POSTING PRESENTATIONS AT MEDICAL MEETINGS ON THE INTERNET

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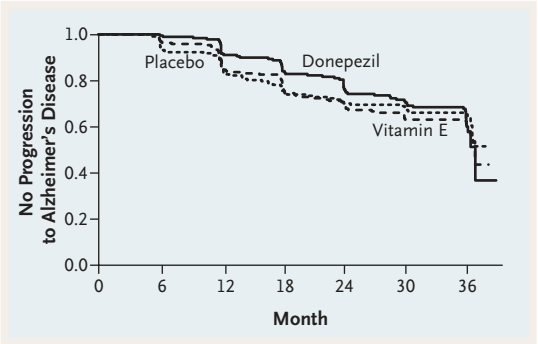
This Week in the Journal

JUNE 9, 2005

ORIGINAL ARTICLE

Vitamin E and Donepezil for the Treatment of Mild Cognitive Impairment



In this randomized trial involving patients with mild cognitive impairment, vitamin E did not reduce the rate of progression to Alzheimer's disease. Although an initial benefit of donepezil was observed during the first year, over the course of the three-year study the rate of progression to Alzheimer's disease was similar in patients treated with donepezil and those treated with placebo. The side effects of donepezil included diarrhea, nausea, muscle cramps, and insomnia.

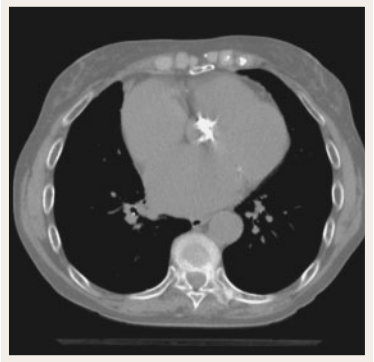
SEE P. 2379; EDITORIAL, P. 2439; CME, P. 2465

ORIGINAL ARTICLE

Intensive Lipid-Lowering Therapy in Calcific Aortic Stenosis

Calcific aortic stenosis, a relatively common problem in the elderly, has been found to be associated with atherosclerosis and hypercholesterolemia. This study found that, contrary to expectations, intensive lipid-lowering therapy with atorvastatin, which reduced low-density lipoprotein cholesterol levels to a mean of 63±23 mg per deciliter, had no effect on the progression of aortic stenosis (as measured by the aortic-jet velocity) or on aortic-valve calcification (as measured by helical computed tomographic scanning).

SEE P. 2389; EDITORIAL, P. 2441; CME, P. 2466



ORIGINAL ARTICLE

Conventional vs. Endovascular Repair of Abdominal Aortic Aneurysms

Endovascular repair of abdominal aortic aneurysms avoids much of the risk associated with conventional surgical repair. In two randomized trials, this technique has been shown to be associated with lower rates of perioperative morbidity and mortality. Longer-term follow-up data from one of these trials (the Dutch Randomized Endovascular Aneurysm Management [DREAM] trial) show that the survival advantage of endovascular repair is not sustained after the first postoperative year.

SEE P. 2398; EDITORIAL, P. 2443

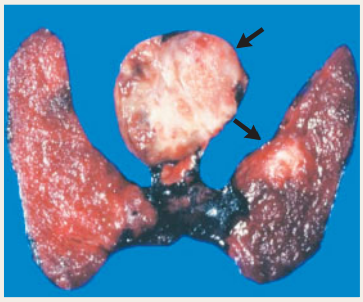
ORIGINAL ARTICLE

Clonal Independence in Multifocal Papillary Thyroid Carcinoma

Whether individual foci in multifocal papillary thyroid cancer arise independently or are metastases of a primary tumor within the gland is uncertain. This investigation used the phenomenon of X-chromosome inactivation in women to study such foci. In five tumors, all foci had different patterns of X-chromosome inactivation, indicating their independent origin; results in five other tumors were indeterminate.

The independent origins of multiple foci of neoplasia in multifocal papillary thyroid cancer suggest environmental or inherent susceptibility to thyroid cancer. These kinds of tumors may require aggressive treatment.

SEE P. 2406; PERSPECTIVE, P. 2376



MECHANISMS OF DISEASE

Calpains and Disease

Calpains are members of a large family of Ca^{2+}-dependent proteolytic enzymes. Some are tissue-specific; others are ubiquitous. Poised to digest numerous intracellular proteins, their potential to cause or contribute to disease is considerable. This review outlines the structure and function of calpains and their involvement in a type of muscular dystrophy, type 2 diabetes, cataracts, and Alzheimer's disease.

SEE P. 2413

CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL

A Woman with Back and Leg Pain and Respiratory Failure

A 22-year-old woman was transferred because of respiratory failure. She had a history of sickle cell anemia and had back and leg pain the day before she was admitted to another hospital, where respiratory failure developed while she was being treated for a sickle cell crisis. After transfer, assisted ventilation, nitric oxide, and exchange transfusion did not reverse the hypoxemia, and the patient died. An autopsy was performed.

SEE P. 2425; CME, P. 2467

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Controlling the Expression of Oncogenes in Lung Cancer

A new mechanism for regulating *RAS* oncogenes has been uncovered, which may have relevance to the pathogenesis of lung cancer.

SEE P. 2446

Managing Conflict at the End of Life

M. Gregg Bloche, M.D., J.D.

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

The media spectacle that surrounded the dying of Terri Schiavo is now two months past, and America's culture warriors have moved on to other battlegrounds. Much has been said about which political players won or lost and whether key voting blocs will care. But does the experience offer any useful lessons about the subject in dispute — decision making at the end of life?

There is a widespread perception that the law failed Terri Schiavo, her family, and the country by not yielding a quick, clear resolution. Cultural conservatives and others who rallied to the side of Schiavo's parents condemn the courts for failing to keep her alive. Many who backed her husband's efforts to withdraw her feeding tube urge increased use of advance directives and living wills — and safeguards against challenges to the judgment of surrogate decision makers. Both sides look to the law to set clear rules, though the two sides differ sharply on what those rules should be.

Almost forgotten in this debate, and ignored in press coverage of the Schiavo affair, is the peripheral role of law when end-of-life dilemmas arise. The law sets some limits: active killing, for example, is impermissible, and clear advance directives must be followed if they have been properly given. In most U.S. jurisdictions, suicide is unlawful, as is the assistance of physicians in self-killing. But within these bounds, end-of-life questions are almost always resolved in the private sphere, by patients, their physicians, and their family members, working with nurses, social workers, and members of the clergy.

Dr. Bloche is a professor of law at Georgetown University and a guest scholar at the Brookings Institution, both in Washington, D.C., and an adjunct professor at the Bloomberg School of Public Health, Johns Hopkins University, Baltimore.

In tens of thousands of cases each year, patients and families handle catastrophic illness or injury without going to court. They do so with unsung courage, in the face of fear, anguish, and sometimes bitterness. Every loss of a loved one is, in part, a loss of hope — hope for healing of old rifts and fulfillment of thwarted possibilities. Anger and denial are common, especially when relationships were conflict-ridden beforehand. Cast-off parents, rival siblings, children who never measured up to their parents' expectations bring much to the bedside beyond their religious and philosophical leanings.

Anger, denial, and other nonrational influences can lock family members into warring stances over whether to treat a devastating illness aggressively or discontinue life-sustaining measures. What is remarkable, given the intensity of the feelings at stake, is how rarely such conflicts make their way to court. It is a measure of how discreetly such squabbles are handled that we know little about how often they arise. And it is a measure of people's character under this pressure that families usually come together to make these judgments or to honor the preferences their loved ones have expressed.

This is for the good: to rend families asunder at the end of a loved one's life does spiritual violence to all concerned. Within wide boundaries, we are committed to honoring patients' clearly stated wishes. This commitment not only safeguards patients' liberty and dignity; it protects against family strife when a patient's intentions are clear. When the patient's wishes are unstated and illness precludes asking about them, it is important to limit the possibilities for family conflict and lasting anger. Enabling families to mourn and move on — and discouraging them from playing out old resentments as end-of-life battles — should be a clinical and social priority.

The law can help to pursue this goal by making

it difficult for any one party to impose a decision when family members or others concerned disagree with it. Answers dictated by the law yield clear winners and losers, heightening long-term resentments and inviting further strife. A large literature suggests that solutions crafted by the parties to a conflict come with a sense of shared ownership that dampens discord.[1] By making it harder to invoke a court's final say, law can encourage conversation aimed at reconciliation or, at least, mutual accommodation. The proposition that law should promise quick, clear answers is a recipe for intensified family and social strife, since we are nowhere near national consensus on what the answers should be.

Some features of current law support the family-friendly resolution of agonizing end-of-life questions. The law's inquiry into what the patient would want when there is neither a clearly stated prior preference nor a designated surrogate decision maker is a legal fiction, since a person in a persistent vegetative state or a similarly incapacitated condition cannot formulate a preference. But this fiction sends the right moral message. It centers problem-solving conversation on something family members and friends have in common — their commitment to the patient's interests. This focus, in turn, encourages more mature handling of old wounds and resentments, even if they cannot be resolved.

Efforts to enmesh the law in end-of-life choices through detailed advance directives and the formal selection of surrogate decision makers risk stoking conflict. Advance directives cannot anticipate all scenarios, and the law's commands can crowd out benevolent feelings.[2] The more detailed the directive, the greater the possibilities for lawyerly argument about its application to situations that its author did not precisely foresee. Opting for a surrogate decision maker solves this problem but introduces another: selection of one person may hurt or offend others, reawakening old resentments. When conflicts seem likely, these risks are worth taking; when ties among loved ones are strong and cooperation prevails, the case for involving the law is weaker.

Resort to the courts by warring family members to try to narrow the acceptable range of end-of-life choices on religious grounds is more worrisome. The clinical options at issue in the Schiavo litigation — removing the feeding tube and allowing a patient with a devastating brain injury to die or continuing tube feedings and embarking on an almost certainly futile therapeutic course — were both within the range allowed by ethics and law. To their credit, the judges who heard the case declined to narrow this range. The escalation of a family dispute through fiery religious references represented an attempt to reduce the latitude allowed by law. A Florida bishop's op-ed piece invoked "the passion of Terry Schiavo," and some insisted that, to God, stopping tube feedings is murder. The attempt failed, but the incendiary language set a new standard for family divisiveness at the end of life.

End-of-life choices shouldn't be treated as purely ethical questions.

What, then, are the lessons of the Schiavo affair for the management of end-of-life conflict? First, we should keep in mind that the affair represented an extraordinary exception: the overwhelming majority of such cases are handled privately, by patients, family members, and caregivers. We should take pride in this fact and not overstate the problems to be solved.

Second, the overarching goal of courts, clinical caregivers, and others with a say in end-of-life disputes should be to pursue private, family-friendly accommodation within the wide limits set by law. Caregivers should, of course, defer to advance directives and to properly designated surrogate decision makers. They should, moreover, encourage patients to make their end-of-life preferences known to those closest to them, preferably through standardized means. But in so doing, caregivers should assert themselves gently: to push too hard for a living will or advance directive is to put patient trust at risk, particularly in this era of escalating worry about pressure to cut costs.

In addition, caregivers should encourage conversation about end-of-life questions among patients, family members, and others who are closely involved. And when the clinical picture takes a catastrophic turn and a patient can no longer formulate preferences, caregivers should give high priority to detecting hints of discord. At the first sign of tension, physicians, nurses, and social workers should

become active listeners in search of smoldering feelings that might give rise to conflict.

If and when conflict erupts, end-of-life choices shouldn't be treated as purely ethical questions, divorced from the regrets and resentments involved. Psychiatric and social-work consultation should be part of the management plan, and mediation merits study as an approach. Mediators' methods of listening, exploring parties' needs, reframing problems, and proposing solutions have been well honed in work with divorcing couples, estranged business partners, and others in life-transforming crises.[3] These skills are well suited to the work of guiding warring family members toward agreement on end-of-life choices for their loved one.

At times, physicians, and even insurers, become parties to these conflicts. Financial incentives, real or perceived, can shape positions and sow distrust. Cost-control strategies that engage caregivers in covert rationing can have toxic effects,[4] particularly when medical futility is at issue. Our national unwillingness to acknowledge the conflict between efforts to limit medical spending and insistence on all possibly beneficial care worsens this toxicity. Good mediation technique can help to clarify misunderstandings, soften anger, and ease irrational distrust. But it cannot finesse contradictions that, as a country, we refuse to face.

For the last six years of Terri Schiavo's life, Robert Lynch, the local Catholic bishop, tried unsuccessfully to meet with her parents and husband to reach a solution through mediation. As their personal struggle became an international spectacle, Lynch broke with the Church hierarchy by refusing to side with the parents. Instead, he called on "both sides [to] step back" and to try for "a heroic moment of concern for the feelings of each other."[5] In a public appeal that was ignored by all sides, he said: "The legacy of Terri's situation should not be that of those who love her the most, loathing the actions of one another."[5] Schiavo's legacy has turned out to be worse than he feared. After her death, her parents and husband continued to battle — over access to her remains.

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Patients in a Persistent Vegetative State — A Dutch Perspective

Inez de Beaufort, Ph.D.

He is 31 years old now — Henk-Jan, the son of Gerard and Ineke Stinissen. He is the son Ineke Stinissen never knew. In 1974, as the result of a dramatic medical error that occurred during emergency cesarean delivery, she was left in a persistent vegetative state. Through most of Henk-Jan's life, his mother's condition and treatment have been matters of an emotionally fraught public controversy. In 1985, his father sought publicity — in a television program called "How Long Should Dying Last?" — for his view that his wife's artificial nutrition and hydration should be stopped. In 1987, he went to court to demand that the nursing home stop the feeding. On January 9, 1990, it was finally discontinued, and Ineke Stinissen died 10 days later. Behind these few facts lies a long, sad story that has been widely debated in the Netherlands. Why stop the feeding? Why not? Why now? The questions echo today, in the wake of the recent case of Terri Schiavo.

Dr. de Beaufort is a professor of health care ethics at the Erasmus Medical Center of Erasmus University, Rotterdam, the Netherlands.

In the first hearing of the Stinissen case and again on appeal, the Dutch courts focused on the following questions: Are artificial nutrition and hydration medical treatment or basic care? If the former, is this treatment futile? And who is authorized to decide whether to stop treatment? The husband argued that he was his wife's legal representative and that the doctors were treating his wife without permission. The courts judged that Gerard Stinissen had the right to take his wife to another nursing home where the doctors might have different views. It was decided that artificial feeding was mainly medical, although an element of basic care was acknowledged to be involved. Given the varying opinions of doctors about the question of futility, the courts considered it inappropriate to come to a conclusion on that aspect.

Ultimately, the nursing home clinicians consulted outside ethicists and physicians. When the attending physicians declared that they were now prepared to stop treatment but feared that the process of dying would be terrible, the consulting physicians convinced them that it need not be.[1]

"This is a fate worse than death, but we don't want to lose him."

The day after artificial feeding was stopped, members of a prolife Christian patient movement started a legal action, claiming that they could represent Ineke Stinissen's interests because her husband and doctors had neglected to do so. They argued that no one can judge the quality of life of incompetent patients and questioned the peculiar determination of the nursing home that now, after 15 years, it was suddenly futile to continue treatment. Their argument was rejected by the courts, which did not consider them representatives of the patient.

Nevertheless, the debate with regard to patients in a persistent vegetative state continued. The Royal Dutch Medical Association and the Health Council published reports on the topic,[2,3] both of which turned the question around. Instead of asking whether it is acceptable to stop treatment, they asked whether it is justified to continue treatment. Both organizations answered in the negative, concluding that such treatment is medically futile.

In the Netherlands, the withdrawal of artificial nutrition and hydration from a patient in a persistent vegetative state is not considered euthanasia, which is a category that is restricted to measures taken by a doctor to actively end the life of a person at his or her explicit request — actions that are considered to be justified only as a means of ending unbearable and hopeless suffering. The distinction between euthanasia and discontinuing medically futile treatment was stressed in the Stinissen case — in part because the development of the Dutch euthanasia law was at a delicate stage, and its proponents feared that the Stinissen debate would jeopardize that process. And indeed, people who believed that artificial nutrition and hydration are necessary medical treatment or essential care and who therefore regarded stopping such treatment as a means of killing conflated this practice with euthanasia to bolster their argument that both should be banned.

There now seems to be a consensus in the Netherlands that artificial hydration and nutrition are medically futile for patients in a persistent vegetative state and therefore can and should be stopped. There are legal rules for proxy consent to this action. Under Dutch law, a doctor generally cannot treat someone without his or her consent. In the case of an incompetent patient, the legal representative makes the decision regarding the continuation of treatment. It is illegal to defy a negative advance directive (a refusal of treatment). If there is no written directive, the wish of the person is to be reconstructed with the help of persons close to him or her.

Since artificial feeding is considered medically futile for patients in a persistent vegetative state, the burden of judgment rests on the doctors, although the assent of the family is sought. As soon as Dutch doctors are certain about the diagnosis and the prognosis, they discuss the situation with family members, so that the family can adjust to the idea that the point of no return has been reached. But real life can be unruly. If the patient's family does not agree to the withdrawal of treatment, and if there is no statement of the patient's wishes, treatment is sometimes continued. In 2003, there were 32 patients in a vegetative state in Dutch nursing homes. Of the 30 patients whose data were analyzed, 26 had survived for more than a year, 5 of

them for more than 10 years.[4] The heartbreaking dilemma for the families of these patients was perhaps expressed best by the parents of a boy who had been in such a state for more than 16 years: "This is a fate worse than death, but we don't want to lose him."[1]

There are two possible explanations for the practice of continuing to treat such patients, which is at odds with the general view in the Netherlands. First, although such treatment is considered futile by many people, others (who might include the patient's doctors and relatives) disagree. They may believe that such a life has an objective value that does not depend on the subjective experiences of the patient (or the absence thereof). This view is respected. Second, physicians, on the strict condition that they will not be inflicting suffering on the patient, are prepared to "err" on the side of the family, providing what they consider to be futile treatment at the request of and for the benefit of the family, at least for a while. Patience may be essential for a family torn between hope and acceptance. Here the question "Why stop now?" becomes pertinent. What is it about today that is so different from yesterday or a month ago? Sometimes, no adequate answer can be given.

We must be cautious when comparing the Dutch debate about Ineke Stinissen with the U.S. debate about Terri Schiavo, although some considerations were central to both: the role of the patient's wishes, the nature and quality of life in a persistent vegetative state and the subjective or objective meaning attached to such a life, and the distinction between basic care and treatment in relation to futility. The differences between the two cases seem to lie primarily in the way the disagreements were handled.

Like most of their American counterparts, Dutch patients and doctors generally try to solve their moral problems among themselves, within the existing social and legal boundaries, rather than ask judges to cut the Gordian knot. The Dutch are, generally speaking, not of a litigious disposition, and doctors may even regard the need for a judicial decision as an indication of their own shortcomings of skill in communication or deliberation. The people involved will keep talking until a mutually acceptable solution is reached. This approach suits a pluralist society, in which we try to show respect for others and demand respect for our own views. When cases involving end-of-life decisions are brought to court in the Netherlands, they — unlike the Schiavo case — are usually put forward as test cases after the decisions have been made. They are used primarily to change or challenge policies.

Of course, politics have always been well represented in debates about end-of-life decisions. The government of the Netherlands supports both financially and morally several extensive Dutch research projects on such decisions, knowing that the world is looking over the shoulders of the Dutch — particularly given our reputation as iconoclasts with respect to end-of-life decision making. Therefore, the Dutch people look over the shoulders of the doctors, dissecting their decisions under the microscope of public opinion. Although we in the Netherlands may try to respect others' choices regarding dying and to recognize the private nature of these choices, the exploitation of human tragedies for political purposes is all too familiar. We have held our breath as moral hurricanes swept over the country and harsh words were spoken in the political arena and in the media. But we have, so far, not witnessed any politicization as extreme as that seen in the Terri Schiavo case.

Most of us, given the opportunity, would want to orchestrate the way in which we die. The tragic stories of Terri Schiavo and Ineke Stinissen teach us that we must think seriously about — and communicate to our family members and friends — what we would want if we were ever left "awake but not aware."

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The Multiplicity of Thyroid Nodules and Carcinomas

Robert D. Utiger, M.D.

Related article, page 2406

About 5 percent of adults in the United States have palpable thyroid nodules. Most of these nodules are at least 2 cm in their longest dimension, and their frequency doesn't change much with age. Ultrasonography, unquestionably the most sensitive thyroid-imaging test, reveals smaller nodules in many more people, and the frequency of these nodules does increase with age. A thyroid nodule may represent any of a variety of entities (see diagram).[1,2] Among the nodules that are evaluated by biopsy, about 95 percent are benign. Since there is selection for biopsy, mostly on the basis of size (the criterion is usually ≥1 cm in the longest dimension), the proportion of all nodules that are benign is probably higher.

Benign thyroid nodules are most often hyperplastic nodules of a multinodular goiter, but some are thyroid adenomas, and a few are cysts. Some hyperplastic nodules and adenomas have mutations that result in constitutive activation of the thyrotropin receptor or of intracellular signaling, leading to hyperthyroidism. For this reason, thyroid function should be assessed by measurement of serum thyrotropin in all patients with a thyroid nodule, but the result will usually be normal.

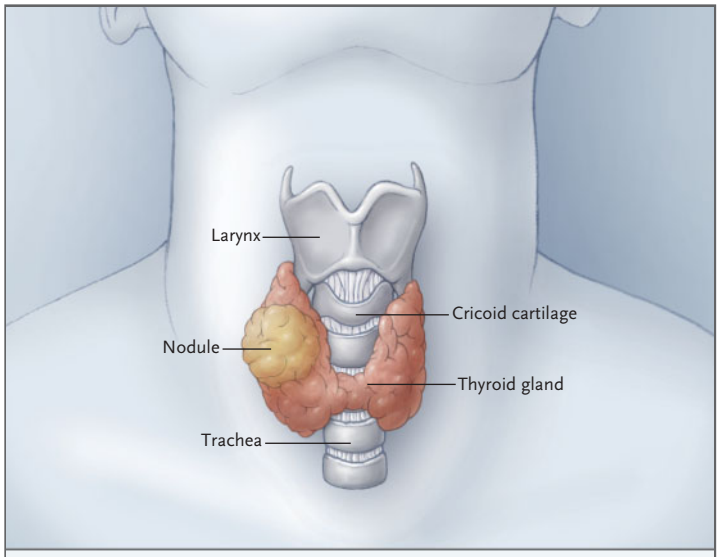
There is no reliable noninvasive way to distinguish a benign thyroid nodule from a thyroid carcinoma — it cannot be done by means of palpation, imaging, or biochemical testing. Rarely, there is evidence of invasion (vocal-cord paralysis) or metastasis (cervical lymphadenopathy) or a family history of medullary thyroid carcinoma, making it likely that the nodule is a carcinoma. The presence of multiple nodules does not decrease the likelihood that one of them is a carcinoma, as was once thought. Serum calcitonin concentrations are high in all patients with medullary thyroid carcinoma, but this type of carcinoma is rare (see diagram), and the concentrations may also be high in patients with other thyroid disorders.

Dr. Utiger is a clinical professor of medicine at Harvard Medical School and Brigham and Women's Hospital, Boston.

Thyroid biopsy is — with some caveats — a reliable way to distinguish a benign nodule from a carcinoma. Nodules that are papillary carcinomas, medullary carcinomas, or anaplastic carcinomas (about 5 percent overall) are usually identified through biopsy.[1] These diagnoses are based on the cytologic appearance of individual cells. This is also the case for most benign nodules (75 percent). One caveat is that too few cells may be obtained (in 10 percent of cases). Another is that follicular adenomas and carcinomas (the remaining 10 percent of cases) cannot be distinguished from one another; the same is true for Hürthle-cell tumors. When too few cells are obtained, the biopsy is usually repeated, often with similar results. The distinction between a follicular adenoma and a follicular carcinoma is based on the absence or presence, respectively, of invasion of the capsule of the nodule or of blood vessels or lymphatics within the nodule. Tissue sections are needed in order to search for these findings; about one in five of these nodules proves to be a carcinoma.

The widespread use of thyroid biopsy has increased the proportion of surgically removed nodules that turn out to be carcinomas, but there is room for improvement. There are likely to be cellular or molecular differences among the common types of thyroid nodules, but so far, attempts to improve the diagnostic accuracy of biopsy on the basis of such differences have been unsuccessful. Advances are especially needed for distinguishing between follicular adenomas and both follicular carcinomas and the follicular-variant papillary carcinomas.

Papillary carcinomas are often multifocal. Most of the additional foci are microscopic; the frequency of detection no doubt depends on the diligence



Benign Nodules (95%)	Carcinomas (5%)
Hyperplastic nodules (85%)	Papillary (81%)
Adenomas (15%)	Follicular and Hürthle-cell (14%)
Cysts (<1%)	Medullary (3%)
	Anaplastic (2%)

Common Varieties of Thyroid Nodules.

with which the thyroid is examined by a pathologist. Are these carcinomas intrathyroidal metastases from the primary tumor, spreading through lymphatics not only within the ipsilateral thyroid lobe but also through the thyroid isthmus to the contralateral lobe? Or are they individual carcinomas? That they are often found at autopsy in the absence of a large carcinoma is evidence that they are individual tumors.

Better evidence is provided by the results of the molecular studies reported by Shattuck et al. in this issue of the *Journal* (pages 2406–2412). The investigators asked whether different foci of papillary carcinoma in the same woman had the same X chromosome, whether maternally or paternally derived, as determined by the presence of a polymorphism in the X-linked androgen-receptor gene. For the results to be informative, the women had to be heterozygous for the polymorphism. Among the 10 women who met this criterion, there were 5 in whom different foci of carcinoma consisted of monoclonal populations of cells with one or the other polymorphism. In other words, these five women had two different papillary carcinomas. The carcinomas in the other five women could have been derived from a single clone or from independent clones in which the same X chromosome, whether maternally or paternally inherited, happened to be inactivated. All these carcinomas were 0.4 cm or larger, but it is likely that the more numerous smaller carcinomas (measuring 0.1 cm or less) are also independent.

The findings of Shattuck et al. have nothing to do with the pathogenesis of papillary carcinoma. (There are mutations and gene rearrangements that are specific for papillary carcinoma, but none have been found in more than about half the tumors, and such genetic changes may not be tumor-initiating events.) The fact that multiple nodules with the histopathological features of papillary carcinoma are monoclonal suggests that they are indeed carcinomas that can grow and spread. This evidence supports the clinical finding that patients with papillary carcinoma who undergo thyroid lobectomy are more likely than those who undergo near-total thyroidectomy to have recurrent carcinoma in the remaining lobe as well as elsewhere.[3]

The American Cancer Society estimates that thyroid carcinoma will be diagnosed in 25,690 people in the United States this year[4]; the annual estimate has been increasing faster than the population for several decades. Whether this is a true increase or an increase in ascertainment is not known. The proportion of these carcinomas that are papillary carcinomas is increasing as well. The average tumor size at detection is probably decreasing, because more tumors are being detected by means of imaging. That augurs well for the prognosis, because a larger tumor — along with older age and the presence of distant disease at the time of diagnosis — is the most important determinant of a poor outcome.[3] This is not to say that treatment is unimportant; the outcome is better when treatment consists of near-total thyroidectomy and the administration of radioiodine, as compared with thyroid lobectomy and no radioiodine. The conclusion that both the average tumor size at diagnosis and treatment are changing for the better is supported by the slower increase in mortality than in the rate of new cases.

Whether the frequency of benign thyroid nod-

ules — the "background" against which thyroid carcinomas are identified — is increasing more rapidly than the U.S. population is not known. If it is, the cause might be decreasing iodide intake, which is the best-known risk factor for multinodular goiter and which is known to have occurred in the United States in the past several decades.[5] At the same time, the proportion of nodules that are carcinomas might decrease, increasing the need to find precise noninvasive methods of diagnosis.

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Vitamin E and Donepezil for the Treatment of Mild Cognitive Impairment

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ABSTRACT

BACKGROUND

Mild cognitive impairment is a transitional state between the cognitive changes of normal aging and early Alzheimer's disease.

METHODS

In a double-blind study, we evaluated subjects with the amnestic subtype of mild cognitive impairment. Subjects were randomly assigned to receive 2000 IU of vitamin E daily, 10 mg of donepezil daily, or placebo for three years. The primary outcome was clinically possible or probable Alzheimer's disease; secondary outcomes were cognition and function.

RESULTS

A total of 769 subjects were enrolled, and possible or probable Alzheimer's disease developed in 212. The overall rate of progression from mild cognitive impairment to Alzheimer's disease was 16 percent per year. As compared with the placebo group, there were no significant differences in the probability of progression to Alzheimer's disease in the vitamin E group (hazard ratio, 1.02; 95 percent confidence interval, 0.74 to 1.41; P=0.91) or the donepezil group (hazard ratio, 0.80; 95 percent confidence interval, 0.57 to 1.13; P=0.42) during the three years of treatment. Prespecified analyses of the treatment effects at 6-month intervals showed that as compared with the placebo group, the donepezil group had a reduced likelihood of progression to Alzheimer's disease during the first 12 months of the study (P=0.04), a finding supported by the secondary outcome measures. Among carriers of one or more apolipoprotein E ϵ4 alleles, the benefit of donepezil was evident throughout the three-year follow-up. There were no significant differences in the rate of progression to Alzheimer's disease between the vitamin E and placebo groups at any point, either among all patients or among apolipoprotein E ϵ4 carriers.

CONCLUSIONS

Vitamin E had no benefit in patients with mild cognitive impairment. Although donepezil therapy was associated with a lower rate of progression to Alzheimer's disease during the first 12 months of treatment, the rate of progression to Alzheimer's disease after three years was not lower among patients treated with donepezil than among those given placebo.

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Mild cognitive impairment represents a transitional state between the cognitive changes of normal aging and the earliest clinical features of Alzheimer's disease.[1] Amnestic mild cognitive impairment refers to the subtype that has a primary memory component, either alone (single domain) or in conjunction with other cognitive-domain impairments (multiple domain), but of insufficient severity to constitute dementia.[2-6] Previous research has shown that the rate of progression to clinically diagnosable Alzheimer's disease is 10 to 15 percent per year among persons who meet the criteria for the amnestic form of mild cognitive impairment, in contrast to a rate of 1 to 2 percent per year among normal elderly persons.[7] Approximately 80 percent of those who meet the criteria for amnestic mild cognitive impairment will have Alzheimer's disease within six years, and the presence of one or more apolipoprotein (*APOE*) ε4 alleles is associated with a more rapid rate of progression.[8,9] Thus, preventing the progression of mild cognitive impairment to Alzheimer's disease is likely to provide substantial benefit.

Oxidative damage accompanies Alzheimer's disease, and cholinesterase inhibitors are recommended for use in mild-to-moderate Alzheimer's disease.[10] The Alzheimer's Disease Cooperative Study (ADCS)[11] showed that treatment with the antioxidant vitamin E could delay the time to important milestones in patients with moderately severe Alzheimer's disease. The present study was designed to determine whether treatment with vitamin E or donepezil, the most widely used cholinesterase inhibitor available at the time the study was designed, could delay the clinical diagnosis of Alzheimer's disease in subjects with the amnestic form of mild cognitive impairment.

METHODS

PARTICIPANTS

We enrolled 769 subjects from 69 ADCS sites in the United States and Canada.[12] The criteria for inclusion were amnestic mild cognitive impairment of a degenerative nature (insidious onset and gradual progression),[7] impaired memory, a Logical Memory delayed-recall score approximately 1.5 to 2 SD below an education-adjusted norm, a Clinical Dementia Rating (CDR) of 0.5, a score of 24 to 30 on the Mini–Mental State Examination (MMSE), and an age of 55 to 90 years. Detailed inclusion and exclusion criteria are presented in the Supplementary Appendix, available with the full text of this article at www.nejm.org.

STUDY DESIGN

In this multicenter, randomized, double-blind, placebo-controlled, parallel-group study, which was conducted between March 1999 and January 2004, subjects with amnestic mild cognitive impairment were randomly assigned to receive 2000 IU of vitamin E, placebo donepezil, and a multivitamin daily; 10 mg of donepezil, placebo vitamin E, and a multivitamin daily; or placebo vitamin E, placebo donepezil, and a multivitamin daily. The multivitamin contained 15 IU of vitamin E. The initial dose of donepezil was 5 mg daily, and the dose was increased to 10 mg after six weeks. The initial dose of vitamin E was 1000 IU daily, and the dose was increased to 2000 IU (1000 IU twice daily) after six weeks. If a subject had difficulty tolerating the higher dose of vitamin E or donepezil, the investigator could reduce the dose of either medication temporarily and then rechallenge with the higher dose.

We used an adaptive allocation scheme for the treatment assignment, with the MMSE score, age, and *APOE* ε4 status as balancing covariates. The study was designed by the mild-cognitive-impairment protocol committee of the ADCS and was executed and analyzed by the ADCS investigators. Fifty percent of the funding was provided by the National Institute on Aging, with the other 50 percent coming from Pfizer and Eisai. Pfizer and Eisai served in an advisory capacity for the study, but final decisions concerning all phases of the study were made by the ADCS investigators. The study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki, and the U.S. Code of Federal Regulations title 21 Part 50 (Protection of Human Subjects) and title 21 Part 56 (Institutional Review Boards). Written informed consent was obtained from all participants and study partners who had knowledge of the participants' functional activities. A data and safety monitoring board reviewed the blinded safety data every three months during the trial.

EFFICACY MEASURES

The primary end point was the time to the development of possible or probable Alzheimer's disease, defined according to the clinical criteria of the National Institute of Neurological and Communicative Diseases and Stroke and the Alzheimer's

Disease and Related Disorders Association.[13] On verification by a central review committee that a participant met these clinical criteria for Alzheimer's disease, the participant stopped taking donepezil or matching placebo in a blinded fashion and was offered open-label donepezil until he or she completed the study at month 36.

Secondary measures were also assessed, including the scores for the MMSE; the Alzheimer's Disease Assessment Scale, cognitive subscale (ADAS-Cog); the global CDR; the CDR sum of boxes (the sum of individual CDR domain scores); the ADCS Mild Cognitive Impairment Activities of Daily Living Scale; the Global Deterioration Scale; and a neuropsychological battery consisting of the New York University paragraph-recall test, the Symbol Digit Modalities Test, the category-fluency test, a number-cancellation test, the Boston Naming Test, the digits-backward test, the clock-drawing test, and a maze-tracing task.[12,14]

STATISTICAL ANALYSIS

The primary analysis was conducted according to the intention-to-treat principle in order to determine whether there was a significant reduction in the time to progression to Alzheimer's disease among subjects treated with either vitamin E or donepezil as compared with those given placebo. The Cox proportional-hazards model was used, and baseline variables (age, the MMSE score, and the *APOE* genotype) were included in the analysis as covariates. Two primary analyses were conducted, one comparing the vitamin E and placebo groups, and one comparing the donepezil and placebo groups. The Hochberg method[15] was used to adjust the two P values for multiple comparisons. The Schoenfeld residuals test was used to test for nonproportional hazards.[16] A z-test (the difference in the proportions divided by the standard error of the difference) was used to compare estimated survival rates at various points on the Kaplan–Meier curves (at 6, 12, 18, 24, 30, and 36 months). The Hochberg method was used to adjust the six P values for multiple comparisons.

Hazard ratios derived from the Cox analysis were used to compare the risk of progression in the donepezil or vitamin E group with that in the placebo group for the entire cohort and for the subgroup of *APOE* ϵ4 carriers. In the 12- and 24-month analyses, data were censored at 388 and 749 days, respectively. The hazard-ratio analyses were secondary, and the resulting P values were not adjusted for multiple comparisons. Baseline characteristics among the three groups were compared with the use of Wilcoxon's rank-sum test or Fisher's exact test, as appropriate. For the statistical evaluation of main effects, a P value of less than 0.05 was considered to indicate statistical significance, and for interaction effects, a P value of less than 0.10 was used.

The secondary outcomes were examined with the use of analysis of covariance for the change in scores without correction for multiple comparisons, and missing values were imputed with the use of a projection method appropriate for assessing responses among subjects with neurodegenerative diseases.[17] As part of the secondary analyses, several cognitive-domain scores for memory (consisting of the ADAS immediate and delayed word-recall scores and the New York University immediate and delayed paragraph-recall scores), executive function (the digits-backward test, Symbol Digit Modalities Test, and number-cancellation test), language (the Boston Naming Test and category-fluency test), and visuospatial skills (the clock-drawing test) were calculated in addition to an overall composite cognitive-function score. The cognitive-domain and overall composite scores were calculated as the weighted sum of the individual standardized test scores. The individual test scores were standardized by dividing each score by the standard deviation of the baseline scores. Weights were calculated as the reciprocal of the sum of the correlation coefficients between the tests in each domain at baseline.

The annual rates of progression to dementia were calculated with the use of a life-table analysis. An analysis based on a logistic-regression model was conducted to determine whether missing data from subjects who were lost to follow-up were missing completely at random[18] and, if so, could be ignored.

RESULTS

STUDY POPULATION

A total of 790 subjects underwent randomization, and 769 completed the baseline assessment. There were no significant differences among the three groups in baseline demographic or psychometric characteristics (Table 1).

PRIMARY OUTCOME MEASURES

A total of 214 participants had progression to dementia, with 212 being classified as having possi-

Table 1. Baseline Characteristics of the Subjects.*

Variable	Placebo Group (N=259)	Donepezil Group (N=253)	Vitamin E Group (N=257)	All Subjects (N=769)
Age — yr	72.9±7.6	73.1±7.1	72.8±7.3	72.9±7.3
Female sex — no. (%)	121 (47)	112 (44)	119 (46)	352 (46)
APOE ε4 carrier — no. (%)	136 (53)	147 (58)	141 (55)	424 (55)
ADAS-Cog score				
Original	11.03±4.2	11.28±4.5	11.48±4.4	11.26±4.4
Modified	17.40±6.0	17.72±6.2	18.04±6.0	17.72±6.1
MMSE score	27.35±1.8	27.25±1.8	27.20±1.9	27.27±1.8
CDR sum-of-boxes score	1.87±0.8	1.80±0.8	1.78±0.8	1.82±0.8
Score on Global Deterioration Scale	2.72±0.6	2.66±0.6	2.64±0.6	2.67±0.6
Score on Activities of Daily Living Scale	45.87±5.2	46.49±4.3	45.82±4.6	46.06±4.7

* Plus–minus values are means ±SD. A total of 2264 subjects were screened. The primary reason for exclusion was failure to meet cutoff scores for the Logical Memory paragraph. Scores for the original cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) can range from 0 to 70, and scores for the modified ADAS-Cog can range from 0 to 85, with higher scores indicating poorer function. Scores for the Mini–Mental State Examination (MMSE) can range from 0 to 30, with higher scores indicating better function. Scores for the Clinical Dementia Rating (CDR) sum of boxes can range from 0 to 18, with lower scores indicating better performance. Scores for the Global Deterioration Scale can range from 1 to 7, with higher scores indicating poorer function. Scores for the Activities of Daily Living Scale can range from 0 to 53, with higher scores indicating better function.

ble or probable Alzheimer's disease, 1 as having mixed dementia, and 1 as having primary progressive aphasia. The overall rate of progression to Alzheimer's disease was 16 percent per year.

During the three years of the trial, there were no significant differences in the probability of progression from mild cognitive impairment to Alzheimer's disease on the basis of the Cox analysis between the vitamin E group and the placebo group (hazard ratio, 1.02; 95 percent confidence interval, 0.74 to 1.41; P=0.91) or the donepezil group and the placebo group (hazard ratio, 0.80; 95 percent confidence interval, 0.57 to 1.13; P=0.42) (Fig. 1A). The Schoenfeld residuals test of nonproportional hazards was significant (P=0.001 for the comparison of the donepezil group with the placebo group and P=0.01 for the comparison of the vitamin E group with the placebo group), indicating that the proportional-hazards assumption for the Cox model was not met. The 36-month analysis was therefore followed by a prespecified assessment of the treatment effects at each six-month evaluation point. This analysis showed that there were no significant differences between the vitamin E and placebo groups at any time during the trial.

The risk of progression to Alzheimer's disease was lower in the donepezil group than in the placebo group for the first 12 months of the trial (P=0.004 at 6 months and P=0.04 at 12 months by a z-test adjusted for comparisons at multiple points) (Fig. 1B). A total of 38 subjects in the placebo group had progression to Alzheimer's disease in the first 12 months, as compared with 33 in the vitamin E group and 16 in the donepezil group. By 36 months, however, the numbers of subjects with progression to Alzheimer's disease did not differ significantly among the three groups: 73 in the placebo group, 76 in the vitamin E group, and 63 in the donepezil group. For the comparison that included all subjects, the hazard ratios for progression to Alzheimer's disease were lower in the donepezil group than the placebo group during year 1 (P=0.004) and during years 1 and 2 (P=0.03), but not during the entire three years of the study (P=0.21; P values not adjusted for comparisons at multiple points).

SECONDARY OUTCOME MEASURES

There were few significant differences in cognitive function from baseline between the vitamin E and placebo groups. The exceptions were in the scores for the executive, language, and overall cognitive scores, and these were confined to the first 18 months of the study. There were more differences in the change from baseline scores between the donepezil group and the placebo group, as shown in Table 2; they included the scores for the MMSE, CDR sum of boxes, Global Deterioration Scale, and modified ADAS-Cog, as well as memory, language, and overall cognitive scores, but with one exception, they were also confined to the first 18 months of the study.

APOE ε4 CARRIERS

Possession of the *APOE* ε4 allele was a major predictor of progression to Alzheimer's disease in all three groups, with 76 percent of the cases of progression to Alzheimer's disease occurring among *APOE* ε4 carriers (P<0.001) (Fig. 1C). There were 136 carriers in the placebo group, 147 in the donepezil group, and 141 in the vitamin E group (Table 1). The curves for the vitamin E and placebo groups separated slightly during the first year, then merged again (P=0.77) (Fig. 1D). In this secondary analysis, it was observed that the curves for the donepezil and placebo groups had separated by six

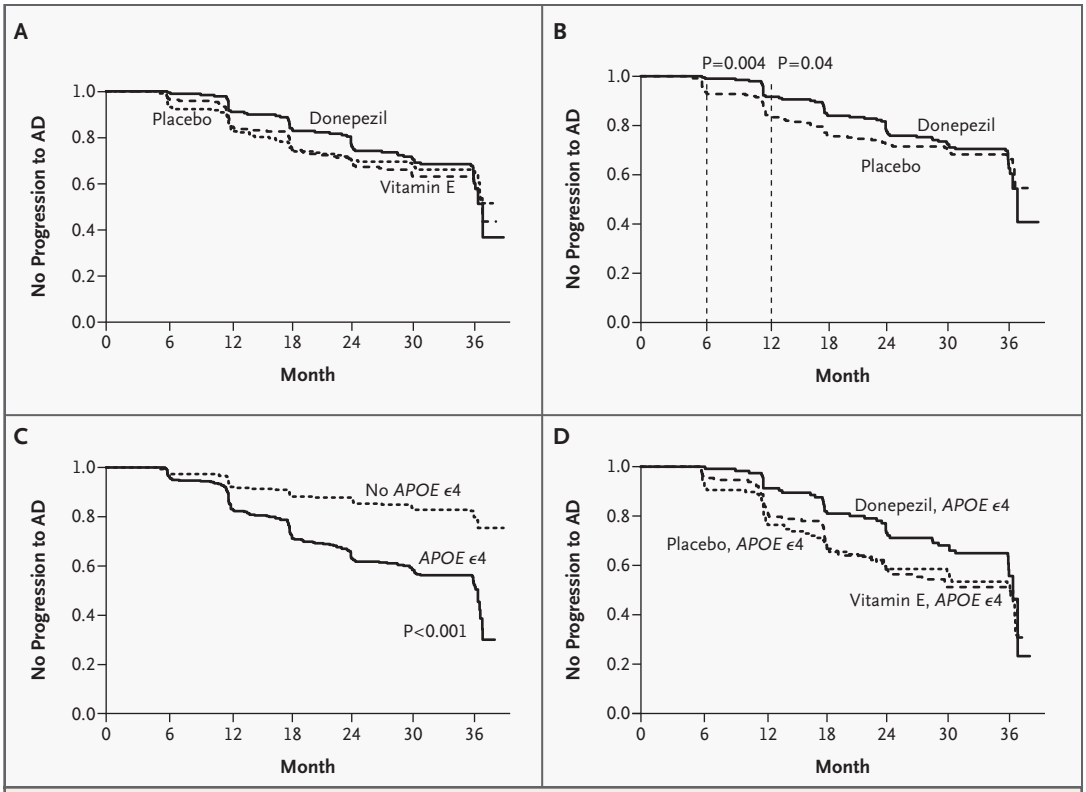

Figure 1. Kaplan–Meier Estimates of the Rate of Progression from Mild Cognitive Impairment to Alzheimer's Disease (AD).
Panel A shows the survival estimates in all three groups during the three-year study. Panel B shows the results of prespecified comparisons involving z-tests at 6 months (P=0.004) and 12 months (P=0.04). Panel C shows the effect of *APOE* ϵ4 carrier status on the rate of progression to Alzheimer's disease, and Panel D shows the effect of treatment among *APOE* ϵ4 carriers. Comparisons were adjusted for multiple comparisons with the use of the Hochberg method.

months and remained apart during the remainder of the trial (P=0.04), with donepezil treatment reducing the risk of progression to Alzheimer's disease by approximately one third at year 3 among subjects with one or more *APOE* ϵ4 alleles (Table 3).

OUTCOMES AND ADVERSE EVENTS

Adverse events in the donepezil group included muscle cramps, gastrointestinal symptoms, and sleep disturbances (Table 4). Twenty-three deaths occurred during the study (17 during the double-blind phase and 6 during the open-label phase), and all were judged to be unrelated to treatment. During the double-blind phase, seven subjects died in the donepezil group and five subjects died in each of the other two groups (P=0.79).

A total of 230 subjects discontinued treatment during the double-blind phase: 92 in the donepezil group, 72 in the vitamin E group, and 66 in the placebo group (P=0.90). Among the leading reasons for discontinuation besides death were adverse events in the case of 47 subjects and withdrawal of consent in the case of 105 subjects.

EFFECT OF MISSING DATA

To assess the effect of missing data, we compared the baseline values between the 230 subjects who withdrew during the double-blind phase and the 539 subjects who progressed to open-label treatment or completed the double-blind phase. There were no significant differences in demographic characteristics or neuropsychological measures. A contingency-table analysis of the number of subjects according to the treatment group and period of withdrawal indicated a trend toward more early dropouts (at the three- and six-month visits) in the donepezil group than in the placebo group (P=0.07). The results of an evaluation of the assumption that the missing data were missing com-

Table 2. Changes from Baseline in Cognitive and Functional Measures.*

Test	Change in Score from Baseline					
	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo
Cognitive and functional measures						
MMSE						
Donepezil	0.06±2.03†	−0.31±2.25‡	−0.52±2.46‡	−0.98±2.54‡	−1.47±3.04	−2.31±3.72
Vitamin E	−0.53±2.28	−0.54±2.28	−0.96±2.61	−1.21±2.78	−1.75±3.09	−2.20±3.64
Placebo	−0.36±2.02	−0.80±2.34	−1.02±2.61	−1.49±2.90	−1.77±3.24	−2.75±4.04
Activities of Daily Living Scale						
Donepezil	−0.21±3.43	−1.41±4.48	−1.78±5.02	−3.09±6.24	−4.44±7.39	−6.26±8.67
Vitamin E	−0.34±4.29	−1.08±4.90	−2.13±5.76	−2.84±6.16	−4.16±7.46	−5.63±8.75
Placebo	−1.06±4.54	−1.44±5.00	−2.34±6.02	−3.43±6.73	−5.00±8.05	−6.39±8.99
CDR sum of boxes						
Donepezil	0.05±0.66	0.25±0.92‡	0.51±1.18‡	0.87±1.55	1.19±1.69	1.60±2.09
Vitamin E	0.17±0.70	0.51±1.21	0.75±1.44	1.02±1.76	1.26±1.89	1.67±2.18
Placebo	0.14±0.86	0.40±1.28	0.72±1.55	0.97±1.76	1.26±2.15	1.64±2.55
Global Deterioration Scale						
Donepezil	−0.01±0.52†	0.11±0.57	0.19±0.66‡	0.32±0.73	0.45±0.78	0.59±0.89
Vitamin E	0.11±0.49	0.21±0.61	0.27±0.73	0.42±0.80	0.51±0.85	0.64±0.96
Placebo	0.07±0.53	0.15±0.65	0.27±0.73	0.38±0.81	0.48±0.87	0.56±0.99
ADAS-Cog (original)						
Donepezil	−0.61±3.79	0.17±3.73	1.08±4.37	1.22±4.79	2.71±5.21	3.68±5.95
Vitamin E	−0.16±4.19	0.91±4.21	1.19±4.32	1.93±5.13	3.01±5.57	4.59±6.54
Placebo	−0.13±3.34	0.61±4.10	1.29±4.71	1.49±5.07	2.98±5.62	3.74±6.97
ADAS-Cog (modified)						
Donepezil	−1.23±4.74†	−0.55±5.20‡	0.03±5.64‡	0.35±6.23	2.05±6.74	3.12±7.39
Vitamin E	−0.47±5.06	0.27±5.20	0.49±5.42	1.15±6.37	2.48±6.68	3.98±7.56
Placebo	−0.09±4.38	0.60±4.96	0.99±6.07	1.02±6.27	2.65±7.02	3.72±8.54

pletely at random demonstrated that cognitive scores for the MMSE and the ADAS-Cog and total score for the CDR sum of boxes at each visit were predictive of withdrawal before the next visit, indicating that the missing observations cannot be ignored. To assess the z-test results, we conducted a sensitivity analysis consisting of simulations in which the subjects in the donepezil group who dropped out in the first 12 months were randomly divided into two groups: a group of 40 to match the number of dropouts in the placebo group during this period and a group of 24 excess dropouts. A proportion of the 24 excess dropouts was then selected at random and assumed to have had progression to Alzheimer's disease. That proportion was set at the conservative level of double the rate in the group of subjects who completed the study. This analysis included six excess progression events. In these analyses, the 6- and 12-month z-test results remained significant in favor of the donepezil group over the placebo group. The results at all other times were nonsignificant. Similar analyses were performed for the vitamin E and placebo groups, and the results were uniformly nonsignificant.

DISCUSSION

Over the three years of the study, there were no significant differences in the probability of progression to Alzheimer's disease between either the vitamin E or the donepezil group and the placebo group. However, since the effect of treatments varied during the three years of the trial and assumptions for the primary-analysis model were not met, prespecified group comparisons were carried out at each of the six-month evaluations. These analy-

Table 2. (Continued.)*

Test	Change in Score from Baseline					
	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo
Cognitive domains§						
Memory						
Donepezil	0.01±0.51†	0.00±0.57	−0.03±0.57‡	−0.07±0.59	−0.32±0.60	−0.26±0.60
Vitamin E	−0.10±0.48	−0.08±0.49	−0.12±0.55	−0.12±0.57	−0.43±0.55	−0.31±0.59
Placebo	−0.17±0.47	−0.10±0.51	−0.15±0.52	−0.11±0.55	−0.34±0.55	−0.28±0.62
Executive						
Donepezil	0.09±0.36	0.11±0.40	0.03±0.42	−0.01±0.45	−0.06±0.46	−0.16±0.48
Vitamin E	0.11±0.41‡	0.04±0.41	0.00±0.42	0.03±0.45	0.00±0.47	−0.19±0.48
Placebo	0.04±0.42	0.05±0.44	−0.02±0.45	0.01±0.48	−0.08±0.51	−0.19±0.53
Language						
Donepezil	0.09±0.24†	0.04±0.22‡	0.04±0.24†	−0.03±0.25	−0.06±0.29	−0.11±0.32
Vitamin E	0.07±0.23‡	0.05±0.26‡	0.02±0.28‡	−0.03±0.31	−0.05±0.33	−0.10±0.35
Placebo	0.03±0.23	0.00±0.24	−0.03±0.24	0.00±0.27	−0.04±028	−0.08±0.33
Visuospatial						
Donepezil	0.00±0.32	0.00±0.32	−0.05±0.32	−0.06±0.35	−0.14±0.35	−0.14±0.34
Vitamin E	0.03±0.34	−0.01±0.35	−0.02±0.33	−0.04±0.34	−0.07±0.36	−0.12±0.37
Placebo	−0.01±0.34	0.02±0.32	−0.04±0.36	−0.06±0.39	−0.09±0.39	−0.11±0.39
Overall						
Donepezil	0.18±0.82†	0.15±0.92‡	0.01±0.96†	−0.16±1.03	−0.59±1.18	−0.67±1.24
Vitamin E	0.10±0.81†	0.00±0.90	−0.13±0.94	−0.16±1.07	−0.54±1.14	−0.70±1.21
Placebo	−0.12±0.80	−0.03±0.86	−0.24±0.96	−0.15±1.09	−0.53±1.17	−0.65±1.35

* Scores for the Mini–Mental State Examination (MMSE) can range from 0 to 30, with higher scores indicating better function. Scores for the Activities of Daily Living Scale can range from 0 to 53, with higher scores indicating better function. Scores for the Clinical Dementia Rating (CDR) sum of boxes can range from 0 to 18, with lower scores indicating better performance. Scores for the Global Deterioration Scale can range from 1 to 7, with higher scores indicating poorer function. Scores for the original cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) can range from 0 to 70, and scores for the modified ADAS-Cog can range from 0 to 85, with higher scores indicating poorer function.
† $P<0.01$ for the comparison with the baseline value.
‡ $P<0.05$ for the comparison with the baseline value.
§ The values for the cognitive-domain measures (memory, executive, language, and visuospatial) are standardized composite z scores, with positive numbers indicating improvement. The overall cognitive score was based on the four domain scores and computed as explained in the Methods section.

ses demonstrated that vitamin E had no significant effect during the trial with respect to the development of Alzheimer's disease at any time. The analysis for donepezil, however, demonstrated a reduced likelihood of progression to Alzheimer's disease in the donepezil group, as compared with the placebo group, for the first 12 months of the trial.

These results suggest that donepezil treatment may delay clinical progression to Alzheimer's disease but do not address the question of the underlying mechanism. As shown in Table 2, the overall cognitive function of the subjects with mild cognitive impairment in the donepezil group did not decline on most of the measures during the first 6 to 18 months of the study and thereafter declined at about the same rate as in the placebo group. As a result, the size of the donepezil–placebo treatment effect on the MMSE score was about 0.5 point throughout the 36-month trial. This delay in cognitive decline probably contributed to the slower rate of progression to Alzheimer's disease in the donepezil group. The observed relative reduction in the risk of progression to Alzheimer's disease of 58 percent at one year and 36 percent at two years in the entire cohort is likely to be clinically significant. Although our findings do not provide support for a clear recommendation for the use of donepezil in persons with mild cognitive impairment, they

Table 3. Hazard Ratios for the Risk of Progression to Alzheimer's Disease in the Donepezil and Vitamin E Groups as Compared with the Placebo Group.*

Interval	All Subjects		*APOE* ε4 Carriers	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Donepezil vs. placebo				
First 12 mo	0.42 (0.24–0.76)	0.004	0.34 (0.16–0.69)	0.003
First 24 mo	0.64 (0.44–0.95)	0.03	0.54 (0.35–0.86)	0.009
All 36 mo	0.80 (0.57–1.13)	0.21	0.66 (0.44–0.98)	0.04
Vitamin E vs. placebo				
First 12 mo	0.83 (0.52–1.32)	0.43	0.78 (0.46–1.34)	0.37
First 24 mo	0.95 (0.67–1.36)	0.79	0.95 (0.64–1.41)	0.79
All 36 mo	1.02 (0.74–1.41)	0.91	0.95 (0.66–1.36)	0.77

* CI denotes confidence interval. P values were not adjusted for multiple comparisons. In the donepezil group, when corrected for multiple comparisons, the P value at 24 months for all subjects became nonsignificant (P=0.052), and the P value at 36 months for *APOE* ε4 carriers also became nonsignificant (P=0.078).

could prompt a discussion between the clinician and the patient about this possibility.

We also found that amnestic mild cognitive impairment and the presence of one or more *APOE* ε4 alleles were highly predictive of progression to Alzheimer's disease. Of the 214 diagnoses of dementia, 212 were possible or probable Alzheimer's disease, with 76 percent of the cases of progression to Alzheimer's disease occurring among *APOE* ε4 carriers. The results show that the enrollment criteria for amnestic mild cognitive impairment were highly specific. Furthermore, this study replicated observational studies demonstrating a rate of progression from mild cognitive impairment to Alzheimer's disease of 10 to 15 percent per year.[5,7]

Treatment with vitamin E and donepezil did not produce any unexpected side effects. No episodes of bleeding occurred in the vitamin E group. There were more discontinuations in the donepezil group than in the other two groups, as would be expected from its known side-effect profile.[10,19] Most discontinuations were related to gastrointestinal side effects, sleep disturbances, and muscle cramps. There were slightly more deaths in the donepezil group, but the number was not out of proportion to the number expected among subjects in this age group and was not significantly different from the numbers in the vitamin E and placebo groups.

We used numerous secondary measures, and in general, they appeared to corroborate the overall outcome data concerning the rate and risk of progression from mild cognitive impairment to Alzheimer's disease. Results for language and the overall composite measure showed some effect of vitamin E therapy, but they were of insufficient magnitude to affect the overall performance of the group. In the donepezil group, the results for memory, language, the overall composite measure, and global measures of cognition, disease severity, and stage of dementia paralleled the overall treatment effect of the drug on the risk of progression to Alzheimer's disease.

Table 4. Adverse Events.*

Adverse Event	Donepezil Group	Vitamin E Group	Placebo Group
		percent	
Diarrhea	16.7†	10.2	6.6
Muscle cramps	16.3†	1.2	1.9
Insomnia	10.8†	3.1	1.9
Nausea	8.4†	1.2	1.9
Abnormal dreams	6.8†	0.4	1.6
Bronchitis	6.4	2.4	3.1
Loose stools	6.0‡	2.7	1.6
Vomiting	6.0‡	2.7	1.9
Arthritis	5.2‡	2.0	1.6
Cataract extraction	4.8	5.9	2.7

* The rates are for adverse events that occurred in at least 5 percent of subjects in the donepezil or vitamin E group and at least two times in the placebo group during the double-blind phase.
† P<0.01 for the comparison with the placebo group.
‡ P<0.05 for the comparison with the placebo group.

A major modifying effect of the comparison of donepezil with placebo was the *APOE* ϵ4 carrier status. Most of the treatment effect of donepezil occurred among the *APOE* ϵ4 carriers. In secondary analyses, we observed that when the analysis was confined to the *APOE* ϵ4 carriers, the effect of donepezil was significant at 12, 24, and 36 months. However, there are insufficient data to warrant recommending APOE genotyping in persons with mild cognitive impairment, and our results cannot be used to make this recommendation, since the study was not statistically powered to determine the effects of treatment in separate groups of APOE ϵ4 carriers and noncarriers.

Despite evidence of oxidative stress in patients with Alzheimer's disease and mild cognitive impairment and observational studies suggesting that supplementation with antioxidant vitamins may decrease the risk of Alzheimer's disease, we did not find that vitamin E significantly affected the risk of progression.[20-22] Furthermore, this therapy had only minimal effects on secondary measures.

In summary, this study provides evidence that treatment may delay the clinical diagnosis of Alzheimer's disease. Specifically, the likelihood of Alzheimer's disease was reduced for only the initial 12 months of the study among patients treated with donepezil, as compared with those who received placebo; however, in secondary analyses, it was observed that the effect was more prominent among *APOE* ϵ4 carriers, with a reduction in risk apparent throughout the 36 months of the study. The results of the secondary analyses of cognitive and global measures supported the primary-outcome results.

Our findings suggest that the design of our study and the enrollment criteria are practical and can be used to demonstrate the effects of a given intervention in subjects with amnestic mild cognitive impairment. Other therapeutic agents under development, particularly those designed to prevent Alzheimer's disease or progression to Alzheimer's disease, may be particularly beneficial in subjects with mild cognitive impairment.

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APPENDIX

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ORIGINAL ARTICLE

A Randomized Trial of Intensive Lipid-Lowering Therapy in Calcific Aortic Stenosis

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ABSTRACT

BACKGROUND

Calcific aortic stenosis has many characteristics in common with atherosclerosis, including hypercholesterolemia. We hypothesized that intensive lipid-lowering therapy would halt the progression of calcific aortic stenosis or induce its regression.

METHODS

In this double-blind, placebo-controlled trial, patients with calcific aortic stenosis were randomly assigned to receive either 80 mg of atorvastatin daily or a matched placebo. Aortic-valve stenosis and calcification were assessed with the use of Doppler echocardiography and helical computed tomography, respectively. The primary end points were change in aortic-jet velocity and aortic-valve calcium score.

RESULTS

Seventy-seven patients were assigned to atorvastatin and 78 to placebo, with a median follow-up of 25 months (range, 7 to 36). Serum low-density lipoprotein cholesterol concentrations remained at 130±30 mg per deciliter in the placebo group and fell to 63±23 mg per deciliter in the atorvastatin group ($P<0.001$). Increases in aortic-jet velocity were 0.199±0.210 m per second per year in the atorvastatin group and 0.203±0.208 m per second per year in the placebo group ($P=0.95$; adjusted mean difference, 0.002; 95 percent confidence interval, −0.066 to 0.070 m per second per year). Progression in valvular calcification was 22.3±21.0 percent per year in the atorvastatin group, and 21.7±19.8 percent per year in the placebo group ($P=0.93$; ratio of post-treatment aortic-valve calcium score, 0.998; 95 percent confidence interval, 0.947 to 1.050).

CONCLUSIONS

Intensive lipid-lowering therapy does not halt the progression of calcific aortic stenosis or induce its regression. This study cannot exclude a small reduction in the rate of disease progression or a significant reduction in major clinical end points. Long-term, large-scale, randomized, controlled trials are needed to establish the role of statin therapy in patients with calcific aortic stenosis.

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In the western world, calcific aortic stenosis is the most common form of valvular heart disease, and its incidence increases with age such that 3 percent of adults over 75 years of age have aortic stenosis.[1] It is a gradually progressive disease, characterized by a long asymptomatic phase, lasting several decades, followed by a shorter symptomatic phase associated with severe narrowing of the orifice of the aortic valve. Once symptoms occur, the prognosis is poor and surgery is usually mandated. Calcific aortic stenosis is now the leading indication for valve replacement in North America and Europe. However, there are currently no effective disease-modifying treatments, and the possibility of halting the disease process would represent a therapeutic advance.

Calcific aortic stenosis is mediated by a chronic inflammatory disease process that has many similarities with atherosclerosis and includes inflammatory-cell infiltrates, lipoproteins, lipids, extracellular-bone-matrix proteins, and bone mineral.[2-5] Consistent with these observations, clinical studies have revealed a strong association with coronary artery disease[6,7] and many of its risk factors, including hypercholesterolemia.[1] Disease progression in aortic stenosis is variable and influenced by several factors, including the degree of stenosis,[8] valvular calcification,[9-11] and hypercholesterolemia.[12,13] Indeed, calcific aortic stenosis is a feature of severe homozygous familial hypercholesterolemia,[14] and intensive lipid-lowering therapy with plasmapheresis can reverse valvular stenosis in patients with this disease.[15]

The use of hydroxymethylglutaryl–coenzyme A reductase inhibitors, or statins, is an established treatment for the primary and secondary prevention of coronary artery disease.[16,17] Several studies have shown that these drugs can halt the progression of coronary artery disease[18-20] and reduce coronary calcification.[21-23] Given the clinical association with hypercholesterolemia and coronary artery disease, and the histologic similarities with atheroma, it has been suggested that statin therapy may halt the progression, or even induce regression, of calcific aortic stenosis. This hypothesis is supported by numerous retrospective observational studies[24-29] showing that concomitant statin therapy was associated with a delay in disease progression, demonstrated by a reduction of 0.30 m per second per year in the rate of change in aortic-jet velocity, and of 30 percent per year in valvular calcification.

The aim of the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE) was to establish whether intensive lipid-lowering therapy with 80 mg of atorvastatin daily would halt the progression or induce regression of the aortic-jet velocity on Doppler echocardiography, and of the aortic-valve calcium score on computed tomography (CT), in patients with calcific aortic stenosis.

METHODS

PATIENTS

Patients older than 18 years of age with calcific aortic stenosis, an aortic-jet velocity of at least 2.5 m per second, and aortic-valve calcification on echocardiography[11] were eligible for inclusion. Exclusion criteria were child-bearing potential without contraception, active or chronic liver disease, a history of alcohol or drug abuse, severe mitral-valve stenosis (mitral-valve area, <1 cm^2), severe mitral or aortic regurgitation,[30] left ventricular dysfunction (ejection fraction, <35 percent), a planned aortic-valve replacement, intolerance of statins, statin therapy or a potential benefit from statin therapy (according to the treating physician), a baseline serum total cholesterol concentration of less than 150 mg per deciliter (4.0 mmol per liter), and presence of a permanent pacemaker or cardiodefibrillator. Of the patients screened, 455 were eligible for inclusion, 173 agreed to participate, and 155 ultimately underwent randomization.

STUDY PROTOCOL

Between March 2001 and April 2002, the blinded study coordinator randomly assigned eligible patients by the minimization technique[31] with the use of a dedicated, locked computer program (Edinburgh University) incorporating the following eight variables: age, sex, smoking habit, hypertension, diabetes mellitus, serum cholesterol concentration, aortic-jet velocity, and aortic-valve calcium score. Patients were assigned to either 80 mg of atorvastatin (Lipitor, Pfizer) or matched placebo as a single daily dose. Numbered containers were used.

Patients were assessed at baseline, two months, and six months and every six months thereafter for a minimum of two years. Clinical evaluation included assessment of functional status and adverse events, and the biochemical analysis of blood. Echocardiography and CT were performed at baseline, at each annual visit, and before withdrawal from the study. Patients who underwent randomization and who were subsequently started on open-

label statin therapy by their attending physician were immediately scanned and withdrawn from the study.

Drs. Cowell, Reid, Northridge, and Bloomfield collected the data; Drs. Newby, Northridge, and Boon designed the study and vouch for the data and the analysis; Dr. Prescott analyzed the data; and all investigators participated in writing the article. The drug and the placebo were provided by Pfizer, who had no other input into the study. The investigation conformed to the Declaration of Helsinki and was approved by all regional ethics committees. All patients gave written informed consent.

ECHOCARDIOGRAPHY

Assessment of valvular stenosis was determined by a single dedicated research ultrasonographer. Patients were studied with the use of a 3-MHz transducer for M-mode (single-dimensional) and pulsed and continuous-wave Doppler scanning. All measurements were determined online, averaged from three cardiac cycles (five cycles if the patient was in atrial fibrillation), and recorded onto super-VHS videotape and optical disk according to a standard protocol.

Peak and mean aortic-valve pressure gradients were calculated with the Bernoulli equation, and aortic-valve area was calculated with the continuity equation. The severity of aortic stenosis was determined with echocardiography according to the following standard guidelines: normal is defined by a peak velocity of 1.0 to 2.0 m per second, peak and mean gradients of 0 mm Hg, and a valve area of greater than 2.0 cm^2; mild by a peak velocity of 2.1 to 3.0 m per second, a peak gradient of 16 to 35 mm Hg, a mean gradient of less than 15 mm Hg, and a valve area of 2.0 to 1.3 cm^2; moderate by a peak velocity of 3.1 to 4.0 m per second, a peak gradient of 36 to 64 mm Hg, a mean gradient of 15 to 50 mm Hg, and a valve area of 1.2 to 0.8 cm^2; and severe by a peak velocity of greater than 4.0 m per second, a peak gradient of greater than 64 mm Hg, a mean gradient of greater than 50 mm Hg, and a valve area of less than 0.8 cm^2.

COMPUTED TOMOGRAPHY

CT was performed by a single operator with the use of a double-helix scanner (Twin II Flash, Philips Medical Systems) calibrated against a standard phantom. The region of the aortic valve was scanned with a spiral CT with the use of 2.7-mm slices, a pitch of 0.7, and an increment of 1.4 mm during inspiratory breath–holding sessions. All images were analyzed by a single operator with the use of automated computerized software (Picker Cardiac Scoring), involving a modified Agatston scoring method[32] with a threshold of 90 Hounsfield units to compensate for nongated imaging.

Reproducibility of echocardiography and CT assessments was determined in two subsets of 20 patients, as described elsewhere.[33] Coefficients of reproducibility[34] for aortic-jet velocity and aortic-valve calcium score were 0.32 m per second and 0.07 log arbitrary units (AU), respectively.[33]

STATISTICAL ANALYSIS

The two primary end points were progression of stenosis, determined according to changes in aortic-jet velocity on Doppler echocardiography, and progression of valvular calcification, as measured by CT. Secondary end points were a composite of clinical end points (death from cardiovascular causes, aortic-valve replacement, or hospitalization attributable to severe aortic stenosis), aortic-valve replacement, death from any cause, hospitalization for any cause, and hospitalization for cardiovascular causes. On the basis of standard deviations of 0.32 m per second per year[8,29,35] and 1100 AU per year,[32] we calculated that the planned sample size of 75 patients per group would give the study a power of 80 percent at a 5 percent significance level to detect a difference in the primary end points of 0.15 m per second per year in aortic-jet velocity and 500 AU per year in aortic-valve calcium score. These differences are equivalent to a reduction of more than 30 percent in the rate of progression of aortic stenosis. This would exclude a clinically significant effect in the majority of older patients with established disease, although a smaller effect may be clinically relevant in younger patients with mild aortic stenosis.

The data-monitoring committee conducted two interim assessments of safety and an interim assessment of efficacy one year after enrollment began. The trial was to be terminated early in the event of a negative effect of treatment (i.e., $P<0.05$) or a strong benefit of treatment (i.e., $P<0.001$). On the recommendation of the data-monitoring committee, the trial continued until the study was completed.

Analyses were performed using SPSS software, version 12.0, and SAS software, version 8e. Intention-to-treat analyses were used for all clinical outcome variables. Disease progression was deter-

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Atorvastatin (N=77)	Placebo (N=78)
Age — yr	68±11	68±10
Male sex — %	68	72
Hypertension — no.	48	54
Hyperlipidemia — no.	8	5
Diabetes mellitus — no.	3	4
Current smoker — no.	21	22
Coronary heart disease — no.	18	21
Cerebrovascular disease — no.	9	11
Peripheral vascular disease — no.	5	13
Drug history — no.		
Aspirin	43	40
ACE inhibitor	12	14
Beta-blocker	21	27
Warfarin	8	12
Height — cm	168±9	169±8
Weight — kg	79±15	80±15
Heart rate — bpm	68±11	66±12
Systolic blood pressure — mm Hg	144±18	144±21
Diastolic blood pressure — mm Hg	82±10	81±12
Biochemistry†		
Total cholesterol — mg/dl	220±38	217±34
LDL cholesterol — mg/dl	137±34	133±30
Cholesterol:HDL ratio	4.1±1.1	4.1±1.4
Urea — mg/dl	38±13	43±13
Creatinine — mg/dl	1.07±0.25	1.08±0.26
Glucose — mg/dl	91±19	95±21
Sinus rhythm — %	94	92
Atrial fibrillation — %	6	8
Romhilt–Estes score — median (interquartile range)	1 (0–3)	2 (1–4)
Tricuspid aortic valve — %	96	97
Bicuspid aortic valve — %	4	3
Aortic-jet velocity — m/sec	3.39±0.62	3.45±0.67
Peak gradient — mm Hg	47.8±17.4	49.5±19.5
Aortic-valve area — cm^2	1.03±0.4	1.02±0.41
Aortic-valve calcium score — median AU (interquartile range)	5424 (2750–9689)	6221 (3037–9575)
Log aortic-valve calcium score — log AU	3.7±0.5	3.7±0.6

* Plus–minus values are means ±SD. ACE denotes angiotensin-converting enzyme, LDL low-density lipoprotein, HDL high-density lipoprotein, and AU arbitrary units.

† To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for urea to millimoles per liter, multiply by 0.357. To convert values for creatinine to micromoles per liter, multiply by 88.4. To convert values for glucose to millimoles per liter, multiply by 0.05551.

mined primarily by dividing the change between the baseline and final scans by the duration of follow-up. Treatment comparisons for the continuous outcome variables were based on an analysis of covariance, with the prerandomization level of a variable used as a covariate. In a confirmatory analysis of the primary end points, random-coefficient models were fitted to incorporate all observations.[36] In the subgroup analyses, interaction terms between treatment and subgroup have been added to a model incorporating prerandomization level, treatment, and subgroup to identify factors that were associated with a differential treatment effect within subgroups. Categorical variables have been analyzed using Fisher's exact test. Two-tailed tests were used throughout. Two-sided P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Seventy-seven patients were assigned to atorvastatin and 78 to placebo, with a median follow-up of 25 months (range, 7 to 36). As a consequence of minimization, baseline characteristics were well matched (Table 1). Mean aortic-jet velocity was 3.43±0.64 m per second (range, 2.5 to 5.0), and the median aortic-valve calcium score was 5920 AU (interquartile range, 2485 to 14,231). Of the 155 patients, 119 had mild-to-moderate aortic stenosis (aortic-jet velocity, 2.5 to 3.9 m per second), and 36 had severe stenosis (aortic-jet velocity, ≥4.0 m per second).

SERUM CHOLESTEROL CONCENTRATIONS

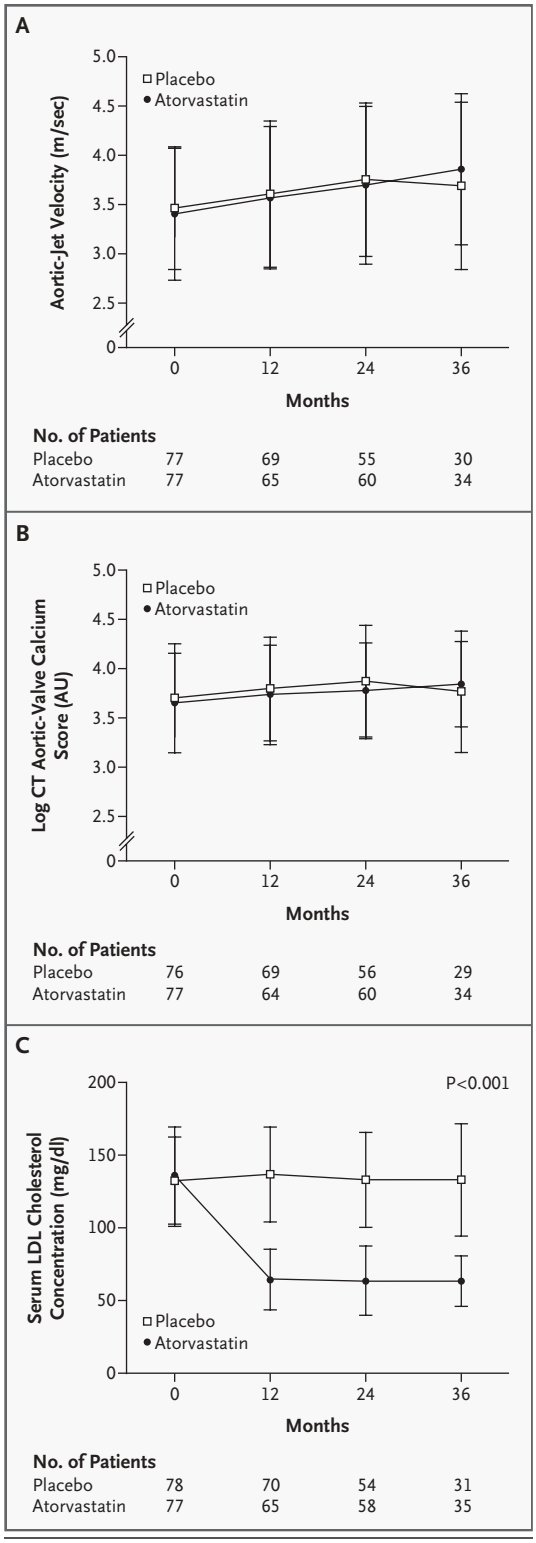
The mean serum low-density lipoprotein (LDL) cholesterol concentration remained at 130±30 mg per deciliter (3.4±0.8 mmol per liter) in the placebo group and decreased by 53 percent to 63±23 mg per deciliter (1.7±0.6 mmol per liter) in the atorvastatin group (P<0.001) (Fig. 1C). Serum total cholesterol was 209±35 mg per deciliter (5.5±0.9 mmol per liter) and 132±27 mg per deciliter (3.5±0.7 mmol per liter) in the placebo and atorvastatin groups, respectively (P<0.001), and is in keeping with 97 percent adherence to the study treatment in both groups, which was confirmed by a pill count.

EFFECT OF ATORVASTATIN ON DISEASE PROGRESSION

Intensive lipid-lowering therapy with 80 mg of atorvastatin daily had no effect on the rate of change in

Figure 1. Progression in Aortic-Valve Stenosis and Serum LDL Cholesterol Concentrations in Patients Treated with Intensive Atorvastatin Therapy or Matched Placebo.

Patients received 80 mg of atorvastatin daily or matched placebo. LDL denotes low-density lipoprotein, CT computed tomography, and AU arbitrary units. I bars indicate SDs.



aortic-jet velocity or valvular calcification (Table 2). Progression in valvular calcification was 22.3±21.0 percent per year in the atorvastatin group, and 21.7±19.8 percent per year in the placebo group (P=0.93; ratio of post-treatment aortic-valve calcium score, 0.998; 95 percent confidence interval, 0.947 to 1.050). We also performed a longitudinal analysis of the rate of change over time for the two treatment groups with the use of a mixed-effects linear model.[36] This showed no difference in the rate of disease progression, with point estimates and 95 percent confidence intervals for the treatment difference that were similar to those shown in Table 2 (mean difference in the rate of change of aortic-jet velocity [the change in the atorvastatin group minus that in the placebo group], 0.008 m per second per year [−0.058 to 0.075]; mean difference in rate of change of aortic-valve calcium score, 71 AU per year [−524 to 666]). Serum LDL cholesterol concentrations did not correlate with disease progression demonstrated on echocardiography (r=0.021, P=0.81) or CT (r=−0.109, P=0.21). The proportion of patients reaching secondary clinical end points seemed to be less in the atorvastatin group, but none of the comparisons achieved statistical significance (Table 3).

SUBGROUP ANALYSES

Prespecified subgroup analysis of the primary endpoint data was conducted in patients with mild-to-moderate aortic stenosis (aortic-jet velocity, <4.0 m per second) and severe aortic stenosis (aortic-jet velocity, ≥4.0 m per second) at baseline. As anticipated from earlier studies, patients with severe stenosis at baseline progressed more rapidly (P=0.04), but the study findings were consistent regardless of the severity of stenosis at baseline (Table 4).

Likewise, the length of follow-up did not influence outcome. In those followed for more than 24

Table 2. Progression from Baseline of Aortic-Valve Stenosis on Echocardiography and Computed Tomography.*

Variable	All Patients	Atorvastatin	Placebo	Adjusted Difference: Atorvastatin – Placebo (95% CI)	P Value
Echocardiography					
No. of patients	134	65	69		
Change in aortic-jet velocity (m/sec/yr)	0.201±0.208	0.199±0.210	0.203±0.208	0.002 (–0.066 to 0.070)	0.95
Increase in peak gradient (mm Hg/yr)	6.52±7.24	6.48±7.43	6.56±7.10	0.21 (–2.02 to 2.45)	0.85
Change in aortic-valve area (cm^2/yr)	–0.081±0.107	–0.079±0.107	–0.083±0.107	0.007 (–0.026 to 0.040)	0.68
Computed tomography					
No. of patients	133	64	69		
Absolute change in aortic-valve calcium score (AU/yr)	1608±1865	1564±1956	1648±1790	85 (–554 to 723)	0.80
Change in log aortic-valve calcium score (per yr)	0.20±0.16	0.20±0.16	0.20±0.15	0.00 (–0.05 to 0.05)	0.93

* Plus–minus values are means ±SD. CI denotes confidence interval, and AU arbitrary units.

Table 3. Number of Patients Reaching Secondary End Points.

Secondary End Point	Atorvastatin (N=77)	Placebo (N=78)	P Value (Fisher's Exact Test)
Composite*	13	21	0.19
Death from cardiovascular causes	3	3	1.00
Aortic-valve replacement	11	19	0.17
Hospitalization for severe aortic stenosis	3	5	0.73
Death from any cause	3	5	0.73
Hospitalization for any cause	10	12	0.84

* The composite end point was death from cardiovascular causes, aortic-valve replacement, or hospitalization for severe aortic stenosis.

months (median, 33), the increase in aortic-jet velocity was 0.21±0.20 m per second per year in the atorvastatin group and 0.17±0.14 m per second per year in the placebo group (Table 4). In those followed for 24 months or less (median, 23), the increase in aortic-jet velocity was 0.19±0.22 m per second per year in the atorvastatin group and 0.23±0.25 m per second per year in the placebo group.

ADVERSE EVENTS

There were similar rates of adverse events in the two treatment groups. Four patients (5 percent) in the placebo group and seven patients (9 percent) in the atorvastatin group discontinued the study drug (P=0.52 by Fisher's exact test), predominantly as a result of gastrointestinal symptoms. Three patients in the atorvastatin group had an increase in the creatine kinase level to more than five times the upper limit of the normal range, without symptoms of myositis; one of these patients was withdrawn at the request of the data-monitoring committee. There were no cases of rhabdomyolysis and no serious adverse events.

DISCUSSION

In this randomized, double-blind, placebo-controlled, parallel-group trial of lipid-lowering therapy in patients with calcific aortic stenosis, a single coordinating center used a consistent and reproducible approach to assess the severity of aortic stenosis.[33] We have clearly shown that high-dose atorvastatin reduces serum LDL cholesterol concentrations by more than a factor of two, as anticipated,[37] but it does not halt the progression or induce regression of the valvular disease process. This was shown with the use of two distinct measures of disease severity — aortic-jet velocity assessed with Doppler echocardiography and valvular calcification assessed with helical CT. Moreover, there was no relationship between serum LDL cholesterol concentrations and the progression of aortic stenosis, nor did high-dose atorvastatin have a demonstrable effect on clinical end points. Thus, regardless of the method of assessing disease progression, we have consistently shown that aortic stenosis progresses despite intensive reductions in serum cholesterol concentrations.

The minimization technique helped ensure that there were no baseline inequalities between the

Table 4. Subgroup Analyses of Disease Progression According to Aortic-Jet Velocity.*

Characteristic	Atorvastatin				Placebo			
	No.	Baseline Value	No.	Rate of Change	No.	Baseline Value	No.	Rate of Change
		m/sec		*m/sec/yr*		*m/sec*		*m/sec/yr*
Baseline severity of stenosis†								
Mild to moderate	58	3.12±0.43	49	0.17±0.21	61	3.18±0.44	55	0.19±0.20
Severe	19	4.24±0.21	16	0.27±0.21	17	4.45±0.26	14	0.27±0.23
Duration of follow-up								
≤24 Mo‡	30	3.49±0.69	30	0.19±0.22	37	3.64±0.67	37	0.23±0.25
>24 Mo§	35	3.31±0.55	35	0.21±0.20	32	3.28±0.61	32	0.17±0.14

* Plus–minus values are means ±SD. P=0.57 for the interaction of treatment and the baseline severity of stenosis, and P=0.41 for the interaction of treatment and the duration of follow-up.
† Patients with mild-to-moderate aortic stenosis had an aortic-jet velocity of less than 4.0 m per second, and those with severe stenosis an aortic-jet velocity of at least 4.0 m per second.
‡ The median follow-up was 23 months.
§ The median follow-up was 33 months.

treatment groups. Several factors may have influenced our ability to detect an effect of statin therapy on the progression of aortic stenosis in this trial. First, as a consequence of our inclusion criteria, we recruited some patients with severe disease and an aortic-jet velocity of at least 4 m per second, and it could be argued that lipid-lowering therapy is unlikely to influence disease progression at such an advanced stage. We therefore conducted a prespecified subgroup analysis excluding patients with a baseline aortic-jet velocity of 4 m per second or more. Our findings were consistent regardless of the severity of stenosis at baseline — atorvastatin had no effect on disease progression, even in the majority of patients with mild-to-moderate stenosis. We excluded patients with an aortic-jet velocity of less than 2.5 m per second, and we acknowledge that intervening at this earlier stage of the disease process may have been more beneficial. However, such patients do not commonly present to routine clinical practice, and their identification would potentially require population screening.

Second, two years of treatment may not have been sufficient to influence the natural history of the disease. We assessed this possibility by determining if patients with a longer follow-up showed a treatment benefit. In patients who underwent nearly three years of treatment with intensive statin therapy, no trend toward a beneficial effect of atorvastatin was apparent. Therefore, we do not believe that the lack of an effect was due to an inadequate treatment period.

Finally, our study was designed to detect a substantial delay in disease progression and was not powered to assess meaningful effects on clinical end points, such as valve replacement and cardiovascular death. Although we can exclude a treatment benefit of the magnitude previously reported in retrospective observational studies (a reduction in the aortic-jet velocity of 0.30 m per second per year[29] and valvular calcification of 30 percent per year[24,26]), the 95 percent confidence intervals indicate that we may have missed a modest treatment benefit (a delay in disease progression of <0.07 m per second per year for aortic-jet velocity and <5 percent per year for valvular calcification). Although such modest reductions are unlikely to be meaningful in the majority of older patients, a small decrease in disease progression may be clinically important in younger patients with mild disease that may progress over many years.

Given the strength of the data linking aortic stenosis with atherosclerosis and hypercholesterolemia, why have we failed to halt the progression of calcific aortic stenosis? One potential explanation is that, although these features may drive the initiation of aortic stenosis, disease progression may depend on other factors. The aortic valve is subject to continuous dynamic mechanical stress, and the plasticity and structure of the leaflets can have an overriding influence, as is the case with a bicuspid valve. Moreover, in contrast to atherosclerosis, aortic stenosis is associated with a virtual absence of smooth-muscle-cell proliferation and lipid-laden

macrophages[2] and is dominated by earlier and more extensive mineralization. Decreasing the lipid pool and strengthening the fibrous cap may be less relevant to the progression of aortic stenosis than they are for the reduction in atherosclerotic-plaque rupture with statin therapy in patients with coronary heart disease.

Because of the association between aortic stenosis and coronary artery disease, statin therapy in patients with aortic stenosis may confer secondary preventive benefits that are independent of its effects on the valvular disease process. The current study was not powered to assess the benefits of lipid-lowering therapy on cardiovascular end points such as nonfatal and fatal myocardial infarction. It remains a possibility that aortic stenosis and sclerosis[38] may be important markers of occult vascular disease and may identify patients who would gain from the preventive benefits of statin therapy.

We conclude that intensive lipid-lowering therapy with 80 mg of atorvastatin daily does not halt the progression of calcific aortic stenosis or induce its regression. Nevertheless, this trial does not rule out a small but potentially relevant reduction in the rate of disease progression or a significant reduction in major clinical end points. Our study reinforces the need for a long-term, large-scale, randomized, controlled trial of intensive lipid-lowering therapy in patients with calcific aortic stenosis, particularly in those with early, mild disease. In the meantime, we do not recommend statin therapy for patients with calcific aortic stenosis in the absence of coexisting vascular disease.

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APPENDIX

The following participated in the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE): **Research team:** L. Anderson, C. Bell, M. Bland, J. Burton, S. Cameron, N. Cruden, J. Cunningham, H. Cuthbertson, L. Flint, M. Henderson, D. Lyle, M. O'Donnell, F. Paterson, K. Paterson, S. Robinson, H. Spence, J. Tickner, A. White. **Collaborating centers (all in the United Kingdom):** *Borders General Hospital, Melrose* — P. Broadhurst, C. Norris, P. Leslie, J. Gaddie; *Eastern General Hospital, Edinburgh* — A. Elder; *Royal Infirmary, Edinburgh* — K. Fox, N. Grubb, A. Flapan, H. Miller, N. Uren; *Falkirk and District Royal Infirmary, Falkirk* — A. Hargreaves, P. McSorely; *Queen Margaret Hospital, Dunfermline* — D. MacLeod; *Roodland's Hospital, Haddington* — A. Flapan; *St. Johns' Hospital, Livingston* — J. Irving, A. Jacob; *Royal Infirmary, Stirling* — A. Bridges, S. Glen; *Wellcome Trust Clinical Research Facility, Edinburgh; Western General Hospital, Edinburgh* — M. Denvir, T. Shaw, I. Starkey. **Pharmacy:** *Royal Infirmary, Edinburgh* — B. Booth; *Freeman Hospital, Newcastle-upon-Tyne, United Kingdom* — A. Heed. **Medical Statistics:** *University of Edinburgh, Edinburgh* — T. Forster.

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CLINICAL TRIAL REGISTRATION

The *Journal* encourages investigators to register their clinical trials in a public trials registry. The members of the International Committee of Medical Journal Editors plan to consider clinical trials for publication only if they have been registered (see N Engl J Med 2004;351:1250-1). The National Library of Medicine's www.clinicaltrials.gov is a free registry, open to all investigators, that meets the committee's requirements.

ORIGINAL ARTICLE

Two-Year Outcomes after Conventional or Endovascular Repair of Abdominal Aortic Aneurysms

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ABSTRACT

BACKGROUND

Two randomized trials have shown better outcomes with elective endovascular repair of abdominal aortic aneurysms than with conventional open repair in the first month after the procedure. We investigated whether this advantage is sustained beyond the perioperative period.

METHODS

We conducted a multicenter, randomized trial comparing open repair with endovascular repair in 351 patients who had received a diagnosis of abdominal aortic aneurysm of at least 5 cm in diameter and who were considered suitable candidates for both techniques. Survival after randomization was calculated with the use of Kaplan–Meier analysis and compared with the use of the log-rank test on an intention-to-treat-basis.

RESULTS

Two years after randomization, the cumulative survival rates were 89.6 percent for open repair and 89.7 percent for endovascular repair (difference, −0.1 percentage point; 95 percent confidence interval, −6.8 to 6.7 percentage points). The cumulative rates of aneurysm-related death were 5.7 percent for open repair and 2.1 percent for endovascular repair (difference, 3.7 percentage points; 95 percent confidence interval, −0.5 to 7.9 percentage points). This advantage of endovascular repair over open repair was entirely accounted for by events occurring in the perioperative period, with no significant difference in subsequent aneurysm-related mortality. The rate of survival free of moderate or severe complications was also similar in the two groups at two years (at 65.9 percent for open repair and 65.6 percent for endovascular repair; difference, 0.3 percentage point; 95 percent confidence interval, −10.0 to 10.6 percentage points).

CONCLUSIONS

The perioperative survival advantage with endovascular repair as compared with open repair is not sustained after the first postoperative year.

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*The members of the DREAM Trial Group are listed in the Appendix.

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Two randomized trials have demonstrated better outcomes with elective endovascular repair of abdominal aortic aneurysms than with conventional open repair in the first month after the procedure.[1,2] The reported in-hospital mortality rates in these two trials were 4.6 percent and 6.0 percent for open repair and 1.6 percent and 1.2 percent for endovascular repair, respectively. Although the relevance of a reduction in perioperative risk should not be underestimated from the patient's perspective, the improvement in early survival with the use of a less invasive technique is not surprising.[3] Consequently, both reports stressed the need for longer-term data before a decision could be reached about which therapy is better in patients who are suitable candidates for either procedure.

Findings in uncontrolled long-term studies of endovascular aneurysm repair have suggested that the early advantage of endovascular over open repair may not persist over time.[4,5] Endovascular repair appeared to be associated with higher rates of reintervention and complications as well as a continued risk of aneurysm rupture. The Dutch Randomized Endovascular Aneurysm Management (DREAM) trial was conducted to assess the rates of death from any cause and complications in a multicenter, randomized trial comparing elective open and endovascular aneurysm repair.

METHODS

STUDY DESIGN AND PATIENTS

The design and methods of the trial have been described in detail elsewhere.[2,6] In brief, patients referred to surgery clinics at 26 centers in the Netherlands and 4 centers in Belgium who had received a diagnosis of an abdominal aortic aneurysm of at least 5 cm in diameter and who were considered suitable candidates for both techniques were randomly assigned to undergo open or endovascular repair after giving written informed consent. Randomization was carried out centrally with the use of a computer-generated permuted-block sequence and stratified according to study center in blocks of four patients.

The study was performed according to the principles of the Declaration of Helsinki. The institutional review boards of all participating hospitals approved the protocol. The corresponding author assumed full responsibility for the conduct of the trial, had full access to all the data, and controlled the decision to publish. The study was publicly funded, and the sponsor had no role in the study design.

DATA COLLECTION AND FOLLOW-UP

All data were submitted to the trial-coordination center (Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, the Netherlands). Follow-up visits were scheduled 30 days and 6, 12, 18, and 24 months after the procedure. Before hospital discharge and at each follow-up visit, all patients underwent a physical examination, which included calculation of the ankle–brachial blood-pressure index; abdominal helical computed tomographic angiography; and abdominal color duplex ultrasonography. In addition, patients in the endovascular group underwent plain abdominal radiography before hospital discharge and 12 and 24 months postoperatively.

Data acquisition was stopped on March 1, 2005, for this report. For all analyses, data on patients were censored after their last follow-up visit. For the crude survival analysis, however, reports on vital status obtained at any time before the cutoff date were also incorporated.

END POINTS

The primary end point of the trial was a composite of operative mortality and moderate or severe complications, as discussed in the initial report on the results of the trial.[2] Mortality and complications at two years were predetermined secondary end points in the original trial design. The outcome events that we analyzed were deaths from all causes, aneurysm-related deaths, complications, and reinterventions.

The cause and exact date of death were determined by assessment of death certificates and by contacting the physicians involved (surgeons and general practitioners) and patients' relatives if necessary. Aneurysm-related death was defined as death resulting from aneurysm rupture, graft infection, or thrombosis; any death occurring within 30 days after the original procedure or a reintervention; or any death occurring more than 30 days after the original procedure or a reintervention but during the same admission.

Complications were classified and graded according to the reporting standards of the Ad Hoc Committee for Standardized Reporting Practices in Vascular Surgery of the Society for Vascular Surgery/International Society for Cardiovascular Surgery.[7,8] Three severity grades (mild, moderate, and

severe) were distinguished. Mild complications were not considered in this analysis. A reintervention was defined as any surgical or endovascular procedure performed after the primary aneurysm-repair procedure and related to the aneurysm or the primary procedure, including incisional hernia repairs but exclusive of procedures involving superficial wound complications. An outcome adjudication committee, consisting of five vascular surgeons, assessed the type and severity of each complication and reintervention in a blinded fashion and independently from each other. Disagreements were resolved in a plenary consensus meeting.

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Open Repair (N=178)	Endovascular Repair (N=173)
Age — yr	69.6±6.8	70.7±6.6
Male sex — no. (%)	161 (90.4)	161 (93.1)
Mild, moderate, or severe SVS/ISCVS risk-factor score — %†		
Diabetes mellitus	9.6	10.4
Tobacco use	55.1	64.2
Hypertension	54.5	58.4
Hyperlipidemia	52.6	47.0
Carotid artery disease	15.2	14.5
Cardiac disease	46.6	41.0
Renal disease	8.4	7.5
Pulmonary disease	18.5	27.7
Total SVS/ISCVS risk-factor score†	4.5±2.5	4.4±2.5
FEV_1 — liters/sec	2.6±0.7	2.5±0.7
Body-mass index	26.6±4.1	26.3±3.4
ASA class — no. (%)		
I (healthy status)	44 (24.7)	37 (21.4)
II (mild systemic disease)	110 (61.8)	122 (70.5)
III (severe systemic disease)	24 (13.5)	14 (8.1)
Medication use — no. (%)		
Beta-blockers	92 (51.7)	76 (43.9)
Statins‡	72 (41.9)	63 (37.3)
Antiplatelet agents	72 (40.4)	70 (40.5)
Angiotensin-converting–enzyme inhibitors	50 (28.1)	58 (33.5)
Calcium-channel blockers	32 (18.0)	30 (17.3)
Anticoagulants	27 (15.2)	20 (11.6)

* Plus–minus values are means ±SD. There were no significant differences between the groups. FEV_1 denotes forced expiratory volume in one second, and ASA American Society of Anesthesiologists. The body-mass index is the weight in kilograms divided by the square of the height in meters. Because of rounding, not all percentages total 100.

† The Society for Vascular Surgery/International Society for Cardiovascular Surgery (SVS/ISCVS) risk-factor score is calculated for eight domains, and scores for each domain can range from 0 (no risk factors) to 3 (severe risk factors).[7] Total scores can range from 0 to 24, with higher scores indicating greater risk.

‡ No information on the use of statins was available for six patients in the open-repair group and four patients in the endovascular-repair group.

STATISTICAL ANALYSIS

All data were analyzed according to the intention-to-treat principle. Kaplan–Meier analysis was used to analyze survival and other end points, and differences between groups were compared with the use of the log-rank test. Cox proportional-hazards regression was used to estimate hazard ratios for the analysis of reintervention rates. Means (±SD) were used to describe continuous variables. Differences between groups were compared with the use of the Mann–Whitney U test for continuous variables and Fisher's exact test for proportions. All reported P values are two-sided and are not adjusted for multiple testing.

RESULTS

CHARACTERISTICS OF THE PATIENTS AND TREATMENT ASSIGNMENTS

Between November 2000 and December 2003, 178 patients were randomly assigned to undergo open repair and 173 to undergo endovascular repair. Six patients did not undergo aneurysm repair after randomization: four declined treatment (three assigned to open repair and one to endovascular repair), one died from a ruptured abdominal aortic aneurysm before undergoing open repair, and one died from pneumonia before undergoing endovascular repair. There were six crossovers: five patients who were randomly assigned to undergo open repair underwent endovascular repair, and one patient assigned to endovascular repair underwent open repair. Overall, the operation was started according to the randomized assignment in 96.6 percent of patients (339 of 351).

The baseline characteristics of the patients are given in Table 1. Demographic characteristics, the prevalence of coexisting conditions, cardiovascular-risk profiles, the distribution of American Society of Anesthesiologists risk classes, and medication use were similar in the two groups.

The median interval between randomization and the procedure was 39 days in both the open-repair group (range, 4 to 260) and the endovascular-repair group (range, 1 to 183; P=0.76); 92.6 percent

of patients (325 of 351) underwent aneurysm repair within 3 months after randomization. The mean duration of follow-up was 21 months in the open-repair group (range, 0 to 39) and 22 months in the endovascular-repair group (range, 1 to 42). A total of 6 patients were lost to follow-up during the first year (follow-up 98.3 percent complete) and 19 during the first two years (follow-up 94.6 percent complete).

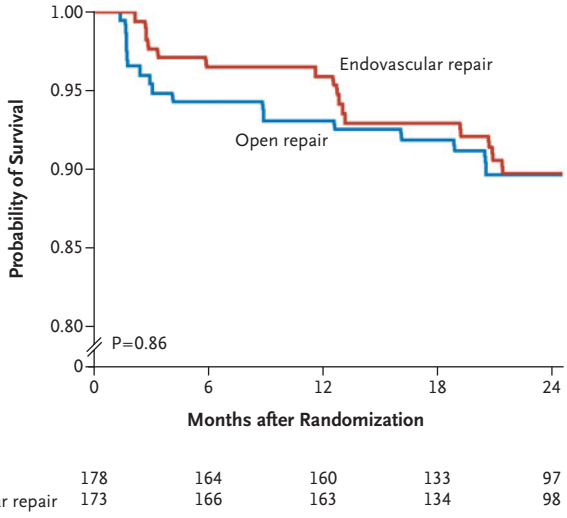


Figure 1. Kaplan–Meier Estimates of Survival among Patients Assigned to Undergo Open or Endovascular Aneurysm Repair.

MORTALITY

Two years after randomization, the cumulative survival rates were 89.6 percent for open repair and 89.7 percent for endovascular repair, for a difference of −0.1 percentage point (95 percent confidence interval, −6.8 to 6.7 percentage points; P=0.86) (Fig. 1). The small but apparent survival advantage in the first year after endovascular repair did not reach statistical significance (P=0.15) and appeared to be based entirely on a decreased rate of in-hospital (perioperative) mortality.

There was one preoperative death and eight in-hospital deaths in the open-repair group and one preoperative and two in-hospital deaths in the endovascular-repair group (Table 2). Taking into account the patients who declined treatment (three in the open-repair group and one in the endovascular-repair group), there were 166 discharges after open repair and 169 discharges after endovascular repair. The causes of death are listed in Table 2. After discharge, there were more deaths from cardiovascular causes in the endovascular-repair group than in the open-repair group (six vs. three), although this difference was not significant (P=0.50).

There was an unexplained cluster of deaths in the endovascular-repair group approximately one year after randomization (Fig. 1). None of these deaths were considered to be aneurysm-related as defined in the Methods section; two of the deaths were due to heart failure, one to acute cardiac arrest, one to stroke, and one to aspiration pneumonia in a patient with metastatic carcinoma of the bladder.

ANEURYSM-RELATED MORTALITY

The cumulative rates of aneurysm-related death two years after randomization were 5.7 percent in the open-repair group and 2.1 percent in the endovascular-repair group, for a difference of 3.7 percentage points (95 percent confidence interval, −0.5 to 7.9 percentage points; P=0.05). The difference in aneurysm-related mortality at two years was based entirely on the difference in in-hospital (perioperative) mortality. After discharge, only one additional aneurysm-related death occurred in each group (Table 2).

COMPLICATIONS

Two years after randomization, the rates of survival free of severe events were 80.6 percent for open repair and 83.1 percent for endovascular repair, for a difference of −2.5 percentage points (95 percent confidence interval, −10.9 to 5.9 percentage points; P=0.39) (Fig. 2). As with the data on aneurysm-related mortality, the difference in the rate of survival free from severe events at two years was based entirely on the difference in in-hospital events. The rates of survival free of moderate or severe events two years after randomization were 65.9 percent for open repair and 65.6 percent for endovascular repair, for a difference of 0.3 percentage point (95 percent confidence interval, −10.0 to 10.6 percentage points; P=0.88).

There were no documented postoperative aneurysm ruptures. However, in two patients who died after endovascular repair, the possibility of aneurysm rupture was considered but not proved (Table 2).

Kaplan–Meier estimates of the likelihood of freedom from reintervention are shown in Figure 3. In the first nine months after randomization, the rate of reintervention after endovascular repair was al-

Table 2. Causes of Death after Open and Endovascular Repair of Abdominal Aortic Aneurysm.

Cause of Death	Before Surgery*		In the Hospital†		After Discharge		Overall	
	Open Repair (N=178)	Endovascular Repair (N=173)	Open Repair (N=174)	Endovascular Repair (N=171)	Open Repair (N=166)	Endovascular Repair (N=169)	Open Repair (N=178)	Endovascular Repair (N=173)
	number of patients							
All causes	1	1	8	2	9	17	18	20
Cardiovascular causes	0	0	2	1	3	6	5	7
Myocardial infarction	0	0	1	1	0	1	1	2
Cardiac arrest	0	0	1	0	2	2	3	2
Congestive heart failure	0	0	0	0	0	2	0	2
Stroke	0	0	0	0	1	1	1	1
Aneurysm-related, noncardiovascular causes	1	0	6‡	1§	1¶	1‖	8	2
Cancer	0	0	0	0	2	4	2	4
Other	0	1	0	0	1**	4††	1	5
Unknown	0	0	0	0	2‡‡	2§§	2	2

* Two patients died before undergoing the assigned operation: one patient with preexistent pulmonary fibrosis assigned to undergo endovascular repair died from pneumonia 84 days after randomization, and one patient assigned to undergo open repair died from a ruptured abdominal aortic aneurysm.
† In-hospital data were reported previously.[2] All 10 in-hospital deaths were aneurysm-related by definition. None of the nine deaths from cardiovascular causes after discharge were aneurysm-related.
‡ The causes of death were as follows: infection of the prosthesis, anastomotic bleeding, ischemic bowel, intraoperative anaphylactic shock, multiorgan failure after repair of a burst abdomen, and progressive dementia and refusal to eat or drink leading to respiratory insufficiency and death.
§ The cause of death was bilateral pneumonia.
¶ The cause of death was peritonitis resulting from an iatrogenic bowel lesion during repeated operation to correct prosthetic malalignment.
‖ The cause of death was an infected endograft.
** The cause of death was pneumonia.
†† The causes of death were as follows: peritonitis, pulmonary embolism, respiratory insufficiency, and general deterioration related to old age.
‡‡ No data were available on the cause of death.
§§ Both patients died suddenly, 33 and 41 months after the procedure. A ruptured aneurysm was considered a possible cause of death, but in neither patient was a postmortem examination performed. Both patients had evidence of a shrinking aneurysm sac on their last (24-month) follow-up computed tomographic scan.

most three times the rate after open repair (hazard ratio, 2.9; 95 percent confidence interval, 1.1 to 6.2; P=0.03). Thereafter, reintervention rates were roughly parallel (hazard ratio, 1.1; 95 percent confidence interval, 0.1 to 9.3; P=0.95).

DISCUSSION

We found that by the end of the first year after randomization, the previously reported perioperative survival advantage of endovascular aneurysm repair over open repair was no longer apparent.[2] Although a lower rate of aneurysm-related death after endovascular repair did appear to be maintained during the first two years, in terms of overall survival, this was cancelled out by excess mortality from other causes, including cardiovascular causes, in the first two years after discharge.

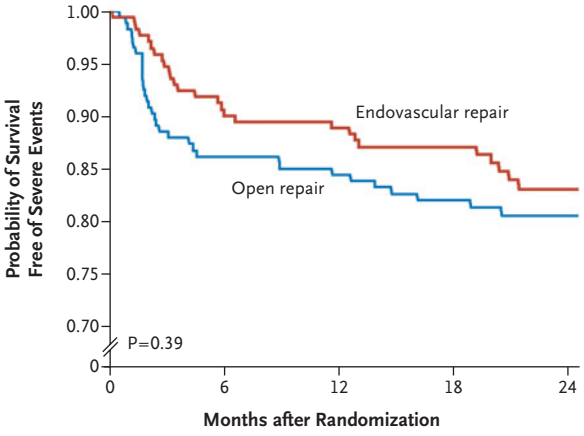
One other randomized trial, the Endovascular Aneurysm Repair (EVAR-1) trial, has compared the results of endovascular aneurysm repair with those of open repair.[1] Whereas the early results of the two trials were similar, the long-term results of EVAR-1 are not yet available and thus cannot be compared with our findings.

Our results are similar to those of two recently reported retrospective, controlled studies comparing endovascular and open repair.[9,10] In both studies, the respective one-year survival rates after open and endovascular repair were approximately 92 and 95 percent, and the respective two-year survival rates were approximately 88 and 89 percent, all of which are very close to our findings. The rates of aneurysm-related death two years after open and endovascular repair were 4.2 and 0.9 percent, respectively, in the study by Cao et al.,[10] as compared

with 5.7 and 2.1 percent, respectively, in our study. It is possible that the prospective nature of our study allowed for more complete detection of aneurysm-related deaths. The difference in reintervention rates between the groups in our study is also similar to that reported in both retrospective studies. In one study, the divergence of reintervention rates did not start until after two years of follow-up,[10] whereas in our study, there was no significant difference in reintervention rates beyond nine months after randomization. This variation may depend on how aggressively certain complications are addressed.

Although our findings — and those in the other trials discussed above — suggest that endovascular aneurysm repair may provide an early survival advantage over conventional surgery, it appears that this advantage is lost by the end of the first year. It is unknown whether the durability of the endovascular graft will jeopardize long-term outcomes. Although nonrandomized, follow-up studies of patients who have undergone aneurysm repair have failed to show a long-term advantage of open over endovascular repair,[9,10] concerns persist, since the rates of aneurysm-related death and reintervention after endovascular repair have been reported to continue to increase over time.[4,11] The overall survival curves in our trial appeared to converge in the second year after randomization. Our 2-year data do not exclude the possibility that these curves will actually cross, resulting in a higher rate of death for endovascular repair than for open repair after 24 months.

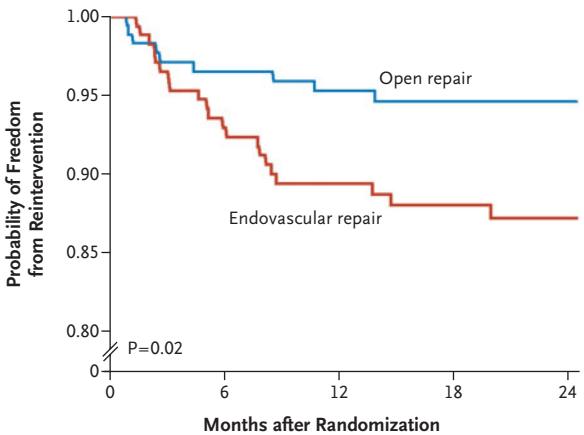
There may be two possible explanations for the convergence of survival curves in our study. One is that patients who have survived the stress of open repair may be somewhat less likely to die in the first few months after surgery than patients who have undergone endovascular repair, since the latter group has not been subjected to a conventional surgical procedure. In other words, the survival advantage resulting from a less-invasive approach to aneurysm repair may largely be based on postponing death among higher-risk patients from the perioperative period to the subsequent months. Although patients in our trial had to be eligible to undergo conventional open aneurysm repair before they could undergo randomization, the health of patients with abdominal aortic aneurysms is often seriously compromised by other types of cardiovascular disease. In our study, 58 percent of the deaths (22 of 38) were due to either cardiovascular causes or causes related to aneurysm repair. This finding is in accordance with those of other follow-up studies of aneurysm repair.[12,13]



No. at Risk

Open repair	178	150	146	121	89
Endovascular repair	173	155	151	125	91

Figure 2. Kaplan–Meier Estimates of Survival Free of Severe Events among Patients Assigned to Undergo Open or Endovascular Aneurysm Repair.



No. at Risk

Open repair	178	158	153	128	93
Endovascular repair	173	155	147	119	88

Figure 3. Kaplan–Meier Estimates of Freedom from Reintervention among Patients Assigned to Undergo Open or Endovascular Aneurysm Repair.

Another possible explanation for the convergence of survival curves is the failure of endovascular repair to prevent rupture of the aneurysm. However, endograft failure is unlikely to occur during the first two years after implantation, and such fail-

ure would be reflected by a convergence of the rates of aneurysm-related death — an effect that was not found in our analysis. Although a grouping of deaths was seen in the endovascular-repair group about one year after randomization, the causes of death were not related to the aneurysm. Furthermore, the apparent grouping of these deaths was seen in a Kaplan–Meier survival analysis that measured the time from randomization, rather than the time from the procedure, indicating that this grouping of deaths was not related to the course after intervention. Only one patient in the endovascular-repair group died of an aneurysm-related cause (an infected endograft) after hospital discharge. Whether the rate of graft failure will increase with further follow-up remains to be seen.

In patients undergoing endovascular repair, efforts should be made to maintain the survival advantage associated with avoiding conventional surgery. This effort may at least in part be a matter of strict risk-factor management. Beta-blockers, antiplatelet agents, and statins were each being used in less than 50 percent of our patients at baseline. Clearly, less-than-optimal medication was used in view of current guidelines on risk management for patients with manifestations of atherosclerosis.[14-16] Of course, better perioperative and postoperative management of risk factors could also improve the results of open aneurysm repair.

In conclusion, the two-year results of the DREAM trial indicate that the perioperative survival advantage with endovascular repair as compared with open repair is limited to the first postoperative year.

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APPENDIX

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ORIGINAL ARTICLE

Independent Clonal Origins of Distinct Tumor Foci in Multifocal Papillary Thyroid Carcinoma

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ABSTRACT

BACKGROUND

Papillary thyroid carcinoma is frequently multifocal. We investigated whether noncontiguous tumor foci arise from intraglandular metastases from a single primary tumor or originate as unrelated clones derived from independent precursors.

METHODS

Using a polymerase-chain-reaction assay involving the human androgen receptor gene (*HUMARA*), we analyzed the patterns of X-chromosome inactivation of multiple distinct foci of well-differentiated multifocal papillary thyroid cancer from 17 women.

RESULTS

Multiple thyroid tumor foci from 10 of 17 patients yielded DNA of adequate quality and were heterozygous for the *HUMARA* polymorphism and hence suitable for analysis. A single X chromosome was inactivated in each focus, consistent with its monoclonality. When the specific monoclonal configurations of each patient's discrete tumor foci were compared, discordant patterns indicative of independent origins were observed among the tumors from five patients; results in the remaining five were consistent with either a shared or independent clonal origin.

CONCLUSIONS

Individual tumor foci in patients with multifocal papillary thyroid cancer often arise as independent tumors.

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Papillary thyroid carcinoma is the most common cancer of the thyroid gland,[1,2] with approximately 20,000 cases annually in the United States. Papillary cancer often presents as a thyroid nodule that does not take up radioactive iodine or as an enlarged lymph node containing a metastasis. The pathogenesis of papillary thyroid cancer involves participation of the *RET*, *NTRK1*, *RAS*, and *BRAF* genes.[3-13]

In patients undergoing surgical treatment for papillary thyroid cancer, pathological analysis commonly identifies multiple noncontiguous tumor foci in individual glands. Estimates of the frequency of such multicentric tumors vary, depending on the techniques used, and range between 18 and 87 percent.[14-19] A "primary" tumor greater than 1 cm in diameter is typical; most of the additional foci measure less than 1 cm in diameter and are termed "microcarcinomas."[19,20] Multifocal tumors have been associated with increased risks of lymph-node and distant metastases, persistent local disease after initial treatment, and regional recurrence.[14,16,17,19,21] All these features suggest that patients with multifocal papillary thyroid cancer should receive aggressive treatment.[22]

Despite attempts to establish whether multiple intrathyroidal tumors are metastases of a primary thyroid tumor or arise independently,[16,19,23,24] the question remains unresolved.[25] The phenomenon of X-chromosome inactivation, in which either the maternal or paternal X chromosome in women is inactivated, makes it possible to determine whether cells in separate tumor foci arose from a single source or from different precursors.[26] Patterns of X-chromosome inactivation can be used to determine whether a tumor arose from one or multiple progenitor cells because the inactivated X chromosome is stably transmitted from parent cell to progeny cell. For this reason, all the cells in a clonal population have the same inactivated X chromosome — maternal or paternal.[27]

The event that randomly inactivates the maternal or paternal X chromosome occurs early in embryogenesis, long before the onset of tumor formation; the inactivation is stable in tumor cells and not ordinarily subject to selection during tumorigenesis. Consequently, for the purpose of determining the origins of tumor cells, studies of patterns of X-chromosome inactivation have advantages over methods that compare specific changes in DNA or gene expression in papillary carcinoma. Such changes could arise as late events in separate subclones of one original tumor[13,28] and lead to the mistaken interpretation that clonally related tumor foci are unrelated.

Analyses of papillary thyroid cancers involving patterns of X-chromosome inactivation[29] and reports of unique clonal genetic alterations, such as *RET* and *NTRK1* gene rearrangements and *BRAF* mutations,[3,6-8,13,30,31] have established that many (and probably all) such tumors are monoclonal neoplasms. To investigate whether distinct tumors in multifocal papillary thyroid cancer arise independently, we compared the specific patterns of monoclonal X-chromosome inactivation of tumor foci in patients with this cancer.

METHODS

TUMOR SAMPLES

Tumor samples from women who underwent thyroidectomy for the treatment of papillary thyroid carcinoma were obtained in accordance with protocols approved by the institutional review board of each center. Patients provided informed consent as dictated by these protocols. Patients with multiple distinct foci of papillary thyroid cancer were selected for study, and identification of tumor tissue and normal tissue in each sample was determined by one endocrine pathologist. The sizes and locations of distinct tumor foci for all 10 patients suitable for analysis are shown in Table 1. Paraffin blocks were cut into 12-μm sections and placed on clean, uncoated microscope slides. Samples were deparaffinized in xylene and rehydrated in graded ethanol. Large tumors in which the tumor margins could easily be visualized were microdissected grossly from the slide, whereas small tumors or those with substantial inflammatory or stromal components were subjected to laser-capture microdissection (Pix Cell II, Arcturus) to capture an enriched population of neoplastic cells.

DNA EXTRACTION

Grossly dissected tissue was incubated in 100 to 200 μl of TE9 (0.5 M TRIS, 0.2 M EDTA, 0.01 M sodium chloride, and 1 percent sodium dodecyl sulfate; pH 9.0) plus 0.2 mg of proteinase K (Invitrogen) for four nights at 55°C. Fresh proteinase K was added daily. Laser-capture microdissection caps were incubated upside down for two nights at 37°C, centrifuged, and subjected to digestion for two additional days at 55°C. Fresh proteinase K was added daily. Chelex 100 resin (Bio-Rad) was added to each

Table 1. Specific Clonal X-Chromosome Patterns and Characteristics of Multifocal Papillary Thyroid Carcinomas.*

Patient No. and Tumor	Tumor Size	Location of Tumor in Thyroid	Unmethylated *HUMARA* Allele
	cm		
6			
6A	1.0	Left (adjacent)†	S
6B	1.8	Left (adjacent)†	S
6C	1.5	Right	S
7			
7A	2.2	Left	S
7B	0.6	Left	S
8			
8A	2.5	Left	L
8C	0.4	Right	S
9			
9A	2.1	Left	L
9B	1.5	Right	S
10			
10A	1.1	Isthmus	L
10B	0.8	Right lower	S
12			
12A	1.3	Left upper	S
12B	1.3	Left middle	L
14			
14A	1.6	Left	L
14B	1.0	Left	L
14C	0.6	Right	S
15			
15A	1.3	Left	L
15B	0.5	Right	L
16			
16A	1.4	Right	S
16B	1.1	Left	S
17			
17A	0.8	Right upper	L
17B	0.8	Left upper	L

* For each patient, two *HUMARA* alleles — a smaller allele (S) and a larger allele (L) — are distinguishable owing to variations in the number of CAG-repeat units. Every tumor focus shows a monoclonal pattern in which one of these alleles, representing the uniformly active X chromosome, is unmethylated and therefore selectively lost in the assay. The tumor foci in Patients 8, 9, 10, 12, and 14 showed an opposing pattern for the allele that was monoclonally activated and unmethylated.

† Two tumors that were in separate but abutting capsules were defined as adjacent.

sample and incubated for one hour, and the supernatant was removed. DNA was extracted with the use of phenol–chloroform and concentrated by means of ethanol precipitation. The DNA was resuspended in TRIS–EDTA (10 mM TRIS hydrochloride and 1 mM EDTA; pH 8.0).

HUMARA DIGESTION ASSAY

We used a polymerase-chain-reaction (PCR) assay for X-chromosome inactivation based on the X-linked human androgen receptor gene (*HUMARA*). In a monoclonal tumor from a woman, all tumor cells have the same combination of active and inactive X chromosomes. These maternally and paternally inherited chromosomes can be distinguished by polymorphisms — in this case a marked variation in the number of tandemly repeated CAG units within *HUMARA*. With the use of primers that flank the CAG repeats, two PCR products of different size can be amplified from a heterozygous patient's genomic DNA. The primers that flank the *HUMARA* CAG repeat sequence also flank a *Hpa*II restriction site (CCGG) that is methylated and thereby protected from digestion when present on an inactive X chromosome, but unmethylated and thereby susceptible to cleavage when on an active X chromosome. Therefore, when *Hpa*II-treated DNA is subjected to PCR with these *HUMARA* primers, most copies of the inactive allele remain intact and are amplified, whereas most copies of the active allele are cleaved by *Hpa*II and thus unable to yield a PCR product.

Most women in the general population are heterozygous at the polymorphic site and thus amenable to analysis, and the small size of the *HUMARA* region containing the polymorphism and the restriction site makes it possible to analyze fixed and paraffin-embedded tissue samples by means of PCR. In DNA from a monoclonal tumor, one of the *HUMARA* alleles is preferentially unmethylated and therefore lost, and clonally related metastases from such a tumor have the same pattern of X-chromosome inactivation as the primary tumor. If, however, multiple tumor foci arise independently, the maternal X chromosome will be inactivated in some foci and the paternal X will be inactivated in others.

Half the DNA from each sample was digested in a 20-μl reaction mixture with 12 U of *Hpa*II (New England Biolabs) at 37°C for 12 to 16 hours. The other half was subjected to mock digestion without the enzyme. After incubation, the restriction enzyme was inactivated at 95°C for 10 minutes.

A 50-μl PCR reaction mixture contained 1× PCR buffer (15 mM TRIS hydrochloride, pH 8.0, and 50 mM potassium chloride), 1.5 mM magnesium chloride, 200 μM of each deoxynucleotide triphosphate, 40 pmol of each primer, 2 U of Amplitaq Gold DNA Polymerase (Applied Biosystems), and 5.0 μl of the digested or mock-digested DNA. Primers used were 5′TCCTATGACACCATTTGGG3′ bearing a fluorescent TET tag on the 5′ end and 5′CTCTACGATGGGCTTGGGAGAAC3′. Thermocycling consisted of denaturation at 95°C for 10 minutes; 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 10 minutes. Samples underwent electrophoresis on an ABI Prism 377 Sequencer, and data were analyzed with the use of GeneScan and Genotyper software (Applied Biosystems).

To account for differences in gel loading and PCR-amplification efficiency, a ratio of the heights of the allele peaks in the tumor and the normal samples was calculated with the use of the following formula: (peak 1 height of undigested sample ÷ peak 2 height of undigested sample) ÷ (peak 1 height of digested sample ÷ peak 2 height of digested sample). A ratio of more than 2.0 or less than 0.5, representing a preferential loss of intensity in the digested sample of 50 percent of one of the two alleles present in the normal sample, was scored as a monoclonal pattern, and the unmethylated allele was noted.

SODIUM BISULFITE TREATMENT AND METHYLATION-SPECIFIC PCR

A methylation-specific PCR assay was also used to examine clonality at the *HUMARA* locus. Sodium bisulfite treatment of DNA converts unmethylated cytosine to uracil but does not affect methylated cytosine. This conversion results in a difference in the sequence of methylated and unmethylated alleles. In DNA from a monoclonal tumor, PCR amplification with the use of primer sets specific for these different nucleotides makes it possible to determine which of two *HUMARA* alleles, which differ in the number of CAG-repeat units, is methylated and which allele is unmethylated.

DNA was treated with sodium bisulfite overnight, purified with the use of the Wizard DNA Clean-Up System (Promega), and concentrated by means of ethanol precipitation according to the protocol of Frommer et al.[32] Two 50-μl PCR reaction mixtures containing 1× PCR buffer, 1.5 mM magnesium chloride, 200 μM of each deoxynucleotide triphosphate, 40 pmol of each primer, 2 U of Amplitaq Gold DNA Polymerase (Applied Biosystems), and 2 μl of sodium bisulfite–treated DNA were performed for each tumor DNA sample. In the first, a fluorescently labeled forward primer specific for the bisulfite-treated, methylated *HUMARA* DNA sequence was used, and in the second, a forward primer specific for the bisulfite-treated, unmethylated sequence was used. PCR primers were unmethylated forward primer 5′GGTTGTGAGTGTAGTATTTTTTGGT3′, methylated forward primer 5′CGAGCGTAGTATTTTTCGGC3′, and universal reverse primer 5′TAAAAAAAACCATCCTCACC3′.[33] Thermocycling consisted of denaturation for 95°C for 10 minutes; 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 10 minutes. Samples underwent electrophoresis on an ABI Prism 377 Sequencer, and data were analyzed with the use of GeneScan and Genotyper software (Applied Biosystems).

RESULTS

Samples of multifocal papillary thyroid carcinoma from 17 women who underwent thyroidectomy for the treatment of papillary cancer were analyzed. Slides containing paraffin-embedded sections from tumors were microdissected manually or with the use of laser-capture microdissection, depending on the size of the tumor and the amount of stromal and inflammatory components, to ensure that the specimen contained a predominance (typically more than 90 percent by means of visual inspection) of neoplastic cells. Tumor DNA was analyzed for X-chromosome–inactivation status with the use of sodium bisulfite treatment followed by methylation-specific PCR, digestion with methylation-specific restriction enzymes followed by PCR, or both. Seven tumors from five patients yielded interpretable results with both procedures and showed consistent agreement in the resulting patterns of X-chromosome inactivation.

Multiple foci of tumor from 10 patients yielded DNA of adequate quality and quantity and were heterozygous for the analyzed polymorphism in exon 1 of *HUMARA* and hence suitable for analysis (Table 1). These foci showed monoclonal patterns of X-chromosome inactivation consistent with previous findings of monoclonality in papillary thyroid cancer (and thus also confirmed that our dissection yielded a highly purified population of tumor

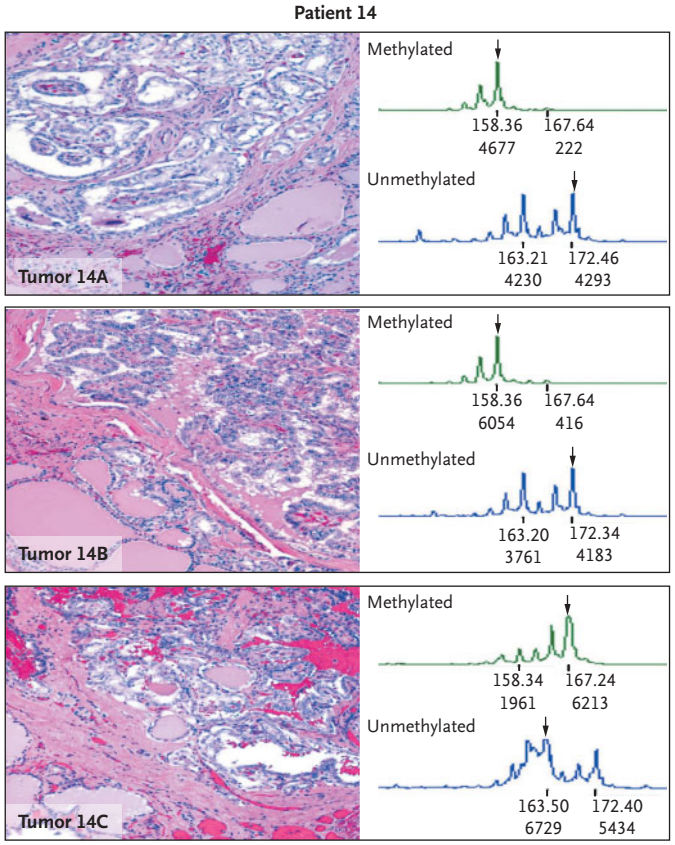

Figure 1. Discordant Patterns of X-Chromosome Inactivation in Distinct Tumor Foci from a Single Patient with Multifocal Papillary Thyroid Carcinoma, as Revealed by Methylation-Specific PCR.

Shown in the left-hand panels are photomicrographs (stained with hematoxylin and eosin) of three tumor foci from Patient 14. Even though the three discrete carcinomas have a similar microscopic appearance, they do not share an identical pattern of X-chromosome inactivation. For each tumor, the corresponding plot (right hand panels) is a quantitative representation of the size and amount of fluorescent PCR products amplified from tumor DNA when analyzed on an automated sequencer. Products are plotted from left to right, from smaller (representing alleles with fewer CAG repeats) to larger (alleles with greater numbers of CAG repeats). Because of the location of the primers, products are regularly 5 bp larger with the use of the unmethylated primers than they are with the use of the methylated primers. The height of the peaks corresponds to the amount of product present. Methylation-sensitive PCR analysis of the *HUMARA* gene shows that in tumors 14A and 14B, the smaller allele is methylated and therefore inactivated, whereas the larger allele is unmethylated. Tumor 14C shows the opposite pattern, with the larger allele methylated and the smaller allele unmethylated. Arrows indicate the methylated allele in the methylated-specific reaction or the unmethylated allele in the unmethylated-specific reaction. The numbers directly beneath the peaks (e.g., 158.36) indicate the estimated allele size (in base pairs); the number beneath each allele size is the corresponding peak height (e.g., 4677), as quantified by Genotyper software. The discordant patterns of X-chromosome inactivation indicate that tumor focus 14C originated independently from foci 14A and 14B. Foci 14A and 14B may also have separate origins and share a pattern of X-chromosome inactivation by chance (such concordance is expected in 50 percent of independently originating tumors), or they could be clonally related.

cells). When the patterns of X-chromosome inactivation were compared among foci from an individual patient, the foci from Patients 8, 9, 10, 12, and 14 had discordant patterns: in some, the maternal X chromosome was inactivated, and in others, it was the paternal X chromosome (Fig. 1 and 2). This finding is strong evidence that these patients' physically distinct papillary thyroid cancer foci arose as separate events from different clonal progenitor cells.

In the remaining five sets of samples (from Patients 6, 7, 15, 16, and 17), there were identical monoclonal patterns in each of two or three tumor foci. The interpretation of these findings is less definitive; although such findings are consistent with the presence of a shared clonal relationship, it is equally possible that the small number of foci in these five sample sets had separate clonal origins but happened by chance to share the same inactivated X chromosome. The ability of the assay to detect clonal independence in the latter five cases could have also been overridden by the highly skewed distribution of inactivated maternal and paternal X chromosomes normally found in some women or the possibility that large, contiguous patches of cells in some normal thyroid glands could have the same inactivated X chromosome owing to cell-migration patterns during organogenesis.[34]

DISCUSSION

The presence of multiple foci of papillary thyroid carcinoma is a common clinical finding, but the origin of these foci is unsettled.[25] They may be intraglandular metastases from a single primary tumor, or each tumor may arise from a distinct progenitor cell. Evidence from previous studies has lent support to both arguments.

Multifocal thyroid disease has been associated with distant metastases in some (but not all) studies,[14,16,17,19] suggesting that multifocal disease carries an increased risk of metastases. Iida et al. noted that many of the small foci are histologically identical to a larger cancer nodule in the same gland,[14] suggesting that the smaller tumors are metastases of the larger tumor. Another factor providing support for this possibility is that the thyroid has a unique lymphatic drainage system, with the two lobes and the isthmus enclosed in a capsule containing an abundant network of intralobular lymphatic vessels. The lymphatic vessels that arise between the thyroid follicles, anastomosing and penetrating into the capsule throughout the gland,[35]

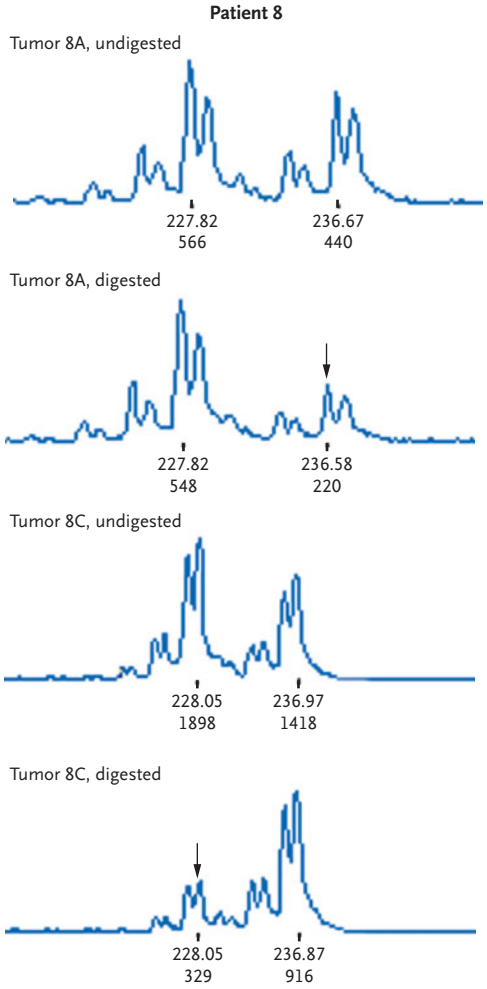

Figure 2. Distinct Tumors with Opposing Patterns of X-Chromosome Inactivation in a Single Patient, as Revealed by Methylation-Sensitive Enzyme Digestion.

The plots quantitatively represent the fluorescent PCR products amplified from digested tumor DNA from Patient 8 when analyzed on an automated sequencer. As expected, in tumor foci 8A and 8C, PCR amplification of the undigested DNA yields the same two alleles. In tumor focus 8A, digestion with the methylation-sensitive enzyme *Hpa*II selectively targets the unmethylated larger *HUMARA* allele (indicated by the arrow), whereas in tumor focus 8C, the unmethylated smaller allele is preferentially digested (arrow). The number directly beneath the peaks (e.g., 227.82) indicates the estimated allele size (in base pairs); the number beneath each allele size is the corresponding peak height (e.g., 566), as quantified by Genotyper software. These discordant patterns of X-chromosome inactivation indicate that the two tumors did not arise from a common progenitor and had independent origins.

would allow tumor metastases ready access to other parts of the gland.

That each tumor focus may have an independent origin was suggested in three cases described as "multinodular" papillary thyroid cancer that included an undifferentiated tumor[24] and by the finding of transcripts representing distinct *RET-PTC* rearrangements within such foci.[23] However, *RET* rearrangement may not always be an early, initiating event in sporadic papillary thyroid cancer.[13,28] That *RET* rearrangements can occur late in the evolution of established tumor clones is supported by the finding that several different *RET-PTC* transcripts can be present within a single tumor focus.[23,28] Thus, reported patterns of *RET* rearrangement have not been a definitive means of determining the origins of multifocal papillary thyroid cancer. Since inactivation of the X chromosome is independent of neoplastic selection and occurs before cell transformation, determination of X-chromosome inactivation can accurately determine whether tumor cells originate from a single precursor or multiple precursors.[24,36-39] Our results with methods based on inactivation of the X chromosome in well-characterized, multifocal papillary thyroid carcinomas favor the independent clonal origins of the distinct foci in some (and possibly most) of these cases.

The finding that multifocal tumors in papillary thyroid cancer have independent origins has implications for pathogenesis. Since neoplastic transformation is usually a rare event, it is unlikely that many cells within the same gland would undergo transformation independently without some predisposing influence, such as an environmental insult, a mutation, or polymorphisms. Exposure to radiation is one well-known predisposing factor,[40] but the frequent presence of multifocal papillary thyroid cancer in patients who have not been exposed to radiation suggests that there are other influences.

Our findings imply that any thyroid tissue remaining after surgery to treat papillary thyroid cancer in patients with multifocal disease may contain — or be likely to develop — additional foci of cancer that could become recurrences. Establishing that papillary-cancer foci may have independent origins provides theoretical support for the appropriateness of bilateral thyroidectomy and radioablation of remaining tissue.

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CORRECTION

Independent Clonal Origins of Distinct Tumor Foci in Multifocal Papillary Thyroid Carcinoma

Independent Clonal Origins of Distinct Tumor Foci in Multifocal Papillary Thyroid Carcinoma . On page 2409, in the left-hand column, line 8 of the first paragraph should have read ``5'TCTGTTCCAGAGCGTGCGCGAAGT3','' rather than ``5'TCCTATGACACCATTTGGG3','' as printed.

REVIEW ARTICLE

MECHANISMS OF DISEASE

Calpains and Disease

Mayana Zatz, Ph.D., and Alessandra Starling, Ph.D.

Calpains are Ca^{2+}-dependent cysteine proteases (proteolytic enzymes with cysteine in the catalytic site) that modulate cellular function. In humans, 14 independent genes encode members of the calpain superfamily. Some calpain proteases are confined to specific tissues, whereas others are ubiquitous. Tissue-specific calpains have been implicated in diabetes, cataracts, multiple sclerosis, cancer, Duchenne's muscular dystrophy, and Alzheimer's disease and are known to cause at least one disorder, autosomal recessive limb-girdle muscular dystrophy type 2A (LGMD2A). We will review recent findings on the role of the calpain superfamily in these disorders, focusing on LGMD2A, the most extensively characterized human calpainopathy.

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N Engl J Med 2005;352:2413-23.

PROPERTIES OF CALPAINS

Two members of the calpain superfamily, calpains 1 (μ-calpain) and 2 (m-calpain), have been extensively studied.[1] Each of these calpains, which differ in their sensitivity to Ca^{2+}, consists of two different polypeptide subunits. The larger subunit (80 kD) has catalytic activity, whereas the smaller, 30-kD, subunit has a regulatory function (Fig. 1).

The large subunits of μ-calpain and m-calpain are encoded by the *CAPN1* and *CAPN2* genes, respectively. Calpains 3, 8a, 9, 11, 12, and 13 also have 80-kD and 30-kD subunits. The 80-kD subunit has four domains: domain I is the N-terminal anchoring α-helix domain and is important for regulating the activity and dissociation of the subunit[2-5]; domain II, a catalytic domain, has two subdomains in the absence of Ca^{2+}[6,7]; domain III binds Ca^{2+} and phospholipids[8]; and domain IV, also called the penta–EF-hand domain (an EF-hand unit consists of two peptide helixes connected by a Ca^{2+}-binding loop), is important for dimer formation.[9] The 30-kD regulatory subunit of calpains 1, 2, and 9 consists of two domains: domain V is the N-terminal, glycine-rich, hydrophobic domain and domain VI the penta–EF-hand domain, which is similar to domain IV of the catalytic subunit.[6,7] The 80-kD subunit of the other calpains (3, 8a, 11, 12, and 13) do not interact with the 30-kD subunit, although they do have domain IV. Calpains 5, 6, 7, 8b, 10, and 15 are atypical calpains in that some of their domains have been deleted or replaced. They lack domain IV and therefore presumably do not associate with the 30-kD subunit.

There are two classes of calpains: one (comprising calpains 1, 2, 5, 7, 10, 13, and 15) is ubiquitous in cytosol; the other (comprising calpains 3, 6, 8, 9, 11, and 12) occurs only or mainly in certain tissues.[10] For example, calpain 8 is stomach-specific, and calpain 3 (CAPN3) is largely specific to skeletal muscle,[1,11] although it is also present in cardiac muscle and the liver.[12] The amino acid sequence of skeletal-muscle CAPN3 is similar to the sequences of the ubiquitous μ-calpain and m-calpain (which are structural variants that catalyze the same reaction), but it contains three specific insertion sequences.

The mechanisms by which calpains are activated and identify their protein targets are complex and poorly understood. Calpain activity is regulated by a ubiquitous spe-

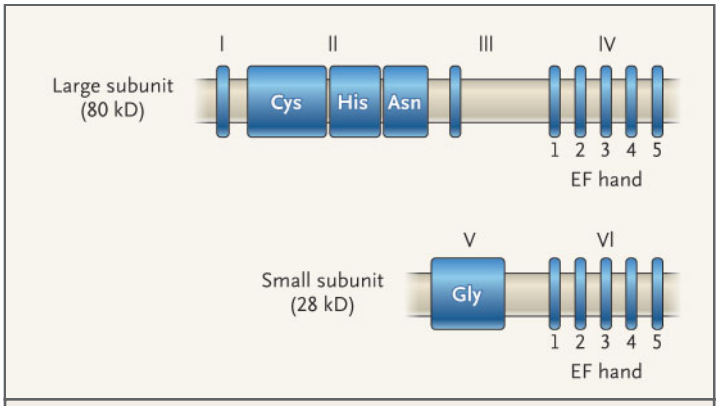

Figure 1. Schematic Representation of the Domain Structure of the Large and Small Subunits of m-Calpain.

μ-Calpain has a similar heterodimeric structure, but it differs in its sensitivity to Ca^{2+}. Adapted from a diagram provided by Dr. Hiroyuki Sorimachi, Laboratory of Biological Function, University of Tokyo, Tokyo.

cific inhibitor, calpastatin.[13,14] The calpain–calpastatin interaction is important in regulation of the activity of μ-calpain and m-calpain,[15] but the nature of this regulation in living cells is not understood. Studies using calpastatin have shown that calpains are clearly involved in some types of apoptosis in specific cell types and in response to certain apoptotic signals.[12]

An intricate strategy for the regulation of calpain activity seems necessary because calpain is an abundant cytoplasmic protease that can cleave many intracellular signaling and structural proteins. Membrane localization of calpains is an important mechanism for regulating their activity. In early work, it was thought that when a calpain binds to the plasma membrane, it is transformed from an inactive, proenzyme into an active, proteolytic enzyme by autolysis. However, other findings indicate that both μ-calpain and m-calpain are active proteolytic enzymes before autolysis and that interaction with a membrane may bind calpains to their substrates, rather than promote autolysis.[16-23]

Once activated on the membrane, the calpain presumably diffuses into the cytosol and becomes resistant to the inhibitory action of calpastatin.[24] Substrate proteins are digested by the activated calpain on the membrane or in the cytosol.[25] According to Gil-Parrado et al.,[26] calpain activity is regulated not only by calpastatin but also by differential intracellular localization. In contrast, dissociation of the subunits that constitute a calpain appears to be less critical to its regulation. Ca^{2+} levels required to initiate autolysis are as high as or even slightly higher than the levels required for proteolytic activity and are much greater than the free Ca^{2+} levels in living cells.[20,27,28] A solution to this paradox appeared when it was discovered that the presence of phospholipids, with phosphatidylinositol, lowered the Ca^{2+} levels required for autolysis of μ-calpain and m-calpain.[29,30] Other studies have shown that autolysis of these two calpains is an intermolecular process,[31] rather than an intramolecular process, as previously thought.[16]

μ-Calpain and m-calpain have diverse functions.[11,32] They catalyze the proteolysis of proteins involved in cytoskeletal remodeling, cell-cycle regulation, signal transduction, cell differentiation, apoptosis and necrosis, embryonic development, and vesicular trafficking.[3,4,32] For this reason, calpain activity has to be tightly regulated both temporarily and spatially to be effective and limited in scope.

CALPAIN-ASSOCIATED DISEASES

A number of pathologic conditions have been associated with disturbances of the calpain system. They include type 2 diabetes, cataracts, Duchenne's muscular dystrophy, Parkinson's disease, Alzheimer's disease, rheumatoid arthritis, ischemia, stroke and brain trauma, various platelet syndromes, hypertension, liver dysfunction, and some types of cancer (Table 1). Several oncogenes and tumor-suppressor gene products are substrates for members of the calpain family.[48] Mutations in the *CAPN3* gene cause LGMD2A, indicating that the protein that it encodes is important for muscle function.

LIMB-GIRDLE MUSCULAR DYSTROPHY TYPE 2A

LGMD2A (Online Mendelian Inheritance in Man number 253600) is one of the limb-girdle muscular dystrophies, a heterogeneous group of genetically determined progressive disorders of skeletal muscle. Seventeen genes responsible for various types of limb-girdle muscular dystrophy have been mapped,[49,50] and the products of most of them have been identified (Fig. 2). LGMD2A, the most prevalent form, accounts for at least 30 percent of all cases.

Molecular Mechanisms

LGMD2A is caused by mutations in the *CAPN3* (also called *p94*) gene,[51] which encodes CAPN3, the largely skeletal-muscle–specific member of the calpain superfamily. CAPN3, located both in the cytosol and in the nucleus, requires extremely low levels of Ca^{2+} for activation. In skeletal-muscle

Table 1. Examples of Pathologic Conditions That Have Been Associated with the Calpains.*

Diseases	Observations	References
Limb-girdle muscular dystrophy type 2A	This disease is associated with mutations in the gene encoding calpain 3 (*CAPN3*) and probable loss of CAPN3 proteolytic activity.	Ono et al.[33]
Gastric cancer	This type of cancer is associated with down-regulation of *CAPN9*.	Yoshikawa et al.[34]
Type 2 diabetes mellitus	Mutations in intron 3 of *CAPN10* are associated with an increased incidence of type 2 diabetes in some populations.	Horikawa et al.[35]
Duchenne's and Becker's muscular dystrophies	These dystrophies are associated with the absence or deficiency of dystrophin, a membrane-associated protein, resulting in an increased Ca^{2+} level in muscle, loss of Ca^{2+} homeostasis, and inappropriate calpain activity.	Tidball and Spencer[36]
Alzheimer's disease	There is an increased amount of m-calpain in the cytosolic but not the membranous fraction of the brain and in the neurofibrillary tangles of the brain.	Nixon and Mohan,[37] Tsuji et al.[38]
Cataract formation	Ca^{2+} influx activates m-calpain, the predominant calpain in the lens, cleaving α- and β-crystallins but not γ-crystallins; the crystallin fragments aggregate to form cataracts.	Shearer et al.[39]
Myocardial infarction	Ca^{2+} homeostasis is lost in ischemic areas, triggering inappropriate calpain activity; desmin and α-spectrin are degraded in ischemic heart tissue by synthetic calpain inhibitors; protein and mRNA levels of m-calpain and μ-calpain increase after myocardial infarction.	Papp et al.,[40] Sandmann et al.,[41] Tsuji et al.,[42] Yoshida et al.[43]
Multiple sclerosis	Levels of the 150-kD calpain-specific degradation product of α-spectrin increase 50% in human multiple sclerosis plaques; degradation of the 68-kD neurofilament protein is inhibited by a synthetic calpain inhibitor.	Shields et al.,[44] Banik et al.[45]
Obsessive–compulsive disorder	Erythrocytes from patients with obsessive–compulsive disorder have significantly higher calpain activities than normal controls, a finding that could not be attributed to differences in memory function.	Mundo et al.[46]
Neuronal ischemia (stroke)	Calpastatin is degraded by calpain to a membrane-bound 50-kD polypeptide in ischemic brain tissue; calpains participate in both apoptosis and necrosis in tissue damage in ischemic areas.	Blomgren et al.[47]

* Data are from a review by Goll et al.[12]

cells, CAPN3 binds specifically to certain regions of titin, a giant structural muscle protein; this binding seems to stabilize CAPN3 and thereby prevent its autolysis. Mutations in the *CAPN3* gene result in a cascade of events leading to CAPN3 deficiency and, eventually, to muscular dystrophy, but the underlying mechanisms are unknown.[12,52] Several studies have suggested that the loss of CAPN3 processing activity, but not its hyperactivation or a defect in its structure, causes LGMD2A.[53,54] According to other studies,[55] CAPN3 deficiency causes myonuclear apoptosis (i.e., death of muscle-cell nuclei) and profound perturbation of the IκBα–nuclear factor (nuclear factor of kappa light-chain gene enhancer in B cells inhibitor α) pathway, such that a failure in CAPN3-dependent IκBα proteolysis results in accumulation of IκBα in the cytoplasm and nucleus and sequestration of nuclear factor-κB in the cytoplasm, both of which culminate in the apoptosis of muscle cells.[55]

It is unlikely that an influx of extracellular Ca^{2+} into the sarcoplasm causes muscle fibers to degenerate, because CAPN3 is localized primarily in the myofibril and not on the sarcolemma.[13,56] Animal models and findings before the onset of muscular dystrophy in young carriers of *CAPN3* mutations suggest a role for CAPN3 in muscle maturation and sarcomere remodeling.[57-59] In vitro, CAPN3 cleaves filamin C, which is specific to muscle, and regulates its ability to interact with γ- and δ-sarcoglycans, two proteins of the dystrophin–glycoprotein complex (dystrophin anchors muscle membranes to actin filaments in myofibrils).[60] Since CAPN3 belongs to a large family of Ca^{2+}-dependent proteases, at least seven of which are expressed in muscle, the calpain superfamily could form a proteolytic network in muscle and other cells. In this way, a defect in CAPN3 could distort the calpain system, leading to overactivation of other calpains.[14]

Clinical Features

Patients with LGMD2A have symmetrical and selective involvement of proximal limb-girdle muscles. They have normal intelligence and no cardiac or facial disturbances. The disease shows wide intrafamilial and interfamilial clinical variability.[49,61-65]

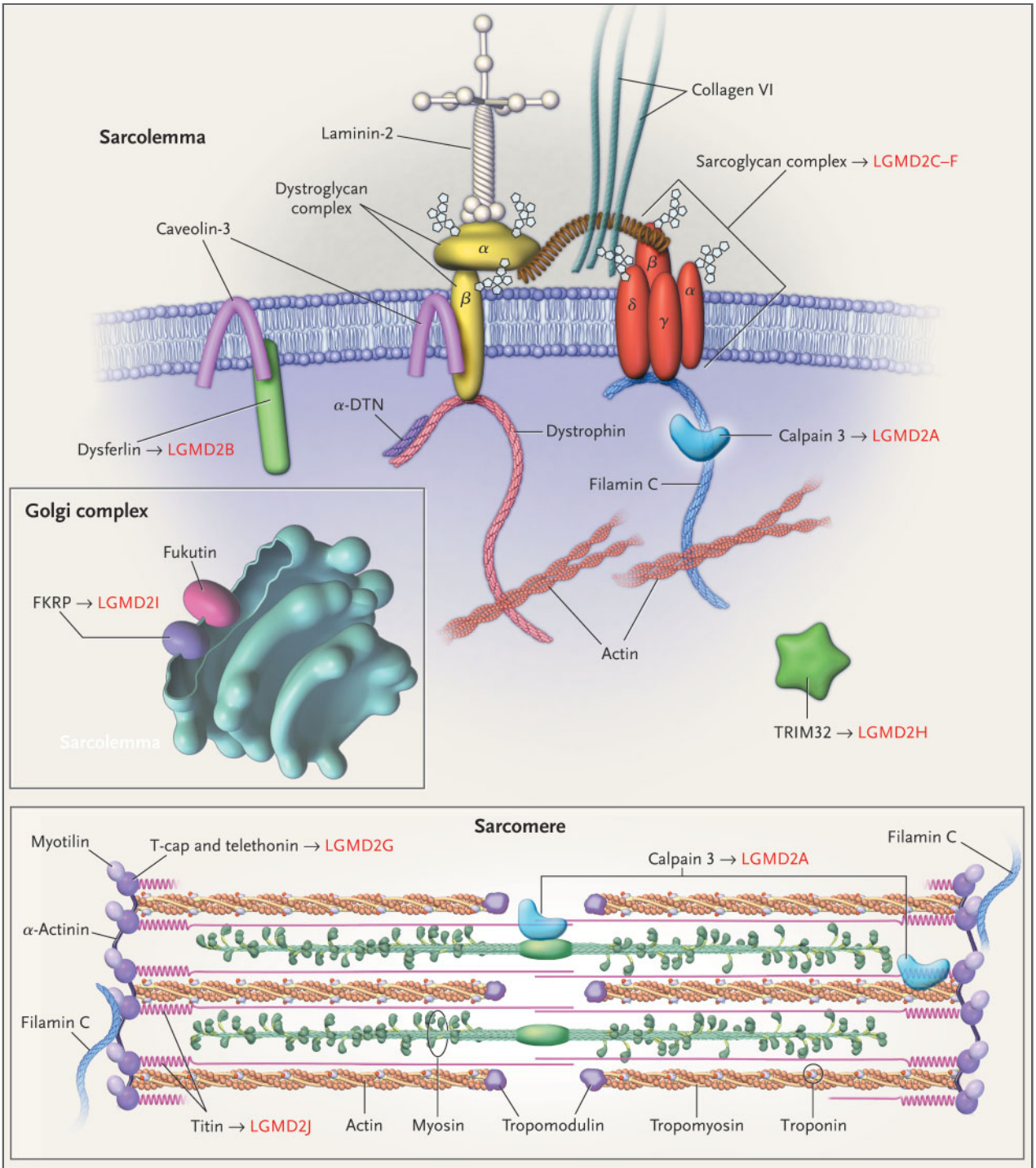

Figure 2. Schematic Representations of the Proteins Associated with Limb-Girdle Muscular Dystrophies (Top), of the Golgi Complex (Inset), and of the Structure of Muscle Fibers (Bottom).

LGMD2 denotes limb-girdle muscular dystrophy type 2, α-DTN α-dystrobrevin, FKRP fukutin-related protein, TRIM32 tripartite motif–containing protein 32, and T-cap titin-cap. The variants responsible for different subtypes of limb-girdle muscular dystrophy are shown in red. Adapted from a diagram provided by Peter Hackman and Bjarne Udd, Neurology Department, Vaasa Central Hospital, Vaasa, Finland.

Findings on examination of muscle-biopsy specimens are consistent with a dystrophic or myopathic process (Fig. 3). The serum level of creatine kinase is moderately or markedly increased, particularly in the active phase of the disorder.[42,66,67] However, we have seen patients with a normal serum level of creatine kinase or a neurogenic pattern on electromyography,[68] suggesting that the spectrum of variability in this calpainopathy might be broader than suspected and that a normal creatine kinase level should not be considered a reason to rule out a possible diagnosis of LGMD2A, even in ambulatory patients.

The age at onset ranges from 2 to 40 years, but the disease usually first appears in the second or third decade of life, with the development of proximal weakness in the lower limbs. Although early-onset cases are usually more severe, cases with a later onset may occasionally involve rapid progression and inability to walk by the third decade. Use of a wheelchair may become necessary during the second through fifth decades, but some patients may remain ambulatory after the sixth decade.[49,61,62,64] A more rapid progression in male patients than in female patients has been observed among Brazilian and Italian patients, an intriguing observation for which we have no explanation.[62,69]

CAPN3 Mutations, *CAPN3* Protein Analysis, and Diagnostic Applications

The *CAPN3* gene encompasses 24 exons and covers a 50-kb genomic region. It is expressed as a 3.5-kb transcript, which encodes a 94-kD translated protein (Fig. 3). More than 130 *CAPN3* mutations, with a distribution that varies according to ethnic group, have been identified to date (Leiden Database at http://www.dmd.nl/capn3_home.html)[61] (Fig. 4). Ten of 13 mutations found in Japanese patients were not found in other populations.[63] Others are prevalent in isolated communities (such as the Basque mutation, which is also the most prevalent in Brazil) as a result of a founder effect.[70,71] We also observed that 80 percent of the identified Brazilian mutations were concentrated in only six exons (Fig. 4). A similar result was recently found in an Italian study.[69]

Western blots of muscle CAPN3 in LGMD2A can show a total or partial deficiency of CAPN3 or, more rarely, no detectable CAPN3 (Fig. 3), with no direct correlation between the amount of CAPN3 and the severity of the phenotype. CAPN3 was detectable at very low levels or was undetectable in patients with LGMD2A whose disease ranged from

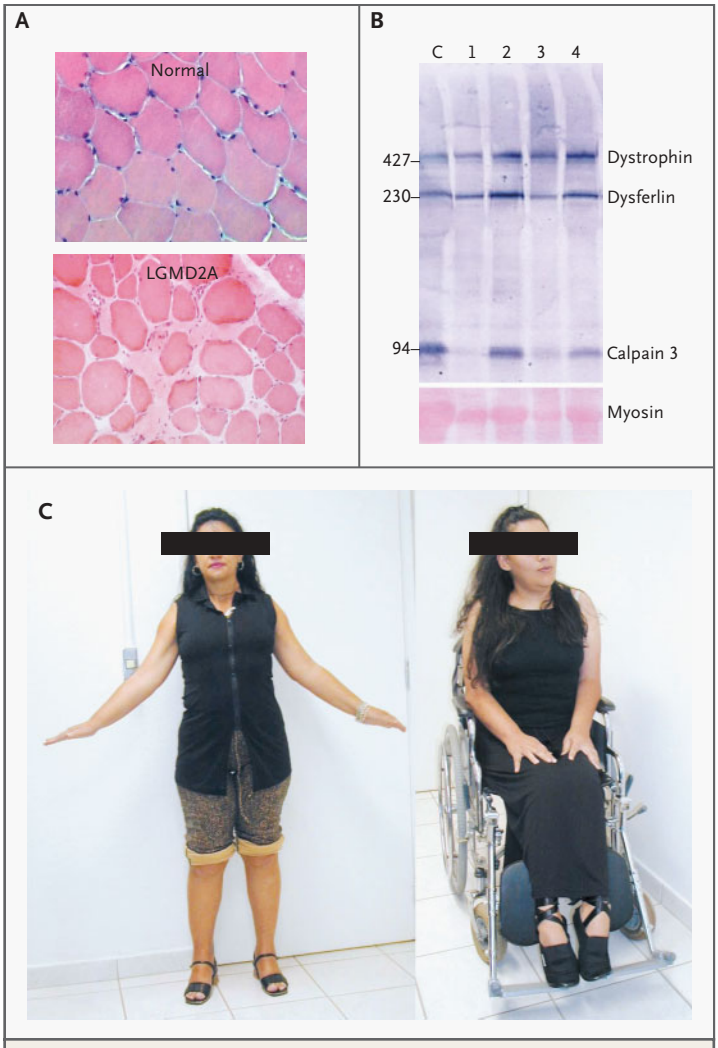

Figure 3. Histopathologic Features, Results of Muscle-Protein Analysis, and Clinical Characteristics in Limb-Girdle Muscular Dystrophy Type 2A.

Panel A shows the histologic features of normal muscle and muscle from a patient with limb-girdle muscular dystrophy type 2A (LGMD2A) and calpainopathy (hematoxylin–eosin stain). In the latter specimen, there is substantial variation in fiber size, with very large fibers surrounded by small groups of atrophic fibers, connective-tissue infiltration, and splitting. Panel B shows the results of multiplex Western blot analysis of the muscle proteins dystrophin, dysferlin, and calpain 3 from a normal control subject (lane C) and four unrelated patients with LGMD2A (lanes 1 through 4) (performed with monoclonal antibody Calp3C/12A2, which recognizes the 94-kD band of calpain 3). Myosin content served as a control for the amount of muscle protein loaded in the Ponceau-prestained blot. The blot shows almost complete absence of calpain 3 in Patient 1, partial deficiency in Patients 3 and 4, and a normal amount of calpain 3 in Patient 2; there was no clinical correlation with the severity of the phenotype. Image courtesy of Dr. Mariz Vainzof, Human Genome Center, Department of Biology, University of São Paulo, São Paulo. Panel C shows affected sisters homozygous for a mutation in *CAPN3* resulting in the replacement of arginine with glutamine at position 769 of the CAPN3 protein. The intrafamilial variability of LGMD2A is evident: the older sister (left) is still ambulatory at the age of 47 years, whereas the younger sister (right), who is currently 33 years of age, has used a wheelchair since the age of 18.

mild to severe,[64,72,73] whereas normal or almost normal 94-kD CAPN3 bands were found in 10 to 20 percent of patients with missense mutations in one or both alleles.[62,64,72] CAPN3 levels in muscle are normal in some forms of limb-girdle muscular dystrophy, such as sarcoglycanopathy (in which sarcoglycan, part of the dystrophin–glycoprotein complex, is affected) and telethoninopathy (which is a disorder of the Z-disk protein of sarcomeres).[73,74] However, a secondary reduction is seen in dysferlinopathy (in which dysferlin, which seems essential for maintaining the structural integrity of the muscle-fiber plasma membrane, is affected), suggesting a possible association between CAPN3 and dysferlin[73,75,76] as well as between CAPN3 and muscle proteins related to some other forms of limb-girdle muscular dystrophy, such as LGMD2I (also known as fukutin-related protein limb-girdle dystrophy)[77,78] and titinopathy (caused by mutations in the gene encoding titin).[79-82] CAPN3 may influence the role of titin in sarcomere formation through proteolytic cleavage, and some mutations that reduce CAPN3–titin interactions might destabilize and inactivate CAPN3 or remove it from its endogenous substrates.[59] In short, a normal amount of CAPN3 on Western blotting may be found in calpainopathies, whereas calpain may be reduced in amount or absent in other forms of LGMD, as a secondary effect. Therefore, for diagnostic purposes, muscle CAPN3 results should always be confirmed by mutation analysis, and screening for mutations should take into account the ethnic origin of affected patients.[62,63,69]

Genotype–Phenotype Correlation

Despite the marked clinical variability in LGMD2A, missense mutations are usually associated with a milder phenotype than are null mu-

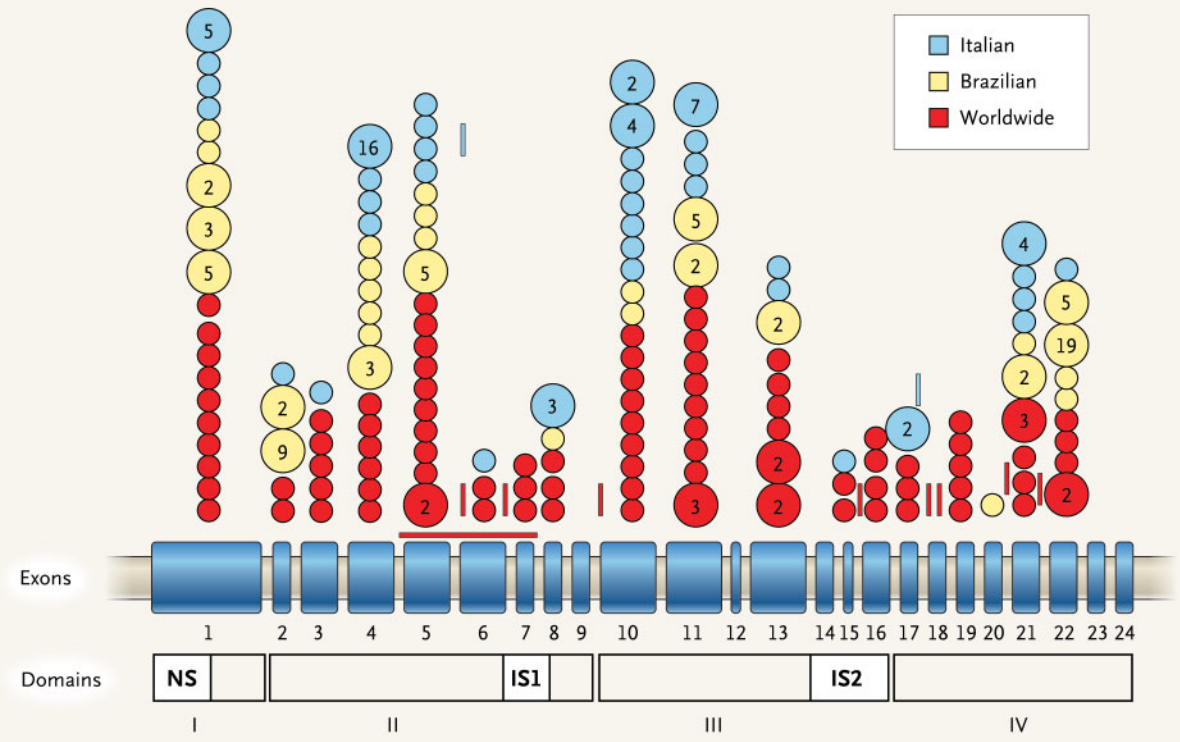

Figure 4. Distributions of *CAPN3* Gene Mutations Worldwide and in the Brazilian and Italian Populations.

Small circles represent mutations identified only once within the given population and large circles mutations identified more than once, with numbers indicating the number of mutations identified. For example, the Basque mutation (the replacement of AG at positions 2362 and 2363 in exon 22 by TCATCT) was found in 19 of 100 alleles from 50 patients with limb-girdle muscular dystrophy type 2A in Brazil. The horizontal lines represent deletions and the vertical lines insertions. NS denotes N-terminal insertion sequence, and IS insertion sequence.

tations.[61,64,66,68,72,83-85] Studies of patients with compound heterozygosity for missense or null mutations, however, showed that one null mutation is enough to result in a more severe phenotype.[62] Null mutations are more often associated with absence of muscle CAPN3, and missense mutations are more often associated with a partial deficiency,[61,62,64] although patients carrying the same null mutation may have total or partial CAPN3 deficiency.[62] Moreover, the clinical course in patients with apparently normal CAPN3 levels in muscle is not mild, suggesting that although the CAPN3 protein is present, its normal autocatalytic activity is lost. Moreover, the observation that patients with LGMD2A may have an apparently normal amount of calpain suggests that the occurrence of this calpainopathy might be underestimated by protein analysis.

Location of the Second CAPN3 Mutation

In about 10 percent of patients with LGMD2A, only one mutated allele is detectable,[61,62,64] suggesting that the second allele has mutations in noncoding regions. We recently identified a family with five affected siblings who carried an in-frame 3-bp deletion in one allele of *CAPN3*. The clinical course in these patients varied from mild to severe, but all of them had very high serum levels of creatine kinase (50 to 100 times the normal level). Muscle-biopsy specimens from two brothers had normal CAPN3 levels. Linkage analysis ruled out the possibility that the second mutation lay in the *CAPN3* gene. Subsequently, we identified a pathogenic mutation in another gene (*XK*) responsible for their clinical course (unpublished data). We are not aware of other patients carrying one mutation in *CAPN3* and a second nonallelic mutation, but the likelihood of finding a patient with two nonallelic or unrelated mutations (double heterozygotes) is at least of the same order of magnitude as the likelihood of finding compound heterozygotes, who are very frequent among patients with LGMD2A.[61,62,64]

TYPE 2 DIABETES

Type 2 diabetes affects more than 135 million people worldwide. Determining the genetic risk factors that increase susceptibility to type 2 diabetes will improve our understanding of the mechanism underlying this disorder and perhaps lead to better therapies. The association of the gene encoding calpain 10 (*CAPN10*), located at 2q37.3, with type 2 diabetes was initially reported by Horikawa and associates.[35] Among the known calpains, calpain 10 is atypical, containing a domain III–like structure in place of the usual domain IV (Fig. 1). So far, eight splice variants have been identified, among them three with no protease activity. The calpain 10 protein is ubiquitous in adult and fetal human tissues.[35]

A polymorphism within intron 3 of *CAPN10* affects its translation to messenger RNA (mRNA).[86] Decreased levels of calpain 10 mRNA were observed in association with the G/G genotype, leading to up-regulation of protein kinase C activity. Since down-regulation of this kinase is important for proper phosphorylation of insulin receptors,[87] this polymorphism could cause insulin resistance. Elevated free fatty acid levels are also associated with some variants of *CAPN10*.[88] Calpain 10 may have a role in the actin reorganization that is required for insulin-stimulated translocation of insulin-responsive glucose transporter 4 to the plasma membrane in adipocytes,[89] suggesting that there is a link between calpain 10 activity and type 2 diabetes.

More recently, Johnson and associates[90] investigated the role of calpain 10 in relation to the type 2 ryanodine receptor (RyR2), which is a Ca^{2+}-release channel on the sarcoplasmic reticulum that may be a central molecule in the control of programmed cell death. They suggested that calpain 10 mediates the death pathway of pancreatic beta cells and that RyR2 plays an essential role in suppressing this pathway. According to these investigators, a novel apoptosis pathway is initiated when Ca^{2+} flux through RyR2 is blocked. This finding clearly defines a physiologic role for calpain 10 within a specific tissue. Sato and coauthors[91] observed that the levels of *CAPN10* transcripts in the white cells of rats before and after the onset of type 2 diabetes were significantly lower than those in control animals. They suggest that *CAPN10* could be a potential candidate gene for predictive type 2 diabetes tests in humans.

ALZHEIMER'S DISEASE

The typical brain lesions in Alzheimer's disease are deposits of β-amyloid peptides in extracellular amyloid plaques and intracellular neurofibrillar tangles composed of the hyperphosphorylated microtubule-associated tau protein.[12] Calpains are not directly related to the production of β-amyloid peptides.[92] However, Ca^{2+} and m-calpain levels are elevated,[38,93,94] and autolysis of μ-calpain to its 76- and 78-kD forms is enhanced[95] in brain tissue from patients with Alzheimer's disease. The regulatory

protein p35, which aids in the development of neural tissue, is cleaved by calpains in brain tissue from patients with Alzheimer's disease into a 25-kD form (p25), activating cyclin-dependent kinase 5.[96] According to Selkoe,[97] p25 is responsible for the hyperphosphorylation of tau in the intracellular neurofibrillary tangles in the brain in Alzheimer's disease. This hyperphosphorylation makes tau highly resistant to degradation by μ-calpain.[98] Finally, because the neurofilament proteins are excellent substrates for the calpains, the calpains have an important role in the necrotic neuronal death that accompanies Alzheimer's disease.[12]

CATARACTS

More than 75 percent of cases of cataracts involve elevated levels of Ca^{2+}. The activation of calpains triggered by these high levels of Ca^{2+} suggests a mechanism for the formation of cataracts. Currently, five calpains have been found in the lens: calpain 1 (μ-calpain), which is expressed only at low levels[11]; Lp85, which is not yet well characterized[99]; and the major enzymes — m-calpain,[39] calpain 10,[100] and Lp82.[101] The role of the calpains in the development of cataracts in humans is not as well documented as it is in rats; in animal models of cataractogenesis, m-calpain appears to be the major calpain involved.[102] It is the only calpain active in human lenses, and human crystallins are substrates for m-calpain in vitro.

Cataract formation in the lenses of young rats is probably one of the best-documented examples of a relationship between inappropriate calpain activity and tissue lesions.[103] A wide variety of insults can cause a large influx of Ca^{2+} into the lens, with levels of Ca^{2+} high enough to trigger the proteolytic activity of m-calpains.[104] The m-calpain in the lens cleaves the N-terminal region of the lens proteins α-crystallin and β-crystallin. The truncated crystallin aggregates resist additional proteolysis and form cataracts, which scatter light. The understanding that calpains are involved in some types of human cataracts and their underlying pathogenic mechanisms will be important in the search for calpain inhibitors as anticataract agents, as recently suggested by Nakamura et al.[105]

CALPAIN INHIBITORS

More than 50 endogenous and exogenous inhibitors of the calpains have been described. They include cellular and extracellular proteins and drugs such as iodoacetate, iodoacetamide, and N-ethylmaleimide, which are inhibitors of cysteine proteases.[12,106,107] The intracellular level of calpastatin correlates directly with calpain activation,[108] and the affinity of calpastatin for the activated forms of the calpains is greater than its affinity for the proenzyme,[109] indicating that both structural and conformational changes due to autolysis favor formation of the enzyme–inhibitor complex. The inhibitory efficiency of calpastatin and its level are important in the prevention of calpain-mediated lesions. Phosphorylation of calpastatin may alter its efficiency and specificity in isolated cells and skeletal muscle.[110-112] This potential inhibitor has possible therapeutic applications in diseases involving the calpains.[12,113]

Intramuscular administration of the synthetic calpain inhibitor leupeptin to dystrophic mdx mice apparently prevents decreases in muscle-fiber diameter, suggesting that this protease inhibitor could prevent the loss of muscle mass in dystrophic mice.[114] In rats, leupeptin, calpain inhibitor 1, and caspase 3 inhibitors reduce infarct size and postischemic apoptosis in hearts without modifying contractile recovery.[115] Other studies suggest that treatment with leupeptin may rescue motor neurons from cell death and improve muscle function after nerve injury.[116,117] The possibility that calpain inhibitors can restore cognition and synaptic transmission in transgenic models of Alzheimer's disease is being tested.[113]

Further elucidation of the roles of members of the calpain superfamily and of potential calpain inhibitors will be important for delineating the various approaches to be used in the treatment of diseases related to these proteins.

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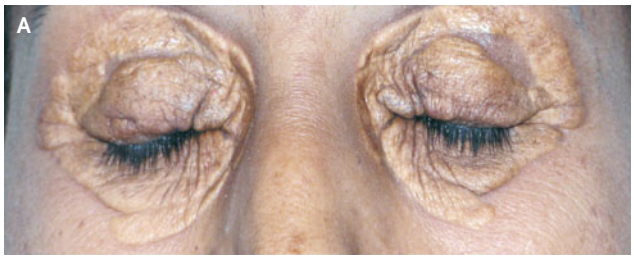
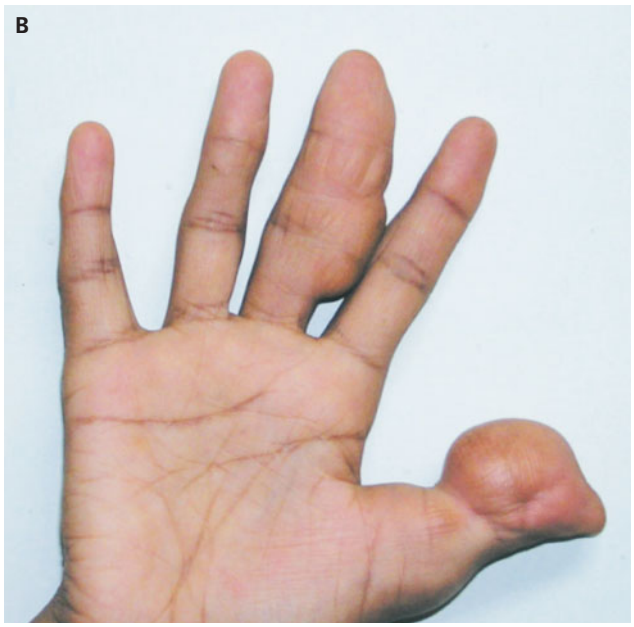
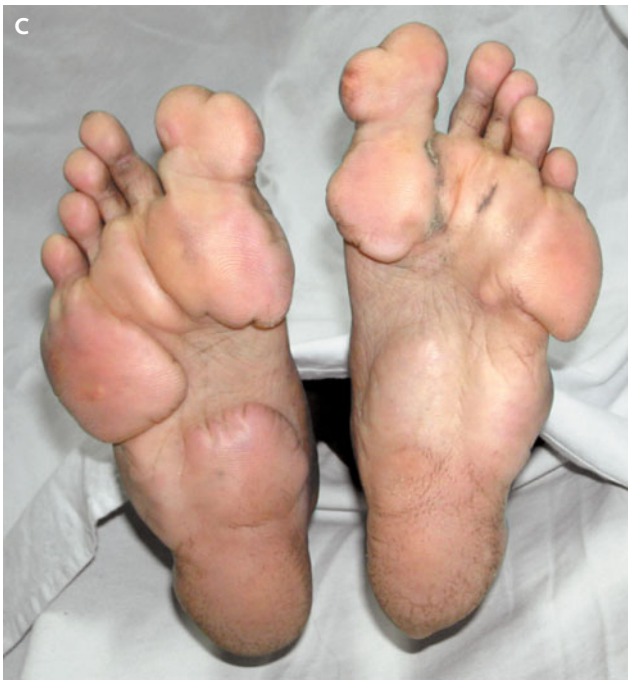
IMAGES IN CLINICAL MEDICINE

Xanthomatous Pseudospectacles in Familial Hypercholesterolemia







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A 40-year-old woman presented with exertional chest pain and was found to have familial hypercholesterolemia (type IIa) and severe three-vessel coronary artery disease. Xanthoma palpebrarum was noted on physical examination, manifested as pseudospectacles (Panel A). The patient also had associated tuberous xanthomas of tumorous proportion on the buttocks, hands, and feet (Panels B and C). Her lipid profile revealed the following levels: total cholesterol, 550 mg per deciliter (14.22 mmol per liter); triglycerides, 180 mg per deciliter (2.03 mmol per liter); high-density lipoprotein cholesterol, 34 mg per deciliter (0.88 mmol per liter); and low-density lipoprotein cholesterol, 480 mg per deciliter (12.41 mmol per liter).

Xanthomas are an important manifestation of altered lipid metabolism. Morbidity and mortality that are associated with the condition are related to atherosclerotic disease. Tuberous xanthomas are small, firm, painless nodules of a reddish yellow color that can coalesce to form multilobated tumors. They usually develop in pressure areas and are associated with increased levels of low-density lipoprotein cholesterol.

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CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL

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Case 17-2005: A 22-Year-Old Woman with Back and Leg Pain and Respiratory Failure

Benjamin D. Medoff, M.D., Jo-Anne O. Shepard, M.D., R. Neal Smith, M.D., and Alexander Kratz, M.D., Ph.D.

PRESENTATION OF CASE

A 22-year-old black woman was transferred to this hospital because of respiratory failure.

A diagnosis of sickle cell anemia had been made when the patient was a child, at six years of age. She had last been hospitalized for a sickle cell crisis 12 years earlier. One month before admission, she had bilateral arm pain after jogging and went to a local hospital. A chest radiographic study obtained with a portable unit showed no abnormalities except for bibasilar hazy opacities, which were thought to be due to overlying soft tissues. Laboratory test results are shown in Table 1. Morphine sulfate, hydrocodone, acetaminophen, and fluids were administered, and the symptoms resolved.

Six days before admission, the patient fell backward while ice-skating. She did not have head trauma. The day after the fall, she had mild nausea and a headache that was relieved by ibuprofen. Two days before admission, while shopping in the frozen-food section of a grocery, she experienced low back pain that radiated to her thighs and the back of her knees. The pain was constant and interfered with walking. She took ibuprofen that evening, which provided some relief. At 2 a.m., she awoke with increased pain and, four hours later, was seen in the emergency room of another hospital.

In the emergency room, the patient reported severe pain (10 on a scale of 1 to 10, with 1 being the lowest level of pain and 10 the highest) in her back, thighs, and knees. She was nauseated and had vomited once; she did not have chest pain, shortness of breath, fever, chills, abdominal pain, dysuria, constipation, or diarrhea.

The patient was a student and resided with her parents in the southern United States. She was working in New England for the summer. She was not sexually active, occasionally drank alcohol, and did not smoke. She had no allergies and took folic acid (1 mg per day). Her parents both carried the sickle cell trait.

On physical examination, she was alert, oriented, and breathing normally. The blood pressure was 132/78 mm Hg, the pulse 116 beats per minute, the respiratory rate 20 breaths per minute, and the temperature 36.1°C. The oxygen saturation was 100 percent while the patient was breathing two liters of oxygen by nasal cannula. The lungs were clear, and the remainder of the examination revealed no abnormalities. The urine was clear, and testing showed a pH of 5 and a specific gravity of 1.015. The urine was pos-

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itive for urobilinogen (1+); there were 0 to 2 white cells and rare red cells per high-power field. Other laboratory values are shown in Tables 1 and 2. A chest radiographic study showed opacities in the left lower lobe of the lungs.

Treatment consisting of intravenous meperidine hydrochloride, diphenhydramine, morphine sulfate, ketorolac tromethamine, and fentanyl was administered. The patient received 2 liters of normal saline, and a third liter was begun. After three hours, the oxygen saturation decreased to 98 percent, and oxygen was increased to 3 liters by nasal cannula. She continued to have severe pain.

Five and a half hours after admission, the oxygen saturation decreased to 76 percent with a respiratory rate of 24 breaths per minute. Blood gas results are shown in Table 3. The lungs were clear. Oxygen (100 percent) was administered to the patient with a nonrebreathing mask, and 25 minutes later the respiratory rate was 14 breaths per minute and oxygen saturation 100 percent. Over the course of the next hour, she reported a need to cough, and the respiratory rate increased to 45 breaths per minute; rales were heard bilaterally, and the oxygen saturation decreased to 73 percent. A chest radiograph revealed increased interstitial markings and an enlarged cardiac silhouette, as compared with the results of the radiograph obtained 10 hours earlier. Continuous positive airway pressure was begun at 7.5 cm of water. Oxygen saturation improved to 98 percent. Furosemide and additional morphine sulfate were given. Specimens of blood and urine were sent for culture, and 1 unit of packed red cells was transfused.

Ten hours after the patient presented, the oxygen saturation decreased to 40 percent and the trachea was intubated; vomitus was noted in the trachea during the procedure. She was transferred to the intensive care unit. A chest radiograph obtained after intubation revealed the endotracheal tube in proper position; an infiltrate was present in the right lung. An attempt at placement of a Swan–Ganz line was unsuccessful. A second unit of packed red cells was transfused, and ceftriaxone, clindamycin, gentamicin, and hydrocortisone were administered. Over the next three hours her respiratory status continued to deteriorate (Table 3). She was placed in the prone position, sedated and paralyzed with vecuronium, with ventilation settings set at pressure control ventilation of 25 cm of water, at 100 percent oxygen, tidal volume of 500 to 550 cc, positive end-expiratory pressure (PEEP) of 22 cm of water, peak inspiratory pressure of 47 cm of water, and a respiratory rate of 20 breaths per minute. Over the next eight hours, the oxygen saturation ranged from 95 to 97 percent. Transport to this hospital was arranged for the patient.

She was admitted to the medical intensive care unit of this hospital 23.5 hours after her presentation to the other hospital. She remained sedated and paralyzed in the prone position. The temperature was 35.7°C, the blood pressure 155/93 mm Hg, the pulse 130 beats per minute, the respiratory rate set at 24 breaths per minute, and the mean arterial pressure 116 mm Hg. There was no scleral icterus, the neck was supple, and coarse breathing sounds were heard bilaterally without crackles. No heart murmur, rub, or gallop was heard. Laboratory tests

Table 1. Hematologic Laboratory Values.*

Variable	Six Months before Admission	One Month before Admission	First Hospital			This Hospital		
			0.5 hr	7 hr	17 hr	23.5 hr (on Admission)	27.5 hr	31 hr
Hematocrit (%)	29.9	26.3	16.9	16.9	26.6	22.6		
Hemoglobin (g/dl)	10.1	9.3	5.6	5.7	8.4	8.4		
White cells (per mm³)	8600	15,400	17,200	20,970		6300		
Hemoglobin A (%)							52.3	73.6
Hemoglobin S (%)							35.6	19.8
Hemoglobin A_2 (%)							1.9	2.4
Hemoglobin F (%)							10.2	4.2

* All time periods are relative to the patient's admission to the first hospital. She received a transfusion at 17 hours after the first admission and an exchange transfusion of 5 units of red cells at 31 hours.

results are in Tables 1, 2, and 3. A chest radiograph obtained with a portable device showed endotracheal and esophagogastric tubes in place and bilateral patchy airspace opacities. Specimens of blood, sputum, nasal swab, and stool were sent for culture, and ceftriaxone, azithromycin, and vancomycin were administered. Nitric oxide treatment was begun. A pulmonary arterial line was placed; the mean pulmonary-artery pressure was 46 mm Hg, and the pulmonary-artery occlusion pressure was 18 mm Hg.

Four hours after admission, the temperature was 36.1°C, blood pressure 110/66 mm Hg, pulse 117 beats per minute, pulmonary-artery pressure 58/38 mm Hg, central venous pressure 17 mm Hg, and pulmonary-artery occlusion pressure 18 mm Hg. The results of hemoglobin electrophoresis are shown in Table 1. An echocardiogram revealed an estimated left ventricular ejection fraction of 52 percent, mild pulmonary insufficiency, and an estimated right ventricular systolic pressure of 54 mm Hg. The right ventricle was dilated, and the interatrial septum was stretched and displaced to the left, which was consistent with right atrial volume overload.

Eight hours after admission, an automated exchange transfusion of 5 units of packed red cells was performed. The results of repeated hemoglobin electrophoresis are shown in Table 1. Computed tomography of the brain showed opacified sinuses

Table 2. Chemistry Laboratory Values.*

Variable	First Hospital		This Hospital	
	0.5 hr	15 hr	23.5 hr (on Admission)	43 hr
Glucose (mg/dl)	138		165	176
Sodium (mmol/liter)	133		134	132
Potassium (mmol/liter)	3.7		3.9	5.6
Chloride (mmol/liter)	100		111	111
Carbon dioxide (mmol/liter)	26		21.5	19.5
Urea nitrogen (mg/dl)	4		11	23
Creatinine (mg/dl)	0.6		0.7	1.8
Creatine kinase (U/liter)	198	209	305	
Alkaline phosphatase (U/liter)	98		329	1114
Aspartate aminotransferase (U/liter)	69		108	180
Alanine aminotransferase (U/liter)	85		64	70
Lactate dehydrogenase (U/liter)		1147		2970
Total bilirubin (mg/dl)	1.0		1.7	6.5
Direct bilirubin (mg/dl)	0.3		0.8	3.3
Protein (g/dl)	7.5		5.7	5.1
Albumin	4.0		2.5	1.9
Globulin			3.2	3.2

* All time periods are relative to the patient's admission to the first hospital. To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for urea nitrogen to millimoles per liter, multiply by 0.357. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for total and direct bilirubin to micromoles per liter, multiply by 17.1.

Table 3. Arterial Blood Gas Values.*

Variable	First Hospital			This Hospital				
	5.5 hr	13 hr	22.5 hr	23.5 hr (on Admission)	25.5 hr	36.5 hr	40.5 hr	44 hr
pH	7.34	7.39	7.39	7.35	7.31	7.24	7.19	7.14
Fraction of inspired oxygen	3 liters by cannula	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oxygen saturation (%)	76	87	95	94	100	90	81	78
Partial pressure of oxygen (mm Hg)	59	52	62	80	65	63	61	47
Partial pressure of carbon dioxide (mm Hg)	44	40	38	44	47	54	55	61
Bicarbonate (meq/liter)	23	24	22				20	20
Positive end-expiratory pressure (cm of water)		22	22	22	22	20	28	28
Peak inspiratory pressure (cm of water)		47	47	47	42	40		
Nitric oxide (parts per million)					20	20	20	40
Osmolality (mOsm/kg)				282				
Base deficit	1.9	0.5						
Tidal volume (cc)		500		350	390	360	400	420

* All time periods are relative to the patient's admission to the first hospital.

and mild soft-tissue edema, with no intracranial hemorrhage or infarction. The results of screening panels for toxins were negative. Over the next 12 hours, the patient's respiratory status continued to deteriorate (Table 3). The results of laboratory tests performed during this time in her hospital stay are shown in Table 2. Twenty hours after her admission to this hospital, 2 units of 5 percent albumin were administered. Ninety minutes later, cannulation for extracorporeal membrane oxygenation was initiated at the bedside. During the procedure, the patient's blood pressure dropped, transiently responded to the administration of norepinephrine and vasopressin, and then became unresponsive to maximal treatment with norepinephrine, vasopressin, phenylephrine, dopamine, epinephrine, and atropine. Cardiopulmonary resuscitation was unsuccessful, and the patient was pronounced dead 23 hours after her admission.

An autopsy was performed.

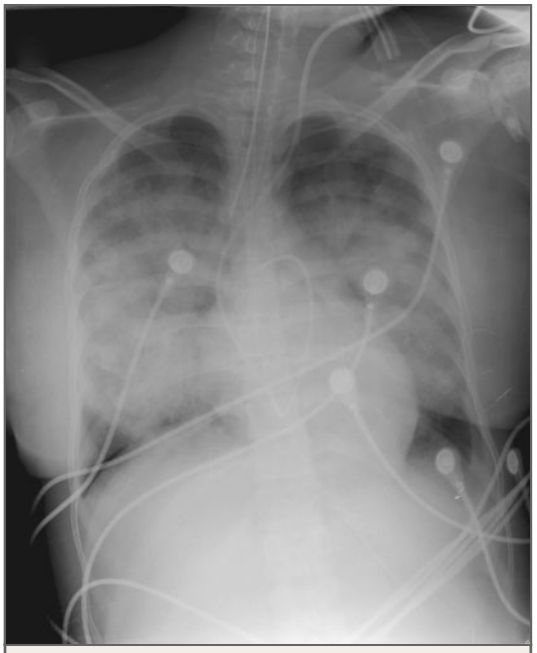

Figure 1. Image Obtained from Portable Chest Radiography.

The bilateral patchy opacities are consistent with pneumonia, edema, or hemorrhage. An endotracheal tube, nasogastric tube, and Swan–Ganz catheter are in place.

DIFFERENTIAL DIAGNOSIS

Dr. Benjamin D. Medoff: May we review the radiology studies?

Dr. Jo-Anne O. Shepard: A chest radiograph obtained on admission to this hospital with a portable device (Fig. 1) shows an endotracheal tube in appropriate position and a nasogastric tube in the stomach. There were bilateral, confluent air-space opacities throughout both lungs in a symmetric fashion that might have represented edema, pneumonia, or pulmonary hemorrhage. There were no pleural effusions. The cardiac silhouette was at the upper limit of normal.

Dr. Medoff: This is a tragic case of a young woman with sickle cell anemia who presented with severe acute respiratory distress syndrome (ARDS). I was peripherally involved in the care of this patient and aware of the final diagnosis. In this discussion, I will focus on the pathophysiology of her disease and the management of catastrophic respiratory failure, especially in the setting of sickle cell anemia.

ARDS

This patient had acute respiratory failure with features that met the consensus criteria for ARDS: bilateral infiltrates on chest radiography, severe hypoxemia (defined by a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen that is less than or equal to 200), and no sign of left atrial hypertension.[1] ARDS occurs when a severe injury to the lung results in damage to the alveolar epithelial and endothelial cell barriers, leading to leakage of fluid into the air spaces, followed by surfactant dysfunction and inflammation.

Although ARDS is most often seen in association with infections and trauma, it can result from numerous uncommon causes that may necessitate specific management strategies (Table 4).[2] One such situation is ARDS in patients with sickle cell anemia, called the acute chest syndrome. The management of this syndrome has unique aspects.

THE ACUTE CHEST SYNDROME

The acute chest syndrome is a life-threatening disorder that is the leading cause of death in people with sickle cell anemia. It is defined as the presence of a new pulmonary infiltrate on chest radiographs, with chest pain, fever, cough, dyspnea, or an elevated white-cell count in a patient with sickle cell anemia. The patient we are discussing today clearly meets these criteria. The acute chest syndrome occurs in the majority of patients with sickle cell disease at least once during their lives and is the second most common cause of hospital admission after painful vaso-occlusive crises.[3-5]

The acute chest syndrome is the result of the occlusion of pulmonary vessels by sickled red cells. Since hypoxemia is the chief stimulus for polymerization of hemoglobin S, lung disease of any type poses a particular threat to the patient with sickle cell anemia. Furthermore, vascular occlusion in the lung leads to hypoxemia and more sickling of red cells, setting up a vicious circle that can dramatically amplify the extent and severity of the crisis.[4] This was probably the scenario in the case of this patient, in whom mild disease escalated rapidly to fulminant respiratory failure.

There are four major precipitants of the acute chest syndrome: infection, bone marrow emboli, thromboembolism, and atelectasis. In this case, since the patient's initial symptom was severe leg pain, rather than dyspnea or cough, bone marrow emboli to the lungs from the release of infarcted marrow into the blood are the most likely cause of the ARDS. In addition, atelectasis, which often develops as a result of the hypoventilation that accompanies rib pain and the use of opiates in patients with vaso-occlusive crises, could have contributed. Pulmonary thromboembolism was also considered; however, this tends to occur with acute hemodynamic collapse in patients with sickle cell anemia since many have chronic pulmonary hypertension.[6,7] This patient's final cardiac arrest was probably due to progressive right ventricular failure, secondary to pulmonary hypertension from the ARDS; however, there could also have been a late thromboembolism.

MANAGEMENT OF ARDS

Basic Principles

The mainstay of treatment for patients with ARDS from any cause is supportive care and mechanical ventilation.[1] Although the ventilator can be lifesaving, it has been shown to be a source of further lung injury in patients with ARDS — so-called ventilator-associated lung injury. The lung collapse that occurs in ARDS reduces the volume of lung available for ventilation, so that the use of normal tidal volumes overdistends the ventilated regions of the lung, causing injury. In addition, the collapsed regions may be exposed to shear forces when adjacent regions of open lung are stretched during tidal ventilation. On the basis of these concepts, currently used management strategies include low-tidal-volume ventilation, limitation of the fraction of inspired oxygen, and lung recruitment with PEEP, all of which were implemented in this patient when she arrived at this hospital.[8]

Table 4. Uncommon Causes of the Acute Respiratory Distress Syndrome.*

Diagnosis	Specific Treatment
Occult infections	Specific antimicrobial therapy
Chlamydia	Corticosteroids for SARS
Cytomegalovirus	
Leptospirosis	
Mycoplasma	
Pneumocystis	
SARS	
Tuberculosis	
Tularemia	
Vasculitis, capillaritis, or diffuse alveolar hemorrhage	High-dose corticosteroids
Antineutrophil cytoplasmic antibody–positive vasculitis	Cytotoxic therapy (cyclophosphamide)
Antiphospholipid-antibody syndrome	
Cryoglobulinemia	
Systemic lupus erythematosus	
Idiopathic inflammation	High-dose corticosteroids
Accelerated usual interstitial pneumonitis	
Acute bronchiolitis obliterans with organizing pneumonia	
Acute eosinophilic pneumonia	
Acute interstitial pneumonia	
Hypersensitivity pneumonitis	
Drug reactions	Supportive therapy
Allergic (eosinophilic) pneumonia	Corticosteroids
Bleomycin toxicity (with oxygen exposure)	
Cocaine or heroin inhalation	
Gemcitabine	
Interleukin-2 therapy	
Transretinoic acid syndrome	
Miscellaneous	Supportive care
Acute chest syndrome	Whole-lung lavage for PAP
Amniotic-fluid embolism	
Exposures (nitrous oxide, paraquat, chlorine gas)	
Fat emboli	
Ovarian hyperstimulation	
PAP	
Transfusion-related acute lung injury	

* SARS denotes severe acute respiratory syndrome, and PAP pulmonary alveolar proteinosis.

Management of Catastrophic ARDS

The most dramatic aspect of this case was the refractory nature of the patient's hypoxemia. In general, I consider cases of ARDS catastrophic when the partial pressure of arterial oxygen remains less than 80 mm Hg despite 100 percent inspired oxygen and PEEP that is greater than 15 cm of water. Although recent strides have been made in our understanding of the management of patients with ARDS,[8] cases like this one fall outside the norm and often require therapies that have minimal data to support their effectiveness. When one is considering treatment for this type of patient, maintaining adequate oxygenation is a matter of life or death, and the risk of ventilator-associated lung injury may have to be ignored. In Table 5, I have listed some

Table 5. Causes of Hypoxemia and Specific Therapies.

Cause of Hypoxemia	Treatment
Hypercarbia	Reduce production Sedation or paralysis or both Cooling Increase clearance Paralysis* Tracheal gas insufflation High-frequency oscillation
Poor ventilation-to-perfusion matching	Positive end-expiratory pressure or recruitment maneuvers Nitric oxide Prone position Paralysis* Extracorporeal membrane oxygenation
Shunt	Positive end-expiratory pressure or recruitment maneuvers Nitric oxide Prone position Paralysis* High-frequency oscillation Extracorporeal membrane oxygenation
Decreased oxygen diffusion	Diuresis under guidance from pulmonary-artery catheter

* Paralysis allows control of the ventilatory cycle by a mechanical ventilator to manipulate ventilatory pattern.

salvage therapies, grouped according to the physiological causes of hypoxemia. I will discuss several of the specific therapies attempted in this case.

Lung Recruitment

ARDS is characterized by areas of edematous lung that are collapsed. These, in turn, create multiple areas of shunting, in which blood flows through areas of lung with minimal or no gas exchange. Recruitment — the opening up — of collapsed regions of the lungs should reduce such shunting and improve oxygenation. In this patient, recruitment of collapsed lung was attempted with use of a combination of PEEP, recruitment maneuvers, and prone positioning. PEEP works by preventing the lung from collapsing during the exhalation phase of mechanical ventilation[9]; however, delivery of a high-pressure (30 to 40 cm of water), prolonged breath (40 to 120 seconds) with the ventilator (the so-called recruitment maneuver) may be required to open up severely affected lungs.[10,11]

Recruitment maneuvers can have dramatic effects on oxygenation in carefully selected patients,[12,13] but there are no data that show improved outcomes with their use.[14] At this hospital, we use recruitment maneuvers in patients who have persistent hypoxemia despite the use of an elevated PEEP level (10 to 15 cm of water). In this case, we performed a recruitment maneuver (the use of ventilation at 40 cm of water pressure for 40 seconds), but there was no improvement in the patient's oxygenation. Turning a patient to the prone position can also frequently result in improved oxygenation, presumably by improving ventilation-to-perfusion matching and recruiting dependent areas of the lung. However, a randomized trial did not show that this approach led to improvements in mortality.[15] A subgroup analysis showed benefit in the most severely ill patients, suggesting that this maneuver may be useful as a salvage therapy for catastrophic ARDS.[16] Unfortunately, there was no benefit in this patient.

Nitric Oxide

Nitric oxide is a potent vasodilator that, when inhaled, causes pulmonary vasodilation. Since the gas is distributed in the areas of the lung with the most ventilation, it should lead to preferential blood flow to the well-ventilated regions, leading to an increase in ventilation-to-perfusion matching.[17] Several trials of nitric oxide in ARDS have shown that it can increase oxygenation in the short term, but they have not shown any effect on mortality or the number of ventilator-free days.[18,19] Nitric oxide, therefore, is not recommended as standard therapy, but it can be used in catastrophic cases in which small improvements can be lifesaving. In addition, nitric oxide has been shown to increase the oxygen affinity of hemoglobin S, making sickling of the red cells less likely to occur,[20] although clinical experience with this therapy is limited.[3] In this patient, because of her persistent hypoxemia and her sickle cell disease, this treatment was instituted, but it did not reverse the problem.

Pulmonary-Artery Catheters

In ARDS, leaking of fluid into the interstitial space can reduce the ability of oxygen to diffuse into the bloodstream. In addition, elevation of pulmonary capillary pressure can increase the movement of fluid out of the vasculature into the interstitium.[21] Accordingly, lowering the cardiac filling pressures with diuresis and colloid infusion has been shown to improve oxygenation in ARDS.[22] In this patient, a pulmonary arterial catheter was placed and used for precise titration of cardiac filling pressures, cardiac output, and mixed venous oxygen saturation during the red-cell exchange and infusion of colloid.

Combination Therapy

Unfortunately, in patients as severely ill as this woman was, a single therapy is rarely enough for stabilization. Combinations of the therapies listed above can have additive effects. A flow chart shows an incremental approach to a patient with catastrophic ARDS, which we followed in this case (Fig. 2). The final measure is the use of extracorporeal membrane oxygenation. Although a previous randomized trial in adults did not show a benefit[23] in terms of mortality, we use extracorporeal membrane oxygenation as a final effort for patients whom we believe will die on standard support. The anecdotal experience at this hospital is that it can have some benefit in carefully selected adult patients with ARDS,[24] and we continue to offer it to patients, such as the one discussed in this case, for whom there are no other options. Unfortunately, she died as preparations were being made to institute this intervention.

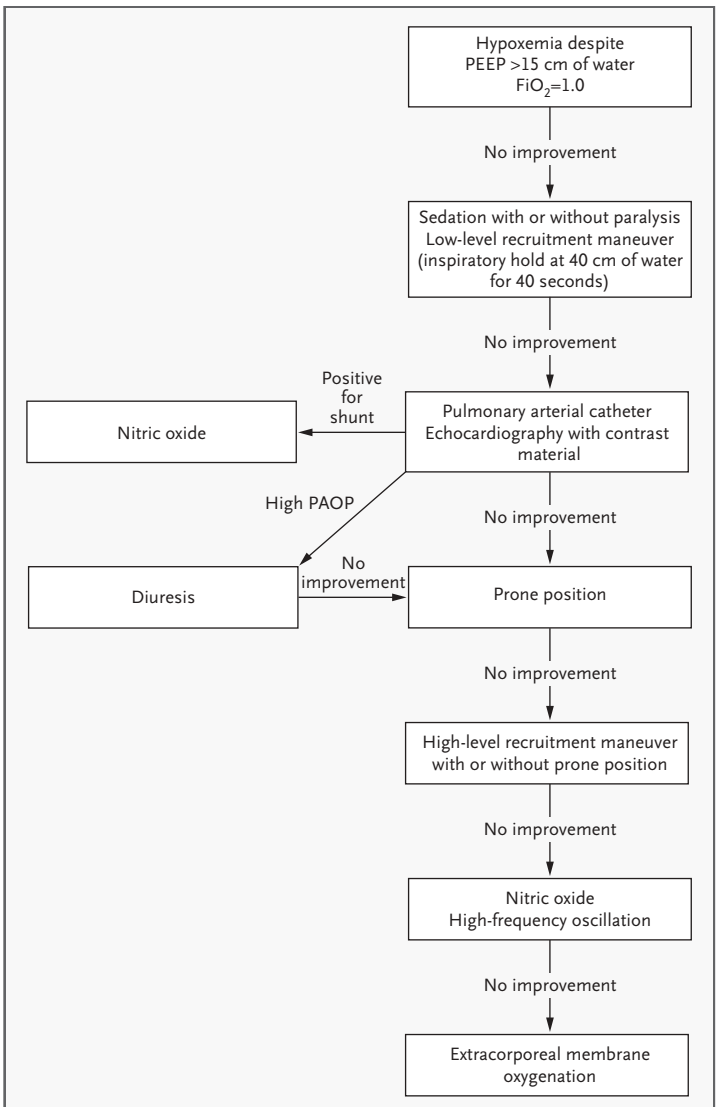

Figure 2. An Incremental Approach to the Management of Catastrophic ARDS.

A high-level recruitment maneuver is used only in patients who are without neurologic disease and bacterial pneumonia and who have adequate blood pressure, filling pressures, and cardiac output. PEEP denotes positive end-expiratory pressure, FiO_2 fraction of inspired oxygen, and PAOP pulmonary-artery occlusion pressure.

MANAGEMENT OF RESPIRATORY FAILURE IN THE ACUTE CHEST SYNDROME

In the acute chest syndrome, the resulting hypoxemia continues to promote red-cell sickling and can lead to rapidly progressive disease, as seen in this case. Early intervention with measures such as incentive spirometry and oxygen supplementation is indicated if oxygen saturation falls more than 3 percent from the patient's baseline level. If symptoms progress, the patient should be aggressively treated with antibiotics (including coverage for atypical pathogens) and fluids. If the patient does not improve, more aggressive options should be considered early, such as the use of exchange transfusion and possibly nitric oxide. Although the criteria for exchange transfusion have not been defined, I would initiate this therapy in patients with rapidly increasing oxygen requirements (every one to three hours), hypoxemia despite delivery of more than 60 percent oxygen by face mask, shock and hypoxemia despite delivery of more than 40 percent oxygen by face mask, a history of pulmonary hypertension or chronic lung disease and in any patient intubated for respiratory failure. Early intervention with mechanical ventilation and even extracorporeal membrane oxygenation[4] may be warranted for seriously ill patients, to prevent hypoxemia and further sickling of cells.

Dr. Nancy Lee Harris (Pathology): Dr. Kratz will discuss the use of transfusion therapy in the acute chest syndrome and in this patient.

TRANSFUSION THERAPY FOR THE ACUTE CHEST SYNDROME

Dr. Alexander Kratz: A crucial intervention in the acute chest syndrome is to reduce the percentage of hemoglobin S in the patient's blood. In an acute setting, two therapeutic interventions are available: transfusion of packed red cells and red-cell exchange.[4,5]

Transfusion of packed red cells can reduce the

percentage of hemoglobin S and increase the oxygen-carrying capacity of the patient's blood, but it can also increase blood volume and viscosity.[25] In contrast, red-cell exchanges allow the replacement of large percentages of the patient's hemoglobin S–containing blood with donor blood containing hemoglobin A, while keeping blood volume and viscosity unchanged. As Dr. Medoff pointed out, there are no randomized trials comparing transfusion with exchange for the acute chest syndrome.[5] However, most centers perform red-cell exchange in a patient who deteriorates despite standard transfusion therapy. This patient had received 2 units of red cells at the other hospital, and her condition had not improved. Therefore, we proceeded to exchange transfusion.

We used an automated instrument that allows the continuous replacement of the patient's hemoglobin S with hemoglobin A. Pheresis will not lead to the total replacement of the patient's red cells with healthy donor cells; the exchange of one blood volume will lead to the replacement of approximately two thirds of the patient's hemoglobin S with hemoglobin A. In a patient such as this woman, we aim to reduce the proportion of hemoglobin S to well below 30 percent. We use blood that has been confirmed to be negative for hemoglobin S and also, usually, that has undergone leukocyte reduction, to minimize the possibility of HLA immunization and of nonhemolytic febrile transfusion reactions. Many centers use red cells that have been matched for minor blood-group antigens in order to prevent the development of alloantibodies.

Patients with sickle cell disease should be very closely monitored for transfusion reactions, because they have often had multiple transfusions and have preformed antibodies to red-cell antigens. It can be difficult sometimes to distinguish a hemolytic transfusion reaction in these patients from sickling-induced hemolysis. A rare complication of blood transfusion or exchange is the syndrome of sickle cell hemolytic transfusion reaction, in which severe anemia develops after the administration of red cells.[26,27]

This patient had already received red-cell transfusions before the results of a hemoglobin electrophoresis performed before the exchange were obtained (Table 1). This study showed 52.3 percent hemoglobin A and 35.6 percent hemoglobin S. She also had an elevated hemoglobin F level, which is known to be protective against sickling and which may explain the relatively benign clinical course of the disease until this episode. After the red-cell exchange, her hemoglobin S level decreased to 19.8 percent. Unfortunately, her condition did not improve.

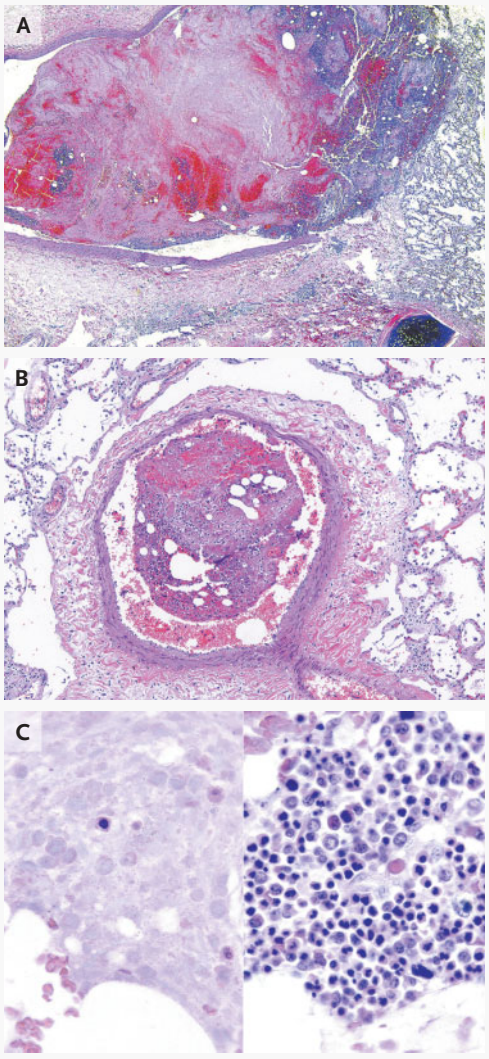

Figure 3. Photomicrographs of Lung and Bone Marrow (Hematoxylin and Eosin).

Bone marrow emboli, consisting of particles of bone marrow surrounded by fibrin, are present in small pulmonary arteries (Panels A and B). In Panel C, a section of bone marrow shows the absence of cellular detail, indicating infarction (left), as compared with a section of normal bone marrow from the same patient (right).

DR. BENJAMIN D. MEDOFF'S DIAGNOSIS

Acute chest syndrome, due to bone marrow emboli.

PATHOLOGICAL DISCUSSION

Dr. R. Neal Smith: At autopsy, the combined weight of the lungs was 1560 g, about 400 g more than normal but less than the weight criterion for diffuse alveolar damage (2000 g), and the lungs were very edematous. Two small, wedge-shaped subpleural infarcts were present in the right upper lobe. Thrombi were seen macroscopically within the small pulmonary arteries of both lungs. Microscopically, these were bone marrow emboli (Fig. 3A and 3B). In addition, fibrin thrombi were identified within alveolar capillaries. A small focus of aspiration pneumonitis was present. Hyaline membranes were not identified.

Although the patient's disease met the clinical criteria for ARDS, the autopsy findings were insufficient for a diagnosis of diffuse alveolar damage, the usual pathological diagnosis that corresponds to ARDS. Massive edema is the first sign of ARDS, leading to diffuse alveolar damage, and appears during the first day. Hyaline membranes follow beginning on day 2. The absence of hyaline membranes may reflect the relatively rapid clinical course in this patient. There was no histologic evidence of pulmonary hypertension, a known complication of sickle cell disease.[28-30]

An evaluation of sections of bone marrow identified extensive infarction (Fig. 3C). There was bilirubin cholelithiasis, a small spleen (32 g) with evidence of old infarcts, prominent sickled red cells (Fig. 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org), and hemosiderosis of the liver; the other organs appeared to be normal.

The cause of death was sickle cell disease with the acute chest syndrome resulting from a vaso-occlusive crisis that caused bone marrow infarction and pulmonary bone marrow emboli.

A Physician: Are there any warning signs that the acute chest syndrome will occur in a given patient?

Dr. Medoff: You have touched on one of the most difficult aspects of the management of this disease. The patients are often only mildly ill, with atelectasis, and then rapidly progress to respiratory failure. I think the pace of the disease is the best predictor. If the oxygen saturations drop despite oxygen supplementation, I would begin aggressive treatment with exchange transfusions. This approach may end up overtreating some patients, but it may help prevent some tragic cases like the one presented today.

ANATOMICAL DIAGNOSIS

Sickle cell disease with the acute chest syndrome, caused by infarction of the bone marrow and embolization to the lungs.

Dr. Medoff reports having received consulting fees from MEDACorp and grant support from Merck. Dr. Kratz reports having received consulting fees from Vitex.

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EDITORIALS

Is This Clinical Trial Fully Registered? — A Statement from the International Committee of Medical Journal Editors

In September 2004, the members of the International Committee of Medical Journal Editors (ICMJE) published a joint editorial aimed at promoting registration of all clinical trials.[1] We stated that we will consider a trial for publication only if it has been registered before the enrollment of the first patient. This policy applies to trials that start recruiting on or after July 1, 2005. Because many ongoing trials were not registered at inception, we will consider for publication ongoing trials that are registered before September 13, 2005. Our goal then and now is to foster a comprehensive, publicly available database of clinical trials. A complete registry of trials would be a fitting way to thank the thousands of participants who have placed themselves at risk by volunteering for clinical trials. They deserve to know that the information that accrues from their altruism is part of the public record, where it is available to guide decisions about patient care, and deserve to know that decisions about their care rest on all of the evidence, not just the trials that authors decided to report and that journal editors decided to publish.

We are not alone in pursuing this goal. The World Health Organization (WHO), through meetings in New York, Mexico City, and Geneva, has brought us close to the goal of a single worldwide standard for the information that trial authors must disclose. Around the world, governments are beginning to legislate mandatory disclosure of all trials. For example, among the bodies considering new legislation is the U.S. Congress, where the proposed Fair Access to Clinical Trials (FACT) Act would expand the current mandate for registration of clinical trials. Many other journals have adopted our policy of requiring trial registration. These initiatives show that trial registration has become a public issue. But, as our deadline for registration approaches, trial authors and sponsors want to be sure that they understand our requirements, so that reports of their research will be eligible for editorial review. The purpose of this joint and simultaneously published editorial is to answer questions about the ICMJE initiative and to bring our position into harmony with that of others who are working toward the same end.

Our definition of a clinical trial remains essentially the same as in our September 2004 editorial: "Any research project that prospectively assigns human subjects to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome." By "medical intervention" we mean any intervention used to modify a health outcome. This definition includes drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. We update our 2004 editorial to state that a trial must have at least one prospectively assigned *concurrent* control or comparison group in order to trigger the requirement for registration.

Among the trials that meet this definition, which need to be registered? The ICMJE wants to ensure public access to all "clinically directive" trials — trials that test a clinical hypothesis about health outcomes (e.g., "Is drug X as effective as drug Y in treating heart failure?"). We have excluded trials from our registration requirement if their primary goal is to assess major unknown toxicity or determine pharmacokinetics (phase 1 trials). In contrast, we think the public deserves to know about trials that could shape the body of evidence about clinical effectiveness or adverse effects. Therefore, we require registration of all trials whose primary purpose is to affect clinical practice (phase 3 trials). Between these two extremes are some clinical trials whose

Table 1. Minimal Registration Data Set.*

	Item	Comment
1.	Unique trial number	The unique trial number will be established be the primary registering entity (the registry).
2.	Trial registration date	The date of registration will be established by the primary registering entity.
3.	Secondary IDs	May be assigned by sponsors or other interested parties (there may be none).
4.	Funding source(s)	Name of the organization(s) that provided funding for the study.
5.	Primary sponsor	The main entity responsible for performing the research.
6.	Secondary sponsor(s)	The secondary entities, if any, responsible for performing the research.
7.	Responsible contact person	Public contact person for the trial, for patients interested in participating.
8.	Research contact person	Person to contact for scientific inquiries about the trial.
9.	Title of the study	Brief title chosen by the research group (can be omitted if the researchers wish).
10.	Official scientific title of the study	This title must include the name of the intervention, the condition being studied, and the outcome (e.g., The International Study of Digoxin and Death from Congestive Heart Failure).
11.	Research ethics review	Has the study at the time of registration received appropriate ethics committee approval (yes/no)? (It is assumed that all registered trials will be approved by an ethics board before commencing.)
12.	Condition	The medical condition being studied (e.g., asthma, myocardial infarction, depression).
13.	Intervention(s)	A description of the study and comparison/control intervention(s). (For a drug or other product registered for public sale anywhere in the world, this is the generic name; for an unregistered drug the generic name or company serial number is acceptable). The duration of the intervention(s) must be specified.
14.	Key inclusion and exclusion criteria	Key patient characteristics that determine eligibility for participation in the study.
15.	Study type	Database should provide drop-down lists for selection. This would include choices for randomized vs. non-randomized, type of masking (e.g., double-blind, single-blind), type of controls (e.g., placebo, active), and group assignment (e.g., parallel, crossover, factorial).
16.	Anticipated trial start date	Estimated enrollment date of the first participant.
17.	Target sample size	The total number of subjects the investigators plan to enroll before closing the trial to new participants.
18.	Recruitment status	Is this information available (yes/no)? (If yes, link to information.)
19.	Primary outcome	The primary outcome that the study was designed to evaluate. Description should include the time at which the outcome is measured (e.g., blood pressure at 12 months).
20.	Key secondary outcomes	The secondary outcomes specified in the protocol. Description should include time of measurement (e.g., creatinine clearance at 6 months).

* The data fields were specified at a meeting convened by the WHO in April 2005; the explanatory comments are largely from the ICMJE.

prespecified goal is to investigate the biology of disease or to provide preliminary data that may lead to larger, clinically directive trials.

We recognize that requiring public registration of trials whose prespecified goal is to investigate the biology of disease or to direct further research might slow the forces that drive innovation. Therefore, each journal editor will decide on a case-by-case basis about reviewing unregistered trials in this category. Authors whose trial is unregistered will have to convince the editor that they had a sound rationale when they decided not to register their trial. The ICMJE will maintain this policy for the next two years. We will then review our experience.

Our September 2004 editorial specified the information that we would require for trial registration. Attendees at a recent meeting of the WHO registration advisory group identified a minimal registration data set of 20 items (Table 1). The WHO-mandated items collectively address every key

requirement that we established in our September 2004 editorial. The ICMJE supports the WHO minimal data set and has adopted it as the ICMJE's requirement: we will consider a trial for publication if the authors register it at inception by completing all 20 fields in the WHO minimal data set. As individual editors, we will review the data in the registration fields when we decide whether to consider the trial for publication. We will consider a registration data set inadequate if it has missing fields or fields that contain uninformative terminology. If an investigator has already registered a clinical trial in a publicly owned, publicly accessible registry using the data fields that we specified in our 2004 editorial, we will consider that registration to be complete as long as each field contains useful information.

Acceptable completion of data fields is an important concern. It shouldn't be, but it is. Many entries in the publicly accessible clinicaltrials.gov database do not provide meaningful information in some key data fields. A search conducted on May 4, 2005 (Zarin D.: personal communication) indicates that certain pharmaceutical-company entries list a meaningless phrase (e.g., "investigational drug") in place of the actual name of the drug, even though a U.S. law requires trial registrants to provide "intervention name" (www.fda.gov/cder/guidance/4856fnl.htm). Many companies and other entities are completing the data fields in a meaningful fashion. Data entries must include information that will be of value to patients and health professionals; the intervention name is needed if one is to search on that intervention.

We recognize that clinical trial registries have many uses, but whatever the use, a worldwide uniform standard for a minimal database is necessary. We have participated in the WHO effort to establish a clinically meaningful trial registration process. The ICMJE supports this ongoing project. When it is complete we will evaluate the process, and if it meets our primary objectives, we will adopt it.

We stated our requirements for an acceptable trial registry in the September 2004 editorial, and they remain the same. The registry must be electronically searchable and accessible to the public at no charge. It must be open to all registrants and not for profit. It must have a mechanism to ensure the validity of the registration data.

The purpose of a clinical trials registry is to promote the public good by ensuring that everyone can find key information about every clinical trial whose principal aim is to shape medical decision-making. We will do what we can to help reach this goal. We urge all parties to register new and ongoing clinical trials. If in doubt about whether a trial is "clinically directive," register it. Don't use meaningless phrases to describe key information. Every trial participant and every investigator should be asking, "Is this clinical trial fully registered?"

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Mild Cognitive Impairment — No Benefit from Vitamin E, Little from Donepezil

Deborah Blacker, M.D., Sc.D.

In this issue of the *Journal*, Petersen et al. present the long-awaited results of a randomized, placebo-controlled trial of donepezil, a standard therapy for Alzheimer's disease, and the widely used antioxidant vitamin E as an early intervention for mild cognitive impairment — essentially the prodromal phase of Alzheimer's disease.[1] The implications of this study for primary care medicine and for public health are enormous. The clear-cut negative findings for vitamin E, which is widely used despite the dearth of evidence of its efficacy, are especially noteworthy. The findings for donepezil, on the other hand, are much less clear.

Mild cognitive impairment is conceived of as a transitional state between normal aging and dementia (or Alzheimer's disease in particular), one in which cognitive deficits are present but function is preserved. As such, it is inherently unstable and its upper and lower boundaries are difficult to delineate. In research such as that of Petersen et al., mild cognitive impairment is defined on the basis of subjective reports of memory loss, objective memory deficits (beyond those expected for age), and intact functional status,[2] whereas in clinical settings, the term is often used to describe patients who present with memory loss but do not have dementia. More recently, the amnestic subtype[3] of mild cognitive impairment has been more carefully defined to identify a subgroup of such patients who have an increased risk of progression to Alzheimer's disease over time, and this is the subtype studied by Petersen and colleagues. Even when defined carefully, however, mild cognitive impairment is a heterogeneous category that includes some persons with the memory changes of normal aging, some with nonprogressive cognitive deficits, some with prodromal Alzheimer's disease, and some with prodromal forms of other neurodegenerative dementias; only those on a course toward Alzheimer's disease are likely to benefit from Alzheimer's disease–specific interventions.

Because new treatments are expected to be better at preserving than restoring nerve function, early recognition of Alzheimer's disease and other neurodegenerative dementias — at the stage of mild cognitive impairment or even earlier, if current efforts at early detection are successful — is a major focus of current research. There is considerable evidence that the pathologic changes of Alzheimer's disease are already well established in the brain in a substantial fraction of those with a research diagnosis of mild cognitive impairment.[4] Thus, intervention to prevent progression of mild cognitive impairment to Alzheimer's disease is probably more accurately viewed as early intervention.

Rather than wait for new agents, Petersen et al. carefully evaluated the ability of two standard treatments for established Alzheimer's disease to slow the progression from mild cognitive impairment to frank Alzheimer's disease. Donepezil is a widely used cholinesterase inhibitor with limited clinical benefits, which are often not detectable in individual patients[5] and are sometimes equated to a delay in progression of approximately six months relative to placebo. Vitamin E was shown in a single high-quality trial (conducted by this same research group) to slow progression in patients with moderate-to-severe Alzheimer's disease.[6] It is widely used for all types of patients with Alzheimer's disease because of its low cost and perceived safety (although the latter has recently been called into question[7,8]). In addition, largely on the basis of theories of oxidative stress, in vitro work,[9] and epidemiologic evidence,[10] vitamin E is widely used for the primary prevention of Alzheimer's disease among persons with normal cognition. However, this approach may soon change as the public adjusts to recent evidence that epidemiologically detected benefits of vitamin E for cardiovascular disease do not stand up to rigorous clinical testing[7] and may even carry risks.[7,8]

Published trials of treatments specifically for mild cognitive impairment have thus far been limited by their small sample sizes, brevity, predominantly industry funding,[11,12] and uniformly negative results. Preliminary results from two as yet unpublished, large clinical trials of another commonly used cholinesterase inhibitor, galantamine, did not show a significant difference between galantamine and placebo in the rate of progression from mild cognitive impairment to Alzheimer's disease over a two-year period.[13,14] Of note, the unexpected finding of an excess risk of death in the

galantamine group in both trials recently led the Food and Drug Administration to issue a safety warning.[15]

The present trial represents a major step forward in the literature on trials of treatment for mild cognitive impairment. More than 700 subjects were enrolled, and they were followed for three years by a federally funded consortium of Alzheimer's Disease Centers with broad experience conducting Alzheimer's disease trials. The biggest news is the disappointing lack of efficacy of vitamin E in a well-powered trial that used the high doses previously shown to slow the progression of Alzheimer's disease. Their detailed analysis of psychometric testing across a wide range of cognitive domains also showed no significant difference between vitamin E and placebo.

The news about donepezil is not quite as disappointing. Although analysis of the primary end point of the study — the rate of progression to Alzheimer's disease within three years — was negative, the study does leave us with some hope. First, as the authors point out, they have shown that this difficult-to-define diagnostic category can be measured and studied, which is no small feat for a syndrome that was delineated less than a decade ago. Second, the rate of progression to Alzheimer's disease was somewhat lower in the donepezil group than in the placebo group during the first year of the study. Alas, by two years even this small effect had worn off. Two possible explanations come to mind: most of the subjects who were going to cross the arbitrary threshold between mild cognitive impairment and Alzheimer's disease had already done so by 12 months, so no differences were detectable after this time, and there was reduced statistical power later in the study as the number of subjects at risk declined owing to death, withdrawal, and the development of Alzheimer's disease. However, the secondary analyses of the psychometric test results suggest that neither of these explanations is the case and, instead, that the benefits really did wear off. Although several of the psychometric tests showed statistically (although not necessarily clinically) significant differences early in the study (e.g., a fraction of an SD unit on a composite memory score), the results in the donepezil group were virtually identical to those in the placebo group after 12 months: the donepezil group really had caught up with the placebo group. The reason for the transient effect of the drug is unclear, and treatment trials for established Alzheimer's disease offer few clues. The longest trial to date, which also lasted three years, also found that few of the differences between the placebo and donepezil groups persisted into the second half of the study.[16] Neither study offers any insight into whether using donepezil in the mild cognitive impairment phase of the illness would have any effect on efficacy once Alzheimer's disease is established.

What of the apparent differential effect between noncarriers and subjects who carried an apolipoprotein E (*APOE*) ε4 allele, a risk factor for Alzheimer's disease in the general population and thus for progression to Alzheimer's disease among those with mild cognitive impairment? As the authors suggest, their findings provide no support for recommending *APOE* testing in the evaluation of patients with mild cognitive impairment. In fact, a closer look at their data reveals no convincing evidence of a difference in treatment effect according to *APOE* ε4 carrier status: the effect of donepezil was similar among *APOE* ε4 carriers and noncarriers (hazard ratio for progression, 0.66, as compared with 0.80 for the entire cohort, with overlapping confidence intervals). These numbers suggest that the observed difference in significance may have been due to analogous differences in statistical power, since Alzheimer's disease developed in about twice as many *APOE* ε4 carriers (who are more likely to be on a course toward Alzheimer's disease) as noncarriers within the three years after enrollment.

What lessons does the study by Petersen et al. offer clinicians and their patients with mild cognitive symptoms? First, symptoms of memory loss in older persons should be taken seriously, since they may represent the beginning of Alzheimer's disease, and — once more effective early interventions are available — it will be critical to ask patients about these symptoms and learn to recognize them as early as possible. Second, at least one standard Alzheimer's disease therapy, donepezil, may offer some benefit, but any such benefit is quite limited and apparently transient. Last and most important, this study puts to rest the hope that early intervention with vitamin E can delay the onset of Alzheimer's disease, joining a group of recent trials of vitamin E with disappointing results.[17]

Despite these largely negative results, the bigger picture remains hopeful. Clinical studies of a wide variety of agents aimed at halting or even reversing the advance of pathologic brain lesions in Alzhei-

mer's disease are under way. There is every reason to expect that at least some of these agents will prove effective and can be deployed early in the hope of stopping the disease process while function remains intact.

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Statins for Aortic Stenosis

Raphael Rosenhek, M.D.

Calcific aortic stenosis is affecting an increasing number of patients in developed countries. It is a progressive disease that leads to a need for aortic-valve replacement when stenosis becomes severe and symptoms develop.[1-4] The growing number of valve-replacement procedures is a burden on health care systems.

The active inflammatory component of calcific aortic-valve disease has been recognized, and similarities with atherosclerotic disease have been identified. Both calcific aortic-valve disease and atherosclerosis are characterized by lipid infiltration, inflammation, neoangiogenesis, and calcification,[5,6] and the two diseases often coexist. Patients with any degree of aortic-valve disease (e.g., aortic sclerosis, mild-to-moderate stenosis, or severe stenosis) have increased cardiovascular morbidity and mortality.[2,7] Also, endothelial dysfunction is present in patients with aortic stenosis.[8]

From these observations, the hypothesis has emerged that statins, which reduce the progression of atherosclerotic disease and significantly improve the clinical outcome among patients with coronary artery disease, might also be beneficial in patients with aortic stenosis. Since aortic stenosis, like atherosclerosis, is an active disease process, it seems plausible that statins might slow its hemodynamic progression. In addition, the use of statins might also lead to a reduction in cardiovascular end points in the group of patients at high risk for vascular complications.

Until now, the effects of statin therapy on the progression of aortic stenosis have been assessed only in retrospective studies. Four such studies used echocardiography to evaluate hemodynamic progression and found a significantly lower rate of progression of aortic stenosis among patients treated with statins.[9-12] Furthermore, an additional retrospective study that used electron-beam computed tomography to determine the degree of valvular cal-

cification identified a lesser degree of aortic-valve calcium accumulation among patients receiving statins.[13] Each of these studies included between 65 and 211 patients, with a mean follow-up time between 21 and 44 months (Table 1). Although these studies consistently described a lower rate of progression of aortic stenosis with statin therapy, they were all limited by their nonrandomized, retrospective nature.

In this issue of the *Journal*, Cowell et al.[14] report the results of a prospective, randomized study of statin therapy in patients with calcific aortic stenosis. A total of 155 patients with aortic stenosis were randomly assigned to receive placebo or statin therapy (80 mg of atorvastatin daily). The hemodynamic progression of aortic stenosis was assessed by serial measurement of aortic-jet velocity with echocardiography, and the rate of change in aortic-valve calcification was measured by serial computed tomography. Information on disease progression was available for 134 patients, of whom 65 received statins, and 69 placebo.

Hemodynamic progression of aortic stenosis did not differ statistically between patients receiving statins and those receiving placebo. This finding was confirmed by the observation of similar changes in the aortic-valve calcification score between patients treated with statins and those who received placebo.

To rule out an effect of the severity of aortic stenosis or of the duration of treatment, subgroup analyses were performed. These showed no differences in disease progression between patients with mild-to-moderate aortic stenosis and those with severe stenosis or between patients with a follow-up duration of 24 months or less and those with longer follow-up.

The study by Cowell and colleagues is of particular importance since it may be the first prospective, randomized study assessing the effect of statins in

Table 1. Characteristics of Studies Assessing the Effects of Statin Therapy on the Progression of Aortic Stenosis.*

Variable	Study					
	Aronow et al.[9]	Novaro et al.[10]	Shavelle et al.[13]	Bellamy et al.[11]	Rosenhek et al.[12]	Cowell et al.[14]
Year of publication	2001	2001	2002	2002	2004	2005
Study design	Retrospective	Retrospective	Retrospective	Retrospective	Retrospective	Prospective
Method	Echo	Echo	EBCT	Echo	Echo	Echo + CT
Mean duration of follow-up (mo)	33	21	30	44	24	25
No. of patients	180	174	65	156	211	134
No. of patients receiving statins	62	57	28	38	50	65
Baseline characteristics of patients						
Mean age (yr)	82±5	68±12	67±10	77±12	70±10	68±11
Female sex (%)	69	56	NA	42	49	30
Hypertension (%)	73	69	35	66	80	51
Diabetes (%)	27	25	12	24	21	4
Coronary artery disease (%)	NA	59	51	35	27	20
Mean total cholesterol (mg/dl)	NA	209	NA	221	222	218
Mean LDL cholesterol (mg/dl)	NA	130	NA	143	142	135
Peak aortic-jet velocity (m/sec)	NA	2.65	NA	2.95	3.96	3.42
Effect of statin on disease progression	Yes	Yes	Yes	Yes	Yes	No

* Echo denotes Doppler echocardiography, EBCT electron-beam computed tomography, CT computed tomography, NA data not available, and LDL low-density lipoprotein.

aortic stenosis. Although the characteristics of the patients in this study and in the retrospective studies[9-13] were similar (Table 1), the present study differs not only because of its prospective design but also because the indications for therapy were different. In the retrospective trials, statin therapy was indicated for the treatment of hyperlipidemia, whereas in the prospective trial, patients in whom statins were indicated for the treatment of hyperlipidemia were excluded.

In the study by Cowell et al., statins were prescribed at a high dose — patients received 80 mg of atorvastatin per day. In the retrospective studies, the doses were probably lower (in the range of the equivalent of 10 to 20 mg of atorvastatin). However, it is improbable that the use of a higher dose of statin was the reason for the negative results of the present study.

The observation periods in the various studies were similar. However, in the retrospective studies, the patients were already receiving therapy at the time of inclusion in the study. Many of these patients had started therapy long before the study. Thus, one cannot rule out the need for longer overall treatment periods to observe an effect of statin therapy. In addition, although all the studies[9-14] were similar in size, they were all relatively small, and it is too early to draw conclusions on the value of statin therapy in aortic stenosis.

In view of the results of the prospective study by Cowell and colleagues, the prescription of statins is not justified for a stenotic aortic valve unless there are also other indications for therapy. This is an important study that underscores the necessity of conducting large randomized trials assessing the effects of statins on both hemodynamic progression and the outcome of aortic stenosis.

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Endovascular Repair of Abdominal Aortic Aneurysm — Round Two

Frank A. Lederle, M.D.

Last year saw the publication of the first randomized comparisons of the endovascular and open techniques for elective repair of abdominal aortic aneurysms. The Dutch Randomized Endovascular Aneurysm Management (DREAM) trial[1] and the British Endovascular Aneurysm Repair Trial 1 (EVAR-1)[2] each showed substantial reductions in 30-day postoperative mortality with endovascular repair, in agreement with the results of large observational studies published before[3,4] and since[5] then. These findings showed that endovascular repair was off to a good start, but there remained an obvious need for data on long-term outcomes.[6] That need has increased in the interim, since the release of the U.S.

Preventive Services Task Force recommendation of one-time ultrasonographic screening for abdominal aortic aneurysm in men 65 to 75 years of age who smoke or have smoked.[7] This segment of the U.S. population is estimated to harbor 360,000 aneurysms.[8] Although in most of these patients the aneurysm will be below the threshold for elective repair of 5.5 cm in diameter and will therefore be managed with periodic imaging surveillance,[6] in many it will not, and for them a procedure must be selected. Recognizing this need, the U.S. Agency for Healthcare Research and Quality has commissioned a technology assessment to compare endovascular and open repair in terms of effectiveness, cost, and quality of life.

The publication of the two-year results of the DREAM trial in this issue of the *Journal*[9] marks the beginning of round two of this comparison. The rather dramatic finding is that the promising trend favoring endovascular repair at 30 days has completely evaporated after 1 year, so that by the time of the report there were a few more deaths in the endovascular-repair group than in the open-repair group. Although the current report focuses primarily on long-term mortality, the study was not designed or powered to assess long-term outcomes, and there were only 38 deaths overall. The primary outcome in DREAM was a composite of 30-day mortality and severe or moderate complications. As stated in the initial report,[1] the differences favoring endovascular repair in terms of the primary outcome and operative mortality alone were not significant, but they were very similar to the significant mortality difference observed in EVAR-1. The mortality data from the new DREAM study are also reminiscent of the data on quality of life and sexual functioning from the first 153 patients who underwent randomization.[10,11] Scores for these measures favored endovascular repair in the early postoperative period, but by six months, scores in the open-repair group equaled or surpassed those in the endovascular-repair group.

In the current report, the authors note that endovascular repair has an advantage in terms of aneurysm-related deaths, based on a P value of 0.05, and argue that this finding is evidence against endovascular repair as the cause of the late deaths. This argument warrants questioning. The observed difference in the rate of aneurysm-related deaths is largely driven by the unequal rates of early postoperative death. It also reflects the high likelihood that late ruptures will be missed when autopsy is required for diagnosis. Because autopsies are now uncommon, sensitive identification of rupture requires that clinical and eyewitness reports be obtained and adjudicated.[12] Inclusion of even one of the two late "possible" ruptures in the endovascular-repair group would eradicate the nominally significant P value. Furthermore, regardless of whether the authors are entitled to a fresh alpha level of 0.05 in a second report,[13,14] that level of significance should probably not be used to draw conclusions about causality with respect to a secondary outcome such as aneurysm-related mortality. Finally, the accompanying confidence intervals do not support significance, suggesting that the P value may have been rounded down.

There are several plausible explanations for the preponderance of late deaths in the endovascular group. The first is the play of chance, given the small number of events. Second, as noted by the authors, open repair may have precipitated the death of frail patients who were likely to die in the coming year. According to this explanation, endovascular repair is beneficial in that, as compared with open repair, it delays death, increasing the area under the mortality curve. Third, endovascular repair may increase late mortality by failing to prevent rupture or by causing complications (resulting in a curve with a steeper slope) and so with longer follow-up might be shown to be inferior. Indeed, it remains to be proven that endovascular repair is beneficial as compared with no treatment, although this proof may be forthcoming in EVAR-2, a study of 319 patients who were not candidates for open repair and who were randomly assigned to endovascular repair or observation.[15]

Determining which explanation for the pattern of late deaths is correct will obviously require more data. The results of both EVAR-1 and EVAR-2, with at least four years of follow-up data for one third of the 1082 patients in EVAR-1, are expected soon.[16] EVAR-1 was designed and powered to compare overall mortality in this analysis, so it is reasonably likely that the trial will show a difference between the two treatments. However, it is less likely that a difference observed in EVAR-1 will be sufficiently robust to withstand meta-analytic buffering by the new DREAM results.

Regardless of the outcome of round two, longer follow-up — to five years and beyond — will be essential to evaluate the durability of endovascular repair. If round two ends in a draw, the final outcome may not be decided until the completion of the

American and French trials,[6] in which patients are still being enrolled. Endovascular repair is rapidly evolving, so if it was not better than open repair several years ago, when patients were being enrolled in the currently reported trials, it might be better now. The American and French trials are both behind schedule in their enrollment. Unlike the situation with DREAM and EVAR-1, endovascular repair is readily available in the United States and France outside of the trials, making recruitment much more difficult. Although it is not surprising that industry-backed Web sites would persuade patients to select a new therapy over randomization, it is disappointing when patients refuse randomization because their physicians recommend that they ask for endovascular repair. Our rush to embrace the new may diminish our ability to learn whether the new is worth embracing. To properly evaluate endovascular repair for abdominal aortic aneurysm, we may need to go a few more rounds.

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CLINICAL IMPLICATIONS OF BASIC RESEARCH

MicroRNA and Lung Cancer

Matthias Eder, M.D., and Michaela Scherr, Ph.D.

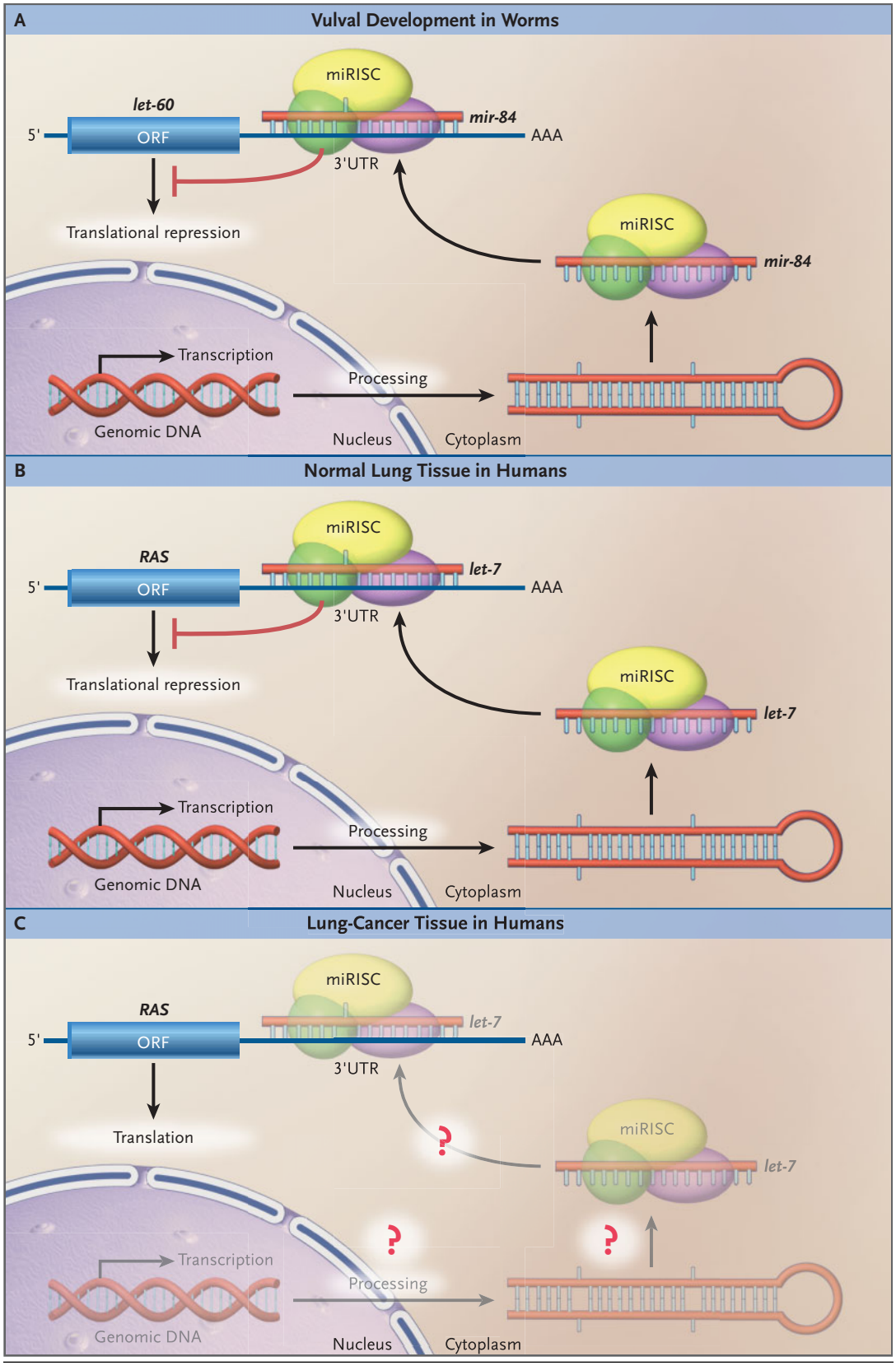
Pioneering work on the nematode *Caenorhabditis elegans* has yielded a wealth of insight into signaling pathways, revealing regulatory mechanisms that are critical to both developmental biology and tumorigenesis. For example, studies of vulval development in the worm were instrumental in identifying components of RAS–mitogen-activated protein kinase signaling. These are highly conserved across species and regulate the growth of normal and malignant cells in mammals. The study of *C. elegans* facilitated another important discovery: the existence of noncoding microRNAs. These tiny fragments of RNA (about 22 nucleotides long) regulate gene expression by hybridizing to complementary sequences in the 3′ untranslated region (3′UTR) of target messenger RNA (mRNA). They can thereby repress the translation of mRNA through an unknown mechanism or increase the instability of mRNA. (RNA interference — a technique that has enjoyed extraordinary success recently as a laboratory tool for manipulating gene expression — uses some of the same molecular machinery.) A recent study[1] by Johnson and colleagues engages both lines of research and may suggest a potential strategy for treating lung cancer in humans.

Johnson et al. first set out to identify new RNA targets of microRNAs in the *let-7* family, which includes *let-7* itself. They used a computer-based screen to identify genes encoding mRNAs with 3′UTRs containing multiple *let-7* complementary sites and homed in on four genes: the nematode *RAS* gene (called *let-60*) and the human *KRAS*, *HRAS*, and *NRAS* genes. They then showed that the expression of a reporter construct is controlled by the *let-60* 3′UTR and by *let-7*. They went on to show that mutations in *let-60* and *let-7* microRNAs can complement each other. Ablating the expression of *let-7* tends to kill the worm; simultaneously ablating *let-7* and repressing *let-60* results in a lower rate of death. Similarly, overexpression of *mir-84* (a member of the *let-7* family) inhibits the development of multiple vulvae caused by a gain-of-function mutation in *let-60*. These data strongly suggest the existence of a reciprocal interaction between *let-7* microRNA species and *let-60* expression in *C. elegans* (Fig. 1).

The authors then investigated the role of *let-7* in the context of human cancer, because the RAS signaling pathway critically affects a cell's propensity for becoming cancerous. Using two different cell lines, they obtained further evidence of a reciprocal interaction: overexpression of *let-7* represses — and inhibition of native *let-7* enhances — the expression of RAS protein. Furthermore, Johnson and colleagues found that *let-7* complementary sites in human *NRAS* and *KRAS* 3′UTRs specifically mediate *let-7*–dependent repression (Fig. 1), which can be abrogated by *let-7* inhibitors. Supporting the relevance of this finding is the fact that the position of genomic regions commonly deleted in lung cancer (which RAS signaling is believed to help initiate) coincides with several human *let-7* genes. Accordingly, microarray analysis of microRNAs revealed specific down-regulation of *let-7* expression in sam-

Figure 1 (facing page). MicroRNA-Mediated Regulation of RAS Expression in *Caenorhabditis elegans* and Humans.

In specific vulval precursor cells from worms (Panel A) and in normal human lung tissue (Panel B), *mir-84*, a member of the *let-7* microRNA family, and *let-7*, respectively, are transcribed, and the transcripts, which have characteristic hairpin structures, are processed into mature microRNAs. These are then incorporated into a silencing complex (called miRISC). MicroRNA species guide miRISC to target mRNAs by hybridizing to complementary sequences in the 3'UTRs of the mRNAs and thereby prevent their translation. A recent study by Johnson and colleagues[1] showed that members of the *let-7* family repress the expression of *RAS* genes and that this mechanism is potentially relevant to the pathogenesis of lung cancer (Panel C). The question marks in Panel C indicate that the reduced expression of *let-7* microRNA in lung cancer may be due to alterations in transcription, processing, or maturation. The abrogation of translational repression results in the overexpression of RAS proteins in lung-cancer cells. Additional details of the mechanism by which microRNA represses gene expression are available elsewhere.[2] ORF denotes open reading frame.



ples of lung but not of breast or colon cancer, as compared with normal adjacent tissue. Finally, direct comparison of three samples of squamous-cell carcinoma of the lung and adjacent normal tissue revealed reduced expression of *let-7* microRNA and concomitant overexpression of *RAS* in the lung carcinomas.

These data are in line with findings from earlier studies demonstrating reduced expression of microRNA in various cancers, such as chronic lymphocytic leukemia and colorectal cancer. In particular, reduced expression of *let-7* in lung cancer indicates a poor prognosis.[3] So, what next? The expression of *RAS* and *let-7* must be analyzed in additional tumor samples, and the relevance of these findings with respect to different subtypes of lung cancer should be investigated. The extent to which altered *let-7* or *RAS* expression tips the balance toward carcinogenesis and tumor survival should be determined. Mechanisms other than genomic rearrangements or deletions that reduce or ablate *let-7* expression, including defective microRNA processing and maturation, should be explored. Although we are far from the point at which we can judge whether augmenting *let-7* expression in lung-cancer cells might prove therapeutic, the findings of Johnson et al. provide inspiration for further work toward this end.

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CORRESPONDENCE

Treatment of Localized Lymphoma

TO THE EDITOR: Reyes and colleagues (March 24 issue)[1] should elaborate on the disadvantages, particularly the long-term side effects, of a regimen of doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) in comparison with a regimen of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). With an increased duration of parenteral chemotherapy (20, rather than 3, days), ACVBP has a complicated schedule and thus poses a compliance problem in nonresearch settings. Furthermore, in many centers, patients need hospitalization to ensure hydration during high-dose methotrexate therapy. ACVBP also causes more mucositis, neutropenia, and thrombocytopenia than does CHOP, and it increases the risk of myelodysplasia and leukemia.[2,3] Generally, treatment with etoposide plus repetitive administration of high-dose alkylating agents is strongly leukemogenic,[4] and a 20-year follow-up showed an increase in the incidence of myelodysplasia.[3]

Bleomycin, used with granulocyte colony-stimulating factor (G-CSF) (which is commonly needed for dose-dense therapy), may precipitate pulmonary fibrosis.[5] Finally, gonadal failure — leading to infertility, osteoporosis, and impaired quality of life[6] — is potentially widespread among young patients.

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TO THE EDITOR: Reyes et al. conclude that ACVBP is superior to CHOP plus radiotherapy for the treatment of low-risk localized lymphoma. However, when the overall rates of local relapse (8.4 percent in the ACVBP group and 6.9 percent in the CHOP-plus-radiotherapy group) are evaluated in detail, it seems that ACVBP is not much better than CHOP plus radiotherapy in achieving control of local disease, which is the aim of radiotherapy. Thus, ACVBP, when compared with CHOP plus radiotherapy, improves control of distant disease but does not affect local control in patients with low-risk localized lymphoma.

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THIS WEEK'S LETTERS

TO THE EDITOR: In the phase 3 Groupe d'Etude des Lymphomes de l'Adulte (GELA) study, reported by Reyes et al., overall and event-free survival favored the ACVBP regimen. However, local failure was more frequent in the ACVBP group (in which 26 patients had a local recurrence, vs. 22 in the CHOP-plus-radiotherapy group). The inferiority of the CHOP-plus-radiotherapy regimen was solely due to the greater number of relapses at distant sites.

In our opinion, the GELA study does not nullify the current practice of providing additional radiotherapy in early stages of aggressive lymphoma,[1,2] especially since other studies have shown beneficial effects on rates of local control with radiotherapy after more intensive chemotherapy.[3] As in many other malignant diseases, local control is a prerequisite for cure as long as salvage options are limited, but in general, statistical proof of this assumption will require the evaluation of many more patients.

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THE AUTHORS REPLY: In response to Tanvetyanon: for more than 20 years, ACVBP has been used in a multicenter setting with 50 to 80 participating centers, including community hospitals, and the results are reproducible.[1] Immediate and late toxic effects of ACVBP are documented in the GELA studies cited by Tanvetyanon. In addition, a survey of 2210 patients who received ACVBP[2] showed than an age greater than 60 years, an elevated lactate dehydrogenase level, and an Eastern Cooperative Oncology Group score above 1 predicted early treatment-related deaths. In our study of low-risk localized lymphoma, we observed only two episodes of grade 4 infection and no deaths from toxic effects. Thus, we believe that ACVBP is a suitable and efficient regimen in young adults.

In the GELA study of second cancers after ACVBP therapy,[3] myelodysplasia occurred principally in elderly patients ($P<0.001$ for the comparison with younger patients). In our study in young adults, ACVBP did not increase the number of secondary cancers when compared with CHOP plus irradiation. Finally, we have not observed in the GELA studies a significant number of cases of pulmonary fibrosis as a result of concomitant administration of G-CSF and bleomycin.[4]

In response to Hoecht and Hinkelbein and to Adli and colleagues: the CHOP-plus-radiotherapy group did have more relapses at distant sites than did the ACVBP group, and this discrepancy translated into a significant difference in the overall number of relapses (78 in the CHOP-plus-radiotherapy group vs. 42 in the ACVBP group). Notably, most patients in the study (67 percent) had stage 1 disease, indicating that local control by irradiation is not sufficient to ensure cure. This conclusion was also reached in the report[5] cited by Hoecht and Hinkelbein, the authors of which state that "adjuvant radiotherapy provides excellent local control, but systemic relapse represents the major cause of treatment failure." We believe that the goal of any first-line treatment of localized lymphoma is to improve survival rather than to control local disease; in our study, 89 deaths were related to progressive lymphoma, 60 of which occurred after CHOP plus radiotherapy. In addition, chemotherapy alone has the advantage of avoiding the long-term sequelae of irradiation, particularly in the frequently involved cervical nodes and Waldeyer's ring. Finally, we assume that the addition of anti-CD20 immunotherapy to chemotherapy should further improve our results, and for this reason we do not recommend adjuvant radiotherapy as first-line treatment.

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Phase 1 Clinical Trials in Oncology

TO THE EDITOR: Horstmann et al. (March 3 issue)[1] assume that a tumor response is of benefit to subjects in phase 1 oncology trials. This assumption is not valid. A complete or partial tumor response in a phase 1 trial is a surrogate end point, which for most agents has not been linked to a clinically meaningful outcome, such as improved survival.[2]

Informing subjects that they have a 10.6 percent chance of a tumor response is potentially misleading unless accompanied by an explicit discussion of clinical end points and whether any connection exists between a tumor response and clinical end points.[3] This discussion should include an explanation that a tumor response is not a cure or a life extender.

Kurzrock and Benjamin's editorial[4] serves only to increase the misrepresentation of phase 1 research.[5] It is important to know that phase 1 research is essential for the development of future treatments. But it is simply misleading to treat an improvement in the rate of tumor response as an increase in the likelihood of direct clinical benefit to subjects.

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TO THE EDITOR: The article by Horstmann et al. and the accompanying editorial indicate rates of clinical benefit higher than those reported in previous meta-analyses. Horng et al.,[1] in a critique of informed consent in phase 1 oncology trials, decried the frequent lack of an explicit statement that efficacy was not to be expected. However, in addition to evidence presented by Horstmann et al., recent phase 1 trials with established drugs have often resulted in high response rates. Among nine trials involving patients with refractory non–small-cell lung cancer that were presented at the meeting of the American Society of Clinical Oncology in May 2002, the reported response rate was 41 percent (range, 0 to 57 percent) in 150 patients, with one drug-related death recorded. Prior estimates of the risks and benefits of phase 1 oncology trials need updating, and insistence on not conveying therapeutic intent in the informed-consent process in all instances is misplaced.

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TO THE EDITOR: In their review of 460 phase 1 oncology trials sponsored by the Cancer Therapy Evaluation Program between 1991 and 2002, Horstmann et al. report that the overall toxicity-related death rate was 0.49 percent, which suggests that these trials are relatively safe, considering that virtually all participants have a deadly disease and have exhausted the conventional treatments.[1]

We analyzed the data from 363 trials of investigational new drugs, involving 12,395 adults with solid tumors, that were published between 1976 and

1993.[2] A total of 117 toxicity-related deaths (0.94 percent) and 33 early deaths from unknown causes (0.27 percent) were noted. In addition, 36 trials were excluded from the analysis because further clinical development of the drug was not recommended. We found that toxicity-related death occurred in 26 of 1039 patients in these trials (2.5 percent). Thus, the rate of death due to toxic events varies among phase 1 oncology trials and may be higher than suspected.

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TO THE EDITOR: Kurzrock and Benjamin argue that clinical benefit is an objective of phase 1 cancer trials, citing my article as an instance of an opposing "misconception."[1] The misconception is theirs, as is evident in authoritative definitions.[2,3] Moreover, in failing to distinguish between what phase 1 trials are specifically designed to measure (dose–toxicity profiles) and what is incidental to the design (e.g., the possibility of benefit), Kurzrock and Benjamin ignore the way in which the strictures of protocol constrain the goals of medicine. This misunderstanding, known as the "therapeutic misconception,"[4] reinforces the fiction that clinical research is an extension of clinical care, rather than a fundamentally distinct and sometimes contrary enterprise. Patients in early cohorts in these trials who receive, by design, what Kurzrock and Benjamin call "subtherapeutic" doses are not involved in a trial that aims to maximize their clinical benefit. Failure to see this as a conflict between the objectives of science and those of personal care is the reason the therapeutic misconception has been called "the most important threat to the validity of informed consent to research."[5]

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THE AUTHORS REPLY: The letters from Drs. Rothschild and King and from Dr. Muggia demonstrate the complexity of understanding "benefits" in the context of phase 1 oncology trials. As Drs. Rothschild and King suggest, tumor response, the most common measure of the effect of agents used for the treatment of cancer, is indeed a surrogate marker. Although tumor response does not necessarily correlate with clinical benefit, it is predictive of potential benefit, and there is evidence that tumor response is associated with symptom relief, improved quality of life, and increased survival.[1-4]

We agree that information provided to potential participants in phase 1 trials should be comprehensive, contextual, and clear about the uncertain or inconsistent relationship of possible tumor responses to clinically meaningful benefit.

Furthermore, it should be made clear that although some participants in phase 1 trials may benefit clinically, these trials are designed to evaluate safety, not therapeutic effect. There is a difference between the possibility of benefit from an intervention in a trial and the intent of the researchers when designing the trial. In this regard, we disagree with Dr. Muggia and maintain that consent forms should not describe the purpose or intent of phase 1 trials as therapeutic. Nonetheless, we recognize that although institutional review boards, bioethicists, and others might emphasize the intention of a trial, prospective patients may be more interested in possible benefits than in whether or not the trial is intended to be therapeutic. Our data demonstrate that sometimes there is therapeutic benefit, regardless of the intention of the research.

The statement by Drs. Sekine and Tamura that "the rate of death due to toxic events varies among phase 1 oncology trials" is consistent with the findings of our study. The data they cite emphasize two important realities that should be considered with

regard to response or toxicity rates in phase 1 trials: first, different subsets of data have strikingly different benefit and toxicity rates, and second, response and toxicity rates based on published data may be biased. Their data support the view that the details of a trial matter in interpreting the data on response and toxicity. Simply labeling a trial phase 1 is not sufficiently informative about risks and benefits; more specific details about the trial and the intervention are necessary.

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THE EDITORIALISTS REPLY: Rothschild and King's allegation that it is "misleading to treat an improvement in the rate of tumor response as an increase in the likelihood of direct clinical benefit to subjects" is at variance with our clinical experience and the oncology literature. Decades ago, Freireich et al.[1] established that improvement in survival in leukemia could be attributed directly to the duration of a response. A response to chemotherapy in randomized trials improved the quality of life despite significant side effects.[2] Differences in benefit between patients with and those without a response may be obscured, however, by an inadequate definition of a response. For example, patients with gastrointestinal stromal tumors who were treated with imatinib mesylate and who had stable disease according to the criteria of the Response Evaluation Criteria in Solid Tumors group derived a benefit that was indistinguishable from the benefit in those with a partial response.[3] Logic dictates that patients with good performance status and intact organ function — the eligibility criteria for most phase 1 studies — will not die of their cancer unless it progresses.

The perception that, in phase 1 studies, drugs are administered to patients solely to reveal drug toxicity is incorrect, since the objectives of phase 1 trials specifically include describing the response. Oncologists refer patients for phase 1 studies because they determine that participation in those studies offers their patients, whose disease has progressed after recognized therapies, their best chance of benefit. Thus, the primary concern of treating physicians and patients is efficacy. Miller's contention that the scientific restrictions of the protocols interfere with patient care is partially valid. For instance, some patients who might benefit are excluded from phase 1 trials by the eligibility criteria. Low initial doses and small dose increases, resulting from excessive caution about patient safety, can detract from benefit to patients. Nonetheless, as Horstmann et al. have demonstrated, phase 1 studies resulted in stable disease or better in up to 44.7 percent of patients, including those treated at the lower doses.

Increased time before the progression of cancer benefits patients unless the therapy has serious toxic effects. The worse "toxicity" is most often that due to progressive disease. We agree with Muggia, who demonstrates that recent phase 1 trials have higher response rates than previously reported and have extraordinarily low death rates. Although participants in any study should be informed that patients who have a response to therapy may not always benefit, it is misleading to tell patients that there is no clinical benefit from a response and that phase 1 trials have no therapeutic aim.

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The Serotonin Syndrome

TO THE EDITOR: The review of the serotonin syndrome by Boyer and Shannon (March 17 issue)[1] raises some questions. First, how many features make a diagnosis of the serotonin syndrome? This is especially relevant when a patient presents with nonspecific symptoms (agitation, tachycardia, tremor, diarrhea, or a combination) that are often early and transient side effects of selective serotonin-reuptake inhibitors (SSRIs). An overenthusiastic diagnosis of the serotonin syndrome at this stage may actually deprive the patient of the benefits of SSRI treatment for depression.

Second, SSRIs and other proserotonergic agents act, by definition, by raising serotonergic activity in the brain. How and when does one decide that this activity is excessive? As Boyer and Shannon rightly point out, "No laboratory tests confirm the diagnosis of the serotonin syndrome." Hence, the term "serotonin syndrome" might be a misnomer, because we do not know whether it is an excess of serotonin that causes the syndrome, even in its full-blown form.

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TO THE EDITOR: Boyer and Shannon appropriately note Whyte's work describing 2222 overdoses of serotonergic drugs. This work strongly reinforces the value of the spectrum concept of serotonin toxicity[1,2] and demonstrates that mild serotonin toxicity occurs in only 15 percent of SSRI-alone overdoses but that severe and potentially fatal serotonin toxicity occurs in 50 percent of cases in which monoamine oxidase inhibitors (MAOIs) and SSRIs are mixed. It is important to understand that it is only these patients who are at high risk and who may need urgent lifesaving treatment with 5-hydroxytryptamine type 2A receptor antagonists. Intravenous chlorpromazine has been used in 20 such cases, with rapid improvement of symptoms. It is better to think of serotonin toxicity as a form of poisoning (inevitable, if enough is ingested), not as a syndrome, because it is not idiosyncratic. This is why an accurate list of which drugs are actually serotonin-reuptake inhibitors or MAOIs is vital — for example, mirtazapine and nefazodone do not pose a risk with MAOIs.[1]

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TO THE EDITOR: One drug interaction is not included in Table 1 of Boyer and Shannon's review. The authors do mention opiates and meperidine, fentanyl, tramadol, and pentazocine in Table 1, but oxycodone and hydrocodone, administered with SSRIs, are also reported causes of the serotonin syndrome.[1] We have reported four cases from a long-term care facility.[2]

In addition, we surveyed 49 long-term care residents who were given oxycodone or hydrocodone with SSRIs and found 10 cases of possible or probable serotonin syndrome. Since nitroglycerin is a suggested treatment for the serotonin syndrome,[3] we performed an analysis to determine whether nitroglycerin use protected patients against the development of the syndrome. Unexpectedly, nitroglycerin use was associated with a higher risk of the serotonin syndrome (odds ratio, 5.3; P=0.02). Nitroglycerin use may be a surrogate indicator of vascular disease, which is known to be associated with decreased monoamine oxidase A activity, which in turn may increase the risk of the serotonin syndrome.

Frail elderly persons may be at high risk for the serotonin syndrome because of the use of multiple

medications and associated vascular disease. Characterization of the serotonin syndrome in this population is lacking and merits further study.

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TO THE EDITOR: The serotonin syndrome can occur in a broad range of clinical settings; however, experience with its management is scarce.[1] Severe cases require immediate and effective therapy and leave little time for expert consultation. Treatment with cyproheptadine, olanzapine, or chlorpromazine is recommended[1] but seems imprudent in inexperienced hands. Furthermore, these drugs may not be readily available, causing unnecessary delay in treatment.

Recently, we successfully managed a life-threatening presentation of the serotonin syndrome without these drugs. A 72-year-old man using tranylcypromine (60 mg a day) accidentally ingested venlafaxine (300 mg); severe muscular rigidity, delirium, hyperthermia, and respiratory failure developed rapidly. We used propofol[2] to induce sedation and rocuronium, a nondepolarizing agent, to induce muscular paralysis, followed by intubation and ventilation.[3] Within two hours, his temperature returned to 37.0°C. His further recovery was uneventful, and he was discharged 48 hours later. This widely practiced method of inducing anesthesia can be used safely and quickly in any hospital setting with an intensive care unit and does not require expertise with the serotonin syndrome.

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THE AUTHORS REPLY: Dr. Basu questions the point at which signs of serotonergic activity become serotonin toxicity. As we suggest in our article, the diagnosis of the serotonin syndrome depends on a clustering of clinical findings, described in Figure 4 of the article. We do not believe that the semantic changes proposed by Dr. Basu are as important as the need for clinicians to respond to the serotonin syndrome or to recognize that the administration of serotonergic drugs in any person with even one of the clinical signs listed can provoke clinical deterioration.

Dr. Gillman correctly highlights the plurality of signs and symptoms seen in the serotonin syndrome and the presumed effectiveness of treatment with chlorpromazine. We would caution clinicians against dismissing a specific agent as incapable of producing the serotonin syndrome. The condition has occurred in surprising clinical situations and, because of phenotypic variations among individual persons, has been associated with unexpected drugs, including mirtazapine.[1]

Drs. Claassen and Gelissen relate the aggressive action needed to manage severe serotonin syndrome, and the strategy they describe of using sedation (with propofol), control of hyperthermia (with rocuronium), and supportive care (orotracheal intubation) is in line with our recommendations. We disagree, however, that the safe and effective use of antihistamines and antipsychotic agents is beyond the grasp of clinicians, especially since extensive clinical experience with the disease and its treatment

is available from toxicology consultation services and poison-control centers.

We agree with Dr. Gnanadesigan and colleagues that opiate and opioid drugs can result in serotonergic excess, but the leading reference they cite involves a transplant recipient receiving maintenance treatment with cyclosporine and other drugs in whom tremor and hallucinations developed — both classic signs of cyclosporine toxicity.[2] In any case, the serotonin syndrome, as well as many pharmacologic factors and drug interactions, is poorly described in the elderly. We have always regarded nitroglycerin as a poorly conceived treatment for the serotonin syndrome, and we are glad that these authors are gathering clinical evidence of its potential dangers.

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Acute Doxorubicin Cardiotoxicity

TO THE EDITOR: A previously healthy 78-year-old woman presented with facial flushing, palpitations, diarrhea, and generalized weakness. The serum serotonin level was markedly elevated (2233 ng per milliliter; normal, <180), as was urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) (118.7 mg per 24 hours; normal, <6.0), and a computed tomographic (CT) scan revealed a small-bowel mass, 3 cm by 2.5 cm, with multiple hepatic lesions. Fine-needle liver biopsy demonstrated cytokeratin- and chromogranin-staining cells, which are characteristic of carcinoid tumor. Despite monthly injections of octreotide, the patient's flushing persisted, and a CT scan showed enlargement of both small-bowel and liver masses. She subsequently underwent hepatic-artery chemoembolization with use of cisplatin (100 mg), doxorubicin (50 mg), and mitomycin (10 mg).

Acute pulmonary edema necessitating mechanical ventilation developed 30 hours after chemoembolization. This decompensation was associated with lactic acidosis, anuric renal failure (peak serum creatinine level, 4.6 mg per deciliter), elevated cardiac-enzyme levels (creatine kinase, 668 U per liter; creatine kinase MB isoenzyme, 48.8 ng per milliliter; troponin I, 20.64 ng per milliliter [normal, <1.50]), and a markedly increased brain-type natriuretic peptide level (3330 pg per milliliter; normal, <100). Transthoracic echocardiography showed severe global left ventricular dysfunction with an ejection fraction of 10 percent. Cardiac catheterization revealed normal coronary arteries and severely depressed cardiac output (cardiac index, 1.0 liter per minute per square meter; normal, >2.8). An intraaortic balloon pump was placed, and the patient had marked clinical improvement and was able to be extubated 48 hours later. She was discharged one week later receiving carvedilol and enalapril. Another echocardiogram obtained four weeks later showed that the left ventricular ejection fraction had increased to 45 percent.

Hepatic-artery chemoembolization has become an accepted treatment for patients with metastatic carcinoid tumor, providing long-term palliation of symptoms and control of tumor growth.[1] Cardiotoxicity associated with the administration of doxorubicin is most commonly manifested by dose-dependent chronic cardiomyopathy, and acute effects, including left ventricular failure, are rare.[2,3] To our knowledge, there have been no previous reports of acute cardiotoxicity after hepatic-artery chemoembolization with an anthracycline antineoplastic agent.

Evidence of both myocardial necrosis and acute

myocardial dysfunction developed in our patient after the administration of doxorubicin in a single dose well below that typically associated with cardiotoxic effects. The rapid improvement in left ventricular function observed in this patient within four weeks suggests that substantial myocardial stunning was present. Although it is very rare, acute reversible left ventricular failure should be considered in patients in whom respiratory failure or shock develops after the administration of even a single dose of doxorubicin.

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Cabergoline plus Lanreotide for Ectopic Cushing's Syndrome

TO THE EDITOR: The ectopic corticotropin syndrome, a rare cause of chronic endogenous hypercortisolism, accounts for 15 to 20 percent of corticotropin-dependent Cushing's syndrome and 5 to 10 percent of cases of Cushing's syndrome overall.[1,2] This syndrome is often associated with corticotropin-secreting lung carcinoid tumors. The treatment of choice in cases in which the syndrome is associated with lung carcinoid is surgery, but the success rate of surgery is limited owing to the persistence of tumor remnants,[3] which frequently necessitate palliative medical treatment to inhibit adrenal cortisol secretion. Somatostatin analogues have been observed to be effective in controlling carcinoid corticotropin secretion[4]; in contrast, dopamine agonists have not been used in treatment of the ectopic corticotropin syndrome. In this report, we describe a patient with the ectopic corticotropin syndrome due to a lung carcinoid tumor. After surgery failed, the condition was successfully managed with a long-acting somatostatin analogue, together with a long-acting dopamine agonist.

A 35-year-old man had a clinical picture suggestive of the ectopic corticotropin syndrome related to a carcinoid tumor; biochemical and hormonal tests confirmed the diagnosis. Somatostatin-receptor scintigraphy revealed abnormal uptake in the anterobasal region of the left lung, and computed tomography (CT) revealed a lung tumor. Thoracotomy was performed, and the presence of a corticotropin-positive, atypical carcinoid tumor was confirmed. After surgical removal of the tumor, Cushing's syndrome persisted, with increased plasma cortisol levels and increased urinary cortisol excretion. Somatostatin-receptor scintigraphy showed persistently abnormal uptake in the left lung, although chest CT revealed no abnormalities. Since reoperation was not an option, therapy with the somatostatin analogue lanreotide (90 mg per month) was begun, on the basis of the positive findings on scintigraphy. After six months, corticotropin and cortisol secretion decreased but then stopped responding to treatment, and after one year, the lanreotide therapy was stopped. Since reverse-transcriptase–polymerase-chain-reaction analysis of somatostatin-receptor and dopamine-receptor expression in a tumor sam-

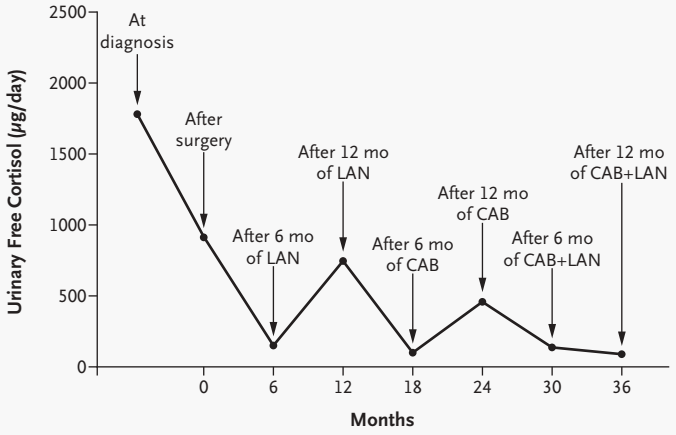

Figure 1. Urinary Cortisol Levels after Various Treatments in a Patient with the Ectopic Corticotropin Syndrome Associated with a Corticotropin-Secreting Lung Carcinoid.

LAN denotes lanreotide, and CAB cabergoline.

ple revealed dopamine D2 receptor expression in addition to expression of somatostatin receptor subtype 5, dopamine-agonist therapy with cabergoline (7 mg per week) was initiated. After six months, corticotropin and cortisol secretion normalized but then stopped responding again, and the administration of cabergoline was stopped after one year. In a final attempt at medical therapy, combined treatment with cabergoline and lanreotide was started on the basis of the documented interaction between the dopamine D2 receptor and the somatostatin receptor subtype 5.[5] Corticotropin and cortisol secretion rapidly normalized and remained normal, as did plasma corticotropin and urinary cortisol levels (Fig. 1).

This case documents the long-term effectiveness of combined treatment with a somatostatin analogue and a dopamine agonist in a patient who no longer had a response to either agent alone and supports the hypothesis that somatostatin and dopamine receptors interact and that somatostatin and dopamine agonists may potentiate actions.

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INSTRUCTIONS FOR LETTERS TO THE EDITOR

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BOOK REVIEWS

THROMBOSIS AND CANCER

Edited by Gilles Lugassy, Anna Falanga, Ajay K. Kakkar, and Frederick R. Rickles. 230 pp. London, Martin Dunitz, 2004. $125. ISBN 1-84184-287-7.

THE ASSOCIATION BETWEEN THROMBOSIS and cancer represents a challenge to scientists and physicians of various disciplines. From a clinician's point of view, progress in this field during the past several years has been substantial. In a patient presenting with venous thrombosis who is otherwise healthy, for example, the likelihood that there is a hidden cancer is now well defined, and we have estimates of the risk of thrombosis among patients with cancer who are exposed to surgery, chemotherapy, or the insertion of a central venous catheter. With the development of new antithrombotic drugs, particularly the low-molecular-weight heparins, preventive and therapeutic strategies are now available, and they are effective, safe, and less inconvenient for the patients than were previous forms of treatment.

The contributors to this book are leading scientists in the field, and some of them conducted the studies under discussion. Hence, the information given is largely firsthand. Eight of the 16 chapters are dedicated to the clinical side of cancer-associated thromboembolism and give detailed, state-of-the-art overviews of epidemiology, risk assessment, prevention, and treatment. In addition to the clear and extensive coverage of current preventive and therapeutic concepts for cancer-related thromboembolism, controversial issues are dealt with comprehensively. For instance, Piccioli and Prandoni address the still-open question of whether or not to screen patients with deep-vein thrombosis for the presence of a hidden cancer, and the treatment of patients with cancer in whom a central venous catheter has been inserted is discussed by several authors. As with many such books, some of the content is dated. For example, long-term anticoagulation therapy with a vitamin K antagonist is no longer the preferred treatment in patients with cancer, since low-molecular-weight heparin has turned out to be more effective than and at least as safe as warfarin.

The mechanisms by which cancer cells promote the activation of coagulation are complex and not entirely understood, and the function of the hemostatic system with respect to tumor growth, angiogenesis, and metastasis is even more obscure. Considering that the book was written for all physicians involved in the treatment of patients with cancer, including general practitioners and internists, one has to admire the editors' courage to devote more than one third of the content to basic science. An excellent overview of the mechanisms of thrombosis in patients with cancer, by Falanga, Marchetti, and Vignoli, is followed by three chapters summarizing the mechanisms of tumor-platelet interactions and the role of both the clotting cascade and the fibrinolytic system in the pathogenesis of neoplasia and tumor metastasis. The basic-science part culminates in a chapter entitled "Tumor Angiogenesis and Blood Coagulation," by Fernandez, Patierno, and Rickles. It is well illustrated and dissects the coagulation cascade, from tissue-factor activation through fibrin deposition, thereby summarizing the ways in which key components of the cascade directly or indirectly contribute to tumor angiogenesis. This chapter is the highlight of this interesting book.

Thrombosis and Cancer is a well-written multiauthored book that, to a large extent, will fulfill its stated purpose — "to provide comprehensive and timely coverage of our current knowledge of cancer-associated thrombosis." It will be of interest to clinicians in many disciplines.

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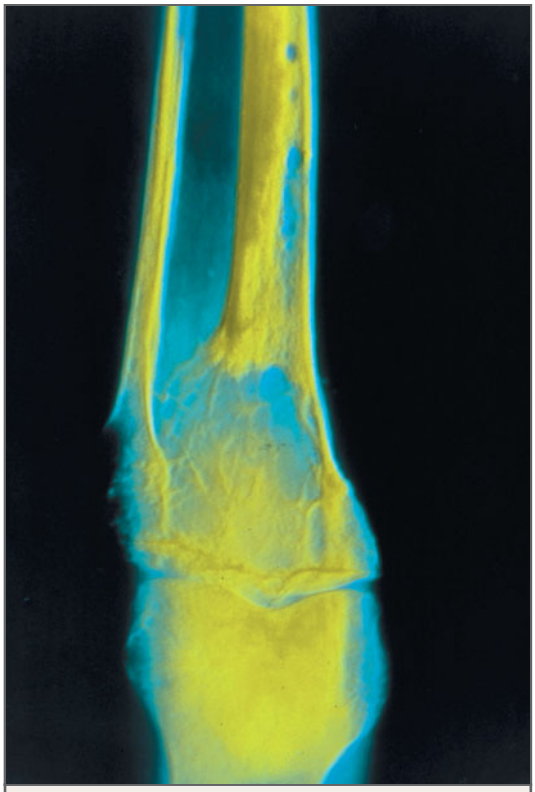
MULTIPLE MYELOMA AND RELATED DISORDERS

Edited by Gösta Gahrton, Brian G.M. Durie, and Diana M. Samson. 466 pp., illustrated. London, Arnold, 2004. $198.50. ISBN 0-340-81010-6.



A Pseudocolor Image of a Radiograph Showing Multiple Myeloma in the Tibia.

Collection CNRI/Phototake.

OUR UNDERSTANDING OF THE PATHOgenesis of multiple myeloma and the treatment of this condition have improved over the last decade to a degree unmatched by the advances of the preceding five decades. The publication of this book — edited by three distinguished members of the "myeloma community" and containing contributions from Europe, North America, and Australia — has been well timed in that it offers a benchmark reference for the specialist in this field and provides the nonspecialist with a comprehensive overview of the discipline at the beginning of an exciting new period.

The book is well structured, and the information within it accessible. It is divided into major sections on history and epidemiology, biology and pathophysiology, clinical investigation, treatment, and complications. Each of these contains several chapters, and there is an additional section that includes six chapters on related disorders.

The chapters are well written, and each offers a clear summary of what is currently known, with clear illustrations (though, sadly, only in black and white, apart from 16 color plates), a useful list of key points, and a comprehensive list of references. The slight overlap among chapters is forgivable, since this allows each to be read as a self-contained unit. For readers who wish to explore further the topics in each chapter, the references identify key primary papers and major review articles. However, the chapter on clinical features spurns this opportunity, since all but 1 of the 113 references are designated a "key primary paper" or a "major review article."

Conventional chemotherapy and high-dose treatment are well covered and discussed in a balanced way. However, there is considerably less discussion of the role of thalidomide in the clinical setting than one would have expected in a 2004 publication, since thalidomide has been used widely for at least a decade both within and outside of clinical trials. There is also less discussion of bortezomib and immunomodulatory drugs than there might have been, although this is perhaps less surprising, given that these emerging agents are at an early phase of clinical development.

Notable highlights are the four chapters on pathophysiology, including an overview of the emerging understanding of the biology of myeloma bone disease. Other highlights include a review of biochemical investigations and their pitfalls (not always the forte of the hematologist), a thought-provoking contribution on supportive care, and an excellent overview of Waldenström's macroglobulinemia, with further good chapters on monoclonal gammopathies of undetermined significance, solitary plasmacytoma, plasma-cell leukemia, amyloidosis, and heavy-chain diseases.

This book attempts to recount the biology and treatment of myeloma from the first case report in 1844 to the introduction of new therapies in the early 21st century. The editors hope, they write, that "the book will be of interest not only to scientists and specialists in the field but also for practitioners and non-specialists." They largely achieve their goals. A copy of this book should be available in ev-

ery hematology department and on the shelf of every specialist in this field.

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PANCREATITIS AND ITS COMPLICATIONS

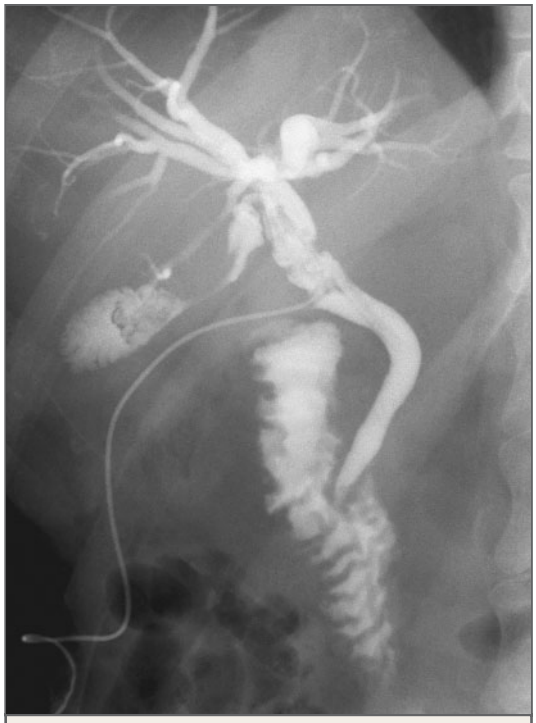
(Clinical Gastroenterology.) Edited by Chris E. Forsmark. 338 pp., illustrated. Totowa, N.J., Humana Press, 2005. $125. ISBN 1-58829-179-0.

THE PANCREAS NEVER SOUNDS A "LUB dub," makes no borborygmi, and is rarely palpable on physical examination. Nonetheless, gram for gram, it is just about the most active and efficient protein-synthesizing factory in the body. Moreover, when activated spontaneously, its vast store of digestive enzymes cannot distinguish between a hamburger and the human retroperitoneum. So when the pancreas is troubled, serious medical problems follow.

Pancreatitis and Its Complications is edited by Chris E. Forsmark of the University of Florida College of Medicine, who comes from a background in clinical academic research and has extensive experience in sophisticated endoscopic imaging and in treatment. The authors of various chapters of this book virtually delineate the global experts in pancreatic disease.

The text is divided into two parts, the first dealing with acute pancreatitis, and the second with chronic pancreatitis. The section on acute pancreatitis is particularly strong in its discussions of epidemiology, pathophysiology, and risk stratification. There is an extensive discussion of the pathophysiology of alcoholic acute pancreatitis and gallstone pancreatitis. Some other causes of acute pancreatitis, such as peptic ulcer disease and acute penetrating or blunt abdominal trauma, are discussed only in passing, unfortunately. The chapter on treatment of severe acute pancreatitis is exceptional, yet it has only a very limited discussion of drug therapies. Indeed, there is twice as much discussion about the role of fasting and nasogastric suction in treating acute pancreatitis as there is discussion devoted to new, albeit disappointing, drug treatments, including octreotide, aprotinin, and gabexate.

Readers should pay particular attention to an exemplary and illuminating chapter dealing with the



Biliary Stones Causing Acute Pancreatitis.

Wellcome Photo Library.

management of pancreatic-fluid collection and pseudocysts in patients with acute pancreatitis. Excellent tables and a particularly helpful and erudite algorithm provide an intelligent and well-balanced approach to the treatment of patients with these common complications of acute pancreatitis.

The second section, on chronic pancreatitis, is also well written and very well illustrated. The tendency has always been, particularly in general medical and surgical circles, to consider chronic pancreatitis as merely the long-standing, painful consequence of multiple bouts of acute pancreatitis. The insidious, and sometimes silent, yet relentless development of chronic pancreatitis with its multiple manifestations is nicely displayed in both text and illustration format.

Four important areas are exceptionally well covered: the pathophysiology and diagnosis of chronic pancreatitis, the use of pancreatic-enzyme preparations, and the endoscopic approach to patients with chronic pancreatitis. David Whitcomb's chapter dealing with the pathophysiology of chronic pancreatitis discusses genetic mutations that are probably associated with the condition, specifically those involving the *CFTR*, *PRSS1*, and *SPINK1* genes. He also discusses the possible contributions of less

appreciated factors, such as tobacco smoking and renal failure, to the development of chronic pancreatitis. Forsmark's chapter on the diagnosis of chronic pancreatitis leaves one wondering why so many of the tests he mentions are simply not available in the United States, including bentiromide (NBT-PABA) and pancreaolauryl tests, triolein breath tests, and secretin and cholecystokinin (CCK) simulation tests. Reluctantly, one has to conclude that American medicine has bypassed these inexpensive and noninvasive tests of pancreatic function for the more invasive and remunerative tests, such as endoscopic retrograde cholangiopancreatography and endoscopic ultrasonography. An important therapeutic point mentioned by contributor Phillip P. Toskes is the use of nonenteric-coated pancreatic enzymes in the treatment of chronic pancreatitis. Unfortunately, most clinicians fail to recognize that for patients with small-duct chronic pancreatitis, the use of nonenteric-coated pancreatic enzymes such as pancrelipase (Viokase 16) can have dramatic beneficial effects on recurrent abdominal pain. Finally, the chapter by Lee McHenry, Glen Lehman, and Stuart Sherman on the endoscopic approach is particularly well written, but it suffers from the lack of color endoscopic photographs.

All in all, this book is an important compendium of the current state of the art and science in treating patients with pancreatitis. It is certainly a "must have" for gastroenterologists and probably for internists and surgeons as well.

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PHARMACOTHERAPY OF OBESITY: OPTIONS AND ALTERNATIVES

Edited by Karl G. Hofbauer, Ulrich Keller, and Olivier Boss. 488 pp., illustrated. Boca Raton, Fla., CRC Press, 2004. $149.95. ISBN 0-415-30321-4.

With the global epidemic of obesity and its coexisting conditions, the need for effective obesity treatments has never been greater. Current behavioral and dietary therapies frequently lead to sufficient weight loss to reduce risk factors such as hypertension and dyslipidemia. However, with the exception of bariatric surgery, treatments resulting in long-term maintenance of lost weight remain elusive. At the same time, advances in basic science have led to a better understanding of the pathways that affect energy balance and to the identification of myriad potential central and peripheral drug targets. Hence, a comprehensive textbook focusing on the pharmacotherapy of obesity is timely.

The target audience of *Pharmacotherapy of Obesity* is physicians and other health care professionals who treat obesity. The book's six sections cover the pathophysiology of obesity, general therapeutic aspects, benefits of weight loss, drugs on the market, drugs in research and development, and treatment alternatives (which include herbal supplements and bariatric surgery). The quality of the chapters and the level of detail vary considerably. The chapter titled "Diet, Physical Activity, and Behavior" is written for the nonspecialist and provides practical advice on behavioral strategies, whereas the chapter titled "The Pathophysiology of Appetite Control" is more appropriate for a graduate student or researcher. A chapter (or at least more information) on the use of medications to treat obesity in children and adolescents would have been helpful.

The inclusion of a chapter on antipsychotic drugs, weight gain, and diabetes is timely. This chapter delves into the mechanisms by which atypical antipsychotic drugs may contribute to insulin resistance and diabetes. In a book targeted to the clinician, more attention might have been paid to potential ways to prevent and treat these consequences, which are discussed in only a few paragraphs. Additional review of the many other psychotropic medications that may contribute to weight gain would also have been useful.

There is much overlap between chapters, and the sometimes-differing interpretations of the same data may be confusing for the uninitiated reader. For example, when trying to determine whether the effect of sibutramine on pulse and blood pressure may change the risk–benefit ratio, a reader of the chapter titled "Basic Considerations and Guidelines for Treatment" is told that "noradrenergic agents have an effect on pulse and blood pressure, but this action tends to be neutralized by the opposite effect from weight loss." However, the reality is that a similar amount of weight loss due to lifestyle changes will have a larger favorable impact on pulse and blood pressure than will weight loss due to the effects of certain weight-loss medications, such as sibutramine. This observation is correctly discussed in the following chapter, titled "Weight Loss and Cardiovascular Risk Factors."

A possible limitation of this book about a fast-changing field is that it may be outdated by the time it

is published. The editors have established a Web site (www.endo-diabasel.ch/BookAnti-obesitydrugs.htm) where they plan to post important developments related to the pharmacotherapy of obesity that may occur between published editions. Readers who are knowledgeable about this field will be acutely aware of the changes that took place while this book was in production. For example, in the chapter on herbal supplements, ephedra and ephedrine–caffeine are still discussed as treatment options, although the medications were withdrawn from the market in April 2004. In the chapter on drug targets, cannabinoid receptors are briefly discussed; however, rimonabant — currently in phase 3 clinical trials and of much interest to the reading public — is listed only by its research compound identifier (SR141716), and only animal data are discussed. Off-label use of medications that are approved for other purposes, such as topiramate and bupropion, gets only a brief, one-paragraph mention. Thus, many of the drugs that might be of most interest to treating physicians are not discussed in depth or the information that is presented is not current enough to be useful.

In sum, this book contains reasonably useful information regarding existing medications, but the information on drugs that are under development is not as useful. Members of the target audience — practicing clinicians — are not likely to find this book very useful as a manual for treating their obese patients, although students and researchers will find information that may be of use to them.

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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.*

NEUROANATOMICAL DISSECTION: HUMAN BRAIN AND SPINAL CORD

The course will be offered in Milwaukee, July 21–23. It is sponsored by Marquette University.

Contact Marquette University, College or Health Sciences, P.O. Box 1881, Milwaukee, WI 53201; or call (414) 288-3093; or see http://www.marquette.edu/chs/ce/neuroce.html.

NATIONAL ASSOCIATION FOR THE RELIEF OF PAGET'S DISEASE

The following conferences will be offered in Oxford, England, United Kingdom: "Advances in the Molecular Pharmacology and Therapeutics of Bone Disease" (July 6 and 7) and "International Symposium on Paget's Disease" (July 8 and 9).

Contact Janet Crompton, The Old White Hart, North Nibley, Dursley, Glos GL 11 6DS, United Kingdom; or call (44) 1453 549929; or fax (44) 1453 548919; or see http://www.paget.org.uk; or e-mail janetcrompton@compuserve.com.

HARVARD SCHOOL OF PUBLIC HEALTH

A meeting entitled "Medicine and Compassion: A Special Program on Expanding Compassion in Practice and Medical Training" will be held in Boston, July 7–9.

Contact Harvard School of Public Health, 677 Huntington Ave., CCPE, Department A, Boston, MA 02115-6096; or call (617) 384-8692; or see http://www.hsph.harvard.edu/ccpe/programs/compassion.html; or e-mail contedu@hsph.harvard.edu.

UNIVERSITY OF PENNSYLVANIA

The 15th annual "Dermatopathology: Self-Assessment and Board Review" will be held in Philadelphia, July 9 and 10.

Contact Department of Dermatopathology, Hospital of the University of Pennsylvania, 2nd Floor, Maloney Bldg., 3600 Spruce St., Philadelphia, PA 19104; or call (215) 662-4497; or fax (215) 349-5615; or e-mail susan.lamey@uphs.upenn.edu.

4TH INTERNATIONAL MEETING ON INTENSIVE CARDIAC CARE

The meeting will be held in Tel Aviv, Israel, Sept. 27–29. Deadline for submission of abstracts is July 10.

Contact Secretariat, 4th International Meeting on Intensive Cardiac Care, P.O. Box 574, Jerusalem 91004, Israel; or call (972) 2 6520574; or fax (972) 2 6520558; or e-mail seminars@isas.co.il; or see http://www.cardiac-care2005.com.

NEUROLOGY FOR THE NON-NEUROLOGIST

The seminar will be held in Cape Cod, Mass., July 15–17. It is sponsored by Medical Education Resources.

Contact Linda Main, 1500 W. Canal Court, Littleton, CO 80120; or call (800) 421-3756 (national) or (303) 798-9682 (Colorado); or fax (303) 798-5731; or e-mail info@mer.org; or see http://www.mer.org.

ASTHMA AND ALLERGY FOUNDATION OF AMERICA

The AAFA is offering free educational materials to patients and health care professionals. The AAFA provides programs and services designed to strengthen the partnership between health care provider and patient.

Contact Mike Tringale, Asthma and Allergy Foundation of America, 1233 20th St., NW, Suite 402, Washington, DC 20036; or call (800) 7-ASTHMA (national) or (202) 466-7643, extension 272 (D.C.); or fax (202) 466-8940; or e-mail mike@aafa.org; or see http://www.aafa.org.

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

The "3rd Annual Meeting" will be held in San Francisco, June 23–25.

Contact International Society for Stem Cell Research, 60 Revere Dr., Suite 500, Northbrook, IL 60062; or call (847) 509-1944; or fax (847) 480-9282; or see http://www.isscr.org; or e-mail isscr@isscr.org.

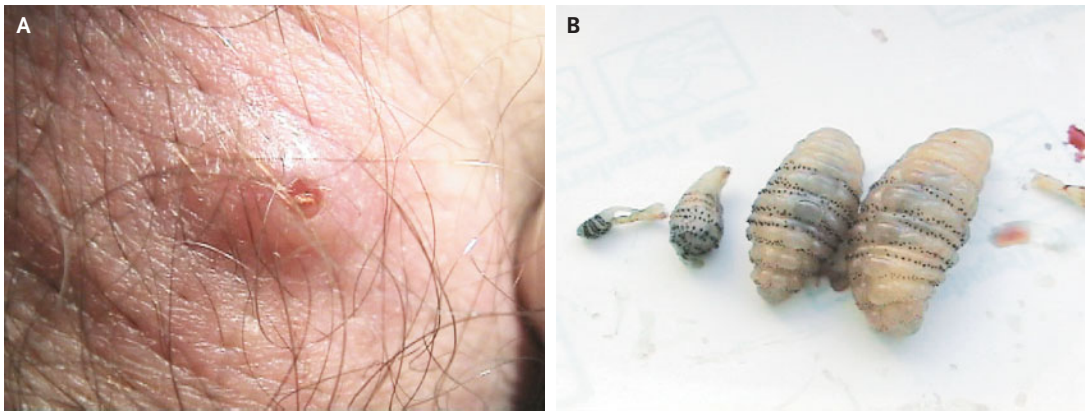
THE PAGET FOUNDATION

The following conference will be held: "Paget's Disease of Bone and Fibrous Dysplasia: Advances and Challenges" (Fort Lauderdale, Fla., Jan. 13 and 14).

Contact The Paget Founation for Paget's Disease of Bone and Related Disorders, 120 Wall St., Suite 1602, New York, NY 10005; or call (212) 509-5335; or e-mail pagetsym@aol.com.

IMAGES IN CLINICAL MEDICINE

Myiasis Due to *Dermatobia hominis* (Human Botfly)



A 65-year-old man presented with skin lesions on his chest and left arm and shoulder six weeks after returning from a vacation in Belize at the beach and in the rain forest. The lesions occasionally stung, drained a dark exudate, and enlarged despite two weeks of treatment with cephalexin. The patient had no constitutional symptoms. Physical examination revealed five nodules of varying sizes with surrounding erythema and a central pore through which a single, moving larva was observed (Panel A). The pores were occluded with petrolatum for two hours. After lidocaine was injected around the nodules, five *Dermatobia hominis* larvae at various developmental stages were extracted with the use of manual pressure and tweezers (Panel B and Video Clip 1). Larvae can also be extracted with suction or surgically. The patient recovered fully.

Distributed throughout Latin America, *D. hominis* begins its life cycle when adults lay eggs on porter zoophilic insects. The eggs hatch in response to the host's body heat, followed by larval penetration into the skin. Mature larvae then emerge from the host and pupate in the soil.

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