugs in relation to osteonecrosis of the jaw that emphasized a particular risk with surgical intervention. The International Myeloma Foundation is working collaboratively with Novartis to raise awareness and develop enhanced guidelines. The full results of the study were presented to the Food and Drug Administration at an Oncology Drug Advisory Committee meeting held on March 4, 2005.[4]

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TO THE EDITOR: Bisphosphonates are powerful osteoclast inhibitors with antitumor and antiangiogenic properties and a half-life of many years. The use of these drugs significantly reduces skeletal-related events in patients with multiple myeloma and other cancers.[1] However, long-term use can result in suppression of bone turnover and compromised healing of even physiologic microinjuries within bone that occur as a result of day-to-day stresses.[2]

Osteonecrosis of the jaw has been reported recently in patients with cancer who were receiving either pamidronate or zolendronate, or both, and in those receiving alendronate for osteoporosis.[3] Osteonecrosis of the jaw presents as an exposure of the mandible or maxilla that can be either painless or painful. Unlike osteoradionecrosis, osteonecrosis involves the maxilla fairly frequently, and as many as one fifth of cases occur spontaneously. We have seen more than 20 cases of osteonecrosis of the jaw in patients with myeloma at our institution during the past six months, although we had seen very few in previous years; the reasons for this increased incidence are unclear.

Osteonecrosis of the jaw probably results from the inability of hypodynamic and hypovascular bone to meet an increased demand for repair and remodeling owing to physiologic stress (mastication), iatrogenic trauma (tooth extraction or denture injury), or tooth infection in an environment that is trauma-intense and bacteria-laden. Coexisting factors may include the use of other medications with antiangiogenic properties (such as glucocorticoids, thalidomide, and bortezomib in patients with myeloma), diabetes mellitus, irradiation of the jawbone, peripheral vascular disease, and hyperviscosity syndromes. It is hypothesized that benign sequestration of the lingual mandibular plate in healthy persons results from physiologic trauma to the mucosa, leading to hypovascularity and focal bone death.[4] Interestingly, this site is frequently involved in osteonecrosis.

Radical resection appears to be of limited use in cases of osteonecrosis of the jaw and may be contraindicated; the disease may progress despite surgery and cessation of bisphosphonate therapy.[3] Factors such as underlying disease status, prognosis, extent of the lesion, presence or absence of jaw pain, and presence or absence of infection (not just surface bacterial colonization) should be considered when planning further treatment. Once bone resorption has been curtailed, there may be little benefit in giving lower doses of bisphosphonates, especially in patients receiving long-term bisphosphonate therapy.[5]

In our view, until studies in animals and prospective clinical trials shed more light on this condition, patients should be informed of the risk of osteonecrosis. Dentists and oral surgeons should judiciously remove all dental infections before or within a few weeks of the initiation of bisphosphonate therapy in this high-risk population of patients with cancer. Moreover, among patients receiving bisphosphonates in whom dental infections develop, withdrawal of the drugs until the infection is controlled may be warranted.

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TO THE EDITOR: The main known risk factors for osteonecrosis of the jaw are dental procedures, poor dental hygiene, corticosteroid therapy, and local

radiotherapy. More recently, treatment with bisphosphonates, such as pamidronate and zoledronic acid, was reported to be associated with osteonecrosis of the jaw among cancer patients.[1,2] However, other confounding risk factors for osteonecrosis were also noted in published reports, especially dental procedures during therapy.[3] As a consequence, the manufacturer of zoledronic acid recently modified its U.S. post-marketing and precautions information with the following statement regarding dental care: "While on treatment, these patients should avoid invasive dental procedures, if possible" (www.pharma.us.novartis.com).

We report nine cases of well-documented osteonecrosis of the jaw that occurred in patients who were receiving therapy with zoledronic acid but had not undergone dental procedures. In September 2002, we started using zoledronic acid almost exclusively for skeletal protection in patients with cancer. Zoledronic acid was given every three or four weeks at a dose of 4 mg intravenously during a period of 15 minutes. Between December 2003 and July 2004, nine cases of osteonecrosis of the jaw were diagnosed at our institution (four in patients with multiple myeloma and five in patients with metastatic breast cancer) among 194 patients treated with zoledronic acid. Before receiving zoledronic acid, six patients had been treated first with pamidronate (90 mg every three or four weeks). Eight of the nine patients had biopsy-proven osteonecrosis of the jaw. All the cases were diagnosed while the patients were receiving zoledronic acid. The median duration of treatment with pamidronate was 39 months (range, 4 to 58), with a median cumulative dose of 3060 mg (range, 360 to 5520). For zoledronic acid, the median duration of therapy before the appearance of osteonecrosis was 18 months (range, 4 to 22), and the median cumulative dose was 72 mg (range, 36 to 88).

The percentage of cases of osteonecrosis of the jaw that are associated with zoledronic acid is high in our institution (4.6 percent). Nevertheless, our data confirm other previous reports concerning the possible association between bisphosphonates and osteonecrosis of the jaw. From our observations, it is unclear which bisphosphonate, zoledronic acid or pamidronate, is the causal agent. However, our analysis provides more evidence that further investigations should be performed to determine which patients are at increased risk for osteonecrosis of the jaw, what is the optimal and safe duration of treatment, and what recommendations should be made for the follow-up of patients being treated with bisphosphonates.

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THE ABOVE LETTERS WERE REFERRED TO NOVARTIS PHARMACEUTICALS, THE MANUFACTURER OF PAMIDRONATE AND ZOLEDRONIC ACID, WHICH OFFERS THE FOLLOWING REPLY: The letters published in this issue of the *Journal*, as well as case reports published since 2003,[1-3] underscore the fact that osteonecrosis of the jaw is a concern for cancer patients and their physicians. As the developer of Aredia (pamidronate) and Zometa (zoledronic acid), we have obtained expert advice on how to revise the labels for these two drugs. Our labeling recommends a dental examination to identify and correct predisposing conditions before bisphosphonate treatment is started in patients with potential risk factors, including cancer.[4] This approach may help identify and rectify dental problems before or during treatment so that osteonecrosis of the jaw may be prevented or its progression limited. Patients taking bisphosphonates should avoid invasive dental procedures, if possible. Further, more conservative treatments for osteonecrosis of the jaw may also include systemic antibiotics to control or prevent infection, as well as antimicrobial oral rinses.

Dental surgery may exacerbate osteonecrosis of the jaw in patients in whom the condition has developed during bisphosphonate therapy. No data are available to suggest whether discontinuation of bisphosphonate treatment in patients requiring

dental procedures reduces the risk of osteonecrosis. Collaboration between the oncology and dental communities will be important for gaining better insights into the optimal treatment of patients with osteonecrosis of the jaw.

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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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CORRECTION

Osteonecrosis of the Jaw and Bisphosphonates

Osteonecrosis of the Jaw and Bisphosphonates . On page 101, lines 5 through 8 of the letter by Tarassoff and Hei should have read, ``As the developer of Aredia (pamidronate) and Zometa (zoledronic acid), we have implemented labeling revisions for these two drugs with the approval of the Food and Drug Administration and based on best clinical judgment,´´ rather than ``. . . we have obtained expert advice on how to revise the labels for these two drugs,´´ as printed.

N Engl J Med 2005;353:2728-c

BOOK REVIEWS

EMBODYING INEQUALITY: EPIDEMIOLOGIC PERSPECTIVES

(Policy, Politics, Health and Medicine Series.) Edited by Nancy Krieger. 545 pp. Amityville, N.Y., Baywood, 2005. $70. ISBN 0-89503-294-5.

One of the most striking things an American observes on first arriving in a developing country is that poor people are so small and rich people so large. That physical stature so perfectly mirrors social stature is hardly surprising, given that adequate nutrition is a commodity like any other. Indeed, this pattern was evident in the United States until political and economic developments drove down the price of simple carbohydrates and fat, so that calories became literally cheaper than water. Now we increasingly observe the opposite pattern in developed countries, with the poor marked by such physical manifestations of caloric excess as obesity and diabetes. Either way, the body is the physical memory of one's place in the economic order. Inscribed on us all is the detailed record of our lot in life: too little food or too much, the miles walked in good shoes or bad, and the physical and emotional insults and injuries endured. Like the rings of a tree, which are spaced according to the yearly whims of rain and sun, our bodies harbor the permanent marks of leanness or plenty and, thus, indelible physical evidence of our social arrangements.

Embodying Inequality, compiled from papers published in the *International Journal of Health Services*, explores this basic theme. The editor, Harvard social epidemiologist Nancy Krieger, is also the author or coauthor of 4 of the 22 chapters in the book. In addition, she provides a brief preface to each section, as well as an overall introduction that lays out the role of social epidemiology as a scientific subdiscipline and as an intellectual framework for the diverse papers that follow. Economic class is just one of the axes of social distinction that are explored in these chapters, with considerable space also given to sex role and race and with some attention to sexual orientation and physical disability. Using a wide variety of methodologic approaches from history, social science, and public health, the various authors contribute to a remarkably detailed account of how the social becomes biologic. In an era in which the biologic determination of our social arrangements is continually trotted out as the science news item of the day, this perspective is a welcome relief.

The range and quality of the material selected for inclusion are impressive and reflect favorably on the more than three decades of editorial leadership of Johns Hopkins health policy scholar Vicente Navarro at the *International Journal of Health Services*. The added commentary by Krieger is useful in providing a sense of the structure of this collection, revealing common themes, although she lapses at times into platitudes — such as the invocation of an "ecosocial" theory, which, as one might surmise, is sort of a grand theory of everything. Nonetheless, a compelling realization gleaned from this book is that social epidemiology is, indeed, the epidemiology of just about everything, since any factor that could make us sick or healthy is meted out in accordance with our status. After all, if money couldn't buy you health, of what use would it be? And if racial privilege didn't equal more years of life, why would anyone fight to defend it? Thus, in a society of haves and have-nots, the chasm between them is inevitably one of illness and death.

Embodying Inequality lays the groundwork for an etiologic model of the production of health disparity. For those who view health as a basic human right, it is therefore also an etiologic model of injustice.

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UNIVERSAL COVERAGE: THE ELUSIVE QUEST FOR NATIONAL HEALTH INSURANCE

(Conversations in Medicine and Society.) By Rick Mayes. 207 pp. Ann Arbor, University of Michigan Press, 2004. $26.95. ISBN 0-472-11457-3.

Observers of the U.S. health care scene cannot help but be perplexed by the enigma of a system of funding health care that

leaves so many patients financially vulnerable, in spite of per capita spending on medical care that is almost twice that of the average industrialized nation. We tend to look for relatively simplistic explanations of this phenomenon, but most theories are too narrowly focused to provide adequate enlightenment. In contrast, in this book, Rick Mayes provides a political history of the reform movement that demonstrates why it seems almost improbable that national health insurance ever could have reached the threshold of political feasibility.

Mayes begins with President Franklin Roosevelt's decision to abandon his attempt to include national health insurance in his Social Security legislation. Roosevelt feared that the opposition of the politically influential American Medical Association would doom his entire program, a risk that he dared not take. Mayes then describes several other critical junctures at which comprehensive reform again seemed feasible but failed each time, primarily because of a lack of the requisite political alignment of influential interests. The one notable exception was the success during the Johnson administration in enacting the Medicare and Medicaid programs. Mayes ends with a description of the politically inept effort of the Clintons, which never could have produced a consensus.

Perhaps the most important contribution to understanding why the quest for universal coverage remains elusive is Mayes's description of the various incremental measures that were effective in increasing coverage but, ironically, solidly institutionalized the reforms and thus made it more and more difficult to enact a universal system. Even Social Security, as one of the most popular government programs ever, was a factor, since Medicare was a logical expansion of the provision of security in retirement. Labor's eventual support of employer-sponsored coverage, with the acquiescence of business interests, firmly secured the position of the insurance industry as a major player in health care funding. Medicaid nominally fulfills the commitment to provide coverage for patients in poverty, and many believe that tweaking this program would provide the final major increment of bringing in low-income people who currently do not qualify for Medicaid. Although Mayes does fill in the blanks in confronting the complexities of health care reform, nevertheless it is clear why many observers believe that refinement of our current institutions would be adequate and that a comprehensive national health insurance program is not necessary.

Such complacency ignores the fact that many of the tens of millions of people without coverage do suffer adverse health outcomes and even death. Also disconcerting is the fact that the newer, ubiquitous, innovative insurance products are no longer adequate to ensure either financial security or health security. But Mayes does give us hope. Although he acknowledges that critical junctures are rare, he notes that they do occur, especially in response to unmet social needs. Perhaps the deterioration in insurance coverage that has taken place may have brought us much closer to our next critical juncture than most of us realize.

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VULNERABLE POPULATIONS IN THE UNITED STATES

By Leiyu Shi and Gregory D. Stevens. 312 pp. San Francisco, Jossey-Bass, 2005. $58. ISBN 0-7879-6958-3.

Recent findings and trends associated with health disparities, along with their causes, consequences, and potential solutions, are reviewed in *Vulnerable Populations in the United States.* It is an excellent primer for undergraduates and graduate students in public health and for medical students interested in vulnerable populations and health disparities.

The first chapter introduces a general conceptual framework for studying vulnerable populations. After reviewing the many models in the literature that have helped elucidate the social and behavioral determinants of health, Shi and Stevens propose a model of vulnerability that has been developed as an interaction of risk factors, access to care, and quality of care. Risk factors are characterized as both individual and ecologic and identified on three levels: predisposing factors, enabling factors, and need. The model moves beyond familiar approaches by emphasizing multiple interactions both conceptually and in some of the analyses presented later in the book.

Although the conceptual approach used in this study is useful for understanding patterns of health, it has limited use as an approach to health outcomes. Risk factors are particular for specific illnesses, impairments, and deaths that are not specified by

general models. As public health and medicine work to heal the long-standing schism that currently weakens both disciplines, we must move beyond general models to a more specific understanding of the determinants of health outcomes and disease states.

The book focuses on three key areas associated with vulnerability and health disparities: race and ethnic background, social and economic status, and insurance-coverage status. These factors structure the subsequent chapters presenting the determinants of vulnerability, the empirical literature that documents and explains disparities, and the relationship between multiple risk factors. It is refreshing that the authors do not try to reduce any one of the key factors to the others or suggest that one is more important or explains the others. Race and ethnic background, social class, and health insurance coverage are inextricably linked in the history of the United States and in the health of its people.

Most welcome in this book is a chapter that joins the patterns and causes of vulnerability in a description of the programs of public health and medicine that address disparities. Shi and Stevens analyze programs for validity, scope and reach, sustainability, and effectiveness. This chapter is uneven in its treatment of the programs in somewhat surprising ways. For example, the history of Medicare and Medicaid — billion-dollar programs that were created to address health disparities — get one line, whereas some small local programs are described in several paragraphs. The chapter also neglects the history of the effort to link local and philanthropic health systems that has been so critical to the safety net of this country. The change in that system is critical to understanding the current uninsured status of 45 million people.

The last chapter is devoted to resolving health disparities in the United States, beginning with a description of Healthy People 2010, a federal initiative that sets goals for national efforts to improve the health of Americans, including the identification of preventable threats. A wide variety of strategies are discussed, ranging from the very specific (cultural competence in health care) to broad social reforms, such as income redistribution and single-payer health insurance. The book ends on a refreshingly positive note of activism, outlining concrete steps to help eliminate health disparities that students and practitioners can take seriously.

We should look forward to many new editions of this book as the drama of health disparities in our nation continues to unfold. Subsequent editions might be improved by including a discussion of cultural competence in primary-prevention programs. Smoking rates have dramatically decreased among highly educated Americans but have changed very little among the least educated. This growing gap suggests that our smoking-cessation programs may not have been attuned to the realities of all social classes in the United States. A later edition might also take into account that we have become an increasingly global society.

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NOTICES

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

AMERICAN COLLEGE OF CLINICAL PHARMACOLOGY

The "34th Annual Meeting" will be held in Rockville, Md., Sept. 11–13. Deadline for early registration is Aug. 17.

Contact American College of Clinical Pharmacology, 3 Ellinwood Court, New Hartford, NY 13413-1105; or call (315) 768-6117; or fax (315) 768-6119; or see http://www.accp1.org.

2ND INTERNATIONAL CONFERENCE ON BIRTH DEFECTS AND DISABILITIES IN THE DEVELOPING WORLD

The conference will be held in Beijing, Sept. 11–14. Deadline for online registration is Aug. 15.

Contact Chinese Medical Association, International Department, 42 Dongsi Xidajie, Beijing 100710, China; or call (86) 10 6524 9989, extension 2462; or fax (86) 10 6512 3754; or e-mail chenchen@cma.org.cn or chenchen@chinamed.com.cn; or see http://www.chinamed.com.cn/birthdefects/.

ANNUAL INTENSIVE COURSE ON MEDICAL ETHICS

The course will be offered in London, Sept. 19–23.

Contact Bang Nong, Centre for Professional Development, Imperial College, Room 318, Sherfield Bldg., London SW7 2AZ, United Kingdom; or fax (44) 20 7594 6883; or e-mail cpd@imperial.ac.uk; or see http://www.imperial.ac.uk/cpd/medeth.htm.

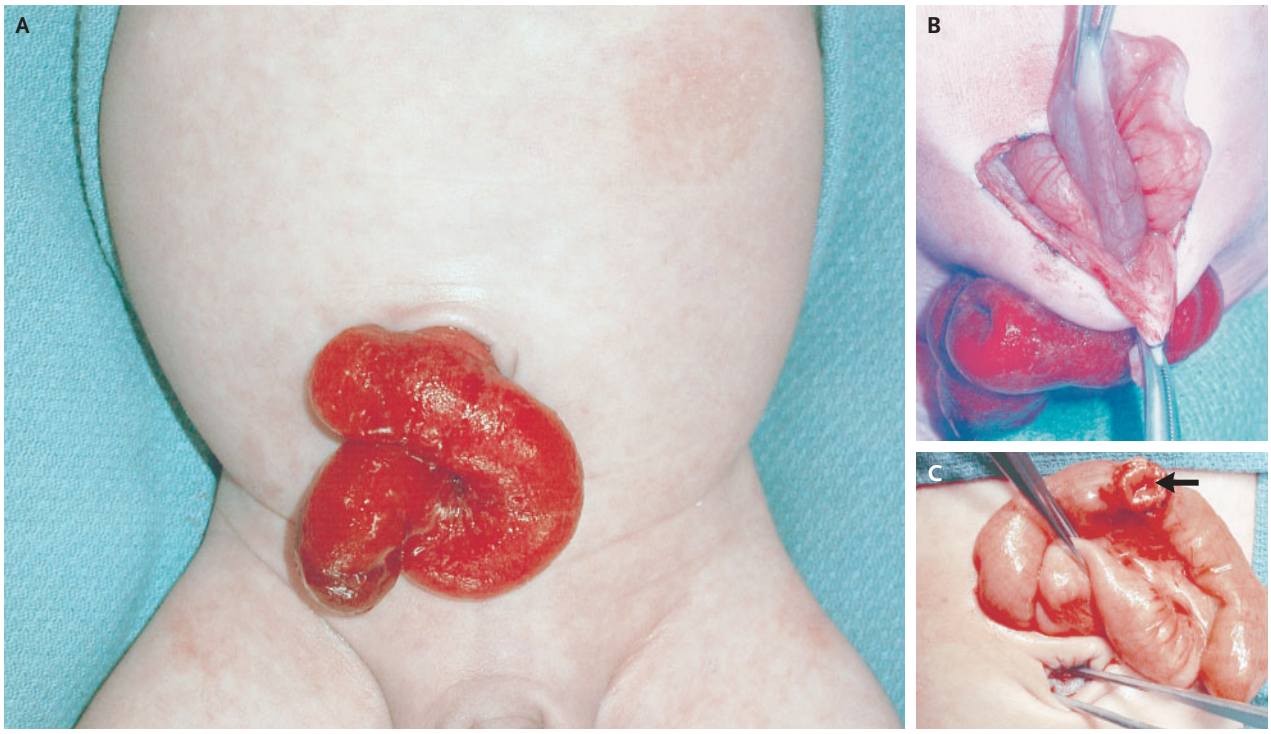
OBESITY AND DIABETES

The three-day training course will be offered in Nicosia, Cyprus, Sept. 20–22. It is offered by Cyprus International Institute for the Environment and Public Health, in association with Harvard School of Public Health.

Contact Lenia Josephides, Cyprus International Institute, P.O. Box 24440, 1703 Nicosia, Cyprus; or call (357) 22 692692; or see http://www.hsph.harvard.edu/cyprus.

IMAGES IN CLINICAL MEDICINE

Small-Bowel Prolapse through a Persistent Omphalomesenteric Duct



A 23-DAY-OLD TERM MALE INFANT PRESENTED TO THE EMERGENCY ROOM with acute small-bowel evisceration through the umbilicus (Panel A). His parents had noted periumbilical erythema and mucus-containing umbilical drainage before the evisceration. The child was taken to the operating room, where, through a supraumbilical incision, a 10-cm length of distal ileum that had prolapsed through a patent omphalomesenteric duct was reduced (Panel B). The duct was released from the umbilicus (Panel C) and closed with sutures (arrow). Inspection of the remaining bowel revealed malrotation, which necessitated a Ladd's procedure to release peritoneal bands; an appendectomy was performed, and the bowel was replaced in an anatomical position to prevent volvulus.

Anomalies in the omphalomesenteric duct occur because of a lack of involution during the ninth week of gestation. Surgical resection of remnants of the duct is required for the treatment of bleeding, intestinal obstruction, intussusception, and as in this case, intestinal prolapse. Associated defects involving intestinal malrotation are rare.

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