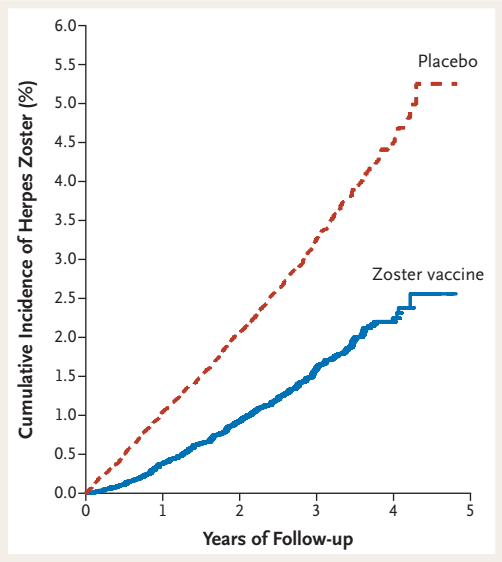
This Week in the Journal

JUNE 2, 2005

ORIGINAL ARTICLE

Vaccination to Prevent Herpes Zoster



Herpes zoster and postherpetic neuralgia occur more often with increasing age. In this controlled trial among 38,546 adults 60 years of age or older, vaccination with a live attenuated varicella–zoster vaccine reduced the incidence of postherpetic neuralgia by 66.5 percent (as compared with placebo) and the incidence of herpes zoster by 51.3 percent.

Vaccination with the highly immunogenic varicella–zoster vaccine was associated with frequent local symptoms but not severe adverse reactions. Boosting immunity to varicella can reduce the incidence and severity of herpes zoster.

SEE P. 2271; PERSPECTIVE, P. 2266; EDITORIAL, P. 2344; CME, P. 2365

ORIGINAL ARTICLE

Influence of Variants of *VKORC1* on the Dose of Warfarin

The gene encoding vitamin K epoxide reductase complex 1 (*VKORC1*) is the target of warfarin, and a variant of the gene may be associated with variations in dose requirements. This study reveals a strong association between *VKORC1* haplotype and the warfarin dose among European Americans. It also suggests that *VKORC1* variants may explain the variations in dose requirements among persons of different ancestries.

SEE P. 2285

ORIGINAL ARTICLE

Inherited Avascular Osteonecrosis of the Femoral Head

The gene encoding type II collagen, *COL2A1*, was found to be mutated in affected members of three families with an inherited form of avascular osteonecrosis of the femoral head. This finding will spur studies of this gene in persons with the idiopathic form of the disease.

SEE P. 2294; PERSPECTIVE, P. 2268

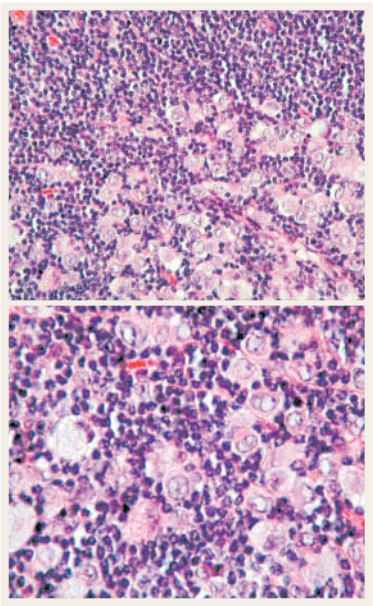
ORIGINAL ARTICLE

Adjuvant Docetaxel for Node-Positive Breast Cancer

This randomized trial included almost 1500 women with breast cancer and positive axillary nodes and compared treatment with doxorubicin and cyclophosphamide combined with either fluorouracil or docetaxel. The rates of disease-free and overall survival were significantly higher among women in the docetaxel group.

The management of breast cancer has improved step by step, beginning with the abandonment of routine radical mastectomy and continuing to large trials, such as this one, that involve new combinations of drugs and new agents that bring incremental improvements to women with breast cancer.

SEE P. 2302; EDITORIAL, P. 2346



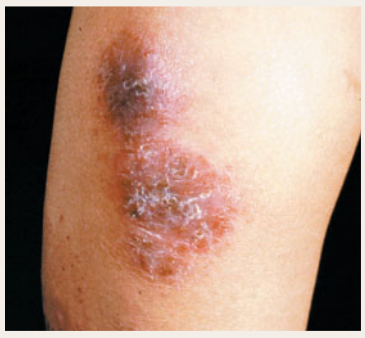
Courtesy of Dr. Richard Berendt, Cross Cancer Institute, Edmonton, Canada.

CLINICAL PRACTICE

Atopic Dermatitis

A 10-year-old girl with atopic dermatitis reports itching that has recently become relentless, resulting in sleep loss. Her mother has been reluctant to treat the girl with topical corticosteroids because she was told that they damage the skin, but she is exhausted and wants relief for her child. How should the problem be managed?

SEE P. 2314; CME, P. 2366



MEDICAL PROGRESS

Brucellosis

Brucellosis has been present for millennia and has managed to elude eradication, even in most developed countries. Brucellosis causes much clinical morbidity as well as an important loss of agricultural productivity in the developing world. In this era of international tourism, brucellosis has become a common imported disease in the developed world. This review article discusses the pathogenesis and treatment of the disease.

SEE P. 2325; CME, P. 2367

CLINICAL PROBLEM-SOLVING

Don't Know Much about History

A 20-year-old man who had immigrated to the United States from Mexico three months earlier presented to the emergency department reporting weight loss and pain in his hip and the right side of his lower back. He had noted decreased appetite for several weeks and had unintentionally lost 16 kg.

SEE P. 2338

PERSPECTIVE

Americans as Survivors

Robert Jay Lifton, M.D.

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

Physicians have always been concerned with how people survive trauma. There has been much interest in the psychology of the survivors of such massive trauma as that inflicted by the Nazis in their death camps, by the atomic bombing of Hiroshima and Nagasaki, and more recently, by the extraordinary earthquake and tsunami in South Asia.

Less noted has been the experience of Americans as survivors of violent collective trauma. We owe this lack of attention to the relative rarity of large-scale killing and dying on American soil and to the fact that in wars fought abroad, suffering has usually been countered by a sense of victorious achievement.

All this changed as a result of the Vietnam War, in which heavy American casualties were followed by defeat; then the terrorist attacks of September 11, 2001, which brought large-scale trauma home to Americans; and, now, the war in Iraq and the anguish and uncertainty associated with it. The collective psychological responses to these events reverberate throughout our society — as they would in any society undergoing similar trauma. These responses can be both painful and a potential source of illumination.

A survivor is one who has been exposed to the possibility of dying or has witnessed the death of others yet remained alive. The responses of survivors vary greatly, depending on the particular encounter with death and on personal traits. But I have found certain psychological patterns to be quite consistent.[1] Survivors struggle with images of death and dying — what I call a "death imprint." They feel a sense of debt to the dead, a need to placate them or carry out their wishes in order to justify their own survival. Survivors embark on an anguished quest for meaning and form, for what Erich Lindemann, in his classic study of the Cocoanut Grove nightclub fire in Boston in 1942, called "an acceptable formulation of [one's] future relationship to the deceased."[2] Human beings are meaning-hungry creatures, and survivors can epitomize this need by undertaking lifelong missions on behalf of the dead.

One can speak of both immediate survivors and more distant survivors, and there are important differences between them. Immediate survivors are those who have been directly exposed to death — as survivors of the attacks on the World Trade Center or of combat in Vietnam or Iraq or as family members of those who were killed. Their responses are visceral; their psyches have often been decimated, and they must struggle to reconstitute a sense of self. They bear wrenching psychological pain. Generally speaking, the nearer one was to the attack, the greater one's anxiety about death. After the attack on the World Trade Center, the levels of fear and of therapeutic need in New York City were found to be considerably higher than those elsewhere in the United States.

Distant survivors of September 11, 2001, or the Vietnam War include Americans in general. Their psychological wounds are less elemental, but their responses may entail considerable passion — a sense of individual and collective fear and vulnerability and feelings of injured national pride and humiliation.

Any experience of survival can connect psychologically with earlier traumas, with the losses and separations of ordinary life. Similarly, large-scale traumas can become intermingled. The war in Vietnam, the attacks on September 11, and the war in Iraq may blend within the individual American psyche, becoming virtually indistinguishable sources of pain and anger. This psychological blurring of the perceptions of events can contribute to collective confusion and a susceptibility to political manipulation.

No event, however destructive, has inherent

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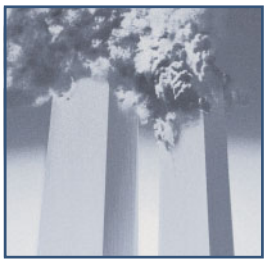
meaning. Rather, the meaning must be constructed by those responding to the event — by both immediate and distant survivors and, later, by historians. Consider, for instance, two responses to the Holocaust. The slogan "Never again!" signaled the emergence, in the late 1960s, of the Jewish Defense League, a political movement that included family members of Holocaust survivors and did not hesitate to act violently against designated enemies. During the same period, in 1969, a group of Auschwitz survivors asked me to join them in protesting the massacre by U.S. soldiers of 500 Vietnamese civilians in the village of My Lai. That atrocity, which had just been reported in the American press, seemed, as one Auschwitz survivor put it, "too close" to his own experience. Antithetical as these two responses were, both expressed the searing emotion that such a powerful encounter with death can evoke.



Survivors may find healing in extracting wisdom from their experience and in combating the destructive forces to which they have been subjected. Hiroshima survivors have traveled the world to tell their stories and mobilize opposition to the nuclear arms race. Similarly, parents whose children have died of leukemia have joined or formed groups to support research aimed at preventing and treating the disease.

But collective survivor missions can also be bound up with mass violence and war. The Nazis drew skillfully on vengeful feelings in response to the humiliating defeat of Germany in World War I, summoning the image of the "stab in the back," the betrayal, by Jews and others as the cause of that defeat. Promising national and individual revitalization, the Nazis derived mythic power from "the fallen" — Germans killed in the war — and at party meetings even called out their names to invoke their presence. There are probably few wars in history that were not fought on the basis of meanings given to the trauma of a previous war — that is, in relation to a mission to undo, reverse, or in some way alter the earlier outcome.

In response to the Vietnam War, the attacks of September 11, 2001, and the current war in Iraq, Americans' emotions as survivors have combined with ideological tendencies in polarizing ways. In the case of Vietnam, even during the war, opposition from U.S. veterans, active service personnel, and the public was widespread. With the American defeat, a broadly shared sense of meaning, experienced psychologically and politically, included the view that the United States should show greater military restraint and avoid wars with unclear goals in remote areas. An opposite meaning, built on similar emotions but a different worldview, held that any such restraint would be a sign of national weakness — the same kind of "weakness" manifested in that war by not making use of the potential of U.S. firepower. Hence, at the end of the first Gulf War in 1991, President George H.W. Bush triumphantly declared, "By God, we've kicked the Vietnam syndrome once and for all!"[3]

Leading advisers of the administration of President George W. Bush were also deeply affected by the defeat in Vietnam, which, as the writer James Mann observed, "led to a preoccupation with first regaining and then maintaining American military power."[4] Thus, in an important collective psychological sense, both the Gulf War and the war in Iraq can be understood as survivor missions in response to the Vietnam War.

In the immediate aftermath of September 11, Americans' shared responses of shock, fear, and anger blurred early differences in survivor meanings. These emotions were experienced by political leaders, who were able to channel them into an official survivor mission of a "war on terrorism" — a series of far-flung military and paramilitary actions, sometimes considered a crusade against evil, without clear limits in time or place. Support for that response, which included the invasion of Afghanistan and the overthrow of a government that had harbored the perpetrators of the attacks, was readily mobilized among Americans, struggling as they were with feelings of humiliation and victimiza-

tion. Yet many moved beyond these feelings to contest the amorphous extension of the survivor mission to Iraq, which they thought had no discernible connection to the attacks on September 11.

An alternative survivor mission had a very different emphasis. Some Americans understood the attacks to be murderous acts of terrorism (even, in terms of the Nuremberg Principles, crimes against humanity) to which the response should be the use of only sufficient force to bring the perpetrators to justice. They raised questions about both the sources of the terrorism (including U.S. policies that might have contributed to Islamist extremism) and the failure of U.S. intelligence and other government agencies to prevent the attacks.

Much of the response embodied in this alternative mission was expressed by family members of those killed in the attacks, when they put pressure on the administration and Congress to conduct an official investigation that would probably not have occurred otherwise. Their mission resonated with large numbers of Americans, as part of a continuing struggle to grasp the meaning of the attacks.

The war in Iraq is being fought as a manifestation of survivor missions stemming from both the Vietnam War and the attacks in 2001. Yet once any war begins, it takes on meanings of its own. The dead become the ultimate moral focus, and the traditional survivors' mission is to ensure that they did not "die in vain" and to complete their work by prosecuting the war ever more vigorously. This survivor mission began with the first American death in the invasion of Iraq and extends to the continuing deaths of American soldiers and civilians at the hands of Iraqi insurgents.

The traditional response to war has been questioned by Americans who have given alternative meanings, psychological and ethical, to deaths in Iraq. They draw a parallel between the wars in Iraq and Vietnam: both are seen as unwinnable counterinsurgencies with unclear goals carried out in alien, hostile environments against enemies who are invisible and highly dangerous. A small but growing group of veterans of Iraq who embrace these alternative meanings have modeled themselves on the antiwar veterans of the Vietnam War.

Most Americans seem to be hovering between traditional and alternative responses to the war in Iraq. Though troubled by deaths in a war whose justifications are seen as contradictory, they are nevertheless loath to admit that Americans may have died in vain.

There have always been survivors critical of war, but frequently they are drowned out by traditional claims to glory. Even Homer's *Iliad*, in many ways a saga that immortalizes the heroic warrior, contains an undercurrent of sadness and suffering, with voices that question the purpose of war and the grotesque deaths and terrible sacrifices incurred. Such feelings reached an apogee in World War I, as expressed by the English soldier poet Wilfred Owen: "Starkly I return to stare upon the ash of all I burned"; "Foreheads of men have bled where no wounds were. / I am the enemy you killed, my friend. / I knew you in this dark." A collective survivor mission has taken shape in the vast antiwar literature created by poets, novelists, and memoirists.

Vietnam veterans with whom I worked in the early 1970s could identify more with veterans of World War I than with those of World War II, who were often their own fathers and who saw themselves as having fought a necessary or "good" war. The alternative survivor mission of American veterans of the Vietnam War took the form of powerful public psychodrama when a number of these veterans threw their medals and decorations onto the steps of the Capitol in a passionate rejection of traditional claims to personal and national glory.[5]

Large-scale killing and dying always lead to struggles with collective trauma. As physicians and as citizens, we do well to examine the ways in which the psychology of the survivor enters into these struggles, since survivors, whether immediate or distant, seek relief and new knowledge from the pain of death and loss. Beyond their psychological effects on individual people, such events can have vast social and historical consequences. The way in which we survive them — the meanings we give them — will have a profound influence on the American future.

1. Lifton RJ. The future of immortality: and other essays for a nuclear age. New York: Basic Books, 1987:231-56.
2. Lindemann E. Symptomatology and management of acute grief. Am J Psychiatry 1944;101:141-8.
3. Bush GHW. Remarks to the American Legislative Exchange Council, March 1, 1991. In: Public papers of the Presidents of the United States, 1991. Book 1. January 1 to June 30, 1991. Washington D.C.: Government Printing Office, 1992:197.
4. Mann J. Rise of the Vulcans: the history of Bush's war cabinet. New York: Viking, 2004:52.
5. Lifton RJ. Home from the war: learning from Vietnam veterans. New York: Other Press, 2005.

Aging, Immunity, and the Varicella–Zoster Virus

Ann Arvin, M.D.

Related article, page 2271

Older people and their physicians are all too well acquainted with herpes zoster — commonly called shingles, from the Latin *cingulum,* or "girdle." Most recognize that aging creates a special vulnerability to this often severely painful skin rash caused by varicella–zoster virus (VZV). VZV is so named because varicella (chickenpox) represents the first encounter between the virus and the host, and herpes zoster represents the second. VZV persists in the sensory ganglia of the cranial nerves and the spinal dorsal-root ganglia after varicella resolves, and it may become reactivated after many decades of latency; molecular analyses of VZV DNA demonstrate that herpes zoster is a reappearance of one's childhood virus. The debilitating pain syndromes that accompany this reactivation have motivated the search for effective medical interventions. In this issue of the *Journal,* Oxman et al. (pages 2271–2284) report a new strategy that uses immunization with a live attenuated VZV vaccine, derived from the Oka strain, to reduce the morbidity associated with herpes zoster in healthy men and women older than 60 years of age. Pediatric Oka vaccines prevent varicella-related illness and death. A higher-potency Oka vaccine now promises to ameliorate recurrent VZV at the other end of the age spectrum.

VZV, like other herpesviruses, has evolved a genetic program that controls virus–host interactions so that its survival in the human population is ensured. The vesicular skin lesions of varicella and herpes zoster contain the high concentrations of infectious VZV that are needed for transmission to susceptible persons. During varicella outbreaks, VZV infects most children in a community, but those born later will escape infection unless the virus is reintroduced.

What perturbs the latent VZV genome is not understood, but reactivations that cause herpes zoster in adults provide a reliable source of transmissible virus. This resurfacing of VZV requires the movement of virions from the neurons, along axons, to the skin, where the virus must evade innate and adaptive host immune responses, spread from cell to cell, and form lesions that eventually penetrate the epidermis. The clinical experience indicates that these mechanisms of VZV immune evasion are most successful in the elderly.

The localized skin rash is not a serious problem, but the prodromal pain associated with herpes zoster is a sign of the active infection of sensory ganglia. The syndrome of zoster sine herpete also suggests direct involvement of the ganglia, and autopsy studies have confirmed the destruction of neurons and satellite cells during VZV reactivation.[1] As a consequence of this pathogenic process, the benefit of antiviral therapy with acyclovir and related agents is limited in patients with herpes zoster. Neurologic damage begins before the characteristic dermatomal rash appears, and postherpetic neuralgia can be prolonged and intractable despite early antiviral therapy and aggressive pain management.

The concept that VZV takes advantage of waning immunity to ensure its persistence in the population by causing herpes zoster is supported by correlations between age and a diminished capacity of peripheral-blood T cells from persons who are immune, and therefore latently infected, to proliferate and produce interferon-γ when stimulated with VZV antigens in vitro. VZV-specific T-cell immunity is elicited by primary VZV infection and is required for the resolution of varicella. Memory CD4 and CD8 T cells that recognize VZV proteins remain readily detectable in younger adults, in whom herpes zoster is relatively rare (see graph). Robust memory-T-cell immunity to VZV may reflect either the extent of the initial expansion of VZV-specific T cells elicited during primary infection or periodic boosting on exposure to varicella or on abortive, subclinical reactivation (or some combination of these mechanisms). Loss of VZV-specific T-cell responses also defines periods of susceptibility to herpes zoster in immunocompromised patients. In contrast, anti-

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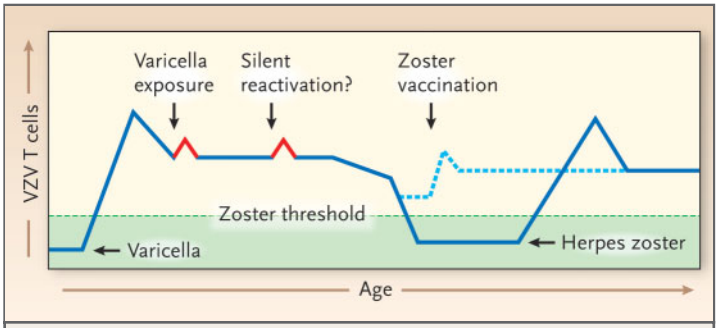

Host Factors in VZV Latency and Reactivation.

Varicella is the primary infection caused by VZV, and its resolution is associated with the induction of VZV-specific memory T cells (blue line). Memory immunity to VZV may be boosted periodically by exposure to varicella or silent reactivation from latency (red peaks). VZV-specific memory T cells decline with age. The decline below a threshold (dashed green line) correlates with an increased risk of zoster. The occurrence of zoster, in turn, is associated with an increase in VZV-specific T cells. The administration of zoster vaccine to older persons may prevent VZV-specific T cells from dropping below the threshold for zoster occurrence (dashed blue line).

VZV IgG antibodies persist, and persons who are at risk for herpes zoster because of declining T-cell responses continue to be protected from varicella. Functionally, VZV-specific memory T cells probably control the later stages of reactivation that produce the typical signs of herpes zoster, rather than preventing latent VZV genomes in the ganglia from beginning to replicate. Over time, waning VZV-specific T-cell immunity may place a person in a "danger zone" for symptomatic VZV reactivation (see graph).

Giving inactivated varicella vaccine to recipients of hematopoietic-cell transplants results in an earlier reconstitution of VZV-specific T-cell immunity and a lower incidence of herpes zoster[2] — demonstrating that VZV-specific T cells play a definitive role in maintaining the virus–host equilibrium in favor of the host. Similarly, the effectiveness of the high-potency Oka vaccine probably results from the restoration of VZV-specific T cells to a level above the threshold for herpes zoster — either by reversing a gradual decline from the original expansion of VZV-specific memory T cells or by substituting immunization for exogenous or endogenous reexposures that boost immunity (see graph). The effect of immunization against VZV demonstrates that naturally acquired immunity can be enhanced in a manner that controls the medical consequences of a persistent viral pathogen. It also suggests that it would be worthwhile to investigate the use of therapeutic vaccines against other herpesviruses in healthy and high-risk patients.

Translating the observations of Oxman et al. into clinical practice will require the licensing of higher-potency VZV vaccines, because the pediatric vaccines derived from the attenuated Oka strain are not adequate for boosting T-cell responses in older adults. Clinical experience is also required to determine the duration of protection, as well as whether repeated doses should be given and, if so, at what intervals. The burden-of-illness end point used by Oxman et al. is a composite of the effects on incidence and morbidity. Although the effect of vaccination on incidence was more limited in persons 70 years of age or older than in those who were 60 to 69 years old, the effect on the burden of illness was substantial in the former group because the severity and duration of pain were worse. More information is needed to determine whether these benefits are sustained in the very old.

What is certain is that although reactivation of VZV is rarely life-threatening, the effect of herpes zoster and postherpetic neuralgia on the quality of life is serious for many older people. According to the 2000 Census, 35.0 million people in the United States were older than 65 — a 12.0 percent increase from 1990 — and this trend will be even more dramatic as the baby boomers begin to turn 60. The possibility that a feared consequence of aging may be minimized or avoided is an important advance.

Dr. Arvin reports having received consultation fees from Merck.

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Type II Collagen and Avascular Necrosis of the Femoral Head

Darwin J. Prockop, M.D., Ph.D.

Related article, page 2294

All of us carry millions of variations in the sequences of our genomes and thousands of mutations in genes, some of which alter the amino acid sequences of proteins. The majority of the variations in genomes and even many of the changes in the sequences of proteins have no apparent effect on the risk of death or on longevity. The challenge of current molecular genetics is to distinguish the neutral variations from mutations that alter the functions of proteins sufficiently to predispose us to disease.

In their study reported in this issue of the *Journal*, Liu et al. (pages 2294–2301) have taken the easiest route to identifying disease-causing mutations: they performed genetic analyses in members of several generations of three families with a well-defined clinical phenotype, avascular necrosis of the femoral head (ANFH). First, they analyzed the short repetitive sequences of DNA (called microsatellites) that are scattered throughout the genome and that can be used to trace the inheritance of short regions of chromosomes as they are passed through different generations of a family. The results indicated that all the family members with ANFH but none of those without the syndrome had inherited the same variant region of chromosome 12. A fortuitous recombination of chromosome 12 in one family narrowed the region to about 5 million bases that contained 21 known genes and 25 potential genes.

This is the point at which many similar genetic studies flounder. If Liu et al. had been unlucky, they would have been left with the daunting task of sequencing more than 40 genes from affected and unaffected members of the families. They then could have faced the even more daunting task of establishing which of the hundreds of changes they probably would have detected actually altered the function of a vital protein sufficiently to lead to ANFH.

The investigators, however, had a striking piece of luck. The genes in the implicated region included the gene encoding type II collagen, which had previously been shown to be sensitive to a large number of disease-causing mutations. Type II collagen is a member of a family of collagens that form long fibers. The molecules of fiber-forming collagens are assembled from three chains that are each more than 1000 amino acids long (see diagram). A distinctive feature of the long collagen chains is that every third amino acid in the sequence is glycine, the smallest amino acid. Any substitution of a larger amino acid for one of the more than 300 "obligate" glycine residues distorts the long, rigid helix formed by the three chains of the protein. Some substitutions of larger amino acids for obligate glycines totally disrupt the triple helix and cause rapid degradation of the protein. Other glycine substitutions produce more subtle distortions of the helix and generate abnormal collagen fibers.

Type II collagen is the most abundant protein found in cartilage, and several hundred different glycine substitutions in type II collagen have been shown to cause genetic diseases of the skeleton. The diseases range from mild chondrodysplasias to lethal disorders. Several hundred similar substitutions of obligate glycines in type I collagen, the most abundant protein of bone and many other tissues, cause bone defects that range from mild to lethal osteogenesis imperfecta and that may include some forms of osteoporosis. To date, hundreds, if not thousands, of genes encoding the type I and type II collagens have been analyzed, and no substitution of an obligate glycine has been detected in an asymptomatic person. The diseases are dominant; there are no glycine substitutions in the normal alleles of patients.

For these reasons, Liu et al. must have been delighted when they sequenced the gene for type II collagen in their patients: they found a serine substitution for an obligate glycine in the same position in two of the families and a similar serine substitution for glycine in another position in a third family. The authors did not directly demonstrate that the mutated type II collagen altered the structure of the collagen matrix in the femoral heads in

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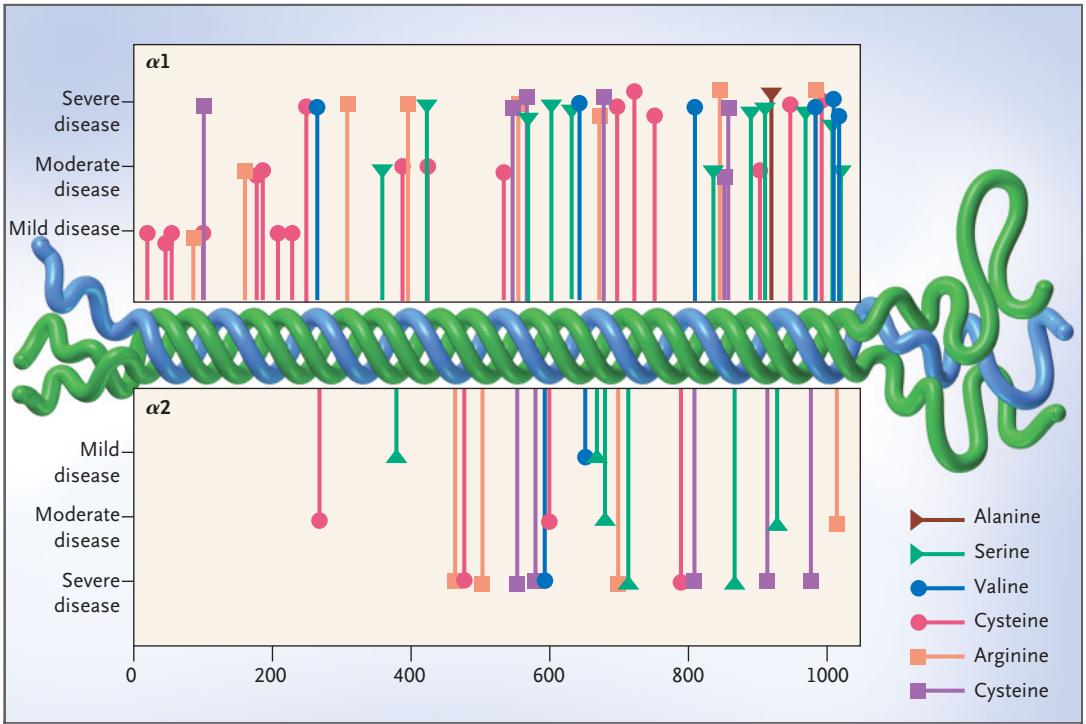

Mutations in Collagen Genes That Cause Skeletal Disease.

The myriad substitutions for glycine residues in the two chains of type I collagen that cause osteogenesis imperfecta illustrate the critical role of the glycine residue in establishing structural integrity. The helix of type I collagen is formed from two $\alpha 1$ chains and one $\alpha 2$ chain; the amino acid sequences of $\alpha 1$ and $\alpha 2$ chains differ slightly from one another. The structure of type II collagen is similar to that of type I, but the helix is formed from three identical $\alpha 1$ chains. As indicated, substitutions of different amino acids at different positions produce either a mild, moderate, or severe form of skeletal diseases. Similar glycine substitutions in type II collagen produce skeletal disorders referred to as chondrodysplasias, but it is more difficult to relate the manifestations of disease to the mutations.

their patients — a technically difficult task. However, their data meet the current criteria for establishing the genetic cause of a disease.

Like most unexpected observations, the data obtained by Liu et al. raise several new and difficult questions. Why did the mutations in type II collagen produce necrosis of the femoral head? Why did the patients lack other signs seen in most patients with mutations in type II collagen, such as short stature and defects of other joints or of the eyes?

In cartilage, type II collagen forms an arcade of tough fibers that entrap proteoglycans. The proteoglycans absorb large amounts of water to distend the arcade, and the distended arcade gives cartilage its resilience to compression. Also, cartilage made up of type II collagen plays a critical role in the development and growth of most bones. Since the patients did not have any generalized skeletal defects, the serine substitutions probably produced only a minor distortion of the collagen helix and minor changes in the collagen fibers they formed. We might therefore guess that the substitutions somehow caused a localized collapse of matrix around blood vessels during the early development of the femoral head. Unfortunately, this hypothesis is difficult to test.

How many of the 300,000 or more patients with ANFH in the United States also have mutations in type II collagen? Liu et al. did not find either of the glycine substitutions in 65 patients with ANFH who did not have a family history of the disease. As they point out, most patients with ANFH have no such family history, have a later age at onset than that in the three families they studied, and have predispos-

ing environmental factors such as the use of steroids or alcohol. The results do suggest that it would be useful to analyze the type II collagen gene in additional patients with ANFH who have an early onset of symptoms and who do not have any obvious predisposing conditions. As Liu et al. note, early diagnosis might improve the results of recently introduced therapies for ANFH, such as surgical decompression of the core of the hip or administration of growth hormone, differentiation factors, or bisphosphonates.

Dr. Prockop reports that the Center for Gene Therapy receives income generated by a DNA sequencing service provided by his laboratory.

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A Vaccine to Prevent Herpes Zoster and Postherpetic Neuralgia in Older Adults

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ABSTRACT

BACKGROUND

The incidence and severity of herpes zoster and postherpetic neuralgia increase with age in association with a progressive decline in cell-mediated immunity to varicella–zoster virus (VZV). We tested the hypothesis that vaccination against VZV would decrease the incidence, severity, or both of herpes zoster and postherpetic neuralgia among older adults.

METHODS

We enrolled 38,546 adults 60 years of age or older in a randomized, double-blind, placebo-controlled trial of an investigational live attenuated Oka/Merck VZV vaccine ("zoster vaccine"). Herpes zoster was diagnosed according to clinical and laboratory criteria. The pain and discomfort associated with herpes zoster were measured repeatedly for six months. The primary end point was the burden of illness due to herpes zoster, a measure affected by the incidence, severity, and duration of the associated pain and discomfort. The secondary end point was the incidence of postherpetic neuralgia.

RESULTS

More than 95 percent of the subjects continued in the study to its completion, with a median of 3.12 years of surveillance for herpes zoster. A total of 957 confirmed cases of herpes zoster (315 among vaccine recipients and 642 among placebo recipients) and 107 cases of postherpetic neuralgia (27 among vaccine recipients and 80 among placebo recipients) were included in the efficacy analysis. The use of the zoster vaccine reduced the burden of illness due to herpes zoster by 61.1 percent ($P<0.001$), reduced the incidence of postherpetic neuralgia by 66.5 percent ($P<0.001$), and reduced the incidence of herpes zoster by 51.3 percent ($P<0.001$). Reactions at the injection site were more frequent among vaccine recipients but were generally mild.

CONCLUSIONS

The zoster vaccine markedly reduced morbidity from herpes zoster and postherpetic neuralgia among older adults.

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Herpes zoster, or shingles, is characterized by unilateral radicular pain and a vesicular rash that is generally limited to a single dermatome.[1,2] Herpes zoster results from reactivation of latent varicella–zoster virus (VZV) within the sensory ganglia.[3,4] The incidence and severity of herpes zoster increase with advancing age; more than half of all persons in whom herpes zoster develops are older than 60 years. Complications occur in almost 50 percent of older persons with herpes zoster.[3-5] The most frequent debilitating complication is postherpetic neuralgia, a neuropathic pain syndrome that persists or develops after the dermatomal rash has healed.[5-9] The frequency and severity of postherpetic neuralgia also increase with increasing age.[9-11] The pain and discomfort associated with herpes zoster can be prolonged and disabling, diminishing the patient's quality of life and ability to function to a degree comparable to that in diseases such as congestive heart failure, myocardial infarction, diabetes mellitus type 2, and major depression.[12] Antiviral therapy reduces the severity and duration of herpes zoster but does not prevent the development of postherpetic neuralgia.[2,11] Postherpetic neuralgia may persist for years and is often refractory to treatment.[13]

Forty years ago, Hope-Simpson proposed that immunity to VZV plays a pivotal role in the pathogenesis of herpes zoster,[3] and subsequent observations support the thesis that cell-mediated immunity to VZV is a major determinant of the risk and severity of herpes zoster.[3,7,11,14-17] Whereas levels of antibody to VZV remain relatively constant with increasing age, the increased incidence and severity of herpes zoster and postherpetic neuralgia among older adults are closely linked to a progressive age-related decline in cell-mediated immunity to VZV.[4-8,14-21] Recurrences of herpes zoster are uncommon among immunocompetent persons, presumably because an episode of herpes zoster boosts immunity to VZV, effectively "immunizing" against a subsequent episode.[3,4,7,8,22]

Previous studies have shown that VZV vaccines can elicit a significant increase in cell-mediated immunity to VZV in immunocompetent older adults[21-25] and reduce the incidence and severity of herpes zoster in recipients of bone marrow allografts.[26,27] These observations led us to hypothesize that immunization of older persons with a VZV vaccine would boost their cell-mediated immunity to VZV and thereby provide protection against herpes zoster and postherpetic neuralgia.[22] The Shingles Prevention Study (Department of Veterans Affairs [VA] Cooperative Study No. 403) was conducted to determine whether vaccination with a live attenuated VZV vaccine would decrease the incidence, severity, or both of herpes zoster and postherpetic neuralgia in adults 60 years of age or older.

METHODS

A detailed description of the methods used in this study is provided in the Supplementary Appendix (available with the full text of this article at www.nejm.org). A brief overview is presented here.

STUDY DESIGN

We conducted a randomized, placebo-controlled, double-blind clinical trial at 22 sites, in which adults 60 years of age or older received either VZV vaccine or placebo. The study was approved by a human rights committee of the VA Cooperative Studies Program (VACSP) and by the local institutional review boards at all study sites. An independent data and safety monitoring board reviewed the safety data and the interim results.

STUDY POPULATION

Eligible subjects had a history of varicella or had resided in the continental United States for at least 30 years. Immunocompromised persons and those unable to adhere to the study protocol were excluded. All subjects provided written informed consent.

INTERVENTION

Subjects received one subcutaneous injection of 0.5 ml of the investigational live attenuated Oka/Merck VZV vaccine ("zoster vaccine") or placebo. The estimated potency at vaccination of the 12 vaccine lots used in the study ranged from 18,700 to 60,000 plaque-forming units per dose. The median potency was 24,600 plaque-forming units, and more than 90 percent of vaccinated subjects received 32,300 plaque-forming units or less.

FOLLOW-UP

Active follow-up and ascertainment of cases of herpes zoster were ensured by an interactive automated telephone-response system, which subjects called monthly. If a subject's responses to a standardized set of questions suggested a possible case of herpes zoster, the subject was instructed to contact the local study site immediately, and a fax con-

taining the subject's response was sent to the site. Subjects who did not call the automated telephone-response system within a pre-established length of time were called by the automated telephone-response system. If this effort to reach the subject failed, the local study site was notified by fax to contact the subject directly. At the end of the study, subjects were asked to report any previously unreported episodes of herpes zoster.

SAFETY EVALUATION

All adverse events occurring within 42 days after vaccination were recorded. Thereafter, only serious adverse events were recorded if reported by the subject and considered by the study physician to be related to the vaccination. Deaths were identified on the basis of reports from family members and during follow-up of missed monthly calls to the automated telephone-response system.

Approximately 300 subjects at each of the study sites were enrolled in a substudy that more closely monitored adverse events. These subjects maintained a daily log of body temperature and a "report card" of symptoms related to the injection site and other clinical symptoms during the 42 days after vaccination. Thereafter, they were followed to identify all hospitalizations.

IDENTIFICATION AND EVALUATION OF SUSPECTED CASES OF HERPES ZOSTER

At enrollment, the subjects were educated with regard to the signs and symptoms of herpes zoster. Those who had a new rash or new unilateral pain were urged to contact their study site immediately. Study personnel attempted to evaluate all subjects with new rashes as soon as possible. Subjects with unilateral rashes and no alternative clinical diagnoses were classified as having "suspected cases of herpes zoster" and were followed according to the study protocol. The evaluating physician offered subjects with clinically diagnosed herpes zoster, without cost, the licensed antiviral drug famciclovir (Famvir, SmithKline Beecham and Novartis Pharmaceuticals), in accordance with the manufacturer's recommendations, and with standard-of-care treatment for pain. Pain management was not specified by the study protocol.

Herpes zoster–associated pain (including unpleasant sensations such as allodynia and pruritus, which are not always characterized as pain by persons with herpes zoster) was measured with the use of the Zoster Brief Pain Inventory, an assessment tool in the form of a questionnaire completed by the subject that was specifically designed to measure pain and discomfort in herpes zoster.[28] This questionnaire and others[29,30] were used to assess the effect of herpes zoster on the subjects' activities of daily living, quality of life, and general health status. Characteristics of the rash, associated complications, and medication use were also recorded. Evaluations based on responses to the questionnaires were repeated over a period of at least 182 days, according to a schedule specified by the study protocol. Digital photographs and specimens for laboratory diagnosis were obtained from subjects with suspected cases of herpes zoster.

CONFIRMATION OF CASES

Before unblinding, each suspected case of herpes zoster was classified as a confirmed case of herpes zoster or as not a confirmed case with the use of a hierarchical algorithm that incorporated the results of the polymerase-chain-reaction (PCR) assay performed at the central laboratory of the study, virus culture at the local virology laboratory, and the final clinical diagnosis of the study's clinical evaluation committee, consisting of five physicians with expertise in herpes zoster.

The PCR assay, designed to detect and discriminate among DNA from wild-type and vaccine strains of VZV and from herpes simplex virus (HSV), could detect approximately 13 copies of DNA from wild-type or the vaccine strain of VZV. The PCR assays included primers and a probe for the human beta-globin gene to verify the presence of cellular DNA in the specimens from the lesions.

If the PCR assay revealed VZV DNA, the suspected case of herpes zoster was classified as a confirmed case; if the assay was positive for beta-globin or HSV DNA and negative for VZV DNA, the case was classified as not a case of herpes zoster. If the specimen obtained for the assay was inadequate (i.e., was negative for both viral and beta-globin DNA) or was missing, the final diagnosis was determined by the isolation of VZV or HSV in the local virology laboratory. In the absence of a valid laboratory diagnosis, the case was classified on the basis of the clinical diagnosis by the clinical evaluation committee.

EFFICACY END POINTS

The primary end point was the burden of illness due to herpes zoster, a severity-by-duration measure of the total pain and discomfort associated with her-

pes zoster in the population of study subjects.[28,31,32] For each confirmed case of herpes zoster, responses to the "worst pain" question in the Zoster Brief Pain Inventory were used to calculate a herpes-zoster severity-of-illness score, defined as the area under the curve (AUC) of herpes-zoster pain plotted against time during the 182-day period after the onset of rash. Subjects in whom herpes zoster developed had severity-of-illness scores ranging from 0 to 1813. Increasing mean scores are highly correlated with a decrease in the health-related quality of life and in functional status among older adults.[28,33] A score of 0 was recorded for subjects in whom herpes zoster did not develop during the study period.

The "herpes-zoster burden-of-illness score" represented the average severity of illness among all subjects in the vaccine or placebo groups; it was calculated as the sum of the herpes-zoster severity-of-illness scores of all members of a group divided by the total number of subjects in the group. The secondary end point was the incidence of postherpetic neuralgia, defined as pain associated with herpes zoster that was rated as 3 or more on a scale ranging from 0 ("no pain") to 10 ("pain as bad as you can imagine"), persisting or appearing more than 90 days after the onset of rash. Scores lower than 3 were not associated with significant decrements in the quality of life or the ability to carry out activities of daily living and were therefore not considered to represent postherpetic neuralgia.[10,28]

STATISTICAL ANALYSIS

A data-analysis plan was completed before the data were unblinded for analysis.[31,34-38] The analysis was performed by the VACSP coordinating center (West Haven, Conn.), with review and approval by the executive committee of the study. Vaccine efficacy with respect to the burden of illness due to herpes zoster (VE_{BOI}) was defined as the relative reduction in the burden-of-illness score in the vaccine group as compared with that in the placebo group and calculated as 1−relative risk (i.e., 1−the herpes-zoster burden-of-illness score in the vaccine group divided by the herpes-zoster burden-of-illness score in the placebo group). The prespecified criteria for the success of the vaccine with respect to the burden of illness due to herpes zoster required a VE_{BOI} point estimate of 47 percent or more and a lower bound of the 95 percent confidence interval greater than 25 percent. A method of assessing the combined effect of disease incidence, severity, and duration, weighted for age group, was used.[31]

Vaccine efficacy with respect to the incidence of postherpetic neuralgia (VE_{PHN}) was defined as the relative reduction in the incidence of postherpetic neuralgia in the vaccine group as compared with that in the placebo group. The prespecified criteria for the success of the vaccine with respect to the incidence of postherpetic neuralgia required a VE_{PHN} point estimate of 62 percent or more and a lower bound of the 95 percent confidence interval greater than 25 percent. The VE_{PHN} was calculated with the use of a conditional exact method weighted for age group.[34-36] The VE_{PHN} was also calculated with the use of alternative definitions of postherpetic neuralgia as pain present for more than 30, 60, 120, and 182 days after the onset of rash caused by herpes zoster. Vaccine efficacy with respect to the incidence of herpes zoster (VE_{HZ}) was calculated similarly.

Efficacy analyses were performed with the use of a follow-up period that excluded the first 30 days after vaccination and excluded subjects who withdrew and those in whom a confirmed case of herpes zoster developed within the first 30 days after vaccination. The results were essentially unchanged when subjects in whom herpes zoster developed during the first 30 days were included. All reported P values are two-sided.

CONDUCT OF THE STUDY

The study was designed by the planning and executive committees of the Shingles Prevention Study Group, the members of which were selected for relevant expertise, with the support of a planning grant from the VACSP to the study chairs (details are provided in the Supplementary Appendix). Merck contributed to the planning process through two nonvoting members on these committees. The statistical methods for analyzing burden of illness were developed and published by Merck statisticians before the initiation of the study.[31] The study was initiated and implemented as a VA Cooperative Study in collaboration with the National Institute of Allergy and Infectious Diseases and Merck. Merck, the holder of the investigational new drug application, participated in the organization of oversight activities and monitored the progress of the study. The Covalent Group, an independent company, was hired by Merck to monitor case report forms and adherence to the study protocol and to report to Merck and the VACSP. The participating investigators and their staff gathered the data. An unblinded statistician at the VACSP coordinating center, who was not involved in the operation of the study, pre-

sented unblinded safety data to the data and safety monitoring board.

Management and consolidation of the data were performed by the VACSP coordinating center. Data-analysis programs were developed and tested by biostatisticians and programmers at the coordinating center and at Merck. The data were analyzed at the coordinating center. The executive committee reviewed and approved the data-analysis plan and all analyses of vaccine efficacy and safety and vouches for the study's results. The writing committee, all members of which were also members of the executive committee, wrote the manuscript and takes responsibility for it.

RESULTS

CHARACTERISTICS OF THE STUDY SUBJECTS

A total of 38,546 subjects were enrolled in the study between November 1998 and September 2001 (Fig. 1). The numbers enrolled at each study site ranged from 1167 to 2508. Follow-up was completed in April 2004. The demographic characteristics of the two study groups were similar (Table 1). The median age in both groups was 69 years; 6.6 percent of the vaccine recipients and 6.9 percent of the placebo recipients were 80 years of age or older. At enrollment, most of the subjects had no health-related limitations on their activities (51.3 percent) or mild health-related limitations (38.6 percent). More than 95 percent of the subjects were actively followed to the end of the study (Fig. 1) and completed a closeout interview. The mean duration of herpes zoster surveillance was 3.13 years (median, 3.12 years; range, 1 day to 4.90 years) with no difference in duration between the groups. Only 0.6 percent of the subjects withdrew from the study or were lost to follow-up; 4.1 percent died during the study.

CONFIRMED CASES FOR THE EFFICACY ANALYSES

More than 3500 rashes that developed in subjects in each treatment group were evaluated clinically but were not considered to be suspected cases of herpes zoster. A total of 1308 subjects with suspected herpes zoster were evaluated according to the protocol (Fig. 1). Among these subjects, 317 (156 in the vaccine group and 161 in the placebo group) were determined not to have herpes zoster. Of these 317 subjects, 49 had rashes that were caused by HSV (24 in the vaccine group and 25 in the placebo group). Closeout interviews did not identify any previously unreported cases of herpes zoster. Of the 1308 suspected cases of herpes zoster, the final diagnosis in 1156 cases (88.4 percent; 417 in the vaccine group and 739 in the placebo group) was based on the results of the PCR assay.

Of the 1308 suspected cases, 984 (75.2 percent) were determined to be confirmed cases. In accordance with the protocol, 24 cases were excluded from the efficacy analyses because they occurred within 30 days of vaccination (6 in the vaccine group and 18 in the placebo group) and 3 because they were a subject's second episode of herpes zoster (Fig. 1). The remaining 957 confirmed cases of herpes zoster (315 in the vaccine group and 642 in the placebo group) constituted the end points of the efficacy analyses. The results of PCR testing were positive for wild-type VZV DNA in more than 93 percent of the confirmed cases of herpes zoster in each study group (Fig. 1). Vaccine virus DNA was not detected in any subjects with suspected herpes zoster.

The rate of use of antiviral medication among subjects with confirmed cases of herpes zoster was similar in the two groups (87.3 percent in the vaccine group and 85.7 percent in the placebo group), as was the proportion in whom treatment was initiated within 72 hours of the onset of rash — in 64.1 percent in the vaccine group and 65.9 percent in the placebo group. The frequency of use of various medications to treat pain resulting from herpes zoster was similar in the two groups, and the average duration of the use of opioids and the average quantity of opioids used among subjects with herpes zoster were greater in the placebo group than in the vaccine group. Thus, differences in the use of pain medication did not inflate the estimates of VE_{BOI} or VE_{PHN}.

BURDEN OF ILLNESS DUE TO HERPES ZOSTER

The herpes-zoster burden-of-illness score was significantly reduced in the vaccine group as compared with the placebo group ($P<0.001$) (Table 2). Overall, VE_{BOI} was 61.1 percent (95 percent confidence interval, 51.1 to 69.1), a result that met the prespecified criteria for success. There were no significant differences in the VE_{BOI} when the results were stratified according to sex or age (Table 2).

INCIDENCE OF POSTHERPETIC NEURALGIA

There were 107 cases of postherpetic neuralgia, 27 in the vaccine group and 80 in the placebo group (0.46 case vs. 1.38 cases per 1000 person-years, respectively; $P<0.001$) (Table 3). Overall, the VE_{PHN}

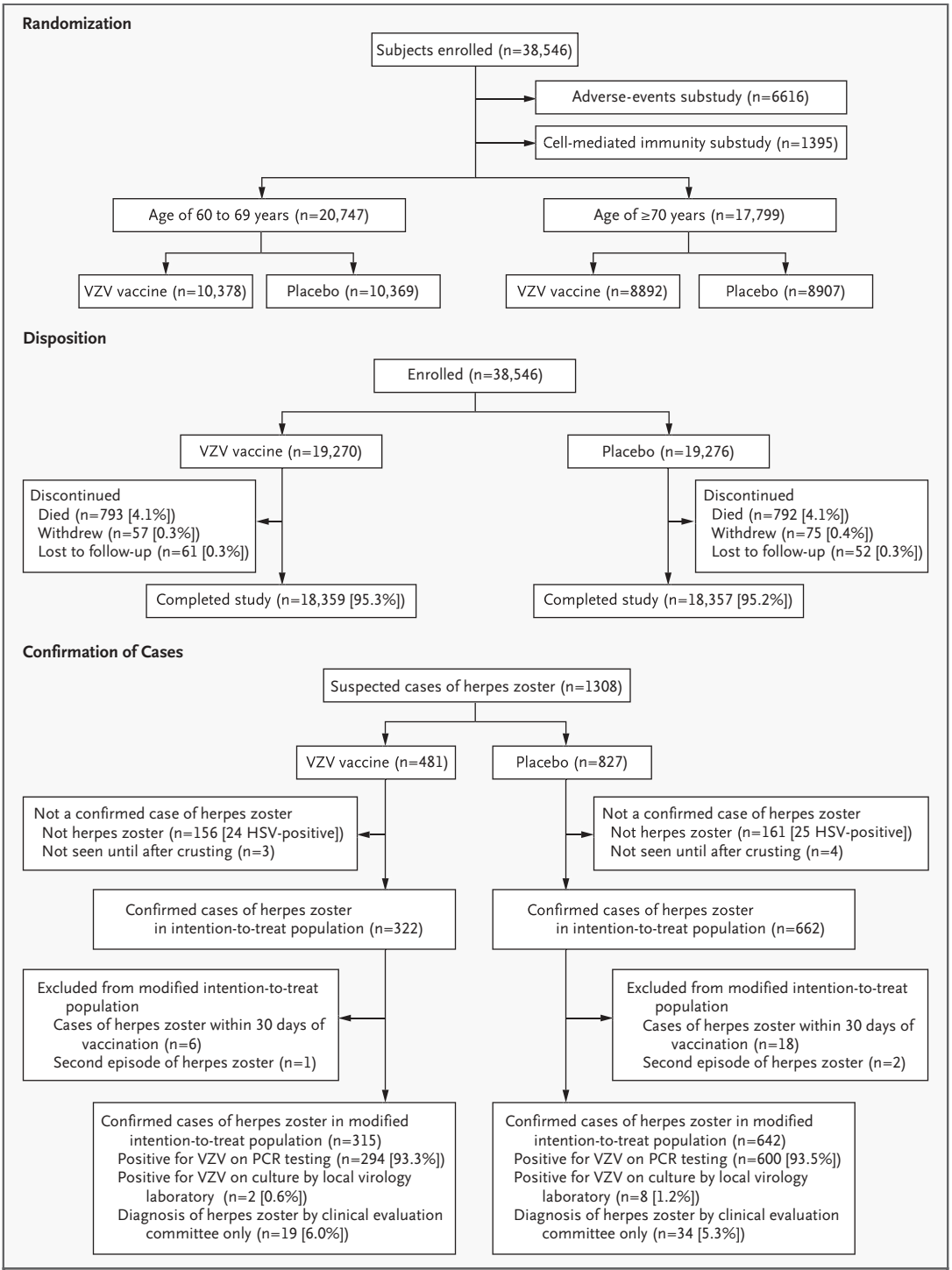

Figure 1. Diagram of the Study Design and Results.

The intention-to-treat population included all subjects who underwent randomization. Efficacy analyses were performed with the use of a follow-up interval that excluded the first 30 days after vaccination and the modified intention-to-treat population, which excluded subjects who withdrew or in whom a confirmed case of herpes zoster developed within the first 30 days after vaccination. For three subjects in whom more than one confirmed case of herpes zoster developed, only the first case was included in the modified intention-to-treat analyses. HSV denotes herpes simplex virus, and VZV varicella–zoster virus.

was 66.5 percent (95 percent confidence interval, 47.5 to 79.2), a result that met the prespecified criteria for success. There were no significant differences in the VE_{PHN} when the results were stratified according to sex or age (Table 3).

The VE_{PHN} did not change appreciably when postherpetic neuralgia was defined with the use of alternative cutoff times for the duration (persistence) of pain (Table 3). In a time-to-event analysis, the cumulative incidence of postherpetic neuralgia was significantly lower in the vaccine group than in the placebo group (P<0.001) (Fig. 2A).

INCIDENCE OF HERPES ZOSTER

The overall incidence of herpes zoster per 1000 person-years was significantly reduced by the zoster vaccine, from 11.12 per 1000 person-years in the placebo group to 5.42 per 1000 person-years in the vaccine group (P<0.001) (Table 2). The VE_{HZ} was 51.3 percent (95 percent confidence interval, 44.2 to 57.6). In a time-to-event analysis, the cumulative incidence of herpes zoster was significantly lower in the vaccine group than in the placebo group (P<0.001) (Fig. 2B). The VE_{HZ} was 37.6 percent among subjects 70 years of age or older and 63.9 percent among younger subjects (P<0.001). There was no difference in VE_{HZ} according to sex.

DURATION AND SEVERITY OF HERPES ZOSTER

The median duration of pain and discomfort among subjects with confirmed cases of herpes zoster was significantly shorter in the vaccine group than in the placebo group (21 days vs. 24 days, P=0.03). Similarly, the mean herpes-zoster severity-of-illness score (AUC) among subjects with confirmed cases of herpes zoster was significantly lower in the vaccine group than in the placebo group (141.2 vs. 180.5, P=0.008). For almost every level of the severity-of-illness score, there were fewer cases of herpes zoster in the vaccine group than in the placebo group. The effect of the zoster vaccine on the severity of illness was greater among older subjects; thus, the VE_{BOI}, the primary end point of the study, was maintained at 55.4 percent.

VACCINE SAFETY IN THE TOTAL STUDY POPULATION

Over the entire study period, the numbers and percentages of deaths were similar in both study groups (Table 4). During the first 42 days after vaccination, the number and types of serious adverse events were similar in the two groups (Table 4), as

Table 1. Baseline Characteristics of the Study Participants.*

Characteristic	Vaccine Group (N=19,270)	Placebo Group (N=19,276)
Demographic		
Age — no. (%)		
60–69 yr	10,378 (53.9)	10,369 (53.8)†
≥70 yr	8,892 (46.1)	8,907 (46.2)
Sex — no. (%)		
Male	11,403 (59.2)	11,357 (58.9)
Female	7,867 (40.8)	7,919 (41.1)
Race — no. (%)‡		
White	18,393 (95.4)	18,381 (95.4)
Black	395 (2.0)	420 (2.2)
Hispanic	265 (1.4)	248 (1.3)
Other or unknown	217 (1.1)	227 (1.2)
General health status		
EuroQol thermometer score§		
Mean ±SD	86.4±11.7	86.3±11.6
Median	90	90
Interquartile range	80–95	80–95
Difficulty walking — no. (%)		
No	11,514 (59.8)	11,402 (59.2)
Rarely	2,464 (12.8)	2,536 (13.2)
Sometimes	3,380 (17.5)	3,475 (18.0)
Often	1,109 (5.8)	1,033 (5.4)
All the time	801 (4.2)	826 (4.3)
Difficulty going places — no. (%)		
No	15,303 (79.4)	15,272 (79.2)
Rarely	2,084 (10.8)	2,079 (10.8)
Sometimes	1,394 (7.2)	1,433 (7.4)
Often	313 (1.6)	327 (1.7)
All the time	174 (0.9)	161 (0.8)
Health-related limitations on activities — no. (%)		
No	9,924 (51.5)	9,862 (51.2)
Mild	7,440 (38.6)	7,423 (38.5)
Moderate	1,637 (8.5)	1,714 (8.9)
Severe	266 (1.4)	273 (1.4)

* Not all subjects responded to every question in the questionnaires. Percentages are rounded.
† One subject was 59 years of age.
‡ Race was self-reported on a questionnaire administered at enrollment.
§ The EuroQol thermometer is a visual-analogue scale for patients to use to rate their overall status from 0 (worst imaginable health) to 100 (best imaginable health). The thermometer is included in the EuroQol questionnaire regarding the quality of life, on which patients graded their general health status in the categories shown.

Table 2. Effect of Zoster Vaccine on the Burden of Illness in Herpes Zoster in the Modified Intention-to-Treat Population.*

Group of Subjects	Vaccine Group			Placebo Group			VE_{BOI} (95% CI)§
	No. of Confirmed Cases/No. of Subjects	BOI Score†	Incidence per 1000 Person-Yr‡	No. of Confirmed Cases/No. of Subjects	BOI Score†	Incidence per 1000 Person-Yr‡	
							%
All subjects	315/19,254	2.21	5.42	642/19,247	5.68	11.12	61.1 (51.1–69.1)
Age							
60–69 yr	122/10,370	1.50	3.90	334/10,356	4.33	10.79	65.5 (51.5–75.5)
≥70 yr	193/8884	3.47	7.18	308/8891	7.78	11.50	55.4 (39.9–66.9)
Sex							
Male	181/11,390	2.09	5.30	361/11,337	5.81	10.65	64.0 (51.4–73.4)
Female	134/7864	2.34	5.58	281/7910	5.47	11.79	57.3 (39.6–69.8)

* Efficacy analyses were performed with the use of a follow-up interval that excluded the first 30 days after vaccination and in a modified intention-to-treat population, which excluded subjects who either withdrew from the study or in whom a confirmed case of herpes zoster developed within the first 30 days after vaccination. Of three subjects in whom more than one case of herpes zoster developed, only the first case was included. VE_{BOI} denotes vaccine efficacy for the burden of illness due to herpes zoster (BOI), and CI confidence interval.

† For the total population and the subgroups stratified according to sex, the BOI score in each treatment group (vaccine or placebo) was the weighted average of the observed BOI stratified according to age, with weights proportional to the total number of subjects within each age group; subjects in whom herpes zoster did not develop were assigned a score of 0 for severity of illness due to herpes zoster on the basis of the Zoster Brief Pain Inventory, a questionnaire developed for the Shingles Prevention Study.

‡ For the total population and for subgroups stratified according to sex, the incidence of herpes zoster in each treatment group was the weighted average of the observed incidence of herpes zoster stratified according to age group, with weights proportional to the total number of person-years of follow-up in each age group.

§ VE_{BOI} for all subjects was the protocol-specified primary end point.

was the distribution of serious adverse events according to body system (data not shown). During this period, varicella-like rashes at the injection site occurred more frequently among those in the vaccine group than among those in the placebo group, but varicella-like rashes at other sites occurred at similar rates in the two groups (Table 4). There were 7 confirmed cases of herpes zoster in the vaccine group and 24 in the placebo group during the first 42 days after vaccination.

Five subjects had serious adverse events that were assessed by site investigators as possibly vaccine-related. Two subjects had received vaccine: a 64-year-old woman who had an exacerbation of asthma two days after receiving the vaccination, and an 80-year-old man in whom symptoms of polymyalgia rheumatica developed on day 3. The remaining three subjects, all men, who had serious adverse events had received placebo: the first subject was 65 years of age and had an anaphylactoid reaction 90 minutes after receiving the vaccination (and 30 minutes after eating peanuts); the second was 69 years of age and received a diagnosis of polymyalgia rheumatica on day 15; and the third was 78 years of age and received a diagnosis of Goodpasture's syndrome on day 52.

ADVERSE-EVENTS SUBSTUDY

In the adverse-events substudy, a significantly greater number of subjects in the vaccine group had one or more adverse events than in the placebo group, reflecting a greater frequency of adverse events at the injection site among subjects in the vaccine group (Table 4). In the vaccine group, the most frequent adverse events at the injection site were erythema (in 35.8 percent of the vaccine group), pain or tenderness (in 34.5 percent), swelling (in 26.2 percent), and pruritus (in 7.1 percent). No other adverse event at the injection site was observed in more than 2 percent of the vaccine recipients. Overall, the proportion of subjects with one or more systemic adverse events was similar in the two groups;

Table 3. Effect of Zoster Vaccine on the Incidence of Postherpetic Neuralgia in the Modified Intention-to-Treat Population.*

Variable	Vaccine Group			Placebo Group			VE_{PHN} (95% CI)
	No. of Confirmed Cases of Herpes Zoster with PHN	No. of Subjects	Incidence per 1000 Person-Yr†	No. of Confirmed Cases of Herpes Zoster with PHN	No. of Subjects	Incidence per 1000 Person-Yr†	%
All subjects	27	19,254	0.46	80	19,247	1.38	66.5 (47.5–79.2)‡
Age							
60–69 yr	8	10,370	0.26	23	10,356	0.74	65.7 (20.4–86.7)
≥70 yr	19	8,884	0.71	57	8,891	2.13	66.8 (43.3–81.3)
Sex							
Male	19	11,390	0.56	51	11,337	1.50	62.8 (35.9–79.3)
Female	8	7,864	0.33	29	7,910	1.22	72.6 (38.6–89.2)
Persistence of PHN among all subjects§							
30 days	81		1.39	196		3.39	58.9 (46.6–68.7)
60 days	45		0.77	113		1.96	60.4 (43.6–72.6)
90 days	27		0.46	80		1.38	66.5 (47.5–79.2)‡
120 days	17		0.29	54		0.93	68.7 (45.2–83.0)
182 days	9		0.16	33		0.57	72.9 (42.1–88.6)

* For the secondary end point, postherpetic neuralgia (PHN) was defined as the pain and discomfort associated with herpes zoster rated as 3 or more, on a scale ranging from 0 (no pain) to 10 (pain as bad as you can imagine), persisting or appearing more than 90 days after the onset of herpes zoster rash. Efficacy analyses were performed with the use of a follow-up interval that excluded the first 30 days after vaccination and the modified intention-to-treat population, which excluded subjects who withdrew or in whom a confirmed case of herpes zoster developed within the first 30 days after vaccination. Of three subjects in whom more than one confirmed case of herpes zoster developed, only the first case was included. VE_{PHN} denotes vaccine efficacy for the incidence of PHN, and CI confidence interval.

† For the total population and the subgroups stratified according to sex, the incidence of PHN in each treatment group (vaccine or placebo) was the weighted average of the observed incidence of PHN stratified according to age group, with weights proportional to the total number of person-years of follow-up in each age group.

‡ VE_{PHN} for all subjects was the protocol-specified secondary end point.

§ PHN was defined as the pain and discomfort associated with herpes zoster that was rated as 3 or more persisting or appearing more than 30, 60, 90, 120, and 182 days after the onset of herpes zoster rash.

however, systemic adverse events assessed as vaccine-related occurred more frequently among vaccine recipients (Table 4).

In the substudy, during the 42 days after vaccination, significantly more subjects in the vaccine group had serious adverse events than in the placebo group (1.9 percent vs. 1.3 percent, respectively; P=0.03); there were no significant differences in the distribution of serious adverse events according to body system or type of event (data not shown). A post hoc, subject-by-subject review of serious adverse events conducted by the writing committee revealed no clinically meaningful differences between the groups in the pathophysiology, nature, timing, intensity, or outcome of these events.

The number of subjects who had one or more hospitalizations was similar in the two groups. No hospitalization among subjects in either group was considered to be related to the vaccine.

DISCUSSION

The pain and discomfort of herpes zoster and postherpetic neuralgia cause substantial morbidity among older adults.[9-12,15,28] Although herpes zoster is not a reportable disease, we estimate that 1 million or more cases occur each year in the United States, a number that is likely to increase as the population ages. Thus, a means of prevention would offer important medical and economic benefits.

The zoster vaccine reduced the burden of illness

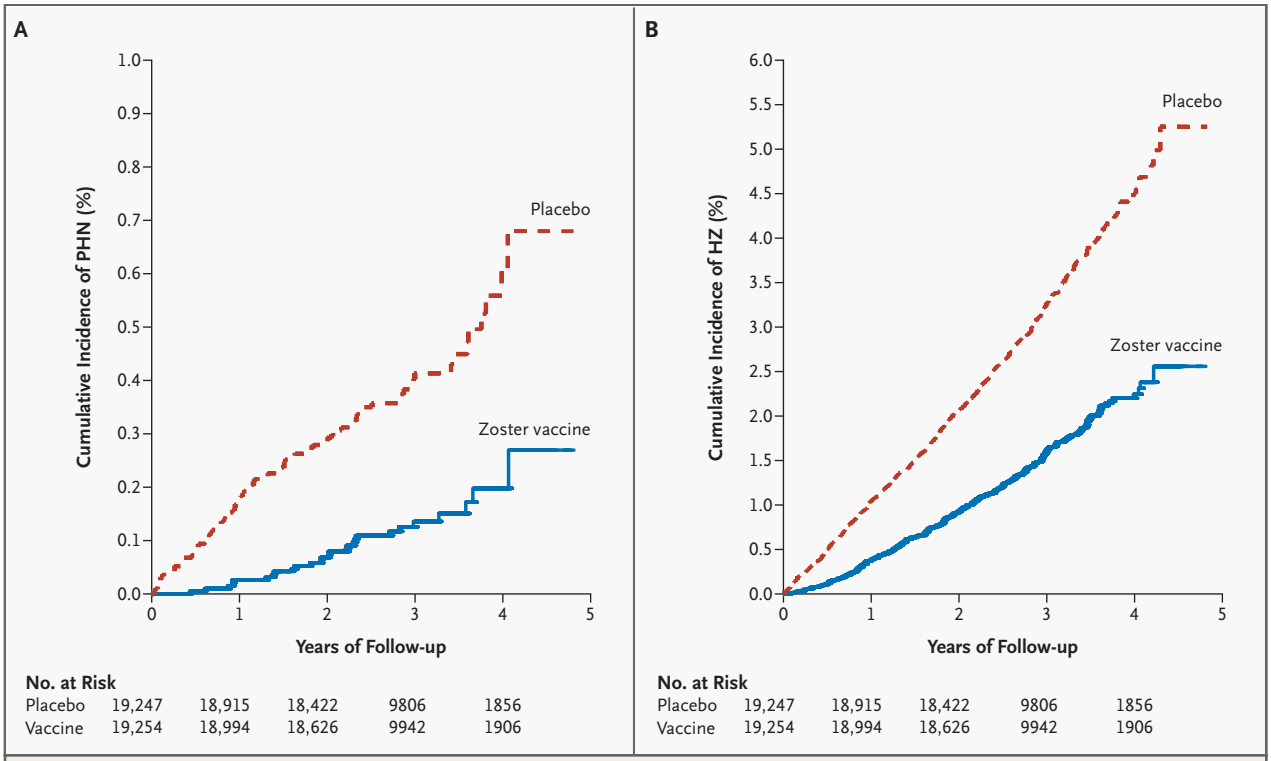

Figure 2. Kaplan–Meier Estimates of the Effect of Zoster Vaccine on the Cumulative Incidence of Postherpetic Neuralgia (Panel A) and Herpes Zoster (Panel B) in the Modified Intention-to-Treat Population.

Incidence rates of postherpetic neuralgia (PHN) and herpes zoster (HZ) were significantly lower in the vaccine group than in the placebo group ($P<0.001$, by a stratified log-rank test that pooled the results of the log-rank test from the two age groups). Cumulative incidence, expressed as a percentage of the subjects at risk, is the probability of the development of the disease during the period from 30 days after vaccination to the follow-up time.

due to herpes zoster among people 60 years of age or older by 61.1 percent and reduced the incidence of postherpetic neuralgia by 66.5 percent. Significant efficacy with respect to the incidence of postherpetic neuralgia was demonstrated, regardless of how postherpetic neuralgia was defined, with a trend toward greater efficacy for postherpetic neuralgia of longer duration. The vaccine also showed significant efficacy for these end points even when the results were stratified according to age and sex. Zoster vaccine also reduced the overall incidence of herpes zoster by 51.3 percent and significantly reduced the pain and discomfort among subjects in whom herpes zoster developed. Although the effect of zoster vaccine on the incidence of herpes zoster was less among older subjects than among younger subjects, the effect of the vaccine on the severity of illness was greater among older subjects, so that the VE_{BOI}, the primary end point of the study, was maintained at 55.4 percent.

We believe that the observed efficacy of the zoster vaccine reflects its ability to boost immunity to VZV in vaccinated subjects — an issue that will require further study. The investigational zoster vaccine had low rates of serious adverse events, systemic adverse events, hospitalization, and death. Results were similar in the two study groups, and local reactions at the vaccination site were generally mild. The greater number of early cases of herpes zoster in the placebo group, as compared with the vaccine group, and the fact that no vaccine virus DNA was detected, indicate that the vaccine did not cause or induce herpes zoster.

The minimum potency of the zoster vaccine administered to subjects in the study was at least 14 times greater than the minimum potency of Varivax (Merck), the vaccine currently licensed to prevent varicella. A preliminary study indicated that potencies of this magnitude are required to elicit a significant increase in the cell-mediated immunity to

Table 4. Adverse Events among All Subjects and among Those in the Adverse-Events Substudy.*

Event	Vaccine Group	Placebo Group	Difference in Risk (95% CI)
			%
All subjects			
No. of subjects	19,270	19,276	
Day of vaccination to end of study	*no. (%)*		
Death	793 (4.1)	795 (4.1)	0.01 (–1.2 to 1.2)†
Death according to age group			
60–69 yr	218 (2.1)	246 (2.4)	–0.80 (–2.0 to 0.4)†
≥70 yr	575 (6.5)	549 (6.2)	0.95 (–1.2 to 3.1)†
Vaccine-related serious adverse event‡	2 (<0.1)	3 (<0.1)	NC
Day of vaccination to day 42			
Death	14 (0.1)	16 (0.1)	–0.01 (–0.1 to 0.1)
One or more serious adverse events	255 (1.4)	254 (1.4)	0.01 (–0.2 to 0.3)
Varicella-like rash at injection site	20 (0.1)	7 (0.04)	0.07 (0.02 to 0.13)§
Varicella-like rash not at injection site	18 (0.1)	14 (0.1)	0.02 (–0.04 to 0.09)
Herpes-zoster–like rash	17 (0.1)	36 (0.2)	–0.10 (–0.18 to –0.03)§
Rash unrelated to herpes zoster	595 (3.2)	620 (3.3)	–0.13 (–0.49 to 0.23)
Confirmed case of herpes zoster	7 (<0.1)	24 (0.1)	–0.09 (–0.16 to –0.03)§
Subjects in the adverse event substudy			
No. of subjects	3345	3271	
Day of vaccination to end of study	*no. (%)*		
Subjects hospitalized	1137 (34.0)	1115 (34.1)	0.1 (–8.8 to 9.0)†
Hospitalization related to herpes zoster	5 (0.2)	6 (0.2)	–0.1 (–0.7 to 0.5)†
Day of vaccination to day 42			
One or more serious adverse events	64 (1.9)	41 (1.3)	0.7 (0.1 to 1.3)§
One or more adverse events	1929 (58.1)	1117 (34.4)	23.7 (21.3 to 26.0)§
One or more systemic adverse events	820 (24.7)	768 (23.6)	1.0 (–1.0 to 3.1)
One or more vaccine-related systemic adverse events‡	209 (6.3)	160 (4.9)	1.4 (0.3 to 2.5)§
Documented temperature 38.3°C or higher	27 (0.8)	27 (0.9)	0.0 (–0.5 to 0.4)
Self-reports of feeling abnormal temperature¶	231 (7.2)	190 (6.0)	1.2 (0.0 to 2.4)
One or more adverse events at injection site‖	1604 (48.3)	539 (16.6)	31.7 (28.3 to 32.6)§
Erythema	1188 (35.8)	227 (7.0)	28.8 (26.9 to 30.6)§
Pain or tenderness	1147 (34.5)	278 (8.5)	26.0 (24.1 to 27.9)§
Swelling	871 (26.2)	147 (4.5)	21.7 (20.1 to 23.4)§
Pruritus	237 (7.1)	33 (1.0)	6.1 (5.2 to 7.1)§
Warmth	57 (1.7)	11 (0.3)	1.4 (0.9 to 1.9)§
Hematoma	53 (1.6)	46 (1.4)	0.2 (–0.4 to 0.8)
Rash	10 (0.3)	3 (0.1)	0.2 (0.0 to 0.5)

* The rates of death and of hospitalization are percentages of subjects in each treatment group. Otherwise, percentages are rates weighted in proportion to the number of subjects with safety follow-up in each age group. NC denotes not calculated. Three subjects who had withdrawn from the study because of worsening health and subsequently died were included in the safety analysis.

† The difference in risk (vaccine group–placebo group) and the 95 percent confidence intervals for deaths and hospitalizations are based on the rates per 1000 subject-years of follow-up to account for differential follow-up among the study participants as a result of staggered enrollment. Otherwise, the differences in risk and 95 percent confidence intervals are based on an asymptotic method for the difference of two binomial proportions where the proportions are weighted according to the number of subjects with safety follow-up in each age group. Negative values for the difference in risk result when the rate in the placebo group is larger than that in the vaccine group.

‡ Events classified as possibly related to vaccination were assessed by a blinded investigator at each site.

§ $P<0.05$ for the comparison with the placebo group.

¶ A temperature of 38.3°C or higher was not documented.

‖ None of the adverse events related to the injection site were considered to be serious adverse events.

VZV among older adults — hence, the need to formulate a high-potency vaccine for this study. We know of no data to suggest that the licensed varicella vaccine would be efficacious in protecting older adults from herpes zoster or postherpetic neuralgia. Thus, we do not recommend the use of the current varicella vaccine in an attempt to protect against herpes zoster and postherpetic neuralgia. The results of our study show that vaccination of immunocompetent persons 60 years of age and older with live attenuated zoster vaccine (Oka/Merck) markedly decreases the morbidity associated with herpes zoster and the incidence of postherpetic neuralgia.

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This article is dedicated to the memory of the late Edgar Hope-Simpson, whose seminal observations and prescient hypotheses on herpes zoster provided our inspiration and rationale, and to the late John Franklin Enders, who taught us what really matters.

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CLINICAL TRIAL REGISTRATION

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ORIGINAL ARTICLE

Effect of *VKORC1* Haplotypes on Transcriptional Regulation and Warfarin Dose

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ABSTRACT

BACKGROUND

The management of warfarin therapy is complicated by a wide variation among patients in drug response. Variants in the gene encoding vitamin K epoxide reductase complex 1 (*VKORC1*) may affect the response to warfarin.

METHODS

We conducted a retrospective study of European-American patients receiving long-term warfarin maintenance therapy. Multiple linear-regression analysis was used to determine the effect of *VKORC1* haplotypes on the warfarin dose. We determined *VKORC1* haplotype frequencies in African-American, European-American, and Asian-American populations and *VKORC1* messenger RNA (mRNA) expression in human liver samples.

RESULTS

We identified 10 common noncoding *VKORC1* single-nucleotide polymorphisms and inferred five major haplotypes. We identified a low-dose haplotype group (A) and a high-dose haplotype group (B). The mean (±SE) maintenance dose of warfarin differed significantly among the three haplotype group combinations, at 2.7±0.2 mg per day for A/A, 4.9±0.2 mg per day for A/B, and 6.2±0.3 mg per day for B/B ($P<0.001$). *VKORC1* haplotype groups A and B explained approximately 25 percent of the variance in dose. Asian Americans had a higher proportion of group A haplotypes and African Americans a higher proportion of group B haplotypes. *VKORC1* mRNA levels varied according to the haplotype combination.

CONCLUSIONS

VKORC1 haplotypes can be used to stratify patients into low-, intermediate-, and high-dose warfarin groups and may explain differences in dose requirements among patients of different ancestries. The molecular mechanism of this warfarin dose response appears to be regulated at the transcriptional level.

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Coumarin-based anticoagulant drugs are the definitive treatment worldwide for the long-term prevention of thromboembolic events. In 2003, a total of 21.2 million prescriptions were written for the oral anticoagulant warfarin (a derivative of coumarin) in the United States alone.[1] However, management of warfarin therapy is challenging in two respects: first, a safe and effective stabilization dose must be determined during the early months of therapy, and second, maintenance doses must be adjusted to compensate for changes in patients' weight, diet, disease state, and concomitant use of other medications. In addition, studies indicate that genetic factors affect outcomes, despite adjustment for these factors. Specifically, patients with the common, functionally defective *2 and *3 allelic variants of the cytochrome P-450 enzyme 2C9 (CYP2C9) require significantly lower maintenance doses, have longer times to dose stabilization, and are at higher risk for serious and life-threatening bleeding than are patients without these variants.[2] Such warfarin sensitivity is easily rationalized because CYP2C9 is responsible for the metabolic clearance of the more pharmacologically potent *S*-enantiomer of warfarin.[3]

In contrast to genetically determined cases of warfarin sensitivity, such as those described above, are rare cases of warfarin resistance. A potential pharmacodynamic mechanism underlying warfarin resistance has been elucidated with the recent discovery of the warfarin target gene, which encodes vitamin K epoxide reductase complex 1 (*VKORC1*).[4,5] This complex recycles reduced vitamin K, which is essential for the post-translational gamma-carboxylation of vitamin K–dependent clotting factors II (prothrombin), VII, IX, and X. Several rare mutations that lead to amino acid changes in the VKORC1 protein have been discovered in warfarin-resistant patients but not in the general population,[4] suggesting that coding-region variants of *VKORC1* are extremely detrimental and that they probably do not explain the typical variability in warfarin dose (2 to 10 mg per day) among individual patients. Recently, a single, noncoding polymorphism was found to be associated with warfarin dose across the normal dose range,[6] suggesting that other regulatory polymorphisms in *VKORC1* might influence the pharmacodynamic response to warfarin.

The purpose of this study was to determine whether other polymorphisms in noncoding regions or their unique haplotype combinations contribute to the variability in the maintenance dose of warfarin. Additional goals were to probe population-specific differences in warfarin dose requirements and to investigate the molecular mechanisms underlying significant *VKORC1* effects.

METHODS

PATIENTS

The study was approved by the human subjects review committees of the University of Washington, Seattle, and Washington University, St. Louis. All the patients who participated in the study provided written informed consent.

The primary study population, from the University of Washington Medical Center, consisted of the same patients previously studied to assess the association between *CYP2C9* variants and anticoagulation-related outcomes.[2] Patients were recruited from pharmacist-run anticoagulation clinics affiliated with the center. Inclusion criteria were a confirmed date of the initial exposure to warfarin, current anticoagulation therapy, and an age of 18 years or older. Exclusion criteria were Asian or African descent (36 patients), management by telephone rather than in person (185), absence of verbal and written consent (5), absence of a blood specimen (3), and absence of a confirmed date of initial exposure to warfarin (11). A total of 186 patients from this population were eligible for the study.

The secondary patient population consisted of patients 18 years of age or older whose warfarin therapy was managed at one of the anticoagulation clinics affiliated with Barnes–Jewish Hospital at Washington University Medical Center, as previously described.[7] Exclusion criteria for this population were non-European ancestral origin (139 patients) and absence of verbal and written consent (17 patients). We prospectively followed 47 patients who had recently begun warfarin therapy until they were taking their maintenance dose. A total of 368 patients from this population were eligible for the study.

COLLECTION OF CLINICAL DATA

Collection of data from the primary patient population consisted of a review of inpatient and outpatient medical records. Two trained abstractors collected data with the use of standardized abstract forms. The anticoagulation database of the University of Washington Medical Center was used to ob-

tain information on the international normalized ratio (INR), daily warfarin dose, and use of prescription drugs and over-the-counter drugs. The daily maintenance dose of warfarin was defined as the dose at three consecutive clinic visits at which the INR measurement was within therapeutic range. The electronic medical-records database of the University of Washington Medical Center was used to obtain information on bleeding events, coexisting conditions, and demographic variables. Blood samples were collected from patients during regularly scheduled office visits.

Data from the secondary patient population were collected by means of structured patient interviews, as previously described in detail,[7] from 2001 to 2004. In brief, patients provided a 5-ml blood sample, demographic and dietary information, a comprehensive list of prescription and over-the-counter drugs, and access to information about their warfarin doses and INR measurements.

POPULATION-SPECIFIC DNA DIVERSITY PANELS

DNA panels consisting of samples from American persons of European, Asian, or African descent were purchased from the Coriell Cell Repository (http://locus.umdnj.edu/nigms). The Asian-American panel consisted of samples from 96 persons from the HD100CHI set (Han People of Los Angeles), 10 from the HD13 set (Southeast Asians), 7 from the HD32 set (Chinese), and 7 from the HD07 set (Japanese). Samples from 96 European-American persons were selected from the HD100CAU set and from 23 European-American persons from the parental generation of the families in the Centre d'Etude du Polymorphisme Humain collection (http://pga.gs.washington.edu/data/sample_description.html). Samples from 96 African-American persons were selected from the HD100AA set.

To explore the functional mechanism of the variability in warfarin dose, we measured *VKORC1* messenger RNA (mRNA) levels in human liver specimens selected from a tissue bank maintained by the University of Washington School of Pharmacy. Basic demographic information on the individual organ donors and methods of tissue procurement have been reported previously.[8,9] All 53 liver specimens used in this study came from European-American donors.

DNA AND mRNA ANALYSES

Because the *VKORC1* gene was identified only recently as the gene encoding the primary warfarin-sensitive component of vitamin K epoxide reductase,[4,5] limited information on polymorphisms within this gene was available. We therefore carried out DNA sequence analysis across the entire genomic region (approximately 11 kb) in samples from our primary patient population to catalogue single-nucleotide polymorphisms (SNPs) comprehensively and to establish the haplotype structure of the *VKORC1* gene. All clinical samples from the primary patient population were subjected to sequence analysis across the extended genomic sequence, which included 5 kb in the upstream promoter region, 4.2 kb of intragenic (intron and exon) sequence, and 2 kb of the 3′ downstream region. Ten common SNPs were identified, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041 of the *VKORC1* reference sequence (GenBank accession number AY587020).

The population-specific diversity samples (European American, African American, and Asian American) were genotyped for the 10 common SNPs identified in the European-American clinical population, with the use of the same method of sequence analysis. In the secondary patient population, four informative SNPs (at positions 861, 5808, 6853, and 9041) were genotyped to differentiate haplotypes H1, H2, H7, H8, and H9, according to the genealogic tree shown in Figure 1.

Total RNA and DNA were extracted from human control liver specimens with Trizol reagent. RNA was reverse-transcribed to yield complementary DNA with the use of poly-dT primers (as described in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Quantitative polymerase-chain-reaction analysis was performed (also as described in the Supplementary Appendix). DNA extracted from liver samples was genotyped at each of the 10 common *VKORC1* SNPs by DNA resequencing (as described above). Haplotypes were inferred, and each sample was classified as one of three haplotype group combinations (A/A, A/B, or B/B, as described in the Supplementary Appendix).

STATISTICAL ANALYSIS

All SNPs identified were tested for deviations from Hardy–Weinberg disequilibrium with the use of a chi-square test. The significance level for all statistical tests was set at $P<0.05$. Haplotypes for each individual sample were estimated with use of the PHASE program (version 2.0),[10] and independent runs were performed for clinical and population-specific samples from each population studied.

We performed multiple linear-regression analy-

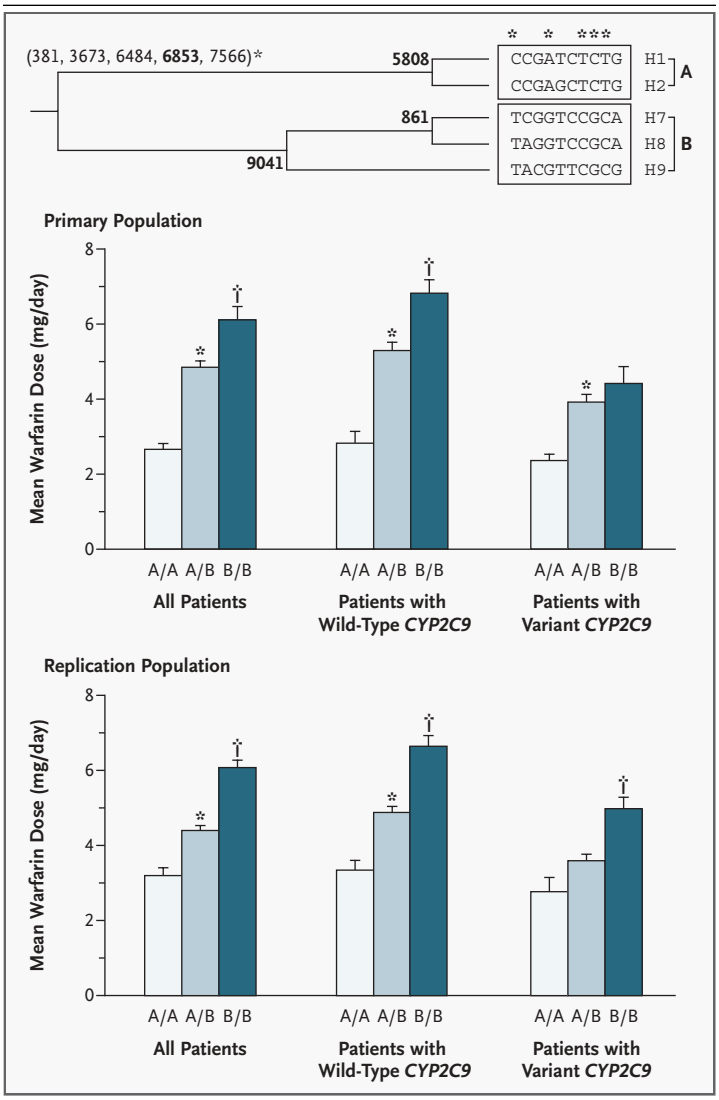

Figure 1. Effect of *VKORC1* Haplotype Combination on Clinical Warfarin Dose.

As shown in the upper panel, common haplotypes (H1, H2, H7, H8, and H9) were clustered with use of the UPGMA method (unweighted pair group method with arithmetic mean); they formed two distinct evolutionarily distant groups, designated A (comprising H1 and H2) and B (comprising H7, H8, and H9). Eight single-nucleotide polymorphisms (SNPs) are labeled at the nodes of the tree, and four SNP sites (shown in boldface) were used to discriminate between each branch and to distinguish groups A and B. Asterisks indicate correlated SNP sets that were significantly associated with warfarin dose. Group A was associated with a low warfarin dose and group B a high warfarin dose. As shown in the middle panel, patients in the primary population were genotyped and assigned a *VKORC1* haplotype combination (A/A, A/B, or B/B). The patients were further classified according to *CYP2C9* genotype (the wild type or either the *2 or *3 variant). The total numbers of patients having a group A combination, a group B combination, or both were 182 (all patients), 124 (wild-type *CYP2C9*), and 58 (variant *CYP2C9*). Four patients could not be assigned either to group A or to group B. As shown in the bottom panel, 357 patients from the replication sample were genotyped and grouped as were those in the primary patient population; 233 had wild-type *CYP2C9* and 124 variant *CYP2C9*. The asterisks in the bottom two panels denote $P<0.05$ for the comparison with combination A/A and the daggers $P<0.05$ for the comparison with combination A/B. The T bars represent standard errors.

sis of the log-transformed maintenance dose, with all patient covariates initially considered. Significant covariates contributing to warfarin dose were age, sex, use or nonuse of amiodarone, use or nonuse of losartan, and *CYP2C9* genotype. The effect size associated with each predictor was calculated as the percentage of the variation in warfarin dose explained by the predictor, divided by the total variance in the regression model.

Genealogic trees were constructed on the basis of the number of differences between haplotypes and with use of the UPGMA clustering method (unweighted pair group method with arithmetic mean). The Kruskal–Wallis test, a distribution-free analysis of variance, was used to assess differences in the warfarin maintenance dose among patients according to their haplotype combination (A/A, A/B, or B/B) and *CYP2C9* genotype (wild-type or variant). Other than the grouping of patients according to *CYP2C9* genotype, no other adjustment for clinical covariates was used. After the overall chi-square test for differences among the three groups had been performed, pairwise comparisons of groups were carried out with use of the asymptotic normality of the total ranks within each group. We applied the Bonferroni correction for each of the three comparisons (A/A vs. A/B, A/B vs. B/B, and A/A vs. B/B).

Data on liver mRNA expression were analyzed after log transformation, and the overall test for group differences was performed by analysis of variance. Pairwise comparisons between groups for significance were performed with the use of Tukey's Studentized range test. Significance levels were set at $P<0.05$. Additional information on the statistical methods used is provided in the Supplementary Appendix.

RESULTS

We found 28 *VKORC1* noncoding SNPs in the primary population (comprising 186 patients) and a single heterozygous, nonsynonymous SNP in the

coding region (genomic position G5432T, encoding Ala41Ser). The patient heterozygous for this polymorphism had the highest overall maintenance dose of warfarin among the patients in the primary population (15.5 mg per day) and was excluded from the other analyses. No other previously reported coding-region SNPs were identified.[4] Of the 28 noncoding SNPs, 10 occurred at a frequency of greater than 5 percent. No deviations from the expected population genotype proportions (predicted by Hardy–Weinberg equilibrium) were detected at these common SNP sites.

Individual tests of each SNP and adjustments for significant covariates revealed that seven SNPs (at positions 381, 3673, 5808, 6484, 6853, 7566, and 9041) were significantly associated with the warfarin dose ($P<0.001$); the strength of the association between the warfarin dose and the other three SNPs (at positions 861, 2653, and 6009) was less significant ($P=0.01$, $P=0.02$, and $P=0.02$, respectively). Of the seven highly significant SNPs, five (at positions 381, 3673, 6484, 6853, and 7566) were strongly correlated with one another (linkage disequilibrium $r^2 \geq 0.9$), and two others (at positions 5808 and 9041) were not correlated with any other SNP in this region. Stepwise regression analysis identified the five highly correlated SNPs as those that were most predictive of the approximately 25 percent variance in warfarin dose. In the same analysis, the *CYP2C9* genotype accounted for 10 percent of the variance in warfarin dose.

The 10 common SNPs were used to infer *VKORC1* haplotypes from the primary sample and the three diversity samples, yielding nine haplotypes (H1 through H9). These, in turn, were used to assign haplotype pairs to each patient or member of a diversity panel. We identified five common haplotypes (those with >5 percent frequency) in the primary sample: H1, H2, H7, H8, and H9 (Table 1).

In the multiple linear regression analysis adjusted for clinically important covariates, four of the five common haplotypes were found to be independently associated with the warfarin dose ($P \leq 0.05$) (Table 1). We identified two haplotypes (H1 and H2) associated with a low warfarin dose requirement (2.9 and 3.0 mg per day) and two haplotypes (H7 and H9) associated with an increased requirement (6.0 and 5.5 mg per day). Results obtained with the use of a generalized linear-score model for haplotypes were similar, as were the results of secondary analyses that excluded the 24 patients receiving amiodarone.

The genealogic tree showing the relationship among the five common haplotypes indicates the emergence of two distinct, highly divergent haplotype groups (Fig. 1). We designated these groups A (comprising haplotypes H1 and H2) and B (comprising H7, H8, and H9) and were able to assign

Table 1. *VKORC1* Haplotype Frequency and Effect on Warfarin Dose among 186 European-American Patients.*

Haplotype Identification Code	Haplotype Sequence†	Frequency of Haplotype in Primary Patient Population		Mean Maintenance Dose among Homozygous Patients (95% CI)‡	P Value
		proportion	*no. of persons*	*mg/day*	
H1	CCGATCTCTG	0.12	43	2.9 (2.2–3.7)	<0.0001
H2	CCGAGCTCTG	0.24	88	3.0 (2.5–3.6)	<0.001
H3	CCGGTCCCCG	0.01	2	NA	NA
H4	CCGGTCCGTG	<0.01	1	NA	NA
H5	TCGAGCTCTG	<0.01	1	NA	NA
H6	TCGGTCCGCG	0	0	NA	NA
H7	TCGGTCCGCA	0.35	132	6.0 (5.2–6.9)	<0.001
H8	TAGGTCCGCA	0.08	28	4.8 (3.4–6.7)	0.76
H9	TACGTTCGCG	0.21	77	5.5 (4.5–6.7)	0.05

* CI denotes confidence interval, and NA not analyzed.
† For each haplotype sequence, the single-nucleotide polymorphisms are listed in sequential order along the *VKORC1* gene, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041.
‡ Analyses were adjusted for age, sex, use or nonuse of amiodarone, use or nonuse of losartan, and *CYP2C9* genotype. The mean warfarin dose among all the patients was 5.15 mg per day (95 percent confidence interval, 4.78 to 5.51).

haplotype group combinations to 182 patients. According to the regression analysis, this higher-order clustering showed that group A contained the haplotypes associated with a low dose of warfarin and group B the haplotypes associated with a high dose of warfarin. As shown in Figure 1, a minimal SNP set composed of four SNPs (at positions 861, 5808, 6853, and 9041) distinguished each of the groups and haplotypes at the terminal ends of this tree.

Patients were assigned a *VKORC1* haplotype group combination (A/A, A/B, or B/B) and then grouped according to *CYP2C9* genotype (the wild type in 124 patients and the *2 or *3 variation in 58). In this analysis, we made no other adjustments for clinical covariates. The warfarin maintenance dose differed significantly among the three *VKORC1* haplotype combinations, at 2.7±0.2 mg per day for A/A, 4.9±0.2 mg per day for A/B, and 6.2±0.3 mg per day for B/B (P<0.001), both within the entire primary patient population and among the patients who were not carriers of *CYP2C9* functional variants (Fig. 1). In the primary population, the overall mean maintenance dose of warfarin (5.1±0.2 mg per day) and range of maintenance doses were typical of those that have been reported in other clinical studies.[7] The average INR was approximately 2.5 and did not differ significantly among the patients classified according to *VKORC1* haplotype combination (lowest P value=0.22) (data not shown).

We carried out a replication study involving a larger, independent population of warfarin-treated European-American patients. We genotyped these patients using the four informative SNP sites that resolved the five common haplotypes. Haplotypes were inferred, major haplotype combinations assigned, and patients subclassified according to *CYP2C9* genotype in the same manner as those in the primary patient population (Fig. 1). These data have been deposited in the Pharmacogenetics and Pharmacogenomics Knowledge Base (accession number PS204853). Stepwise regression analysis indicated that *VKORC1* and *CYP2C9* genotypes accounted for 21 percent and 6 percent, respectively, of the variance in warfarin dose. For all 357 patients in whom a *VKORC1* haplotype could be assigned, there was a significant additive effect: warfarin doses were 3.2±0.2 mg per day for the A/A combination, 4.4±0.1 mg per day for A/B, and 6.1±0.2 mg per day for B/B (P<0.05 for the comparisons between A/A and A/B and between A/B and B/B). We observed a similar additive effect with significant differences between combinations among the 233 patients with wild-type *CYP2C9*. The average INR among these patients was also approximately 2.5 and was not significantly different between any of the haplotype combinations.

The five haplotypes predictive of the warfarin dose accounted for 99 percent and 96 percent of the total haplotypes in the European-American clinical and diversity populations, respectively; there was no significant difference between these populations in the distribution of the two major haplotype groups (group A, 35 percent vs. 37 percent, respectively; group B, 64 percent vs. 58 percent). The five common haplotypes within the European-American population accounted for only 62 percent of the more diverse African-American haplotypes (Table 2). The African-American and Asian-American populations showed significant differences in the frequencies of groups A and B when compared with the European-American population (P<0.001). The frequency of group A haplotypes (predictive of a low warfarin dose) was significantly higher in the Asian-American population (89 percent) and lower in the African-American population (14 percent) than in the European-American population (37 percent) (P<0.001 for both comparisons).

To explore the mechanism of the association between warfarin doses and *VKORC1* polymorphisms, we assayed *VKORC1* mRNA levels in human liver tissue and also determined the major *VKORC1* haplotype group (A/A, A/B, or B/B) of each tissue specimen. A graded and highly significant gene–dose effect was evident (P=0.002). mRNA levels in the B/B (high-dose) group were about three times as high as those in the A/A (low-dose) group (P<0.05) (Fig. 2).

DISCUSSION

In our primary study population, we found that approximately 25 percent of the variance in warfarin dose was explained by the *VKORC1* haplotype alone. Independent verification of a genetic association is critical for determining its validity and importance,[11] so we replicated the association in a second clinical population, in which 21 percent of the variance was explained by the *VKORC1* haplotype. Since *CYP2C9* explained 6 to 10 percent of the variability in these two patient samples, the *VKORC1* genotype appears to be the most important genetic factor determining variability in warfarin dose: in both clinical populations its effect was approximately three times that of the *CYP2C9* genotype.

Table 2. Distribution of *VKORC1* Haplotypes in European-American, African-American, and Asian-American Populations.

Haplotype Identification Code or Group	Haplotype Sequence	Frequency of Haplotype in American Populations*		
		European (N=119)	African (N=96)†	Asian (N=120)†
		proportion (number of haplotypes)		
Haplotype distribution				
H1	CCGATCTCTG	0.12 (28)	0.07 (14)	0.89 (213)
H2	CCGAGCTCTG	0.26 (61)	0.06 (12)	0
H7	TCGGTCCGCA	0.21 (49)	0.42 (80)	0.10 (25)
H8	TAGGTCCGCA	0.14 (34)	0.01 (2)	0
H9	TACGTTCGCG	0.24 (56)	0.06 (11)	0
Other haplotypes	—	0.04 (10)	0.38 (73)‡	0.01 (2)
Group distribution				
Group A (H1, H2)	—	0.37 (89)	0.14 (26)	0.89 (213)
Group B (H7, H8, H9)	—	0.58 (139)	0.49 (93)	0.10 (25)
Total of groups A and B	—	0.96 (228)	0.62 (119)	0.99 (238)

* The total number of haplotypes analyzed is twice the number of persons assessed.
† There were significant differences between the African-American and European-American populations and between the Asian-American and European-American populations, both in terms of haplotype ($P<0.001$) and in terms of group distribution (A or B) ($P<0.001$).
‡ The haplotypes consisted largely of H3 and H6.

We found that haplotypes of *VKORC1* are no more informative than a single segregating SNP chosen from among those at positions 381, 3673, 6484, 6853, and 7566. This finding is consistent with data from a previous study that also showed an association between a SNP in intron 1 of *VKORC1* (C1173T, the SNP at position 6484 in the current study) and the warfarin dose.[6] However, our results explain a larger portion of the interindividual variations in warfarin dose (21 to 25 percent, vs. 14 percent in the previous study) and indicate that *VKORC1* has a proportionally larger effect than *CYP2C9*. (Our results show a threefold effect of *VKORC1* variants when compared with *CYP2C9* variants *2 and *3, whereas the previous study reported that *CYP2C9* had a greater effect than *VKORC1*.) Furthermore, only 2 of our 10 SNPs have been studied previously.[6]

Evidence from various clinical and population studies suggests that persons of Asian, European, and African ancestry tend to require, on average, lower, intermediate, and higher doses of warfarin (approximately 3.0, 5.0, and 6.5 mg per day, respectively).[7,12-14] Because group A haplotypes predicted the low-warfarin-dose phenotype and were relatively common in the Asian-American population, it is likely that the association between ancestral origin and dose is, in part, an effect of the *VKORC1* haplotype. Conversely, the prevalence of group B haplotypes was relatively high in the African-American population, potentially giving rise to the increased dose requirement in this population.

The more diverse distribution of haplotypes among African Americans is consistent with the higher genomic sequence diversity found in populations of African descent.[15,16] These population-specific haplotype differences may be due to demographic effects, such geographic selective pressures, migration, or population bottlenecks and have been observed for other medically relevant genes, such as *ADRB2*.[17] Additional studies involving patients of African and Asian descent who are receiving warfarin will be required to confirm the associations between *VKORC1* haplotype and warfarin dose in these populations, including the influence of haplotypes other than those of groups A and B.

The associations between the A haplotype and reduced mRNA expression and between the B haplotype and increased mRNA expression parallel the effect of these haplotypes on warfarin dose, as would be predicted by a simple, noncompetitive

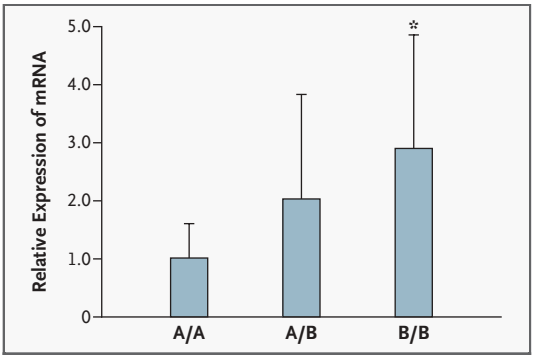

Figure 2. Correlation between *VKORC1* Haplotype Groups and *VKORC1* mRNA Expression.

Levels of *VKORC1* mRNA expression were significantly correlated with haplotype group (P=0.002). All experimental data were normalized to levels of expression of glyceraldehyde-3-phosphate dehydrogenase mRNA and scaled relative to the level of expression in the A/A group (average, 1.49). Six samples in the A/A group, 22 in the A/B group, and 25 in the B/B group were analyzed. Relative expression levels are presented as means; the T bars represent standard deviations. The asterisk denotes P<0.05 for the comparison between haplotype groups A/A and B/B.

model of enzyme inhibition by this anticoagulant.[18] We hypothesize that the level of *VKORC1* mRNA is directed by each haplotype and determines the level of protein synthesis of the vitamin K epoxide reductase complex, which in turn accounts for differences among these patients in their warfarin maintenance-dose requirements. The primary SNP candidates that explain this effect would be those that designate the major haplotype split (the SNPs at positions 381, 3673, 6484, 6853, and 7566) and predict the warfarin maintenance dose. We mapped these SNPs to homologous regions in rats, mice, and dogs to identify potentially conserved, noncoding sequences that encompass these sites. Only two SNPs (at positions 6484 and 6583) from the informative group are conserved; they flank exon 2 but fall outside the canonical regions required for exon splicing. Presumably, these regions act as regulatory sequences that may bind transcription-factor–binding sites, but additional studies will be required to elucidate the mechanism underlying altered *VKORC1* transcription.

The merits of genotyping before or during treatment involving drugs such as warfarin, irinotecan, and thiopurine — the effectiveness of which depends on genetic variants of *CYP2C9* (and now *VKORC1*), *UGT1A1*, and *TPMT*, respectively — is an area of active debate between regulatory authorities and the clinical community.[19] Recently published guidelines suggest initial warfarin doses of 5 to 10 mg per day,[20] but our results suggest that this strategy may expose patients with the A/A *VKORC1* haplotype, who require a low dose of warfarin, to unnecessarily high doses of drug. Because the initial warfarin dose is already individualized according to other clinical data and the dose subsequently adjusted according to the anticoagulation status, it could be inferred that *VKORC1* and *CYP2C9* genotyping may not provide a clinically significant improvement over current practice. However, in our retrospective[2] and prospective[21] studies, we found a significant effect of *CYP2C9* variants on a variety of anticoagulation-related outcomes, despite individualized dosing and frequent monitoring in a specialized anticoagulation clinic at an academic medical center.[1] The current data strongly suggest that analysis of *VKORC1* should be an essential component of prospective studies aimed at investigating the value of genotyping for warfarin therapy. In addition, they provide the detailed genetic information necessary to ensure that such studies are designed appropriately.

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Drs. Rieder and Rettie report having applied for a patent (application serial no. 10/967,879) on the use of *VKORC1* haplotypes and SNPs. Dr. Thummel, an associate dean of the School of Pharmacy at the University of Washington, reports that the school receives financial support from Bristol-Myers Squibb. Dr. McLeod reports having served as a consultant to Veridex, Precision Therapeutics, and Orion Genomics.

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ORIGINAL ARTICLE

Type II Collagen Gene Variants and Inherited Osteonecrosis of the Femoral Head

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ABSTRACT

BACKGROUND

Avascular necrosis of the femoral head (ANFH) causes disability that often requires surgical intervention. Most cases of ANFH are sporadic, but we identified three families in which there was autosomal dominant inheritance of the disease and mapped the chromosomal position of the gene to 12q13.

METHODS

We carried out haplotype analysis in the families, selected candidate genes from the critical interval for ANFH on 12q13, and sequenced the promoter and exonic regions of the type II collagen gene (*COL2A1*) from persons with inherited and sporadic forms of ANFH.

RESULTS

We identified a G→A transition in exon 50 of *COL2A1* in affected members of a four-generation family with ANFH. This transition predicts the replacement of glycine with serine at codon 1170 in a GXY repeat of type II collagen. Another pedigree was shown to harbor the same transition, but the mutant allele occurred on a different haplotype background. In a third family, a G→A transition in exon 33 of the gene, causing a glycine-to-serine change at codon 717, was detected. No mutation was found in the *COL2A1* coding region in sporadic cases of ANFH.

CONCLUSIONS

All the patients with familial ANFH whom we studied carried *COL2A1* mutations. In families with ANFH, haplotype and sequence analysis of the *COL2A1* gene can be used to identify carriers of the mutant allele before the onset of clinical symptoms, allowing the initiation of measures that may delay progression of the disease.

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Drs. Liu and W.-M. Chen contributed equally to this work.

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It has been estimated that 300,000 to 600,000 people in the United States have avascular necrosis of the femoral head (ANFH).[1] Approximately 15,000 new cases of this common and disabling disorder, also called ischemic necrosis of the femoral head or osteonecrosis of the femoral head, are reported annually.[2] The age at the onset of this disease is earlier than that for osteoarthritis; the diagnosis is typically made when patients are between the ages of 30 and 60 years. The clinical manifestations, such as pain on exertion, a limping gait, and a discrepancy in leg length, cause considerable disability. Moreover, nearly 10 percent of the 500,000 total-hip arthroplasties performed each year in the United States involve patients with ANFH.[3] As a result, this disease creates a substantial socioeconomic cost as well as a burden for patients and their families.

Idiopathic osteonecrosis of the femoral head is defined as a disease that causes ischemic osteonecrosis of the femoral head without trauma or sepsis, according to the proposal put forth by the Association Research Circulation Osseous in 1993.[4] In this definition, so-called steroid-induced osteonecrosis and alcohol-associated osteonecrosis are also included in this category. Although the exact pathogenesis of osteonecrosis remains unknown, it appears to involve vascular compromise, cell death, or deficient bone repair.[5]

We recently identified three families with idiopathic ANFH in which there was autosomal dominant inheritance of the disease and reported that the familial ANFH gene mapped to a 15-cM region between *D12S1663* and *D12S1632* on chromosome 12q13.[6] To identify the genes associated with idiopathic osteonecrosis, we scrutinized the region by resequencing the coding and promoter regions of candidate genes.

METHODS

RECRUITMENT

The pedigrees of the three families with ANFH are shown in Figure 1. The disease was diagnosed in affected persons by clinical and radiologic criteria. The chief symptom reported by most of these patients was pain in the groin. Physical examination revealed that the patients had no other skeletal anomalies. Routine plain-film radiography of the pelvis and hip joints was used for ANFH staging, according to Ficat's classification.[7] The genetic study was approved by the institutional research board of the National Health Research Institutes, Taipei, Taiwan. Written informed consent was obtained from all persons who contributed DNA and clinical information to the study. Both affected and unaffected family members were Han Chinese living in Taiwan; their ancestral origin was determined by the investigators on the basis of geography and language.

GENOTYPING AND HAPLOTYPE ANALYSIS

Genotyping was performed with leukocyte DNA for 39 microsatellite repeat markers from chromosome 12 (Table 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). Reactions were performed in 96-well microtiter plates; each well contained 10 ng of DNA template, 6 μl of a polymerase-chain reaction (PCR) amplification mix (True Allele PCR Premix, Applied Biosystems), and 0.06 pmol of each primer in a final volume of 10 μl. The PCR products were analyzed on an ABI Prism 3700 DNA sequencer (Applied Biosystems). GeneScan version 3.6 and Genotyper version 3.5 (Applied Biosystems) for Windows NT were used for allele identification and size assignment. Construction of the haplotypes was performed by the GeneHunter program.[8]

DNA SEQUENCE ANALYSIS

All exons of the type 2 collagen gene (*COL2A1*) from core members of the families (Subjects III-7, III-8, IV-5, IV-6, and IV-7 in Family A; Subjects III-7, III-8, and IV-10 in Family B; and Subjects II-7, II-8, III-12, and III-13 in Family C) were analyzed. In addition, genomic PCR and sequencing of exons 33 and 50 were performed on DNA from all available family members, 65 persons with sporadic ANFH, and 110 controls. Leukocyte DNA was used to amplify the genomic fragments of the *COL2A1* promoter as well as exons and exon–intron junctions in 39 PCR reactions. Oligonucleotide primers were designed with the use of Primer3 software[9] (Table 2 of the Supplementary Appendix). PCR was initiated at 95°C for 10 minutes; this step was followed by 45 cycles of 95°C for 30 seconds, an annealing temperature for 30 seconds, and 72°C for 45 seconds. The final step took place at 72°C for 3 minutes. PCR products were treated with exonuclease I to remove excess primers. The DNA sequencing reaction was performed with the Dye Terminator kit (Applied Biosystems) with the same primers as were used in the PCR amplification, and the reaction products were separated with ABI 3700 or ABI 3730 DNA sequencers.

The results were analyzed with the Phred/Phrap/Consed programs[10-12] (www.phrap.org) and PolyPhred software (version 10).[13]

Allele-specific primer extension was used to confirm patients' heterozygous state. Amplification of exon 50 wild-type sequence was carried out with primer A (5′-AGTCAGGACACTTACAGCAG-3′) plus primer B (5′-GTCCTCCTGGCCCCGTCG-3′), and amplification of exon 50 mutant sequence with primer A plus primer C (5′-GTCCTCCTGGCCCCGTCA-3′). Amplification of exon 33 wild-type sequence was carried out with primer A (5′-TCAGTGGGACTCCCAGGC-3′) plus primer B (5′-GTGCCCAGGGCCTCCAGG-3′), and amplification of exon 50 mutant sequence, with primer A plus primer C (5′-GTGCCCAGGGCCTCCAGA-3′).

Figure 1 (facing page). Pedigrees of the Three Families with ANFH.

Panel A shows the pedigree and results of haplotype analysis of Family A. Short tandem-repeat markers spanning the candidate region for ANFH were used to construct the haplotype. The short tandem-repeat markers used to identify recombination breakpoints and to define the critical region are shown in red, as are the identifying numbers of the subjects involved in that analysis. Asterisks indicate subjects who carried a mutant *COL2A1* allele that was clinically silent at the initial assessment. Panel B shows the pedigree and results of haplotype analysis of Family B. Asterisks indicate subjects who may have carried the mutant allele. Because of space constraints, two affected female subjects whose DNA was not available for haplotype analysis have been omitted from the diagram. Panel C shows the pedigree of Family C. The red box encloses the four core members available for DNA sequence analysis.

RESULTS

CLINICAL CHARACTERISTICS

Three families with ANFH were recruited for the current study (Fig. 1). Two of them (Families A and B) have been described previously.[6] Family C was subsequently added, but only the core family members were available for the current investigation. In conjunction, 65 consecutive patients with sporadic idiopathic ANFH were enlisted during the period from July 2003 to May 2004.

Of the three families with ANFH, there were 32 living members (11 males and 21 females) in whom the disorder had been diagnosed at the time the study was initiated. Twelve members of Family A (4 male and 8 female), 11 members of Family B (6 male and 5 female), and 3 members of Family C (1 male and 2 female) were available for clinical and genetic analyses, and their profiles were compared with those of the 65 patients with sporadic ANFH (55 male and 10 female) (Table 1). Although the sex ratios in the two groups were quite different, we believe that this discrepancy was probably due to ascertainment bias, since we recruited more male patients than female patients from a veterans' hospital. The inherited form of ANFH was associated with a younger age at onset, bilateral hip involvement, and no apparent predisposing conditions. Approximately 85 percent of the patients with sporadic disease had one or more complicating factors, including systemic lupus erythematosus, alcohol consumption, or use of steroid medication. Consumption of alcohol was the single most important nongenetic risk factor associated with the sporadic ANFH.

The clinicopathologic features of a 24-year-old patient are shown in Figure 2. The patient, Subject IV-5 in Family A, presented with groin pain when she was 16 years old. A hip-joint radiograph obtained when she was 21 years old revealed a Ficat's stage II lesion (Fig. 2A), and a magnetic resonance image (MRI) obtained at the same time revealed apparent necrosis of the bone of the femoral head (Fig. 2B). Core-decompression surgery was performed at the age of 21 years. Histopathological examination of the specimen revealed necrosis of bone and marrow tissue — findings consistent with the diagnosis of ANFH (Fig. 2C).

FINE MAPPING OF THE CANDIDATE REGION

To investigate the molecular mechanisms underlying idiopathic osteonecrosis, we took a positional candidate approach to isolate the mutated gene in the families with autosomal dominant ANFH. We first conducted haplotype analysis in two families in which linkage to chromosome 12 had been found and further defined the candidate region for ANFH. In Family A, recombination events were evident in Subjects III-20 and IV-2, and the breakpoints helped to narrow the ANFH-critical region to an 8.2-cM interval between *D12S1301* and *D12S339* (Fig. 1A). We inferred that this genomic segment might harbor the gene for the disease in this family. The affected members of Family B shared, within the family, a haplotype in the ANFH-critical region. However, this disease-associated haplotype, defined by markers between *D12S1663* and *D12S2196*, differed from that of the affected members of Family A. We did

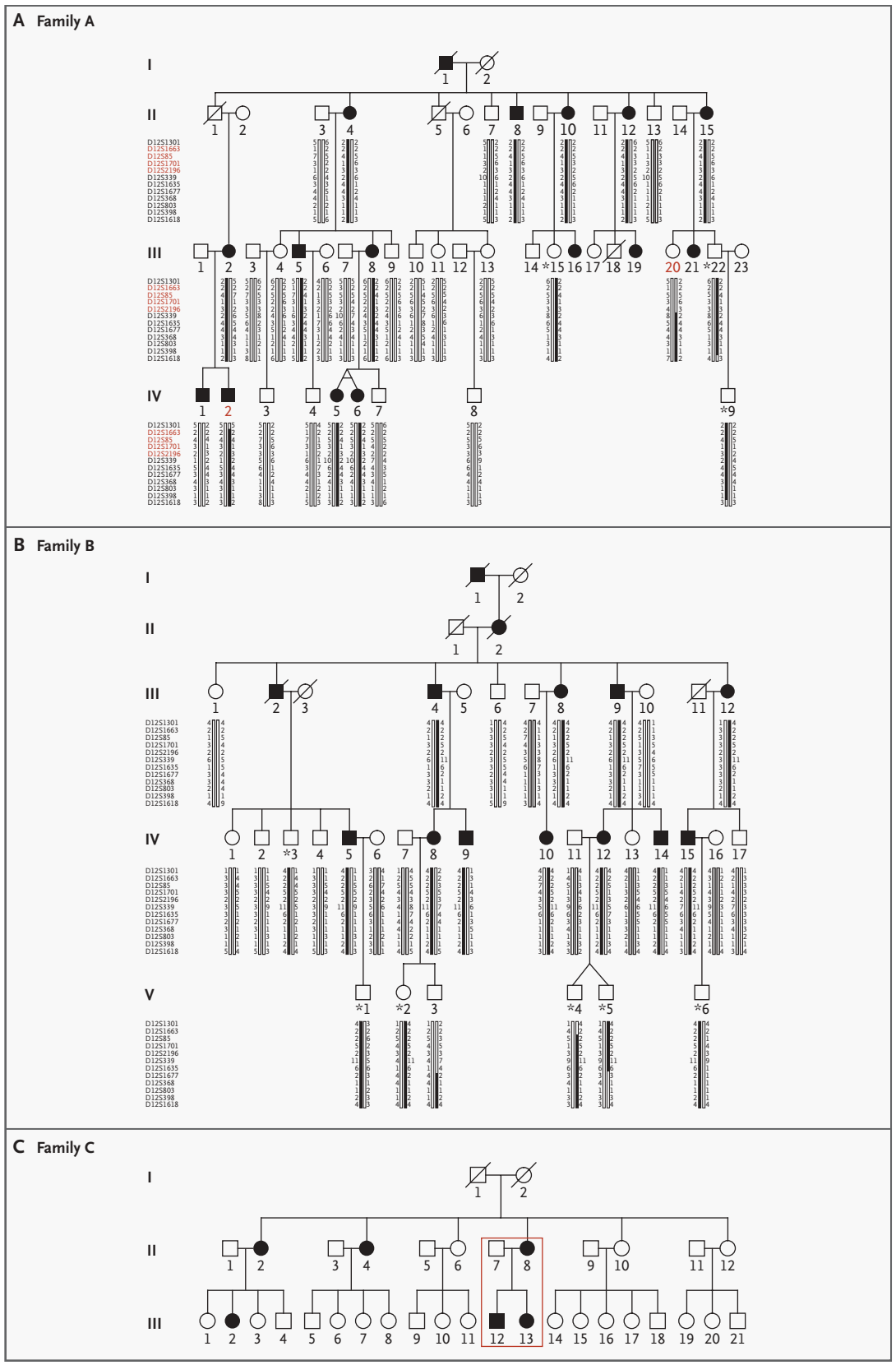

Table 1. Characteristics of the Patients with Familial or Sporadic ANFH.

Characteristic	Family A (N=12)	Family B (N=11)	Family C (N=3)	Sporadic (N=65)
Sex*				
Male	5	8	1	55
Female	11	8	5	10
Age (yr)				
<1–30	4	1	1	8
31–60	4	8	2	50
61–80	4	2	0	7
Associated conditions				
None (idiopathic)	12	11	3	10
Systemic lupus erythematosus	0	0	0	10
Steroid use	0	0	0	6
Alcohol use	0	0	0	31
Steroid and alcohol use	0	0	0	8
Involved site				
Both hips	12	11	3	36
One hip	0	0	0	29

* Affected persons who were not available for clinical evaluation are included in the data on sex.

not carry out haplotype analysis of the third family with ANFH (Family C).

COL2A1 GENE MUTATIONS IN FAMILIAL ANFH

Using Genome Browser (National Center for Biotechnology Information, build 33, April 2003), we identified 21 known genes and 25 predicted genes in the 5.13-Mb critical region between *D12S1301* and *D12S339*. Genes with functions related to bone and cartilage physiology were considered candidates for autosomal dominant ANFH. Among them, *COL2A1* was mapped to 12q13.11–q13.2; it encodes type II collagen, a major structural protein in the extracellular matrix of cartilage. Since type II collagen provides mechanical strength in the cartilage, a mutation in the *COL2A1* gene might be the cause of the hip-joint disorder in the affected families.

We applied a resequencing strategy to investigate this possibility and to uncover any genetic alterations in *COL2A1* that may be associated with ANFH. A 3665G→A transition (GenBank accession number NM_001844) that caused a change from glycine to serine at amino acid position 1170 (GenBank accession number NP_001835) was identified in all 12 available patients from Family A and all 11 available patients from Family B (Fig. 3A). In all the available patients from Family C, a 2306G→A transition (GenBank accession number NM_001844) was identified (Fig. 3B); this transition resulted in a change from glycine to serine in the GXY repeat at amino acid position 717 (GenBank accession number NP_001835) of the predicted protein. The two mutations were not found in either the 110 controls or the 65 persons with sporadic ANFH whom we analyzed. To confirm the heterozygous state of the sequenced mutations, we conducted additional DNA-based diagnostic tests by means of allele-specific primer extension (Fig. 3C) and heteroduplex analysis with the use of single-strand conformational polymorphism and denaturing high-performance liquid chromatography techniques (Fig. 1 and 2 of the Supplementary Appendix). All the assay results are consistent with the conclusion that the patients have a mutant allele as well as a wild-type allele.

DISCUSSION

In this study, we succeeded in using a positional candidate approach to identify the *COL2A1* gene from the chromosome 12q13 region as the gene that is mutated in the inherited form of ANFH. Three points lend support to the idea that the identified mutations in the *COL2A1* gene might be the cause of the disease in the three families we examined. First, the *COL2A1* gene transitions segregated with the disease in these families. Second, the variant sequence did not occur in 220 chromosomes of 110 controls examined. Third, the amino acid change falls at a critical position in the GXY triple-helix repeat of the encoded collagen molecule. Thus, we conclude that the structural alteration in the GXY repeat domain of type II collagen contributes to the pathogenesis of autosomal dominant ANFH.

The clinical features of the three multiplex families are similar to those of the sporadic cases of ANFH seen more commonly in orthopedic practice. Except for the hip joints, the affected persons were otherwise normal, with average height and normal spine development (Fig. 3 of the Supplementary Appendix). Moreover, they had no anomalies of the ocular or auditory system. Notably, the *COL2A1* gene transitions found in the three families with ANFH result in a glycine-to-serine amino acid change in the GXY repeat of the collagen (Fig. 3D). We speculate that, unlike other type II collagenopathies (which are caused by deletion of the gene, trunca-

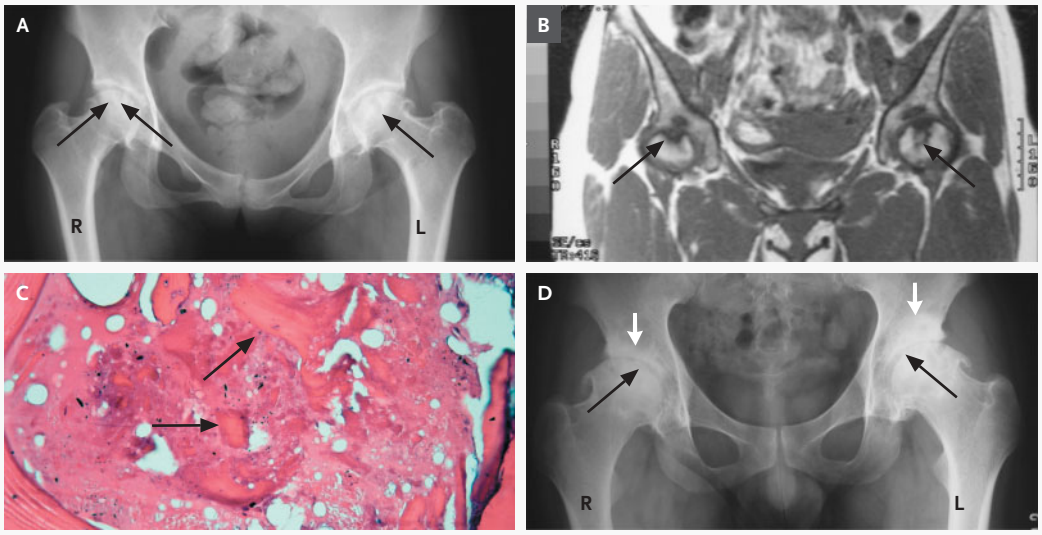

Figure 2. Disease Phenotypes of Autosomal Dominant ANFH.

Panel A shows a radiograph (anteroposterior view) of the pelvis and hip joints of a patient (Subject IV-5 in Family A) with Ficat's stage II ANFH; the radiograph reveals cystic and sclerotic changes in both femoral heads (arrows). Panel B shows a magnetic resonance image of early-stage ANFH in the same patient. The scan shows low-signal segments of both femoral heads (arrows). Examination of the core decompression specimen of the right femoral head (Panel C) revealed several dead bony fragments (arrows) mixed with necrotic marrow tissue and fat cells in the marrow space (hematoxylin and eosin). Panel D shows a radiograph of the pelvis and hip joints of a mutant-allele carrier (Subject III-22 in Family A) with Ficat's stage IV ANFH; the radiograph reveals joint-space narrowing (black arrows) and involvement of acetabulae (white arrows).

tion of the protein, or substitution with a bulky amino acid),[14] the *COL2A1* transitions in familial ANFH might have a milder effect on the structure and function of the protein.

A glycine-to-serine substitution has been reported in several inherited skeletal disorders involving type II collagen, including achondrogenesis or hypochondrogenesis,[15-20] spondyloepiphyseal dysplasia,[21-25] and osteoarthritis[26] (Table 3 of the Supplementary Appendix). It is intriguing that the transitions reported here are distinct from other *COL2A1* mutations and that they result in glycine-to-serine alterations at different positions in the triple-helix domain. Moreover, it is apparent that glycine-to-serine amino acid substitutions in this domain can give rise to diseases with different clinical presentations and varying degrees of severity. It remains to be determined how glycine-to-serine alterations at different positions could affect the posttranslational processing of type II collagen and, consequently, contribute to variable pathophysiological and clinical manifestations. Perhaps, depending on its location in the GXY repeat domain, a specific type of glycine-to-serine substitution might change the fibril structure and interfere with the molecular interactions with other extracellular matrix constituents, such as proteoglycans. In addition, the expression level of the mutant allele relative to the wild-type allele in specific tissues could also have an effect on triple-helix composition and, consequently, clinical severity.

DNA-sequence analysis of the *COL2A1* gene in members of Family A allowed us to identify persons at risk for ANFH. Among 19 persons who had no symptoms at the initial medical examination, 16 had G/G homozygosity at nucleotide position 3665, whereas 3 (Subjects III-15, III-22, and IV-9) had a G→A transition. These three subjects also carried the same haplotype in the critical interval between *D12S1301* and *D12S339* as the patients with ANFH. Groin pain subsequently developed in two of the three subjects (III-15 and III-22, both adults), and a recent radiograph of the hip in Subject III-22 revealed late-stage ANFH (Ficat's stage IV) (Fig. 2D). We conclude that although there is individual variability in the age at onset and in tolerance of the symptoms of the disease, the results of DNA testing coincide with the clinical diagnosis. Moreover,

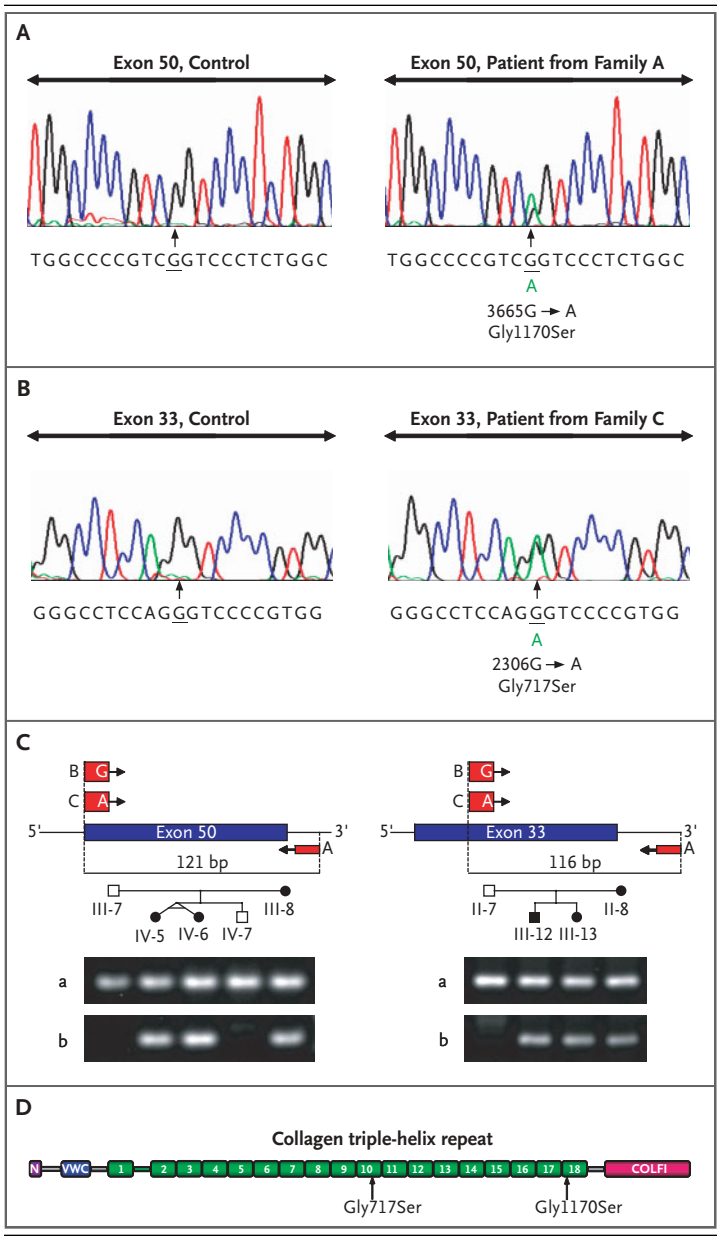

Figure 3. *COL2A1* Mutations in Families with ANFH.

Direct sequencing of fragments amplified by the polymerase chain reaction revealed a 3665G→A substitution in the mutant allele from a patient in Family A (Panel A) and a 2306G→A substitution in the mutant allele from a patient in Family C (Panel B). Panel C shows the results of allele-specific primer extension of the wild-type and G→A mutant sequences. DNA samples annealed with primer pair AB (subpanel a) amplified the wild-type allele and those with primer pair AC (subpanel b) amplified the mutant allele. Affected family members (Subjects III-8, IV-5, and IV-6 of Family A and Subjects II-8, III-12, and III-13 of Family C) were heterozygous for the mutant *COL2A1* sequence. In both reactions, primer A was antisense-strand wild-type sequence, primer B sense-strand wild-type sequence, and primer C sense-strand mutant sequence. Panel D is a schematic representation of type II collagen, showing the positions of the glycine-to-serine mutation in the triple-helix repeat. VWC denotes von Willebrand factor C domain, and COLFI fibrillar collagen C-terminal domain. The diagram is based on annotated messenger RNA of the *COL2A1* gene from the Information Engineering Branch of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/IEB/Research/Acembly).

COL2A1 haplotype and sequence analysis can potentially allow presymptomatic diagnostic testing to be offered to members of families with ANFH.

The third subject (IV-9), currently six years old, does not have the disease, and the most recent radiograph showed normal development of the hips and spine (Fig. 3C of the Supplementary Appendix). As we found in our previous genetic study of two ANFH kindreds,[6] the development of hip-joint problems in carriers of the *COL2A1* mutant allele did not occur until adulthood. Thus, the identification of presymptomatic carriers of ANFH (Subjects III-15, III-22, and IV-9 in Family A and Subjects IV-3, V-1, V-2, V-4, V-5, and V-6 in Family B) provides an opportunity to study the natural history of the disease. Given the genetic basis of the disease, it is now possible to apply radiographic and metabolic indexes, such as measurement of the concentration of collagen fragment in the blood or urine,[27] to monitor the progression of the disease.

The discovery of *COL2A1* as the "causative" disease gene underlying inherited ANFH is unexpected. As its name implies, ANFH is attributed to a compromised vascular supply, but type II collagen is a marker for chondrocytes. Furthermore, mutation of the *COL2A1* gene is known to be the cause of congenital disorders of the skeletal system. The results of the current study, however, suggest that defective cartilage function or abnormal bone homeostasis due to *COL2A1* mutations could also play a role in the pathogenesis of osteonecrosis of the femoral head.

Several strategies have been tested for the preservation of the femoral head in patients with osteonecrosis, including surgical procedures[28] and medication with osteoinductive or angiogenic agents[29] to enhance bone formation and repair.[30] The goal of these approaches is either to reduce the intraosseous

pressure in the femoral head, and thereby restore normal vascular flow, or to enhance regional vascularization. It is known that core decompression for osteonecrosis of the hip has an effect on the natural history and clinical progression of the disease when necrotic involvement of the femoral head is limited.[28] Therefore, molecular analysis of the *COL2A1* gene can be used in families with ANFH to provide an indication for intervention when there is an early sign of osteonecrosis.

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ORIGINAL ARTICLE

Adjuvant Docetaxel for Node-Positive Breast Cancer

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ABSTRACT

BACKGROUND

We compared docetaxel plus doxorubicin and cyclophosphamide (TAC) with fluorouracil plus doxorubicin and cyclophosphamide (FAC) as adjuvant chemotherapy for operable node-positive breast cancer.

METHODS

We randomly assigned 1491 women with axillary node-positive breast cancer to six cycles of treatment with either TAC or FAC as adjuvant chemotherapy after surgery. The primary end point was disease-free survival.

RESULTS

At a median follow-up of 55 months, the estimated rates of disease-free survival at five years were 75 percent among the 745 patients randomly assigned to receive TAC and 68 percent among the 746 randomly assigned to receive FAC, representing a 28 percent reduction in the risk of relapse (P=0.001) in the TAC group. The estimated rates of overall survival at five years were 87 percent and 81 percent, respectively. Treatment with TAC resulted in a 30 percent reduction in the risk of death (P=0.008). The incidence of grade 3 or 4 neutropenia was 65.5 percent in the TAC group and 49.3 percent in the FAC group (P<0.001); rates of febrile neutropenia were 24.7 percent and 2.5 percent, respectively (P<0.001). Grade 3 or 4 infections occurred in 3.9 percent of the patients who received TAC and 2.2 percent of those who received FAC (P=0.05); no deaths occurred as a result of infection. Two patients in each group died during treatment. Congestive heart failure and acute myeloid leukemia occurred in less than 2 percent of the patients in each group. Quality-of-life scores decreased during chemotherapy but returned to baseline levels after treatment.

CONCLUSIONS

Adjuvant chemotherapy with TAC, as compared with FAC, significantly improves the rates of disease-free and overall survival among women with operable node-positive breast cancer.

Adjuvant chemotherapy for breast cancer has undergone a major change over the past two decades. Chemotherapy with a regimen that includes an anthracycline or a combination of cyclophosphamide, methotrexate, and fluorouracil significantly decreases the risks of disease recurrence and death among women with early-stage breast cancer.[1] The overview analysis of the Early Breast Cancer Trialists' Collaborative Group demonstrated that, as compared with standard treatment with cyclophosphamide, methotrexate, and fluorouracil, regimens that contained doxorubicin or epirubicin reduced the annual risk of recurrence of breast cancer by 12 percent and the annual risk of death by 11 percent. Rates of disease-free and overall survival were similar among women treated with either six cycles (spanning 24 weeks) of cyclophosphamide, methotrexate, and fluorouracil or four cycles (12 weeks) of doxorubicin plus cyclophosphamide.[2]

Six cycles of fluorouracil, doxorubicin, and cyclophosphamide (FAC), given in various doses and according to various schedules, or fluorouracil, epirubicin, and cyclophosphamide appear superior to six cycles of cyclophosphamide, methotrexate, and fluorouracil in early-stage breast cancer,[1,3,4] and six cycles of adjuvant fluorouracil, epirubicin, and cyclophosphamide are better than three cycles in terms of disease-free and overall survival.[5] Therefore, at the time this trial was initiated, six cycles of FAC; cyclophosphamide, doxorubicin, and fluorouracil; or fluorouracil, epirubicin, and cyclophosphamide every three weeks were generally accepted as appropriate adjuvant regimens for the treatment of early breast cancer.[6] Although various regimens with fluorouracil, doxorubicin, and cyclophosphamide that differed in schedule and dose were developed,[7-10] no randomized, prospective, comparative trial has demonstrated the superiority of any one regimen.

Docetaxel, an active agent in the treatment of breast cancer,[11] is not cross-resistant with anthracyclines,[12,13] appears to be more active than doxorubicin,[14] and does not interfere with the pharmacokinetics of doxorubicin,[15,16] indicating that, unlike paclitaxel,[17-19] it may not exacerbate doxorubicin-related cardiotoxicity.[20,21] Three large randomized trials involving treatments for metastatic breast cancer found that regimens of docetaxel plus doxorubicin and of docetaxel, doxorubicin, and cyclophosphamide (TAC) have antitumor activity superior to that of doxorubicin plus cyclophosphamide and that of FAC, although survival was not significantly different between the treatment groups in two of the three studies.[21-23]

In 1997, the Breast Cancer International Research Group began a phase 3 trial to compare the docetaxel-containing regimen TAC with a regimen of FAC as adjuvant treatment for women with operable node-positive breast cancer. At a planned interim analysis at 33 months (August 2001), we reported a statistically significant improvement in the rate of disease-free survival among patients treated with TAC as compared with those treated with FAC (hazard ratio, 0.68; P=0.0011).[24] Because the results of this analysis did not meet the predefined P value of less than 0.001 to ascertain a statistically significant difference between TAC and FAC,[25] the independent data monitoring committee recommended that the protocol be amended to include a second interim analysis, to be conducted at the point at which there had been 400 events, in addition to the protocol-specified final analysis after 590 disease-free survival events. The comparison was to be performed at the level of P=0.001 for the primary end point of disease-free survival. We report the results of the second interim analysis, which was performed after a median follow-up period of 55 months (after 399 disease-free survival events).

METHODS

STUDY POPULATION

Women eligible for the study were between 18 and 70 years of age, had a score on the Karnofsky performance scale of 80 percent or more, and had undergone primary surgery (i.e., mastectomy, tumorectomy, or lumpectomy) with axillary-node dissection (sentinel-node biopsy was not routine practice) for unilateral, operable carcinoma of the breast. Patients were randomly assigned to a study group within 60 days after surgery. All patients had at least one axillary lymph node that was positive for cancer on histologic examination. The margins of resected specimens had to be histologically free of invasive adenocarcinoma and ductal carcinoma in situ. A complete staging workup within three months before registration — including bilateral mammography; chest radiography; abdominal ultrasonography, computed tomography, or both; and bone scanning — and an assessment of the left ventricular ejection fraction with the use of multiple gated acquisition scanning or echocardiography were mandatory.

Criteria for exclusion included advanced disease (i.e., T4, N2 or N3, or M1), a history of other cancers, motor or sensory neuropathy of grade 2 or more according to the National Cancer Institute Common Toxicity Criteria, pregnancy, lactation, and any serious illness or medical condition other than breast cancer. Prior therapy with anthracyclines or taxanes was not allowed.

The study was approved by the ethics committees or institutional review boards of all participating institutions. All patients provided written informed consent. The trial was conducted according to Good Clinical Practice and International Conference on Harmonization rules, including verification of source data.

STUDY DESIGN

In this phase 3, multicenter, prospective trial, randomization was stratified according to institution and number of involved axillary lymph nodes per patient (one to three vs. four or more). On day 1 of each of six 21-day cycles, eligible patients received either TAC (50 mg of doxorubicin per square meter of body-surface area in an intravenous infusion for 15 minutes, followed by 500 mg of cyclophosphamide per square meter administered intravenously for 1 to 5 minutes and then, after a 1-hour interval, 75 mg of docetaxel per square meter in an intravenous infusion for 1 hour) or FAC (50 mg of doxorubicin per square meter followed by 500 mg of fluorouracil per square meter, each as an intravenous infusion for 15 minutes, and then 500 mg of cyclophosphamide per square meter in an intravenous infusion for 1 to 5 minutes).

The primary end point was disease-free survival, defined as the time from randomization to the date of a clinical relapse (with histopathologic confirmation or radiologic evidence of tumor recurrence), a second cancer (with the exception of skin cancer other than melanoma, ductal or lobular carcinoma in situ of the breast, or in situ carcinoma of the cervix), or death, whichever occurred first. Secondary end points included overall survival (i.e., the time from randomization until death from any cause), toxic effects, and quality of life.

STUDY PROCEDURES

Concomitant Therapy and Dose Modifications

Patients randomly assigned to receive TAC received dexamethasone premedication (8 mg orally every 12 hours six times beginning the day before treatment started) to prevent docetaxel-related hypersensitivity and fluid retention. All patients were to receive a prophylactic antibiotic (500 mg of ciprofloxacin twice daily on days 5 to 14 of each cycle). Patients in the FAC group received prophylactic antibiotics only after an episode of febrile neutropenia or infection. Primary prophylaxis with granulocyte colony-stimulating factor (G-CSF) was not permitted. However, among patients who had one episode of febrile neutropenia or infection in subsequent cycles, administration of G-CSF was mandatory (150 μg of lenograstim per square meter per day or 5 μg of filgrastim per kilogram of body weight per day on days 4 to 11).

On completion of chemotherapy, tamoxifen (20 mg daily for five years) was administered to patients with estrogen-receptor–positive tumors, progesterone-receptor–positive tumors, or both. Radiotherapy was mandatory after breast-conserving surgery and was administered after mastectomy according to each institution's guidelines.

Dose modifications were planned according to standard toxicity criteria. Discontinuation of treatment was required for patients in whom there were nonhematologic grade 4 toxic effects according to the National Cancer Institute Common Toxicity Criteria, grade 3 toxic effects despite a dose reduction, or clinically significant cardiac events.

Evaluations

Blood counts and general biochemical and clinical assessments, including those for toxic effects, were performed on day 21 of each cycle and then every six months for the first five years of follow-up, after which they were performed annually. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 1.0. Chest radiography was repeated every 12 months for the first 5 years of follow-up. Mammography was repeated annually during follow-up.

Estrogen-receptor and progesterone-receptor status in the tumor was evaluated by immunohistochemical analysis.[26] *HER2/neu* gene amplification was evaluated by fluorescence in situ hybridization, with a positive result defined as a ratio of *HER2/neu* to chromosome 17 of greater than 2.0.[27-30] Assessments of hormone receptors and *HER2/neu* status were performed at the Cross Cancer Institute in Edmonton, Alberta, Canada.

Quality of Life

Quality of life was assessed with the use of the European Organisation for Research and Treatment

of Cancer Quality of Life Questionnaire (QLQ-C30, version 2.0) and the breast-cancer–specific QLQ-BR23 (version 1.0). The QLQ-C30 includes nine multiple-item scales pertaining to symptoms, five to function, and one to overall health — the global health status and quality-of-life scale. The QLQ-BR23 includes 23 questions regarding disease symptoms, treatment-related side effects, body image, sexuality, and future perspective. Patients were asked to complete both questionnaires on seven occasions: at baseline; before cycles 3 and 5; 3 to 4 weeks after the last cycle; and 6, 12, and 24 months after the last cycle.

Statistical Analysis

The trial was designed to have an overall power of 97 percent to detect a 27 percent reduction in the risk of relapse among patients treated with TAC as compared with those treated with FAC, regardless of nodal status. In addition, the study had 90 percent power to detect a 33 percent reduction in the risk of death. At the final analysis (i.e., at the point at which there were 590 patients), the sample size of 1491 patients would allow the detection, with 90 percent power, of a 27 percent reduction in the risk of relapse in favor of treatment with TAC among patients who had one to three positive lymph nodes. For the subgroup of patients with four or more positive nodes, the sample size would provide 80 percent power to detect a 29 percent reduction in the risk of relapse in favor of treatment with TAC.

The primary analysis was conducted according to the intention-to-treat principle, and a stratified log-rank test was used to compare TAC with FAC with respect to both disease-free and overall survival. The number of positive nodes (one to three or four or more) was the only stratification variable in the analysis. Analyses of subgroups according to hormone-receptor status and *HER2/neu* status were prospectively defined but were not powered. Unadjusted analyses and analyses according to the Cox proportional-hazards model (adjusted for age, tumor size, nodal status, hormone-receptor status, and *HER2/neu* status) were performed to estimate disease-free and overall survival. The Kaplan–Meier method was used to calculate probability estimates of disease-free and overall survival. Hypothesis testing was two-sided. Hazard ratios and 95 percent confidence intervals were obtained from the Cox proportional-hazards model. The primary quality-of-life analysis was performed with the use of the scores from the global health status and quality-of-life scale. A repeated-measures mixed-effect analysis of variance was performed to analyze the evolution of the scores on the global health status subscale over time.

The protocol was designed by the study chairs of the Breast Cancer International Research Group in collaboration with Aventis personnel. The data were collected and maintained by the Breast Cancer International Research Group. All analyses were conducted according to the protocol. The efficacy analyses were performed by the independent data monitoring committee; other analyses were conducted by Aventis personnel. Submission of the results for publication was mandated by the independent data monitoring committee. The manuscript was drafted by Dr. Martin and modified after review by the cochairs and other coauthors. A reviewer at Aventis evaluated the manuscript but did not participate in writing it. The final content of the manuscript was determined entirely by the investigators.

RESULTS

PATIENTS

Between June 1997 and June 1999, 1491 women from 20 countries were enrolled in the study. Eleven women (1 who had been randomly assigned to receive TAC and 10 assigned to receive FAC) did not receive any treatment, for the following reasons: 8 withdrew consent, 1 was lost to follow-up, and 2 did not receive treatment for other reasons. In total, 1480 patients (744 in the TAC group and 736 in the FAC group) were treated and were included in the safety analysis. Efficacy analyses were based on the intention-to-treat principle (1491 patients) and on populations of patients who were eligible according to the protocol (1421 patients). Seventy patients (4.7 percent of all those enrolled) — 36 in the TAC group and 34 in the FAC group — were ineligible. The most common reason for ineligibility in both groups was indeterminate hormone-receptor status at randomization (21 women in the TAC group and 19 in the FAC group). The groups were well balanced in terms of demographic and tumor characteristics (Table 1).

TREATMENT

Six treatment cycles were completed by 91.3 percent of the patients in the TAC group and by 96.6 percent of those in the FAC group. Overall, the median relative dose intensities were 99 percent in the TAC group and 98 percent in the FAC group. Treatment

Table 1. Characteristics of the Patients and the Tumors at Baseline.*

Characteristic	TAC Group (N=745)	FAC Group (N=746)
Age — yr		
Median	49	49
Range	26–70	23–70
Menopausal status — no. of women (%)		
Premenopausal†	421 (56.5)	409 (54.8)
Postmenopausal‡	324 (43.5)	337 (45.2)
Primary tumor size — no. of women (%)		
T1, ≤2 cm	296 (39.7)	320 (42.9)
T2, 2–5 cm	392 (52.6)	383 (51.3)
T3, >5 cm	57 (7.7)	43 (5.8)
Nodal status — no. of women (%)		
N1, N2, or N3	467 (62.7)	459 (61.5)
N4 or higher	278 (37.3)	287 (38.5)
Positive estrogen-receptor or progesterone-receptor status — no. of women (%)§	567 (76.1)	565 (75.7)
HER2/neu status — no. of women (%)¶		
Positive	155 (20.8)	164 (22.0)
Unknown	115 (15.4)	114 (15.3)
Breast-conserving surgery — no. of women (%)‖	300 (40.3)	303 (41.2)
With radiotherapy	285 (38.3)	293 (39.8)
Without radiotherapy	15 (2.0)	10 (1.4)
Mastectomy — no. of women (%)‖	444 (59.7)	433 (58.8)
With radiotherapy	227 (30.5)	236 (32.1)
Without radiotherapy	217 (29.2)	197 (26.8)

* TAC denotes docetaxel plus doxorubicin and cyclophosphamide, and FAC fluorouracil plus doxorubicin and cyclophosphamide.
† Premenopausal women were those in whom the last menses had occurred within the previous six months and who had not previously had bilateral ovariectomy or estrogen-replacement therapy (including women of unknown status less than 50 years of age).
‡ Postmenopausal women were those who had had a prior bilateral ovariectomy or in whom more than 12 months had passed since the last menses, with no prior hysterectomy (including women of unknown status 50 years of age or older).
§ For the central review, estrogen-receptor status was assessed with clone 6F11; progesterone-receptor status was assessed with clone 636 with the use of tumor blocks or unstained slides.
¶ *HER2/neu* status was determined by fluorescence in situ hybridization for 1250 patients. Immunohistochemistry with clone CB11 was used for 12 patients. The status of the remaining patients was not assessed owing to a lack of tumor specimens.
‖ Women who had surgery were among the treated patients (744 in the TAC group and 736 in the FAC group).

was modified (by a delay, a dose reduction, or both) for 250 patients in the TAC group (33.6 percent) and 293 in the FAC group (39.8 percent). The most frequent reason for delaying a cycle of treatment was the occurrence of hematologic toxic effects.

Adjuvant radiotherapy[31] was administered to 68.8 percent of the patients in the TAC group and 71.9 percent of those in the FAC group. Among women with hormone-receptor–positive tumors, the rates of compliance with tamoxifen treatment, as planned according to the protocol, were 94.9 percent in the TAC group and 93.7 percent in the FAC group.

EFFICACY

The efficacy analysis was performed after it had been documented that 399 events had been recorded (172 in the TAC group and 227 in the FAC group) as of July 15, 2003, representing a median follow-up period of 55 months (Table 2). Ninety-seven per-

cent of the patients in the study completed at least 45 months of follow-up.

The estimated rates of disease-free survival at five years were 75 percent in the TAC group and 68 percent in the FAC group (P=0.001). This difference was due mainly to the greater number of patients in the FAC group who had relapses of breast cancer at distant sites (Table 2). Similar results were observed in the eligible population as well as in the unadjusted and multivariate analyses (Fig. 1A). After adjustment for nodal status, treatment with TAC, as compared with FAC, was associated with a 28 percent reduction in the risk of relapse (hazard ratio, 0.72; 95 percent confidence interval, 0.59 to 0.88) (Fig. 1A).

The superiority of TAC over FAC was also observed in all planned subgroup analyses, which included the number of involved axillary lymph nodes, hormone-receptor status, and *HER2/neu* status, and was independent of menopausal status (a factor in the sensitivity analysis) (Fig. 2). In the subgroup of patients with one to three positive nodes, treatment with TAC reduced the risk of relapse by 39 percent (hazard ratio, 0.61; 95 percent confidence interval, 0.46 to 0.82; P<0.001). Among women with four or more positive nodes, treatment with TAC reduced the risk of relapse by 17 percent (hazard ratio, 0.83; 95 percent confidence interval, 0.63 to 1.08; P=0.17). Analysis with the Cox model did not detect any difference in the treatment effect between the two nodal-status strata (ratio of hazard ratios, 1.34; P=0.15), suggesting that TAC was superior to FAC, regardless of the number of lymph nodes involved.

Of the 221 deaths, 91 were in the TAC group and 130 in the FAC group; TAC was associated with a 30 percent lower risk of death than was FAC (hazard ratio, 0.70; 95 percent confidence interval, 0.53 to 0.91; P=0.008) (Fig. 1B). The estimated overall survival rates at five years were 87 percent in the TAC group and 81 percent in the FAC group.

Table 2. Analysis of Events According to the Intention-to-Treat Principle.*

Event	TAC Group (N=745)	FAC Group (N=746)
	no. of patients (%)	
None	573 (76.9)	519 (69.6)
Any event	172 (23.1)	227 (30.4)
Relapse of breast cancer	144 (19.3)	197 (26.4)
Local only, regional only, or both	29 (3.9)	39 (5.2)
Distant (with or without local or regional)	115 (15.4)	158 (21.2)
Second primary cancer	20 (2.7)	26 (3.5)
Contralateral breast cancer	7 (0.9)	8 (1.1)
Other cancers	13 (1.7)	18 (2.4)
Death (without evidence of cancer)	8 (1.1)	4 (0.5)
Due to toxic effects, with sepsis	0	0
Due to toxic effects, without sepsis	2 (0.3)	1 (0.1)
Other causes	6 (0.8)	3 (0.4)

* Events are those included in the analysis of disease-free survival. TAC denotes docetaxel plus doxorubicin and cyclophosphamide, and FAC fluorouracil plus doxorubicin and cyclophosphamide.

TOXIC EFFECTS

Overall, the incidence of grade 3 or 4 or severe nonhematologic adverse events, regardless of type, was 36.3 percent in the TAC group and 26.6 percent in the FAC group (P<0.001). The incidence of grade 3 or 4 neutropenia was 65.5 percent in the TAC group and 49.3 percent in the FAC group (P<0.001); febrile neutropenia was observed in 24.7 percent of the patients in the TAC group and 2.5 percent of those in the FAC group (P<0.001) (Table 3). Grade 3 or 4 infections occurred in 3.9 percent of patients treated with TAC and 2.2 percent of those treated with FAC (P=0.05); no deaths occurred as a result of infection (Table 4). The overall incidence of congestive heart failure (including that during follow-up) was 1.6 percent among patients treated with TAC and 0.7 percent for those treated with FAC (P=0.09). As of the cutoff date for this analysis, the only secondary hematologic cancer was acute myeloid leukemia, which developed in two patients in the TAC group and one patient in the FAC group.

QUALITY OF LIFE

All baseline quality-of-life values were similar between the two treatment groups, with a mean score of 72 (on a scale of 0 to 100, with higher scores representing a better quality of life) in both groups on the global health status subscale of the Quality of Life Questionnaire. The mean scores at the end of treatment were 62 in the TAC group (95 percent confidence interval, 61 to 64) and 69 in the FAC group (95 percent confidence interval, 67 to 70). At the first follow-up visit, the quality-of-life scores either returned to or were higher than those at baseline in both groups, with scores of 76 in the TAC group (95 percent confidence interval, 74 to 77) and 75 in the FAC group (95 percent confidence in-

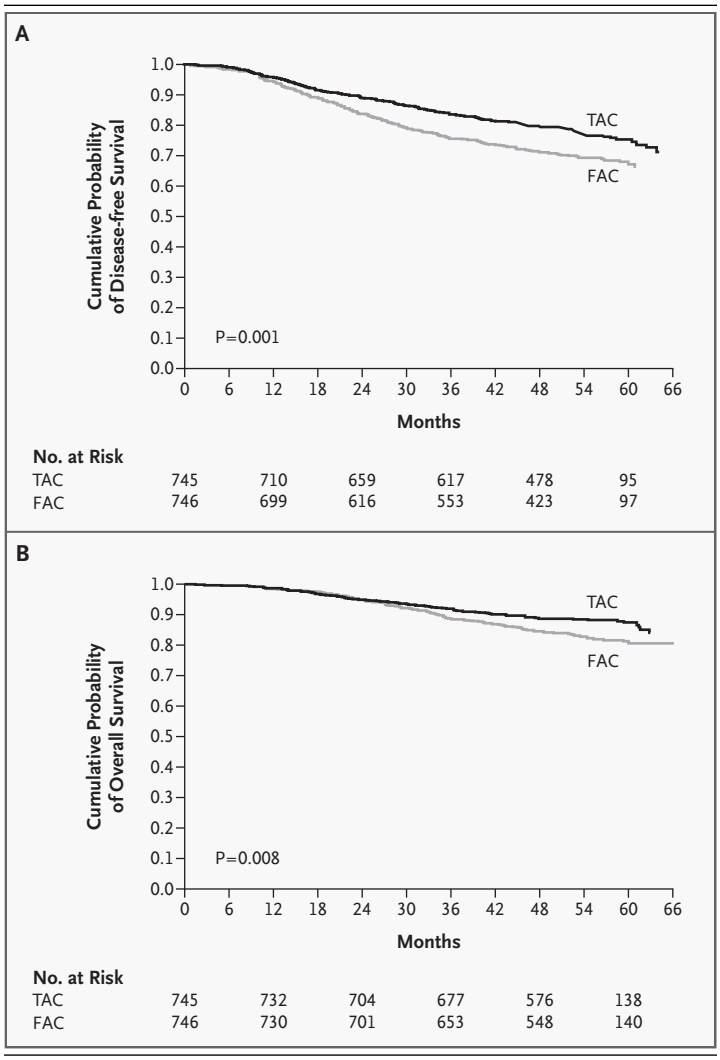

Figure 1. Analysis of Survival Rates in the Two Study Groups.

Panel A shows the rates of disease-free survival. For the 1491 randomly assigned patients included in the intention-to-treat analysis, the hazard ratio, adjusted for nodal status, was 0.72 (95 percent confidence interval, 0.59 to 0.88; P=0.001); unadjusted for nodal status, 0.71 (95 percent confidence interval, 0.59 to 0.87; P<0.001); and with the Cox proportional-hazards model — adjusted for number of positive nodes, age, tumor size, histologic grade, and hormone-receptor and *HER2/neu* status — 0.70 (95 percent confidence interval, 0.58 to 0.86; P<0.001). For the 1421 patients eligible for treatment, the hazard ratio, adjusted for nodal status, was 0.72 (95 percent confidence interval, 0.59 to 0.89; P=0.002). Events occurred in 172 patients (23 percent) in the TAC group and 227 (30 percent) in the FAC group. Data were censored for 573 patients (77 percent) in the TAC group and 519 (70 percent) in the FAC group. Panel B shows the rates of overall survival. For the 1491 randomized patients included in the intention-to-treat analysis, the hazard ratio, adjusted for nodal status, was 0.70 (95 percent confidence interval, 0.53 to 0.91; P=0.008); unadjusted for nodal status, 0.69 (95 percent confidence interval, 0.52 to 0.90; P=0.005); and with the Cox proportional-hazards model, adjusted for the same variables as those listed for Panel A, 0.68 (95 percent confidence interval, 0.52 to 0.89; P=0.004). For the 1421 patients eligible for treatment, the hazard ratio, adjusted for nodal status, was 0.70 (95 percent confidence interval, 0.53 to 0.93; P=0.01). Events occurred in 91 patients (12 percent) in the TAC group and 130 (17 percent) in the FAC group. Data were censored for 654 patients (88 percent) in the TAC group and 616 (83 percent) in the FAC group. P values and confidence intervals are nominal. TAC denotes docetaxel plus doxorubicin and cyclophosphamide, and FAC fluorouracil plus doxorubicin and cyclophosphamide.

terval, 73 to 77). Follow-up quality-of-life measurements were similar between the two groups and similar to baseline values: at six months, the scores were 77 in the TAC group (95 percent confidence interval, 75 to 78) and 75 in the FAC group (95 percent confidence interval, 73 to 77); at the end of two years, they were 78 in the TAC group (95 percent confidence interval, 76 to 79) and 76 in the FAC group (95 percent confidence interval, 74 to 78).

DISCUSSION

This randomized, phase 3 trial of adjuvant chemotherapy in women with operable node-positive breast cancer showed that, at a median follow-up of 55 months, the estimated rate of disease-free survival at 5 years was 75 percent in the TAC group and 68 percent in the FAC group (P=0.001). The relative risk of death was 30 percent lower among women in the TAC group than among those in the FAC group.

Moreover, treatment with TAC, as compared with FAC, was associated with a 28 percent relative reduction in the risk of relapse. The reduction in the risk of relapse did not seem to be driven by nodal status or by hormone-receptor or *HER2/neu* status. A final analysis of this trial at 590 events will be required to confirm and extend the findings of the main and subgroup analyses. Although amenorrhea occurred more frequently among women in the TAC group (61.7 percent) than among those in the FAC group (52.4 percent) (P=0.007), the superior efficacy of TAC over FAC in terms of disease-free survival was independent of menopausal sta-

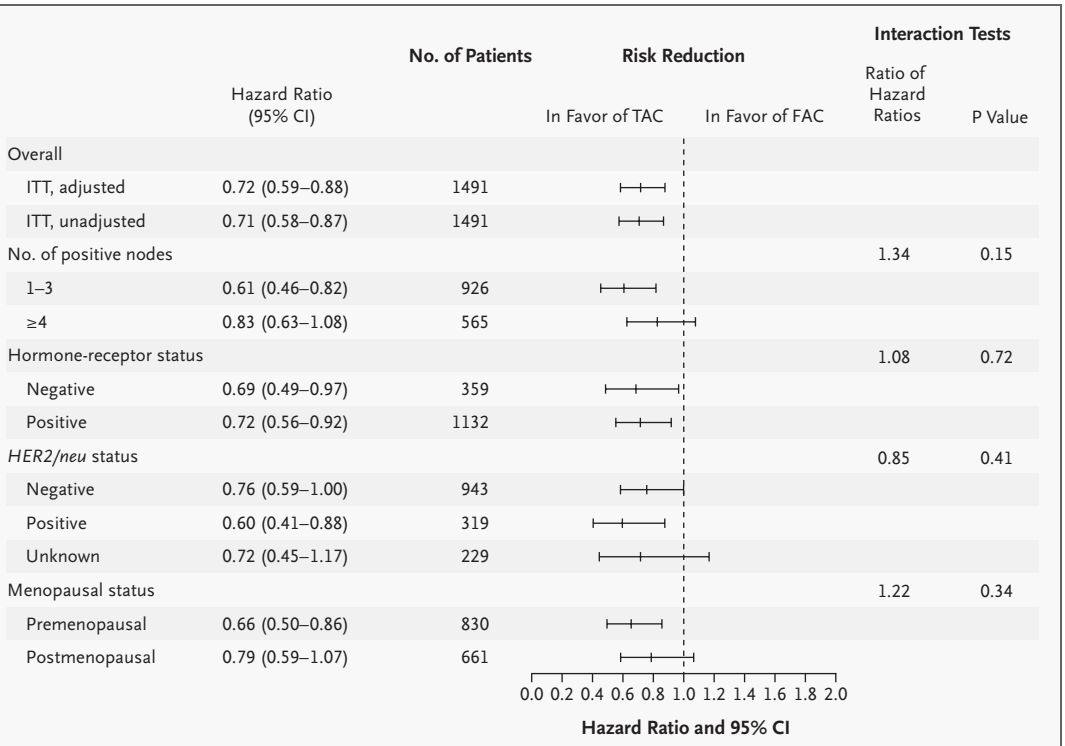

Figure 2. Risk Reduction for Disease-free Survival in the Main Subgroups.

Premenopausal patients included those whose menopausal status was unknown but who were less than 50 years of age; postmenopausal patients included those whose menopausal status was unknown but who were 50 years of age or older. TAC denotes docetaxel plus doxorubicin and cyclophosphamide, FAC fluorouracil plus doxorubicin and cyclophosphamide, ITT intention to treat, and CI confidence interval.

tus. The observation that the benefits of treatment with docetaxel are independent of hormone-receptor status are consistent with the findings of the National Surgical Adjuvant Breast and Bowel Project trial B-27,[32] in which patients with breast cancer who were treated with presurgical doxorubicin plus cyclophosphamide followed by docetaxel had higher rates of complete pathological response than did those treated with doxorubicin plus cyclophosphamide alone, regardless of hormone-receptor status.

The symmetrical design of this trial — in which patients underwent six cycles of treatment with either FAC or TAC, followed by tamoxifen therapy, radiation therapy, or both, as indicated — demonstrates a benefit with the replacement of fluorouracil by docetaxel. Six cycles of three-drug, anthracycline-based regimens are considered among the most effective treatments for node-positive breast cancer.[6] The FAC regimen generally used in North America (two doses of fluorouracil per cycle) was not directly compared with the FAC regimen we selected, and there is no evidence that the omission of a dose of fluorouracil would influence the patients' outcomes. At the dose and schedule used in this trial, FAC is an appropriate control chemotherapeutic regimen. The TAC combination in this trial was also administered at a feasible dose and schedule. In both groups, the dosage of doxorubicin was 50 mg per square meter for six cycles (for a total of 300 mg per square meter).

The Cancer and Leukemia Group B trial 9344 did not demonstrate a benefit with an escalation of the doxorubicin dosage (240, 300, or 360 mg per square meter, delivered over four cycles).[33] Another study of adjuvant therapy[34] showed that administering chemotherapy at shorter intervals (every two weeks vs. every three weeks) significantly improved

Table 3. Adverse Events in the Two Treatment Groups.*

Toxic Effect	TAC Group (N=744)		FAC Group (N=736)		P Value†	
	Overall	Grade 3 or 4 or Severe	Overall	Grade 3 or 4 or Severe	All	Grade 3 or 4
	percent					
Hematologic						
Anemia						
Any	91.5	4.3	71.7	1.6	<0.001	0.003
Need for blood transfusions	4.6	—	1.5	—	<0.001	—
Neutropenia	71.4	65.5	82.0	49.3	<0.001	<0.001
Thrombocytopenia	39.4	2.0	27.7	1.2	<0.001	0.23
Febrile neutropenia‡						
Protocol definition	24.7	—	2.5	—	<0.001	—
NCI CTC definition 2.0	28.8	—	4.4	—	<0.001	—
Neutropenic infection						
Protocol definition§	12.1	—	6.3	—	<0.001	—
NCI CTC definition 2.0	20.4	—	10.8	—	<0.001	—
Nonhematologic						
Alopecia	97.8	—	97.1	—	0.39	—
Asthenia	80.8	11.2	71.2	5.6	<0.001	<0.001
Nausea	80.5	5.1	88.0	9.5	<0.001	0.001
Stomatitis	69.4	7.1	52.9	2.0	<0.001	<0.001
Amenorrhea¶	61.7	—	52.4	—	0.007	—
Vomiting	44.5	4.3	59.2	7.3	<0.001	0.013
Infection	39.4	3.9	36.3	2.2	0.22	0.05
Diarrhea	35.2	3.8	27.9	1.8	0.002	0.02
Peripheral edema	33.7	0.5	12.6	0.1	<0.001	0.37‖
Myalgia	26.7	0.8	9.9	0	<0.001	0.03‖
Skin	26.5	0.8	17.7	0.4	<0.001	0.51‖
Neurosensory effects**	25.5	0	10.2	0	<0.001	—
Anorexia	21.6	2.2	17.7	1.2	0.05	0.17
Arthralgia	19.4	0.5	9.0	0.3	<0.001	0.69‖
Nail disorder	18.5	0.4	14.4	0.1	0.03	0.62‖
Allergy	13.4	1.3	3.7	0.1	<0.001	0.007
Abdominal pain	10.9	0.7	5.3	0	<0.001	0.06‖
Mild-to-severe congestive heart failure	1.6	0.1	0.7	0.1	0.09	1.0‖

* TAC denotes docetaxel plus doxorubicin and cyclophosphamide, and FAC fluorouracil plus doxorubicin and cyclophosphamide.
† P values were calculated with the use of the chi-square test unless otherwise specified.
‡ The study protocol defined febrile neutropenia as fever of grade 2 or more concomitant with grade 4 neutropenia requiring intravenous antibiotics, hospitalization, or both. The National Cancer Institute Common Toxicity Criteria (NCI CTC) definition 2.0 is fever of 38°C or more concomitant with grade 3 or 4 neutropenia.
§ The study protocol defined neutropenic infection as that of grade 2 or more concomitant with grade 3 or 4 neutropenia. The NCI CTC definition 2.0 is infection of any grade concomitant with grade 3 or 4 neutropenia.
¶ Amenorrhea was defined as the absence of menses for at least three months. Percentages were calculated among premenopausal patients (420 in the TAC group and 403 in the FAC group) who could be evaluated for safety and were treated with study drugs.
‖ P values were calculated according to Fisher's exact test.
** Grade 2 neurosensory effects occurred in 3.6 percent of patients in the TAC group and 1.4 percent in the FAC group.

clinical outcomes, raising the possibility that alternative schedules and durations of treatment may further improve outcomes in this setting. A comparison of a dose-dense regimen (treatment administered every two weeks) and the TAC regimen used in the current trial will be part of the National Surgical Adjuvant Breast and Bowel Project trial B-38.

The toxic effects associated with the TAC regimen we used are consistent with those reported in association with TAC in women with advanced breast cancer[20,22] and were manageable with standard supportive measures. Grade 3 or 4 neutropenia was common in both groups (65.5 percent in the TAC group and 49.3 percent in the FAC group, P<0.001). Although the incidence of febrile neutropenia was higher among women treated with TAC (despite the administration of prophylactic ciprofloxacin) than among those treated with FAC (24.7 percent and 2.5 percent), grade 3 or 4 infection was seen in only 3.9 percent of patients in the TAC group, and no deaths due to sepsis occurred. Considering that the rates of febrile neutropenia did not reach the recommended threshold for routine prophylactic administration of G-CSF,[35] the administration of primary prophylaxis with G-CSF should be left to the discretion of the treating physician. However, on the basis of good practice, after an episode of febrile neutropenia, prophylaxis with G-CSF is recommended for all subsequent cycles.

Most patients completed all six treatment cycles (91.3 percent in the TAC group and 96.6 percent in the FAC group), and one third required a delay in or adjustment of treatment (33.6 percent in the TAC group and 39.8 percent in the FAC group). The incidence of congestive heart failure was 1.6 percent among patients treated with TAC, which is consistent with the incidence associated with anthracycline-based adjuvant chemotherapy.[4,33,34]

The tolerability of adjuvant chemotherapy and the magnitude of deterioration in quality of life are important considerations in a woman's decision to undergo treatment. It is reassuring to note that although both chemotherapy regimens in our trial were associated with transient, statistically significant reductions in quality-of-life scores, these scores returned to baseline levels at the first follow-up visit after treatment and were similar between the treatment groups.

In conclusion, this interim analysis of the Breast Cancer International Research Group trial 001 demonstrates a therapeutic advantage of TAC over FAC, but at the expense of increased toxic effects. Furthermore, chemotherapeutic treatment with TAC led to only a transient reduction in quality-of-life scores, which subsequently returned to pretreatment baseline values.

Table 4. Deaths Due to Causes Other Than Breast Cancer or a Second Cancer.*

Deaths	TAC Group (N=745)	FAC Group (N=746)
	no. of patients	
All	10	9
Deaths ≤30 days after last treatment cycle	2	2
Due to toxic effects		
Pulmonary embolism	1†	1†
Due to other causes (unrelated to study drug)		
Pulmonary embolism	1†	0
Hypovolemic shock (hemorrhage during catheter placement)	0	1†
Deaths >30 days after last treatment cycle	8	7
Due to toxic effects		
Sudden cardiac arrest	0	1
Adverse effects on cardiac function	1†	1
Due to other causes (unrelated to study drug)	6‡	4§
Due to additional chemotherapy	1	1

* TAC denotes docetaxel plus doxorubicin and cyclophosphamide, and FAC fluorouracil plus doxorubicin and cyclophosphamide.
† Death occurred before a relapse or second cancer.
‡ Five deaths occurred before a relapse or second cancer.
§ Two deaths occurred before a relapse or second cancer.

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APPENDIX

Additional investigators participating in the Breast Cancer International Research Group trial 001 were as follows: — H. Guixa, E. Mickiewicz, and J. Martinez (Argentina), J. Schuller (Austria), L. Teixeira (Brazil), K. Gelmon, S. Sehdev, Y. Drolet, J. Dufresne, L. Yelle, L. Zibdawi, B. Lesperance, S. Verma, J. Cantin, D. Holland, M. Trudeau, J. Chang, S. Rubin, and S. Allan (Canada), J. Abrahamova and J. Finek (Czech Republic), H. Abd-El-Azim and N. Gad-El-Mawla (Egypt), C. Oberhoff (Germany), V. Georgoulias (Greece), K. Szanto (Hungary), H. Lurie, O. Merimsky, and M. Steiner (Israel), H. Karnicka (Poland), I. Goncalves and M. Chumbo (Portugal), I. Koza (Slovak Republic), P. Ruff (South Africa), A. Pelegri, E. Alba, I. Alvarez, E. Aranda, B. Munarriz, A. Anton, F. Lobo, J.M. Lopez-Vega, M.D. Menendez, A. Murias, J. Cassinello, and J.L. Garcia-Puche (Spain), U. Nylen (Sweden), E. Whipp, and J. Le Vay (United Kingdom), J. Erban, B. Graham, L. Harris, M. O'Rourke, T. Beck, S. Limentani, N. Robert, J. Tongol, F. Schnell, A. Begas, R. Kerns, A. Rosenberg, L. Campos, J. Foster, T. Beeker, N. Iannotti, C. George, and B. Avery (United States), and A. Viola and C. Garbino (Uruguay); design of quality-of-life analyses — H.J. Au (Canada); data-center team of the Breast Cancer International Research Group — V. Bée, D. Borrits, F. Dabbouz-Harrouche, S. de Ducla, S. Gazel, and M.A. Lindsay; study managers — J.P. Aussel, N. Domege, and S. Dumont; statisticians — L. Hatteville and E. Brunel; clinical directors — M. Alakl and A. Yver, who were key Aventis personnel; and independent data monitoring committee — A. Efremidis (Greece), M. Aapro (Switzerland), J. Bryant, E. Mamounas, and E. Perez (United States).

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CLINICAL PRACTICE

Atopic Dermatitis

Hywel C. Williams, Ph.D.

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

A 10-year-old girl with atopic dermatitis reports itching that has recently become relentless, resulting in sleep loss. Her mother has been reluctant to treat the girl with topical corticosteroids, because she was told that they damage the skin, but she is exhausted and wants relief for her child. How should the problem be managed?

THE CLINICAL PROBLEM

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Atopic dermatitis (or atopic eczema) is an itchy, inflammatory skin condition with a predilection for the skin flexures.[1] It is characterized by poorly defined erythema with edema, vesicles, and weeping in the acute stage and skin thickening (lichenification) in the chronic stage (Fig. 1A and 1B). Although termed atopic, up to 60 percent of children with the clinical phenotype do not have demonstrable IgE-mediated sensitivity to allergens,[2] an observation that led the World Allergy Organization to propose a revised nomenclature.[3] Approximately 70 percent of cases of atopic dermatitis start in children under five years of age,[4] although 10 percent of cases seen in hospital settings start in adults.[5] Asthma develops in approximately 30 percent of children with atopic dermatitis, and allergic rhinitis in 35 percent.[6]

DIAGNOSTIC CRITERIA

Atopic dermatitis is difficult to define because of its variable morphology and distribution and its intermittent nature. Several diagnostic criteria have been developed.[7] Consensus criteria for the main clinical features of atopic dermatitis[8] have led to a short list of reliable and valid discriminators that are used worldwide[9] (Table 1).

Assessing disease severity is problematic when there is no objective marker.[10] The many severity scales used in clinical trials are generally not suitable for rapid assessment in the clinic.[11] The presence or absence of sleep disturbance, the number and location of involved sites, and the clinical course are the indicators of severity that probably provide the best basis for making decisions about treatment.[12]

PREVALENCE, COST, AND PROGNOSIS

According to the International Study of Asthma and Allergies in Childhood, the prevalence of symptoms of atopic dermatitis in children six or seven years of age during a one-year period varied from less than 2 percent in Iran and China to approximately 20 percent in Australia, England, and Scandinavia.[13] A high prevalence has also been found in the United States.[14] In the United Kingdom, one population survey of 1760 affected children from one to five years of age found that 84 percent of cases were mild, 14 percent were moderate, and 2 percent were severe.[15]

Studies suggest that atopic dermatitis imposes a high economic burden,[16] with out-of-pocket expenses and overall costs that are similar to those for the treatment of asthma.[17] Causes of family stress related to caring for children with moderate or severe

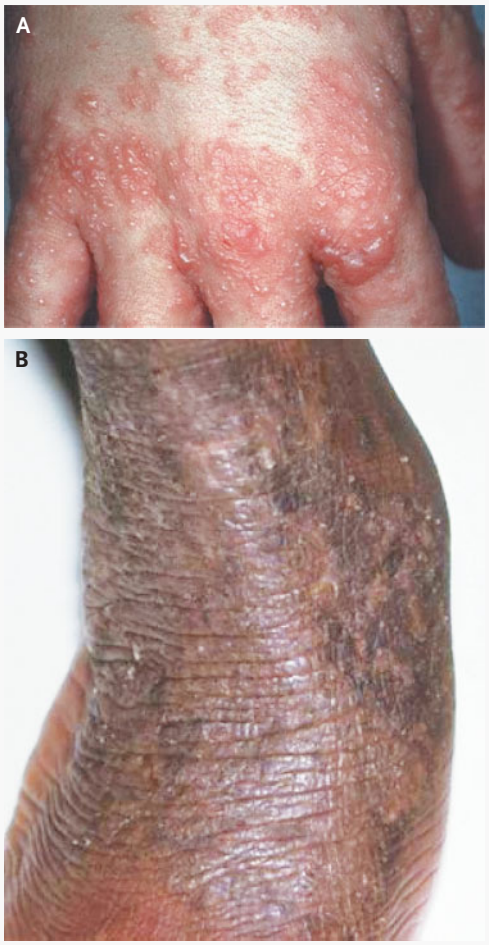

Figure 1. Atopic Dermatitis.
Panel A shows acute atopic dermatitis with intense erythema and vesicles. Panel B shows chronic atopic dermatitis with lichenification (skin thickening and enhancement of skin markings) and scaling on the front of the ankle.

Table 1. Criteria for the Diagnosis of Atopic Dermatitis.*

The diagnosis requires evidence of itchy skin (or parental report of scratching or rubbing) plus three or more of the following:
- History of involvement of the skin creases (e.g., fronts of elbows, backs of knees, fronts of ankles, and areas around the neck or eyes)
- History of asthma or hay fever (or history of atopic disease in a first-degree relative if the child is under four years of age)
- History of generally dry skin in the past year
- Onset in a child under two years of age (criterion not used if the child is under four years of age)
- Visible flexural dermatitis (including dermatitis affecting the cheeks or forehead and outer aspects of limbs in children under four years of age)

* Adapted from Williams et al.[9]

atopic dermatitis (e.g., sleep deprivation, loss of employment, time-consuming treatment, and financial costs) may rival those related to caring for children with diabetes mellitus type 1.[18]

Approximately 60 percent of patients with childhood atopic dermatitis are free of symptoms in early adolescence,[19] although up to 50 percent may have recurrences in adulthood.[20] Early-onset disease, severe early disease, concomitant asthma and hay fever, and a family history of atopic dermatitis may predict a more persistent course.[4] One recent cohort study of 1314 German children showed that the prognosis was related to disease severity and atopic sensitization, as evidenced by elevated serum levels of IgE antibodies to food and inhalant allergens at two years of age.[21]

CAUSES

Atopic dermatitis is probably a complex disease relying on the interplay of several factors.[22] Several genes have been identified that may explain some cases.[23] Genetics alone, however, cannot explain the results of studies of migrant populations that show, for example, that Jamaican children living in London are twice as likely to have atopic dermatitis as Jamaican children living in Jamaica; the increased risk of atopic dermatitis in smaller families and among higher social classes; and the rising prevalence of atopic dermatitis in some countries. These observations suggest a key role for the environment in mediating disease expression.[24] Whereas allergens such as house-dust mites and foods may be important in some cases, nonallergic factors such as rough clothing, *Staphylococcus aureus* infections, exposure to microbes during infancy, excessive heat, and exposure to irritants that disrupt the function of the skin barrier may also be important. Mechanisms for and implications of the possible prevention of atopic dermatitis are reviewed elsewhere.[25,26]

STRATEGIES AND EVIDENCE

DIAGNOSIS

Skin biopsy is of little value in the diagnosis of atopic dermatitis; instead, diagnosis is based on clinical features[7-9] (Table 1). The differential diag-

nosis depends on age and the country of residence (Fig. 2 and Table 2). Because of their high negative predictive value (above 95 percent), negative skin-prick or radioallergosorbent tests for foods and environmental allergens may be useful for assessing the contribution of allergies to disease expression in children with severe disease.[27] Positive tests are less useful, with positive predictive values of about 40 percent.[27]

Concomitant food allergy may be manifested as urticaria and gastrointestinal symptoms and may not necessarily exacerbate atopic dermatitis. Double-blind, placebo-controlled food challenges are the standard for diagnosing associated food allergy, but they are time consuming and not available in many hospitals.

The clinical utility of patch testing with airborne allergens is still unclear.[28] Patch tests are useful for excluding a diagnosis of suspected superimposed allergic contact dermatitis.[29]

TREATMENT

Topical Corticosteroids

One systematic review identified 83 randomized controlled trials of the use of topical corticosteroids in atopic dermatitis.[30] Vehicle-controlled studies lasting less than one month indicate that approximately 80 percent of people report good, excellent, or clear responses with topical corticosteroids, whereas 38 percent of persons in control groups reported such responses.

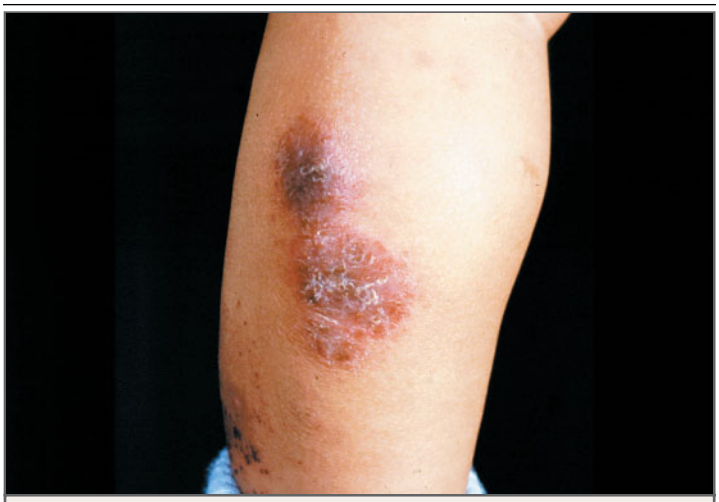

Figure 2. Discoid (Nummular) Eczema in an Infant.
This pattern of eczema is frequently associated with atopic dermatitis and is often confused with ringworm infection.

Potency of topical corticosteroids is classified by the potential for vasoconstriction — a surrogate for clinical efficacy and skin thinning (Table 3). In general, only preparations that have very weak or moderate strength are used on the face and genital area, whereas those that have moderate or potent strength are used on other areas of the body.[31] Lower-potency corticosteroids may be sufficient on all areas of the body in younger children. Preparations are typically used in bursts of three to seven days in order to achieve control. There is little difference in outcome between short-term use of potent preparations or longer use of weaker preparations in children with mild-to-moderate disease.[32] Lichenified atopic dermatitis requires more potent preparations for longer periods.

Long-term studies of moderate-to-potent preparations in children are scarce. One study of 231 children with stabilized atopic dermatitis randomly assigned to receive twice-weekly 0.05 percent fluticasone propionate (plus emollients) or vehicle alone plus emollients for 16 weeks showed that patients in the control group were more likely, by a factor of 8, to have a relapse (95 percent confidence interval, 4.3 to 15.2).[33] A four-month trial of persons 12 to 64 years of age with moderate-to-severe disease showed that the application of fluticasone to previously active and new sites of atopic dermatitis for two consecutive days each week reduced flares significantly, as compared with a group receiving an emollient only.[34]

Reduced efficacy of topical corticosteroids may be related to disease severity rather than to glucocorticoid resistance.[35] There is little evidence that the application of topical corticosteroids twice a day is more effective than once-daily applications,[36] and more frequent use may cause more local side effects.

A main concern with the use of topical corticosteroids is irreversible skin thinning. Although thinning is possible, the concern on the part of patients (and parents) is often well out of proportion to the true risk.[37] Although inappropriate use of potent preparations can cause skin thinning, four 16-week randomized trials did not show any clinically significant skin thinning,[32-34,38] and a 1-year study showed no significant effect on collagen synthesis.[39] A one-year study of unrestricted continual use of a potent corticosteroid on the limbs and trunk, a weak preparation on the face, or both showed that striae developed in 3 of 330 adults with moderate-to-severe atopic dermatitis.[40] Similar studies in children are lacking. Other possible side effects of cor-

Table 2. Differential Diagnosis of Atopic Dermatitis.

Diagnosis	Description
Seborrheic dermatitis of infancy	Red, shiny, relatively well-demarcated eruptions typically involving the diaper area are present in infants four months of age or younger. The lower abdomen and armpits may also be involved, and scalp scaling (cradle cap) may be present. The infant appears comfortable. The condition clears within a few months.
Adult-type seborrheic dermatitis	Poorly defined erythema due to overgrowth of or sensitivity to malassezia yeasts is present in seborrheic areas (i.e., sides of nose, eyebrows, external ear canal, scalp, front of chest, axillae, and groin creases).
Discoid (nummular) eczema	Circular "cracked" areas of erythema 1 to 5 cm in diameter are present initially on the limbs, often with secondary infection (Fig. 2). In children, discoid eczema is most commonly associated with atopic dermatitis and is often confused with tinea (ringworm). In adults, it may be associated with excessive skin dryness and secondary infection with *Staphylococcus aureus*.
Irritant contact dermatitis	Cumulative damage to the skin barrier from irritants such as soaps and detergents is present. The clinical appearance can be identical to that of atopic dermatitis, but location at sites of maximal exposure (e.g., fingers) may be helpful in making the diagnosis. Some degree of irritant contact dermatitis is common in persons with atopic dermatitis (e.g., in babies, around the mouth, owing to saliva and wet food, and in the diaper area, owing to urine).
Allergic contact dermatitis	A hypersensitivity reaction exists after sensitization to specific substances (e.g., the nickel in jewelry, the rubber in gloves, or the glues in some shoes). Localization may suggest this diagnosis, but patch tests are needed to definitively establish it. This diagnosis may coexist with atopic dermatitis.
Frictional lichenoid dermatitis	Shiny papules occur at elbows, knees, and backs of hands, probably related to friction. The diagnosis may be common, and may be more so in patients with atopic dermatitis.
Other exogenous skin conditions	
Scabies	Infestation may produce nonspecific eczematous changes on the entire body. Burrows and pustules on palms, soles, genitalia, and between fingers help to establish diagnosis.
Onchocerciasis	The chronic phase may be accompanied by widespread itching and lichenification of the skin similar to those seen in cases of chronic atopic dermatitis.
Insect bites	Secondary eczematous changes may develop in the area of the bites, especially on the limbs, and may be confused with atopic dermatitis.

ticosteroids include facial telangiectasia and glaucoma from periocular use (rarely reported in adults).

Secondary adrenal suppression and the suppression of growth resulting from systemic absorption of topical corticosteroids are also concerns, although clinically relevant adrenal suppression is very rare.[41] One study involving children with atopic dermatitis did not find any relationship between height velocity and the use of mild-potency as compared with moderate-potency topical corticosteroids.[42] Another study showed biochemical evidence of suppression of the hypothalamic–pituitary–adrenal axis only in children with atopic dermatitis who used potent or very potent topical corticosteroids and in those who had received glucocorticoids from other routes, and not in those who had used topical corticosteroids of mild or moderate strength for a median of 6.9 years.[35]

Emollients

There is no evidence that emollients improve atopic dermatitis directly. However, emollients are widely used because they improve the appearance and symptoms of the dry skin (xerosis) associated with this condition.[30,31,43] One study has shown that emollients may reduce the need for topical corticosteroids by approximately 50 percent,[44] and another study found that emollients enhanced the response to treatment with topical corticosteroids.[45] There is little basis for suggesting the use of one emollient over another, and the preference of the patient is probably the most important factor.[31]

Topical Calcineurin Inhibitors

Topical tacrolimus and pimecrolimus have both been shown to be effective in vehicle-controlled studies. For 1 percent pimecrolimus, the rate ratio

Table 3. Therapeutic Interventions for Atopic Dermatitis.

Intervention	Use	Recommendations	Anticipated Benefits	Potential Harms	Comments
Topical corticosteroids*	First-line treatment for patients of all ages with moderate-to-severe atopic dermatitis	Use the lowest effective potency; use only mild preparations on the face, neck, and intertriginous areas; once daily is probably as effective as twice daily; use for the duration of a flare; use intermittently (e.g., twice a week) Use potent preparations intermittently (e.g., twice a week) to reduce flares in moderate-to-severe disease; do not use continually, because of the possibility of skin thinning	Reduced itching and improvements in sleep, in the appearance of the skin, in self-esteem, and in quality of life (the magnitude of the benefit depends on the potency and on the duration and the site of application, as well as on the type of vehicle base; occlusion may enhance response)	Short-term — stinging on application (for potent preparations) Medium- to long-term — local complications (e.g., skin thinning, striae, glaucoma from prolonged periocular use, contact sensitization, and tolerance) and systemic effects (e.g., suppression of the hypothalamic–pituitary–adrenal axis and Cushing's syndrome)	Optimal methods of use (in terms of potency, frequency, and duration of application) are unclear
Emollients†	First-line treatment for patients of all ages with very mild atopic dermatitis, adjunctive therapy for use with other topical or systemic treatments	Use to reduce symptoms from dry skin associated with atopic dermatitis, especially after inflammation has been treated with topical corticosteroids; thicker emollients are needed for thicker skin such as that on the hands and feet; apply greasier emollients in the direction of the hair to avert occlusion of hair follicles	Reduced skin dryness, itching, and penetration of skin by irritants and allergens; prevention of skin cracking; possible reduced need for topical corticosteroids; and possible enhanced response when used with topical corticosteroids	Stinging on application; a shiny residue on the face and hands may mark objects	Emollients may trap water in the skin (white soft paraffin), introduce water to the skin directly (aqueous cream), or increase the water-holding capacity of the skin (urea); permit patient to choose preparation
Topical tacrolimus	For people over two years of age (0.03% ointment) or 16 years of age or older (0.1% ointment) with moderate-to-severe atopic dermatitis unresponsive to or intolerant of topical corticosteroids	Use twice daily until symptoms resolve; use intermittently or early to prevent flares; do not use when infection is present	Reduced itching and improvements in sleep, in the appearance of the skin, in self-esteem, and in quality of life (magnitude of benefit with the use of 0.1% ointment probably equivalent to that with use of a potent topical corticosteroid, whereas benefit is less with 0.03% ointment)	Short-term — mild stinging or burning on application (approximately 43 percent for 0.1% ointment and 40 percent for 0.03% ointment), normally improves after a week; safety profile based on five years of use appears good Long-term (greater than five years) — safety unknown; use with caution with excess exposure to ultraviolet light	May be especially useful on delicate sites such as the face, neck, and axillae, where local skin thinning from frequent use of topical corticosteroids might be increased; the efficacy is unclear in people unresponsive to or intolerant of topical corticosteroids; can probably be used concurrently with topical corticosteroids applied to other body sites

Topical 1% pimecrolimus	For people over two years of age with mild-to-moderate atopic dermatitis unresponsive to topical corticosteroids	Use twice daily until symptoms resolve; use intermittently to reduce the severity and frequency of flares or use early to prevent flares	Reduced itching and improvements in sleep, in the appearance of the skin, in self-esteem, and in quality of life (magnitude of the benefit is less than that with a potent topical corticosteroid)	Short term — mild stinging or burning on application occurs in approximately 17 percent of patients and normally improves after a week; do not use when infection is present; safety profile based on five years of use appears good Long-term (greater than five years) — safety unknown; use with caution with excess exposure to ultraviolet light	The efficacy is unclear in people unresponsive to or intolerant of topical corticosteroids; may be useful on delicate sites such as the face
Oral antihistamines (nonsedating and sedating)‡	Adjunctive therapy	Unclear	Possible reduced itching and improved sleep	Drowsiness	Frequently used, but evidence of benefit is unconvincing
Refined-coal tar	For patients with mild-to-moderate atopic dermatitis	Use twice daily on affected areas	Reduced itching, redness, and lichenification	Itching and stinging on application in approximately 17 percent of patients; odor; staining of skin and clothes	Data are based on one randomized controlled trial that compared refined-coal tar with 1% hydrocortisone; other tar preparations might also be effective, but irritation and cosmetic acceptability from odor and staining may be an issue
Topical doxepin	Adjunctive therapy for patients older than 12 years	Apply cream thinly 3 or 4 times a day (maximum, 12 g daily)	Short-term reduced itching	Drowsiness; transient stinging and burning on application	There is some evidence of reduced itching in the first 24 to 48 hours, but no evidence that symptoms and disease activity improved over a longer period
Oral corticosteroids§	For patients with a flare of severe atopic dermatitis	Use intermittently	Relief from itching and skin redness and infiltration, and reduced oozing	Short-term — increased appetite, psychosis, dyspepsia Long-term — hypertension, osteoporosis, adrenal suppression, striae, muscle atrophy	There is no evidence from randomized trials, but clinical experience suggests short-term use in instances of severe flare, followed by specialist support; the optimal dosage is unknown

* Three strengths of corticosteroids are available — mild (e.g., 1% hydrocortisone), moderate (e.g., 0.05% clobetasone butyrate), and potent (e.g., 0.05% fluticasone propionate).
† Examples of emollients useful for the treatment of atopic dermatitis include aqueous cream, a 50:50 mixture of white soft paraffin and liquid paraffin, and various proprietary brands.
‡ Nonsedating antihistamines include loratadine, 10 mg, and cetirizine, 10 mg; sedating antihistamines include chlorpheniramine maleate, 4 mg at night, for adults.
§ Prednisolone may be used at a starting dose of 0.5 mg per kilogram of body weight, tapered over two to three weeks.

for the proportion of patients clear or almost clear of atopic dermatitis at three weeks in five vehicle-controlled trials involving 783 patients was 2.72 (95 percent confidence interval, 1.84 to 4.03).[46] For 0.03 percent and 0.1 percent tacrolimus, the rate ratios for the proportion of patients who were clear or who had excellent improvement at 12 weeks were 4.50 (95 percent confidence interval, 2.91 to 6.96) and 5.62 (95 percent confidence interval, 3.67 to 8.61), respectively, in three vehicle-controlled trials involving 656 patients.[46] Short-term studies suggest that 0.1 percent topical tacrolimus may be similar in strength to potent topical corticosteroids,[46] whereas topical pimecrolimus is considerably weaker.[40,47]

Few long-term studies compare intermittent use of topical calcineurin inhibitors with intermittent use of topical corticosteroids. A 12-month vehicle-controlled study of children with atopic dermatitis showed that early use of pimecrolimus reduced the frequency of flares from 51 percent to 28 percent,[48] although early use of mild topical corticosteroids might have shown similar effects.

Topical calcineurin inhibitors do not cause skin thinning. Both tacrolimus and pimecrolimus are associated with mild burning sensations when applied to the skin (Table 3). Five-year studies show a good safety profile for these agents.[49] In the United Kingdom, the National Institute of Clinical Excellence approves the use of topical tacrolimus for children older than two years of age with moderate-to-severe atopic dermatitis not controlled by topical corticosteroids, and of topical pimecrolimus as a second-line option for resistant dermatitis of the head and neck.[50] In the United States, both of these topical calcineurin inhibitors are approved as second-line agents, and the site of application is not restricted for pimecrolimus.

In March 2005, the Food and Drug Administration issued an alert to health care professionals concerning a potential link between topical pimecrolimus and tacrolimus and cancer (mainly lymphoma and skin cancer) on the basis of studies in animals, case reports, and knowledge of how these drugs work.[51,52] The alert emphasizes the importance of using these preparations only as labeled and when first-line treatment has failed or cannot be tolerated.

Other Topical Agents

A study of a refined-coal cream used on one side of the body in adults with mild-to-moderate atopic dermatitis as compared with 1 percent hydrocortisone used on the other side suggested similar efficacy after four weeks.[53] There is insufficient evidence to conclude whether topical cromoglycate preparations are effective.[30,54] Other topical treatments — such as St. John's wort cream, vitamin B_{12}, and licorice gel — whose use is supported by single small, randomized trials require further evaluation before they can be recommended for the treatment of atopic dermatitis.

Oral Antihistamines

Evidence is lacking to support the use of antihistamines for the treatment of atopic dermatitis,[55] although they are sometimes recommended for their sedative effects.[56] Reports on nonsedative antihistamines are conflicting.[30,56,57] The largest study failed to demonstrate any overall benefit from prolonged use of cetirizine in children with atopic dermatitis.[58]

Topical Doxepin

Topical doxepin produces some relief from itching within 48 hours. However, a clinically useful beneficial effect on disease severity has yet to be shown, and drowsiness may be a problem.[30]

Antibiotic Agents

Secondary infection with *S. aureus* is common (Fig. 3) and usually is treated with short courses of antibiotics such as floxacillin, cephalexin, or amoxicillin–clavulanate. One randomized trial found no benefit to prescribing floxacillin continually for four weeks as compared with placebo, and methicillin-resistant strains were more common in those who were prescribed antibiotics.[59] Although combinations of topical corticosteroids and antibiotics are used for atopic dermatitis, no good evidence suggests that they offer additional benefits as compared with topical corticosteroids alone.[30]

Ultraviolet Light

Randomized clinical trials have shown that ultraviolet light (ultraviolet B, narrow-band ultraviolet B, and high-intensity ultraviolet A) is beneficial for atopic dermatitis in the short term.[30] Burning and itching may occur, and carcinogenicity is a long-term concern. Phototherapy is usually used as a second- or third-line treatment.[31]

Immunosuppressive Agents

A brief course of oral corticosteroids (less than three weeks) may be used to control a flare of severe dis-

ease, although data from randomized clinical trials are lacking. Ongoing use of systemic immunosuppressive agents (oral corticosteroids, cyclosporine, azathioprine, mycophenolate, and interferon gamma) is limited by adverse effects and is usually reserved for people with severe disease who do not respond to other measures.[30,43]

Nonpharmacologic Approaches

Avoiding foods suspected to cause flares may be helpful in young children with severe disease, but usually is not helpful in adults.[30,60] Little evidence supports dietary exclusion of milk and eggs in unselected cases.[61] Some evidence supports egg-free diets in infants with atopic dermatitis who produce IgE antibodies to egg protein.[60] No good evidence supports highly restrictive diets, which can sometimes cause malnutrition.[62] Studies have failed to show clinically useful benefits from supplements such as evening primrose oil, borage oil,[63] zinc, pyridoxine, or vitamin E,[30] or from viable lactobacilli (probiotics).[30,64]

Small randomized trials support psychological approaches such as behavior therapy (to reduce the habit of scratching) and relaxation therapy.[30] Parental-education programs and demonstration of topical treatments by caregivers may be helpful.[65,66] Reduction of house-dust-mite allergen can reduce severity scores for atopic dermatitis, but the clinical relevance and sustainability of such reductions is unknown.[30] Impermeable mattress covers are very effective in reducing levels of mite antigens, but they have no clear clinical benefit.[67]

No good evidence supports the use of bandages containing zinc paste. The use of "wet wraps" (an outer dry bandage overlying an inner damp bandage used over either emollients or topical corticosteroids) has become a popular second- or third-line measure for children with resistant atopic dermatitis but is not supported by randomized trials, and enhanced systemic absorption remains a concern.[41] No good data support alternative or complementary therapies such as homeopathy and bioresonance.[30]

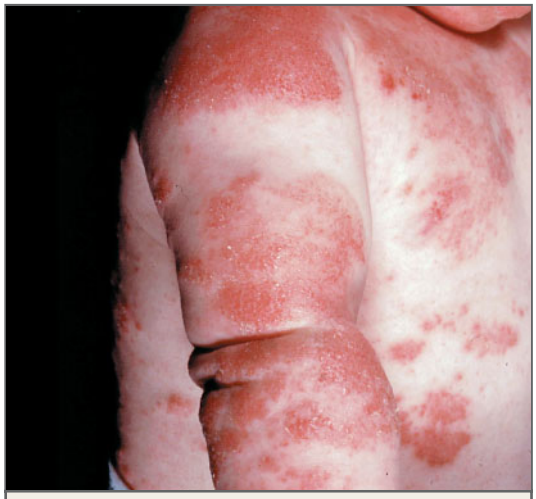

Figure 3. Acute, Secondary Infection in an Infant with Atopic Dermatitis.

Widespread moist, exudative lesions and crusting are present.

AREAS OF UNCERTAINTY

Randomized trials are lacking to assess the benefits of many simple interventions, such as emollients and other nonpharmacologic approaches.[30] The lack of common outcome measures hinders meaningful comparisons across trials.[11] Trials with active comparators are needed to inform choices among agents.[47] Data on the optimal use of topical corticosteroids are needed, along with long-term data on adverse events. Data concerning the long-term safety of topical tacrolimus and pimecrolimus are also needed. The benefits of routine allergy testing require clarification. Moreover, it is unclear whether early aggressive therapy in children with atopic dermatitis alters the natural history of the disease.

GUIDELINES

The American Academy of Dermatology recently published evidence-based guidelines for atopic dermatitis that contain recommendations that are consistent with the evidence summarized in this article.[43] In addition, many useful Web sites are available (Table 4).

CONCLUSIONS AND RECOMMENDATIONS

Patients and families, such as the girl and her mother who are described in the vignette, often have concerns about topical corticosteroids that can be alleviated by appropriate education.[68] Patients and families should be taught about the course of atopic dermatitis; that is, that a single cause and cure are unlikely, although good control is nearly

Table 4. Web Sites with Information about Eczema.

Evidence-based resources

http://www.ncchta.org/execsumm/summ437.htm
National Health Service, United Kingdom, Health Technology Assessment systematic review

http://libraries.nelh.nhs.uk/skin/default.asp
Skin Conditions Specialist Library, National Electronic Library for Health, United Kingdom

http://www.nottingham.ac.uk/dermatology/eczema/index.html
Online diagnostic criteria manual

Information for patients

http://www.aad.org/public/publications/pamphlets/eczemaatopicdermatitis.htm
American Academy of Dermatology

http://www.bad.org.uk/patients/disease/atopic/
British Association of Dermatologists

http://www.dermnetnz.org/dermatitis/treatment.html
DermNet NZ (New Zealand)

Support groups for patients

United States: http://www.nationaleczema.org/
Canada: http://www.eczemahelp.ca/internal.htm
United Kingdom: http://www.eczema.org/
Australia: http://www.eczema.org.au/

always possible. Discussions should be supplemented by written information and a demonstration of the use of topical treatment.

For the girl in the vignette, I would recommend inducing a remission with once-daily application of a potent topical corticosteroid to the limbs and trunk for 10 days before scheduling a second visit to evaluate progress. Although data to support the use of emollients are limited, I would attempt to maintain remission by liberal use of emollients only, with recourse to five-day courses of potent or moderate-strength topical corticosteroids for flares.[33] If such a regimen failed to maintain adequate quality of life, I would introduce "weekend therapy" — that is, the application of a potent corticosteroid to new and previously active sites of atopic dermatitis each Saturday and Sunday evening to reduce flares.[34] Alternatively, intermittent use of topical tacrolimus or pimecrolimus may be used to reduce flares.[50] If facial dermatitis requires continual use of mild topical corticosteroids, I would recommend the use of topical tacrolimus, 0.03 percent, twice daily for three weeks and then once daily until the atopic dermatitis clears up.[50]

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REVIEW ARTICLE

MEDICAL PROGRESS

Brucellosis

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Brucellosis, like tuberculosis, is a chronic granulomatous infection caused by intracellular bacteria and requires combined, protracted antibiotic treatment. The disease causes much clinical morbidity as well as a considerable loss of productivity in animal husbandry in the developing world. In this era of international tourism, brucellosis has become a common imported disease in the developed world.

Brucellosis has been present for millennia[1] and has managed to elude eradication, even in most developed countries.[2,3] A high prevalence in certain geographic areas is well recognized, although largely underestimated (Table 1). The relationship between the disease and individual socioeconomic status is exemplified in the United States, where programs to eradicate brucellosis have successfully limited the annual incidence of the disease, which now occurs predominantly in California and Texas (which account for more than half of the U.S. cases), with relatively high rates of incidence in North Carolina, Illinois, Florida, Wyoming, Iowa, and Arizona. The disease usually presents in Hispanic populations and is probably related to the illegal importation of unpasteurized dairy products from neighboring Mexico, where the disease is endemic.[4,5]

The Bacterium

Brucella belongs to the $\alpha 2$ subdivision of the proteobacteria, along with ochrobactrum, rhizobium, rhodobacter, agrobacterium, bartonella, and rickettsia.[6] The traditional classification of brucella species is largely based on its preferred hosts. There are six classic pathogens, of which four are recognized human zoonoses. The presence of rough or smooth lipopolysaccharide correlates with the virulence of the disease in humans. Two new brucella species, provisionally called *Brucella pinnipediae* and *B. cetaceae*, have been isolated from marine hosts within the past few years.[7,8]

Taxonomic characteristics of brucella species and biotypes[9] are summarized in Table 2. Brucella is a monospecific genus that should be termed *B. melitensis*, and all other species are subtypes, with an interspecies homology above 87 percent. The phenotypic difference and host preference can be attributed to various proteomes, as exemplified by specific outer-membrane protein markers.[10] All brucella species seem to have arisen from a common ancestor to which *B. suis* biotype 3 shares particular similarity.[11] Although the scientific accuracy of this classification cannot be disputed, its practicality has been under scrutiny.

The *B. melitensis* Genome

The complete sequencing of the *B. melitensis* genome was achieved in 2002.[12] The complete sequencing of *B. abortus*[13] and *B. suis*[14] has recently been accomplished as well. *B. melitensis* contains two circular replicons of 1.1 and 2.2 Mb, respectively, with a 57 per-

Table 1. Annual Cases of Human Brucellosis in Various Countries, According to Year.*

Country	1996	1997	1998	1999	2000	2001	2002	2003
Albania	NA	155	376	458	220	NA	NA	NA
Algeria	4356	3,434	2,232	2,223	NA	3,200	NA	2,766
Argentina	NA	676	NA	353	507	NA	296	325
Australia	38	41	45	52	27	NA	40	17
Azerbaijan	NA	624	494	582	654	660	519	407
Bosnia-Herzegovina	NA	NA	NA	NA	NA	7	NA	48
Colombia	53	42	82	42	NA	27	NA	238
Germany	23	25	18	21	27	25	35	27
Greece	NA	254	435	543	545	405	327	222
Iran	NA	NA	NA	17,168	NA	NA	NA	17,765
Israel	235	151	197	163	131	70	56	56
Italy	1896	1,681	1,461	1,324	1,067	923	813	520
Jordan	957	NA	684	432	288	275	219	159
Kyrgyzstan	NA	NA	NA	973	1,219	1,819	1,771	NA
Lebanon	192	429	136	184	NA	NA	NA	NA
Mexico	3362	3,387	3,550	2,719	2,171	3,013	2,851	3,008
Peru	1691	NA	1,269	NA	1,072	372	991	NA
Portugal	866	1,409	816	683	500	381	206	139
Russia	656	461	NA	352	423	508	595	NA
Saudi Arabia	5997	15,933	5,781	NA	NA	NA	NA	NA
Spain	NA	878	1,520	1,519	1,104	887	886	596
Syria	NA	NA	NA	NA	6,487	4,500	NA	23,297
Tajikistan	257	NA	211	NA	851	752	1,071	1,471
Tunisia	490	291	206	355	NA	321	250	128
Turkey	9480	11,812	11,427	11,462	10,742	15,510	17,553	14,435
Turkmenistan	NA	496	NA	NA	264	246	NA	NA
United Kingdom	15	6	7	76	19	26	38	19
United States	112	98	79	82	87	136	125	93
Uzbekistan	707	459	494	480	NA	NA	408	NA

* Data are from the Office International des Epizooties and various national health ministries. These numbers are believed to be a massive underestimation of the true prevalence of the disease. NA denotes not available.

cent GC content and no plasmids; 3197 open reading frames were sequenced, 2487 of which had an assigned function. *B. abortus* biovars 1 and 4 and *B. suis* biotype 1 are remarkably similar to *B. melitensis*. In contrast, *B. suis* biotypes 2 and 4 are composed of two replicons of 1.35 and 1.85 Mb, respectively, whereas *B. suis* biotype 3 is composed of a single circular replicon of 3.3 Mb.

PATHOGENETIC FEATURES

The series of host–microbe interactions that takes place in humans differs in many crucial steps from the pathogenetic mechanisms first recognized in animal models.[15] Brucella is unusual in several ways. First, the bacterium does not bear classic virulence factors, such as exotoxins or endotoxins, and its lipopolysaccharide pathogenicity is not typical. Second, it exhibits a tendency to invade and persist in the human host through inhibition of programmed cell death.[16]

Brucella invades the mucosa, after which phagocytes ingest the organisms. In so-called nonprofessional phagocytes, internalization requires the expenditure of energy, and inhibitors of energy metabolism and receptor-mediated endocytosis can

Table 2. Nomenclature and Characteristics of Brucella Species.

Species	Biotype	Animal Hosts	First Described	Human Virulence*	Species Discrimination
B. melitensis	1–3	Goats, sheep, camels	Bruce, 1887	++++	Fuchsin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 24 hr; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. abortus	1–6, 9	Cows, camels, yaks, buffalo	Bang, 1897	++ to +++	Fuchsin, positive (except biotype 2); thionine, negative (biotypes 1, 2, and 4); safranin inhibition, negative; H_2S production, positive (except biotype 5); urease, positive in 24 hr; CO_2 growth, positive (biotypes 1–4); Tiblisi phage lysis, positive; Weybridge phage lysis, positive
B. suis	1–5	Pigs (biotypes 1–3), wild hares (biotype 2), caribou (biotype 4), reindeer (biotype 4), wild rodents (biotype 5)	Traum, 1914	+	Fuchsin, negative (except biotype 3); thionine, positive; safranin inhibition, positive; H_2S production, positive (biotype 1); urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, positive
B. canis	—	Canines	Carmichael and Bruner, 1968	+	Fuchsin, positive or negative; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. ovis	—	Sheep	Van Drimmelen, 1953	–	Fuchsin, negative for some strains; safranin inhibition, negative; H_2S production, negative; urease, negative; CO_2 growth, positive; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. neotomae	—	Rodents	Stoenner and Lackman, 1957	–	Fuchsin, negative; safranin inhibition, negative; H_2S production, positive; urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, positive or negative; Weybridge phage lysis, positive
B. pinnipediae and *B. cetaceae* (provisional)	—	Minke whales, dolphins, porpoises (pinnipediae), seals (cetaceae)	Ewalt and Ross, 1994	+	Fuchsin, positive; thionine, positive; safranin inhibition, negative; H_2S production, negative; urease, positive; CO_2 growth, negative for pinnipediae and positive for cetaceae; Tiblisi phage lysis, negative; Weybridge phage lysis, positive for pinnipediae and negative for cetaceae

* Virulence is graded on a scale from no virulence (–) to the highest degree of virulence (++++).

suppress this response.[17] Brucella has a two-component system called BvrS/BvrR, which codes for a histidine kinase sensor and controls the expression of molecular determinants necessary for cell invasion.[18] After ingestion, the majority of brucellae are rapidly eliminated by phagolysosome fusion. Of those bacteria, 15 to 30 percent survive[19] in gradually evolving brucellae-containing compartments, in which rapid acidification takes place. How this unique environment is formed is incompletely understood, but it is responsible for limiting antibiotic action and explains the discrepancy between in vitro studies and in vivo events.[20] The induction of the virB operon through a type IV secretion system (a system by which macromolecules are transferred) is of paramount importance during brucella intracellular movement.[21] Replication of the bacterium takes place in the endoplasmic reticulum without affecting host-cell integrity. After replication, brucellae are released with the help of hemolysins and induced cell necrosis (Fig. 1).[22]

THE HOST RESPONSE IN HUMANS

The host response in humans reflects unique features of brucella. Smooth lipopolysaccharide does not activate the alternative complement pathway. Brucella is resistant to damage from polymorphonuclear cells owing to suppression of the myeloperoxidase–hydrogen peroxide–halide system and copper–zinc superoxide dismutase and the production of inhibitors of adenylate monophosphate and guanyl monophosphate. Impaired activity of natural killer cells and impaired macrophage generation of reactive oxygen intermediates and interferon regulatory factors have been documented.[23-25] CD4 lymphocytes play a limited role, acting either by facilitating clonal expansion of other cytolytic cells, as CD8, or by functioning as cytolytic effectors. An increase of γ/δ CD4 and CD8 lymphocytes is characteristic in brucellosis,[26] as is the importance of a Vγ9Vδ T-cell receptor.[27]

Studies using volunteers who have been vaccinated with the Rev 1 vaccine against *B. melitensis* have delineated the evolution of specific antibodies against brucellae. Class M immunoglobulins against lipopolysaccharide appeared during the first week of infection, followed by class G immunoglobulins as early as the second week. Both classes of immunoglobulin peaked during the fourth week, and the use of antibiotics was associated with a decline in both class M and class G titers. Class M titers persisted at levels that were higher than those of class G titers for more than six months, and both classes were present for almost a year. The appearance of class A immunoglobulins in conjunction with class G immunoglobulins for longer than six months was consistent with the presence of chronic disease. Antibody response in brucellosis, although extremely useful diagnostically, plays a limited part in the overall host response.

Interferon-γ has a central role in the pathogenesis of brucellosis[28,29] by activating macrophages, producing reactive oxygen species and nitrogen intermediates; by inducing apoptosis, enhancing cell differentiation and cytokine production; by converting immunoglobulin G to immunoglobulin G2a; and by increasing the expression of antigen-presenting molecules. That interferon-γ has a central role in the evolution of brucellosis is highlighted by the effect of a genetic polymorphism in interferon-γ (the +874A allele). Patients who are homozygous for the +847 allele may be relatively more susceptible to brucellosis and — in an interesting note — to tuberculosis.[30] Typically, serum interferon-γ levels in patients with brucellosis are increased.[31,32]

In contrast, the importance of tumor necrosis factor α (TNF-α) in human brucellosis is the subject of debate. Although the induction of TNF-α was noted in murine models of brucellosis, the inhibition of TNF-α in human disease is an early, crucial step in infection. This inhibition may also be involved in the impaired activation and cytotoxic function of natural killer cells owing to an active bacterial mechanism that involves outer-membrane protein 25, which has been identified as the downregulator of TNF-α.[33] Serum levels of TNF-α were undetectable in patients with active brucellosis in one study,[32] but another study reported that serum levels were increased in a linear fashion with serum levels of interferon-γ and other inflammatory markers.[31] The role of interleukin-12, mainly as a regulator of interferon-γ production, has been extensively studied in animal models and humans.[32,34]

HUMAN DISEASE

Transmission of brucellosis to humans occurs through the consumption of infected, unpasteurized animal-milk products, through direct contact with infected animal parts (such as the placenta by inoculation through ruptures of skin and mucous membranes), and through the inhalation of infected aerosolized particles. Brucellosis is an occupational

disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories. One important epidemiologic step in containing brucellosis in the community is the screening of household members of infected persons.[35]

Consumption of unpasteurized dairy products — especially raw milk, soft cheese, butter, and ice cream — is the most common means of transmission. Hard cheese, yogurt, and sour milk are less hazardous, since both propionic and lactic fermentation takes place. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver and spleen has been implicated in human infection.

Airborne transmission of brucellosis has been studied in the context of using brucella as a biologic weapon. In fact, *B. suis* was the first agent contemplated by the U.S. Army as a potential biologic weapon[36] and is still considered in that category. In a hypothetical attack scenario, it was estimated that release of an aerosolized form of brucella under optimal circumstances for dispersion would cause 82,500 cases of brucellosis and 413 fatalities.[37] Cases of laboratory-acquired brucellosis are the perfect examples of airborne spreading of the disease.[38]

After entering the human body and being taken up by local tissue lymphocytes, brucellae are transferred through regional lymph nodes into the circulation and are subsequently seeded throughout the body, with tropism for the reticuloendothelial system. The period of inoculation usually ranges from two to four weeks.

The classic categorization of brucellosis as acute, subacute, or chronic is subjective and of limited clinical interest. Four species of brucella can cause

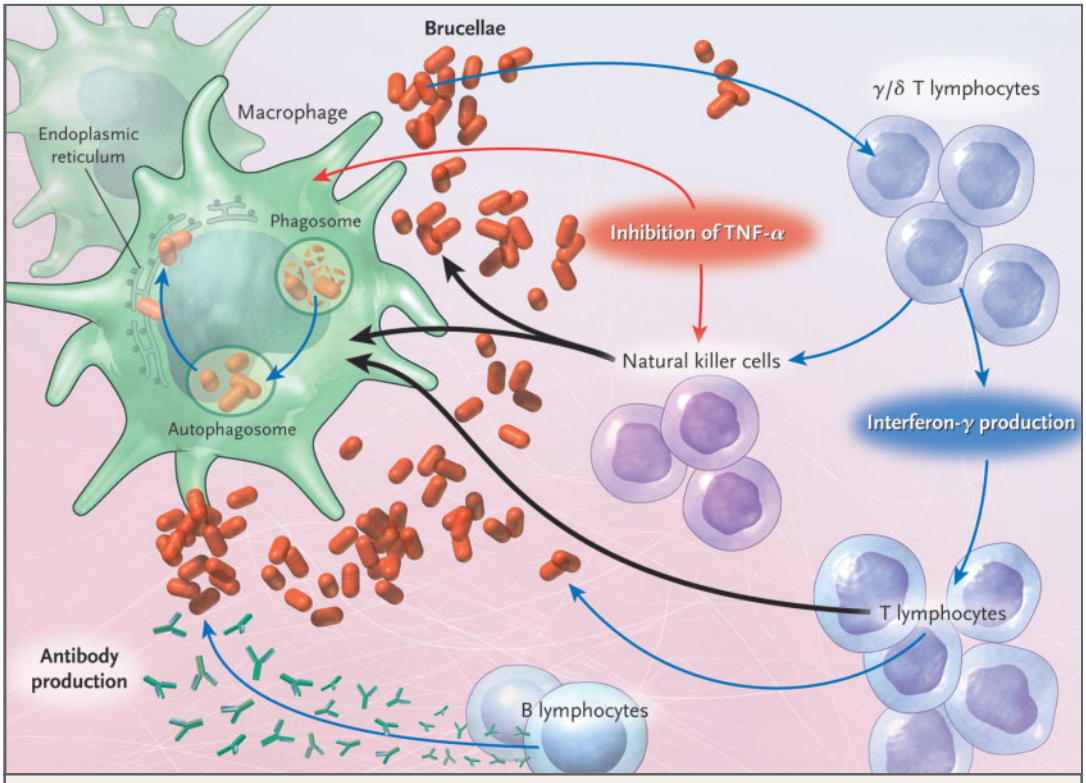

Figure 1. Schematic Representation of Major Events in the Pathogenesis of Brucellosis and the Host Immune Response.
Brucellae enter the macrophages, where the minority of the bacteria survive in specialized evolving compartments and multiply in the endoplasmic reticulum. The inhibition of tumor necrosis factor α (TNF-α) by the bacteria disrupts the bactericidal effect of natural killer cells and macrophages. Interferon-γ production induces a bactericidal effect by natural killer cells and T lymphocytes directly and through macrophage induction. Antibody production by B lymphocytes is also induced but plays a minor role in the immune response. T lymphocytes include both helper and suppressor cells, depending on the stage of the disease. Red arrows indicate negative effect, blue arrows positive effect, and black arrows killing effect.

human disease: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*. Disease from marine species has also emerged.[39] The vast majority of cases worldwide are attributed to *B. melitensis*. A recent study did not report any clinical differences between cases caused by *B. melitensis* and those caused by *B. abortus*.[40] Sufficient data on virulence and clinical presentation of biotypes of *B. melitensis* are lacking, although separate biotypes that predominate in various regions — for example type 2 in northwestern Greece, type 3 in Turkey,[41] and type 1 in Spain[42] — may account for variations in clinical presentation (Table 3).

Human brucellosis is traditionally described as a disease of protean manifestations. However, fever is invariable and can be spiking and accompanied by rigors, if bacteremia is present, or may be relapsing, mild, or protracted. Malodorous perspiration is almost pathognomonic. Constitutional symptoms are generally present. Physical examination is generally nonspecific, though lymphadenopathy, hepatomegaly, or splenomegaly is often present.

Osteoarticular disease is universally the most common complication of brucellosis, and three distinct forms exist — peripheral arthritis, sacroiliitis, and spondylitis. Peripheral arthritis is the most common and is nonerosive, since it usually involves the knees, hips,[43] ankles, and wrists in the context of acute infection. Prosthetic joints can also be affected in peripheral arthritis. Brucellosis has also been proposed as a cause of reactive arthritis. A second form, characterized by sacroiliitis, is readily diagnosed, also usually in the context of acute brucellosis.[44] On the other hand, a third form of osteoarticular disease, spondylitis, remains notoriously difficult to treat and often seems to result in residual damage.[45] The lumbar spine is the usual site of involvement. Spondylitis can be easily diagnosed with plain radiography, in which the characteristic Pons sign (a steplike erosion of the anterosuperior vertebral margin) can be identified, or with scintigraphy and magnetic resonance imaging. The latter imaging technique is popular and produces impressive scans but is costly and not always available. Osteoarticular complications are sometimes linked to a genetic predisposition, with recent data suggesting an association with HLA-B39.[46]

The reproductive system is the second most common site of focal brucellosis. Brucellosis can present as epididymoorchitis in men and is often difficult to differentiate from other local disease.[47] The effect of the local inflammation on subsequent testicular function has not been adequately studied. Brucellosis in pregnancy poses a substantial risk of spontaneous abortion.[48]

Table 3. Clinical Presentation of Human Brucellosis.*

Features	Percentage of Cases
Signs and symptoms	
Fever	91
Constitutive symptoms (e.g., malaise, arthralgias)	26
Hepatomegaly	17
Splenomegaly	16
Lymphadenopathy	7
Complications	
Peripheral arthritis	22 (8 in hips, 7 in knees, 4 in elbows, 4 in wrists, 4 in other locations)†
Sacroiliitis	3
Spondylitis	19 (15 lumbar, 3 dorsal, 1 cervical)
Central nervous system disorders	3
Epididymoorchitis	5.7‡
Vomiting and diarrhea	3
Respiratory disorders	6
Rashes	3
Cardiovascular disorders	0
Laboratory findings	
Hematologic	49 (40 relative lymphocytosis, 5 isolated thrombocytopenia, 2 isolated leukopenia, 2 pancytopenia)
Transaminasemia	24
Positive blood cultures	16
Rate of relapse	4

* Data are from the most recent 100 patients who received the diagnosis of brucellosis at the University Hospital of Ioannina and whose cases were followed for at least a year.
† Some of the patients had polyarthritis.
‡ Data are for 70 male patients.

Hepatitis is common, usually manifesting as mild transaminasemia. Liver abscess and jaundice are rare.[49] Granulomas can be present in liver-biopsy specimens in cases of both *B. melitensis* and *B. abortus*.[50] Ascites is often present, either as a temporary exacerbation of preexisting hepatic disease or as frank peritonitis.[51]

The central nervous system is involved in 5 to 7 percent of cases in most studies, and such com-

plications often have an ominous prognosis. Meningitis, encephalitis, meningoencephalitis, meningovascular disease, brain abscesses, and demyelinating syndromes have all been reported.[52]

Endocarditis remains the principal cause of mortality in the course of brucellosis. It usually involves the aortic valve and typically requires immediate surgical valve replacement. Early recognition, adequate antibiotic treatment, and the absence of signs of heart failure can guide the practitioner toward prolonged, conservative treatment.[53]

Respiratory complications of brucellosis are considered rare. A recent multinational review of cases with respiratory complications indicated that approximately 16 percent of cases had pulmonary involvement that included lobar pneumonia and pleural effusions.[54]

In sum, practically every organ and system of the human body can be affected in brucellosis — a fact that underscores the importance of including brucellosis in the differential diagnosis in areas of endemic disease, even if clinical features are not entirely compatible.

The blood count is often characterized by mild leukopenia and relative lymphocytosis, along with mild anemia and thrombocytopenia. Pancytopenia in brucellosis is multifactorial and is attributed to hypersplenism and bone marrow involvement. Rarely, marked pancytopenia or isolated deficits can be attributed to diffuse intravascular coagulation, hemophagocytosis, or immunologically mediated cellular destruction.[55,56]

SPECIAL SITUATIONS

Relapses, at a rate of about 10 percent, usually occur in the first year after infection,[57] are often milder in severity than the initial disease, and can be treated with a repeated course of the usual antibiotic regimens. Most cases of relapse are caused by inadequate treatment or are associated with characteristics of the initial infection that include a duration of less than 10 days, male sex, bacteremia, and thrombocytopenia.[58] Childhood brucellosis generally exhibits a more benign course in terms of the rate and severity of complications and the response to treatment.[59]

Although the relationship between brucellosis and T-cell–mediated immunity has been well described, brucellosis is not an opportunistic infection in patients who are infected with the human immunodeficiency virus (HIV) or who have AIDS, even in areas of endemic disease. Most patients with HIV infection and brucellosis have a benign clinical course in the early stages of HIV infection, according to the number of CD4+ T lymphocytes.[60]

DIAGNOSIS

The development of a definitive diagnostic test for brucellosis remains an elusive target. Ever since the development of the first serologic test for brucellosis by Bruce more than a century ago, a definitive diagnostic technique has been actively pursued.

The absolute diagnosis of brucellosis requires isolation of the bacterium from blood or tissue samples. The sensitivity of blood culture varies, depending on individual laboratory practices and how actively the obtaining of cultures is pursued. The percentage of cases with positive cultures ranges from 15 to 70 percent.[61] Brucellae are cultured in standard biphasic (solid and liquid) mode or with the Castaneda bottle, which incorporates both solid and liquid mediums in the same container. Automated systems are also reliable in isolating brucella.[62] Blood-culture sensitivity may be improved by a lysis-centrifugation technique.[63] Even with automated systems, subcultures should be performed for at least four weeks. Brucellae are small, gram-negative and oxidase- and urease-positive coccobacilli that resemble fine grains of sand. Catalase tests, which can have positive results for brucella, should not be performed because the technique can cause the nebulization of particles. Species identification is performed on the basis of particular characteristics (Table 2).

Bone marrow cultures are considered the gold standard for the diagnosis of brucellosis, since the relatively high concentration of brucella in the reticuloendothelial system makes it easier to detect the organism. Furthermore, bacterial elimination from the bone marrow is equivalent to microbial eradication.[64] However, harvesting bone marrow for culture remains an invasive, painful technique, and results have not been universally reproducible.

There are two broad categories of serologic methods for diagnosing brucellosis: those based on antibody production against lipopolysaccharide and those based on antibody production against other bacterial antigens. Developed by Bruce, the serum agglutination test remains the most popular diagnostic tool for brucellosis. Titers above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. However, in areas

of endemic disease, using a titer of 1:320 as diagnostic may be more specific. Seroconversion and evolution of the titers can also be used in diagnosis. Drawbacks of the serum agglutination test include the inability to diagnose *B. canis* infections; the appearance of cross-reactions of class M immunoglobulins with *Francisella tularensis*, *Escherichia coli* O116 and O157, *Salmonella urbana*, *Yersinia enterocolitica* O:9, *Vibrio cholerae*, *Xanthomonas maltophilia*, and *Afipia clevelandensis*; and the percentage of cases in which seroconversion does not occur. Lack of seroconversion can be attributed to the performance of tests early in the course of infection, the presence of blocking antibodies, or the so-called "prozone" phenomenon (i.e., the inhibition of agglutination at low dilutions due to an excess of antibodies or to nonspecific serum factors).[65] Some of these shortcomings can be overcome by modifications such as the addition of EDTA, 2-mercaptoethanol, or antihuman globulin. Other variations of agglutination tests[66] have not proven superior. A new dipstick test, however, offers a rapid and reliable diagnostic alternative in acute brucellosis.[67] The superiority of most of the other agglutination tests over the serum agglutination test has not been consistently proven. Serum agglutination tests have a major drawback in that they are not suitable for patient follow-up, since titers can remain high for a prolonged period.[68]

Indirect enzyme-linked immunosorbent assays (ELISAs) typically use cytoplasmic proteins as antigens. ELISA measures class M, G, and A immunoglobulins, which allows for a better interpretation of the clinical situation and overcomes some of the shortcomings of the serum agglutination test. A comparison with the serum agglutination test yields higher sensitivity and specificity.[69] In patients with neurobrucellosis, ELISA offers significant diagnostic advantages over conventional agglutination methods.[70]

All told, antibody profiles do not have specific clinical correlations, and titers often remain high for a protracted period.[71] The asymptomatic patient with an isolated positive titer of class G and A immunoglobulins, or A immunoglobulins only, has not been adequately studied. Variations of ELISA exist, such as competitive ELISA and sandwich ELISA, which may prove useful as a follow-up tool.

The development of a specific polymerase chain reaction (PCR) is a recent advance. PCR is fast, can be performed on any body tissue, and can yield positive results as soon as 10 days after inoculation. It was first developed for brucellosis in 1990, using a 635-bp fragment of *B. abortus* strain 19.[72] Subsequently, two major gene sequences have been used as targets: the 16S rRNA gene sequence,[73] which presents total genus-specific homology and has been satisfactory in clinical settings,[74] and the *BCSP31* gene, which encodes an immunogenic protein of the external membrane of *B. abortus*[75] and has been extensively studied in clinical practice.[76] Cross-reactivity with ochrobactrum is noticed sporadically with both techniques. A comparison of the two techniques showed superiority of the 16S rRNA target in terms of sensitivity.[77]

Nested PCR has proved to have superior specificity and sensitivity, although it is more prone to contamination.[78] Real-time PCR is most likely the diagnostic tool of the future, offering the possibility of results in 30 minutes.[79-81] PCR ELISA is another new promising variation.[82,83] Other variations of PCR exist, such as arbitrarily primed PCR, PCR with random amplification of polymorphic DNA, and a specific multiplex PCR that can concomitantly diagnose brucellosis, Q fever, plague, and anthrax and was developed for purposes of biowarfare defense.[84] Although PCR is very promising, standardization of extraction methods and set-up is lacking, and a better understanding of the clinical significance of the results is still needed.[85]

TREATMENT

Treatment of human brucellosis should involve antibiotics that can penetrate macrophages and can act in the acidic intracellular environment. There is a general need for combined treatment, since all monotherapies are characterized by unacceptably high relapse rates. Practitioners must weigh such questions as the optimal duration of treatment,[86] cost-effective and conveniently administered regimens, favorable pharmacokinetics and pharmacodynamics, and attention to local virulence factors.[87]

The general discrepancy between in vitro findings and in vivo observations precludes the study of resistance patterns of brucellosis or in vitro evaluation of the efficacy of individual antibiotics. Table 4 summarizes information about the various antibiotics that are used to treat brucellosis.

In 1986, the World Health Organization issued guidelines for the treatment of human brucellosis. The guidelines discuss two regimens, both using doxycycline for a period of six weeks, in combination with either streptomycin for two to three weeks

Table 4. Antibiotics Used in the Treatment of Brucellosis in Humans.

Antibiotic	Minimum Inhibitory Concentration (μg/ml)	Dose	Combinations
Doxycycline	0.06–1	100 mg twice daily for 6 wk	Doxycycline combined with streptomycin, with rifampin, with gentamicin, or with ciprofloxacin; doxycycline and streptomycin combined with rifampin or trimethoprim–sulfamethoxazole; doxycycline combined with rifampin and trimethoprim–sulfamethoxazole
Streptomycin	0.25–16	15 mg/kg of body weight intramuscularly for 2–3 wk	Streptomycin and doxycycline; streptomycin and doxycycline combined with rifampin or trimethoprim–sulfamethoxazole
Rifampin	0.1–2	600–1200 mg/day for 6 wk	Rifampin and doxycycline; rifampin and doxycycline combined with streptomycin or trimethoprim–sulfamethoxazole; rifampin and ofloxacin; rifampin and ciprofloxacin
Gentamicin	0.25–2	5 mg/kg/day in 3 divided intravenous doses for 5–7 days	Gentamicin and doxycycline
Trimethoprim–sulfamethoxazole	0.38–8	960 mg twice daily for 6 wk	Trimethoprim–sulfamethoxazole combined with doxycycline, with rifampin, or with streptomycin; trimethoprim–sulfamethoxazole and doxycycline combined with streptomycin or with rifampin
Ofloxacin	0.1–2	400 mg twice daily for 6 wk	Ofloxacin and rifampin
Ciprofloxacin	0.25–1	500 mg twice daily for 6 wk	Ciprofloxacin with doxycycline or rifampin

or rifampin for six weeks. Both combinations are the most popular treatments worldwide, although they are not used universally. The streptomycin-containing regimen is slightly more efficacious in preventing relapse.[88] This may be related to the fact that rifampin down-regulates serum doxycycline levels.[89] However, parenteral administration of streptomycin mandates either hospital admission or the existence of an adequate health care network — both of which are often absent in areas of endemic disease. On the other hand, the use of rifampin in areas in which brucellosis is endemic, where tuberculosis is also usually endemic, raises concern about the development of community resistance to rifampin.

Alternative drug combinations have been used, including other aminoglycosides (e.g., gentamicin and netilmicin).[90] Trimethoprim–sulfamethoxazole is a popular compound in many areas, usually used in triple regimens. Quinolones are an alternative. Various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar efficacy to that of the classic regimens.[91] Only in vitro observations exist for moxifloxacin and levofloxacin.[92] Although quinolones have been used and will continue to be used, the cost of this approach remains a major drawback. The action of macrolides is attenuated in the acidic phagolysosomal environment, and thus these agents are not useful in brucellosis.[93]

Most complications of brucellosis can be adequately treated with standard regimens. The protracted administration of triple regimens is used for neurobrucellosis. The addition of steroids in neurobrucellosis has not proved to be consistently beneficial.[94] A recent meta-analysis of the efficacy of various combinations for spondylitis advocated a duration of treatment of at least three months; the superiority of any particular regimen could not be proved.[95] Quinolones may prove cost-effective in spondylitis, according to preliminary results.[95]

Rifampin is the mainstay of treatment in cases of brucellosis during pregnancy, in various combinations. Brucellosis in children is treated with combinations that are based on rifampin and trimethoprim–sulfamethoxazole and with aminoglycosides.[96]

A human vaccine has not been developed for brucellosis. Although there are adequate scientific and financial tools for such development in some quarters, knowledge is still incomplete about the molecular pathogenesis of brucellosis. Numerous vaccines have been tested in the past, but none have gained wide acceptance.[97] Vaccines derived from the *B. abortus* strain 19 have been used in the former Soviet Union, and strains of *B. abortus* 104M have been used in China. A phenol-insoluble peptidoglycan fraction of *B. melitensis* strain M15 was used in France.[98] Theoretical vaccine targets for the future might use *rfbK* mutations of *B. melitensis*, outer-

membrane protein 25, and the cytoplasmic protein BP26.[99]

THE FUTURE

Eradication of brucellosis depends largely on socioeconomic and political circumstances. Progress in understanding the molecular pathogenesis of the disease, vaccine engineering, and postgenomic approaches may lead to new preventive interventions. Furthermore, the discovery of new pathways in modifying the acidic intracellular environment in which the microbe moves might be used in adjuvant pharmacotherapy. Determination of microbial load might modify treatment planning and the potential for complications.

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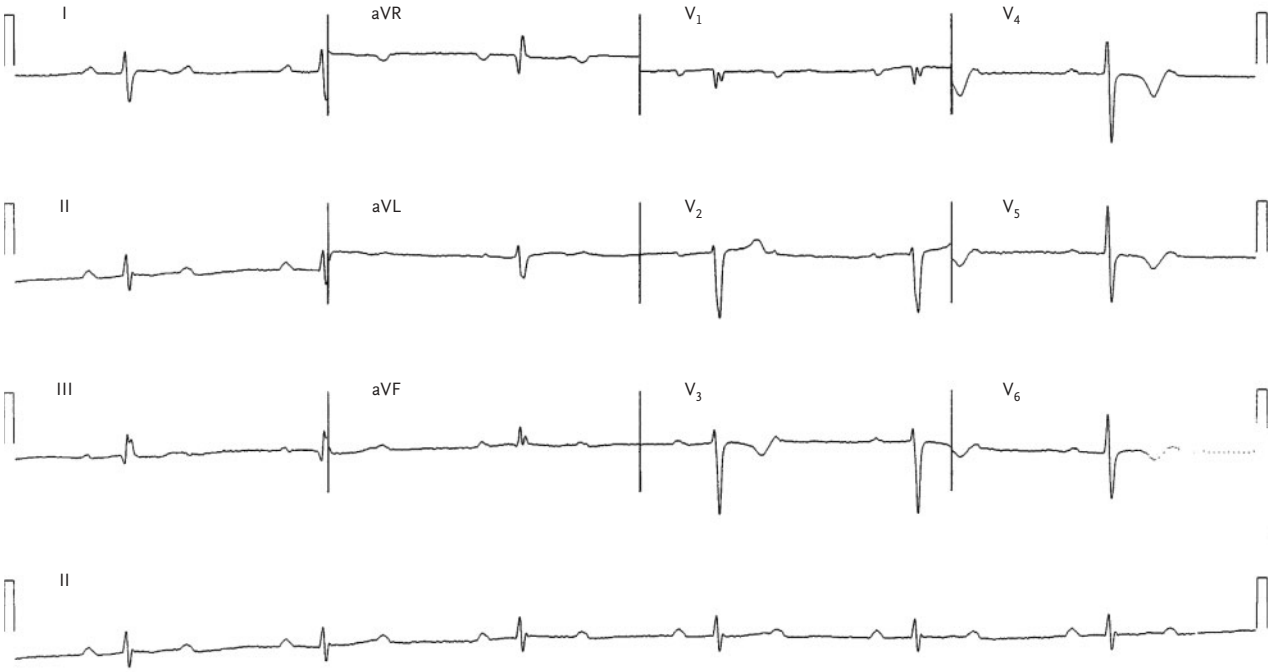
IMAGES IN CLINICAL MEDICINE

A Medical Mystery — Bradycardia



At an office visit in June 2004, a 49-year-old man reported fatigue, arthralgia, and headache, along with a two-day history of chest pain. The patient had hypercholesterolemia, for which he was taking atorvastatin (20 mg daily) and aspirin (81 mg); he had stopped smoking cigarettes 20 years earlier. The patient reported that his father had died at the age of 71 years of myocardial infarction. Physical examination revealed a pulse of 38 beats per minute. Electrocardiography was performed. What is the diagnosis?

Editor's note: We invite our readers to submit their answers at www.nejm.org/mystery. We will publish the diagnosis in the Correspondence section of the July 28, 2005, issue and e-mail it to everyone who submits an answer. All answers must be received by June 16, 2005.

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CLINICAL PROBLEM-SOLVING

Don't Know Much about History

Shelby Dames, M.D., Claude Tonnerre, M.D., Sanjay Saint, M.D., M.P.H., and Stephen R. Jones, M.D.

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

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A 20-year-old man who had immigrated to the United States from Mexico three months earlier presented to the emergency department, reporting that he had had weight loss and pain in his hip and back on the right side. The patient did not speak English; the initial history was obtained with the use of the limited Spanish of team members and with the help of untrained interpreters. Two weeks before presentation, the patient slipped and fell while washing floors. Although he did not recall any pain immediately after the event, pain in the right side of his lower back and hip developed several days later. The pain was described as intermittent and stabbing, and it increased with movement. He noted that he had had decreased appetite for several weeks and had unintentionally lost 16 kg. He also said he had had occasional bitemporal headache, dizziness, and nausea and was easily fatigued and mildly short of breath. He said he did not have fever, chills, night sweats, confusion, diarrhea, constipation, vomiting, urinary urgency, cough, or easy bruising.

Although back pain is frequently benign, several factors — most important, this patient's weight loss — make me worry about serious causes, in particular infection or cancer. One obvious concern is spinal epidural abscess, which might be caused by occult bacteremia, tuberculosis, or a fungal infection, such as coccidioidomycosis. Musculoskeletal pain can be a sign of infective endocarditis. I am also considering amebic liver abscess, since the patient is a Mexican man with pain on the right side of his body. Lymphoma, leukemia, or germ-cell tumors may at times occur with or come to medical attention because of back pain, and they are among the most common cancers at his age. I want to know the patient's travel history, whether he has any risk factors for infection with the human immunodeficiency virus (HIV), and whether he has been exposed to others who have been ill. A physical examination is needed, with particular attention to fever, lymphadenopathy, or masses. In addition, I want to know if the "hip pain" is truly coming from the hip.

The patient had noted a loss of appetite since discontinuing valproic acid three weeks before presentation. He had been taking this medication since he was 13 years of age, after he had had a grand mal seizure. At that time, he was hospitalized for three days in Mexico and was told that he would need to take antiseizure medications for the rest of his life. The patient said that he did not use alcohol, tobacco, or illicit drugs. He had had one female sexual partner. He was living with a cousin and working in a grocery store.

I am puzzled about his association of loss of appetite with the discontinuation of his valproic acid, and I wonder why he stopped taking the medication. The history of seizures in a person originally from Mexico makes it likely that he has neurocysticercosis, which, in areas where the disease is endemic, is a more common cause of seizures than idiopathic epilepsy. It is tempting to apply Occam's razor in an attempt to explain all of this young man's problems with one disease. Neurocysticercosis may involve the spinal cord as well as the brain, and it is possible that he is having radicular pain from a subarachnoid cyst.

The patient was alert but appeared cachectic. His height was 165.1 cm and his weight 47 kg. His temperature was 37.3°C, blood pressure 95/63 mm Hg, pulse 93 beats per minute, and respiratory rate 12 breaths per minute. The results of his lung and cardiac examinations showed no abnormalities. He had palpable splenomegaly and an enlarged liver that spanned 12 cm, but he did not have abdominal tenderness. He did have mild tenderness with percussion of his right lower back and with external rotation of the right hip. A neurologic examination revealed a slow, unsteady gait, global weakness without focal deficits, and normal, symmetric reflexes. The result of a Romberg's test was negative, and there was no tremor or dysmetria. No rash, petechiae, lymphadenopathy, or scleral icterus was noted. Funduscopic and genitourinary examinations were not performed. The remainder of the patient's physical examination revealed no abnormalities.

His chronically ill and wasted appearance cannot be accounted for by a two-week illness; his physical presentation increases my suspicion of cancer or chronic infection. In addition, adrenal insufficiency should be considered, especially since this finding may occur with disseminated tuberculosis or metastatic cancer. The presence of splenomegaly leads me to consider either splenic congestion, usually from portal hypertension due to liver disease, or an infiltrative disease. Diffuse involvement of the liver by cancer (in particular, small-cell lung cancer) can lead to portal hypertension. A lymphoma might cause hepatosplenomegaly without portal hypertension. The absence of peripheral lymphadenopathy makes lymphoma a less likely cause but by no means rules it out. Testing for HIV infection is warranted.

Tuberculosis and a fungal infection remain serious considerations. Extracranial cysticercosis is improbable, since the liver and spleen are not common sites of involvement. Although the absence of fever and heart murmur also makes endocarditis improbable, I would not rule it out in this cachectic man with splenomegaly and back pain. One normal temperature does not rule out intermittent fever, and subtle heart murmurs such as that produced by aortic insufficiency may be easily overlooked.

The patient had a white-cell count of 4000 per cubic milliliter, with 52 percent polymorphonuclear cells, 4 percent band forms, 4 percent monocytes, 39 percent lymphocytes, and 1 percent metamyelocytes. His hemoglobin level was 13.8 g per deciliter, with evidence of teardrop cells, acanthocytes, poikilocytes, and ovalocytes on a peripheral-blood smear. The platelet count was 20,000 per cubic milliliter. The results of a complete chemistry panel showed no abnormal values except for the following: alkaline phosphatase, 199 U per liter (normal range, 40 to 120 U per liter); aspartate transaminase, 147 U per liter (normal range, 16 to 50 U per liter); alanine transaminase, 75 U per liter (normal range, 12 to 61 U per liter); and lactate dehydrogenase, 1115 U per liter (normal range, 313 to 618 U per liter). Coagulation studies were normal. The amylase level was 136 U per liter (normal range, 30 to 110 U per liter), and the lipase level was 419 U per liter (normal range, 23 to 300 U per liter). The level of valproic acid was undetectable, and the results of a toxicologic screen were negative. The erythrocyte sedimentation rate was normal at 4 mm per hour. The C-reactive protein level was 3.1 mg per deciliter (normal range, less than 0.8 mg per deciliter). Radiographs of the patient's chest, right hip, and lumbar spine showed no abnormalities. A computed tomographic (CT) scan of the head showed a calcified lesion, 2 mm in length, in the right frontal lobe. A CT scan of the abdomen and pelvis revealed diffuse hepatic edema and splenomegaly. Blood and urine cultures were obtained.

The presence of misshapen red cells and thrombocytopenia suggests bone marrow invasion, perhaps due to a disseminated infection or tumor. These findings would not be expected in infective endocarditis. The combination of thrombocytopenia and fragmented red cells may also be seen in microangiopathic states such as the hemolytic–uremic syndrome or malignant hypertension, but

none of these fit the clinical context. It is possible that the patient's enlarged spleen is sequestering platelets, but this would not cause damaged red cells to appear in the peripheral blood. Tumor is less probable than infection; no masses were found on imaging studies. Miliary tuberculosis could be responsible for all the clinical findings and should be ruled out. The normal chest radiograph does not rule out this diagnosis, since a substantial proportion of patients with miliary tuberculosis present with a normal chest radiograph. Disseminated tuberculosis and endocarditis are generally associated with a high erythrocyte sedimentation rate; however, a normal erythrocyte sedimentation rate does not rule out these infections. I suggest that a bone marrow biopsy or liver biopsy be performed. I would start the patient on empirical treatment for tuberculosis while awaiting the results of these studies.

The patient was treated with ibuprofen and acetaminophen for his pain. In the hospital, he was noted to have nightly fevers ranging from 38.5°C to 40.5°C. He had occasional chills, but he said that he never felt febrile. On hospital day 2, a new systolic heart murmur was noted, and he began to report pain in his right elbow, similar to that in his right hip. There was no initial growth reported from either set of blood cultures obtained at admission. The test for HIV was negative. A tuberculin skin test was negative, but the control tests for candida and for mumps were also negative. The monospot test for mononucleosis and the acute hepatitis panel for hepatitis A, B, and C were all negative.

Given the finding of fever, it is possible that he has been febrile throughout his illness. Tuberculosis remains my most urgent concern. A negative tuberculin skin test is not helpful in the context of negative controls.

A bone marrow aspirate showed a normocellular marrow with mild myeloid hyperplasia, decreased iron stores, and rare noncaseating granulomas (Fig. 1). A bone marrow smear was negative for acid-fast bacilli. A bone scan obtained with use of technetium-99–labeled methylene diphosphonate scintigraphy revealed mild, asymmetric uptake in the patient's right ankle and right sacroiliac joint, which was believed to be due to degenerative changes; however, osteomyelitis could not be ruled out. Transthoracic and transesophageal echocardiograms showed no evidence of vegetations; the heart function was normal.

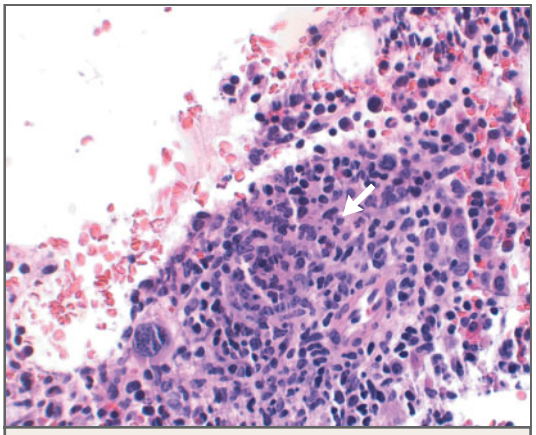

Figure 1. Bone Marrow Aspirate Showing a Noncaseating Granuloma (Arrow) (Hematoxylin and Eosin).

The granulomas found in the bone marrow make it highly probable that the patient has a disseminated granulomatous disease, and tuberculosis is the most likely candidate. It is also possible that he has a systemic fungal infection such as coccidioidomycosis. Other causes of noncaseating granulomas, such as sarcoidosis or Hodgkin's disease, are less probable.

During hospitalization, additional details of the history were obtained with the help of an interpreter. The patient reported that he had worked for three years in a slaughterhouse in Mexico, where he had had daily exposure to carcasses of swine, cattle, and sheep.

This is a critical piece of historical information. If the case had been reframed as that of a "patient with classic fever of unknown origin," then any history of exposure to animals would have been explicitly sought. He might have been a typical abattoir worker of Mexico and, therefore, regularly exposed to *Brucella abortus* and *B. suis* — both of which are endemic in Mexican livestock. His illness, including the hepatosplenomegaly and bone marrow findings, is consistent with brucellosis.

Given this additional history, and the patient's ill appearance, I would empirically treat for brucellosis by adding doxycycline to his current treatment regimen. I would continue empirical treatment for both this and tuberculosis while waiting

for the results of serologic tests for brucella and for cultures.

On the seventh hospital day, serologic testing for brucella was positive, with a titer greater than 1:160. The next day, the repeated blood cultures and bone marrow culture were positive for gram-negative coccobacilli; the organisms were subsequently identified as *B. abortus*. The patient was treated with doxycycline and rifampin. He became afebrile, his heart murmur disappeared, and the pain in his hip, back, and elbow diminished within a few days.

As is often noted, if the diagnosis is not suspected on the basis of the history, it will probably not be made. Taking a thorough history from most patients requires time and patience. Obtaining a history through an interpreter requires extra time and patience. But by doing so, the clinicians caring for this patient were able to make the correct diagnosis.

COMMENTARY

Brucellosis is a zoonotic disease that can involve any organ or system of the body. Most cases in humans are caused by contact with infected animals or animal products, such as unpasteurized milk and cheese.[1] Four species are responsible for most human cases: *B. abortus* (found in cattle), *B. melitensis* (found in sheep and goats), *B. suis* (found in swine), and *B. canis* (found in dogs). The clinical presentation can be acute or progressive, usually starting with nonspecific symptoms, such as fever, sweats, weight loss, arthralgias, and myalgias. In one series of patients infected with *B. melitensis*, the most common findings on physical examination included a diffusely enlarged liver or spleen (present in 38 percent and 22 percent, respectively), osteoarticular abnormalities, such as spondylitis and sacroiliitis (present in 21 percent), relative bradycardia (present in 21 percent), and lymphadenopathy (present in 9 percent).[2] Both cultures and serologic tests are useful for the diagnosis of brucellosis. In the series mentioned above, when cultures were performed under ideal circumstances (defined as an absence of antibiotic treatment in the preceding 72 hours and incubation for at least four weeks), approximately 70 percent were positive for brucella. Recently, higher rates of positive cultures have been reported (91 percent in acute brucellosis and 74 percent in chronic brucellosis).[3]

Although brucellosis remains common in developing countries, the disease is rare in the United States, with about 100 cases reported annually. More than half of these cases have occurred in Hispanic patients, according to the category checked on the survey forms.[4] The incubation period is usually between 7 days and 3 months, although incubation periods as long as 10 months have been reported.[5] A thorough travel history as well as a history of exposure to animals and exotic foods are usually critical to making the diagnosis.[1]

In hindsight, this patient had many classic symptoms of brucellosis; however, it took several days before the clinical team caring for the patient made the correct diagnosis. The initial presentation was compatible with other, more common diseases that the discussant considered, including tuberculosis, endocarditis, fungal infections, hematologic cancers, and an HIV-related illness. The treating clinicians' initial assessment was similar, and their initial evaluation was directed toward a diagnosis based on consideration of these diseases. As the discussant correctly noted, despite a negative tuberculin skin test, the patient could still have had miliary tuberculosis,[6] which can be rapidly fatal if untreated.

After admission, the patient was noted to have nightly fevers. The discussant suggested reframing the problem as fever of unknown origin, although there was no documentation that the patient had had three weeks of fever (as required by the formal criteria[7] for this entity). Nevertheless, the patient did meet most criteria for a comprehensive evaluation for fever of unknown origin, including a physical examination, basic laboratory tests, blood cultures, chest radiography, and abdominal imaging.[8] A critical part of the evaluation also includes a thorough history, which was initially compromised by the language barrier. Fortunately, the use of a professional interpreter eventually allowed the clinical team to discover crucial information — the patient's occupational exposure to the carcasses of swine, cattle, and sheep — thereby alerting the clinicians to the strong possibility of exposure to brucella. After this exposure history became known, serologic studies for brucella were obtained and monitoring of blood cultures was extended, leading to the correct diagnosis.

More than 21 million people in the United States, or 8.1 percent of the population, reported to the 2000 Census Bureau that they spoke English less than very well or not at all, as compared with 6.1 percent of the population in the 1990 U.S. Census.[9]

Furthermore, new regulations requiring adequate access to interpreters for patients covered by Medicare will make the need for interpreters more acute.[10] The use of family members or untrained Spanish-speaking employees is not ideal. Indeed, the use of trained interpreters has been shown to increase access to care,[11] and it may also improve patients' understanding of their disease. Ensuring access to adequate interpreters remains a challenge, but as our case demonstrates, it may be critical to obtaining an accurate history.

It is well recognized that, in the majority of cases, the diagnosis can be made after history taking alone.[12] A study performed three decades ago to determine the relative value of the history, physical examination, and laboratory testing in making diagnoses found that the correct diagnosis was determined after the completion of only the history in 82 percent of patients.[13] A similar study performed in 1992 yielded similar results, with the history leading to the correct diagnosis in 76 percent of the cases.[14] Given the increasing number of non–English-speaking patients in the United States, the use of skilled interpreters to assist with the illness history is more important than ever.

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EDITORIALS

Varicella–Zoster Virus Vaccine — Grown-ups Need It, Too

Donald H. Gilden, M.D.

A few years ago, I had zoster (shingles) — no surprise, since I was over 60 years of age, and zoster is usually a disease of later life. Fortunately, both the rash and the pain disappeared within a few days. I was grateful not to have to endure the dreaded chronic pain of postherpetic neuralgia, which can last for months or years, and there was no complicating zoster paresis, myelopathy, or vasculopathy. I recalled my first encounter with varicella–zoster virus (VZV) as a child with varicella (chickenpox); I had to stay home from school for nearly a week (not so terrible) and could not join my friends to play ball outside (that was terrible). Now, the acute pain of zoster confirmed what I had learned during years of caring for patients with zoster and its complications — that for older adults, all these problems can be far more serious than childhood varicella usually is.

In the United States, zoster affects hundreds of thousands of people annually, most of whom are older than 50 years of age. Because VZV becomes latent in cranial-nerve ganglia, dorsal-root ganglia, and autonomic ganglia along the entire neuraxis, zoster can crop up anywhere on the body. The pain often is long-lasting. In 36.6 percent of patients with zoster who are older than 60 years of age and in 47.5 percent of those older than 70, pain persists for more than one year.[1] The pain is not easily controlled. The large number of topical medications (e.g., capsaicin, ketamine, and lidocaine patches) and oral medications (e.g., gabapentin, carbamazepine, amitriptyline, and even narcotic agents) used to treat postherpetic neuralgia attest to the lack of a single effective medication. Furthermore, in immunocompromised persons, zoster can recur, is more protracted, and is associated with a greater incidence of VZV myelitis (weakness and incontinence) and of vasculopathy (changes in mental status, speech disturbances, seizure, and severe motor deficit), which kills nearly one third of patients.[2]

The status of immunity to VZV among elderly persons reflects the waning cell-mediated immunity to the virus.[3] Thus, zoster may be seen as a continuum, from its occurrence among elderly persons to its greater incidence among patients with more seriously immunocompromised status — those undergoing treatment for cancer, those with AIDS, and recipients of organ transplants. Nevertheless, a waning VZV-specific cell-mediated immunity is stimulated by naturally occurring zoster or immunization against VZV. For example, the frequency of VZV-specific T lymphocytes is higher among older patients who have had zoster than among age-matched controls, and it persists for at least two years.[4] Furthermore, among people 55 to 87 years of age with immunity to VZV who were given the live attenuated Oka-strain VZV vaccine, the frequency of T-cell response to VZV was similar to that among those 35 to 40 years of age. The boost in cell-mediated immunity to VZV was maintained over 24 months, and the dose of the virus and the age of those vaccinated did not significantly influence the response.[5]

These findings set the stage for the large-scale Shingles Prevention Study reported by Oxman and colleagues in this issue of the *Journal*,[6] the goal of which was to determine the effect of vaccination against VZV in preventing zoster. Healthy adults 60 years of age or older (median age, 69 years) were vaccinated with an investigational attenuated Oka/Merck "zoster" vaccine containing 18,700 to 60,000 plaque-forming units of virus — considerably more than the approximately 1350 plaque-forming units in the Oka/Merck VZV vaccine that has been ad-

ministered to American children since approval by the Food and Drug Administration (FDA) in 1995. More than 38,000 recipients of the zoster vaccine were followed closely for three years.

The results are impressive. The double-blind, placebo-controlled study showed a 61 percent reduction in pain and discomfort (the burden of illness) in herpes zoster, and the incidence of postherpetic neuralgia in the vaccine group was reduced by 66 percent. The incidence of zoster in the placebo group was 11.12 per 1000 person-years. This percentage approximates the results of an epidemiologic survey performed a decade ago, which showed an incidence of zoster exceeding 10 cases per 1000 person-years among people older than 75 years.[7] The elderly population has been rapidly increasing — the U.S. Census Bureau conservatively estimated that the population 85 years of age or older increased by 1 million from 1995 to 2005,[8] and the population 60 to 85 years of age increased even more. In fact, the high incidence of zoster found in the placebo group in the Shingles Prevention Study points to an urgent need for effective therapy. Although oral antiviral therapy shortens the duration of zoster and analgesic medications provide some relief of pain, "an ounce of prevention. . . ."

Like the live varicella vaccine administered to children, live zoster vaccine appears to be safe and effective clinically. Serious adverse effects or deaths occurred in 1.4 percent of recipients in both the vaccine and placebo groups. In a subgroup of more than 6000 subjects who kept daily diaries of minor adverse events for a period of 42 days after vaccination, erythema, pain or tenderness, swelling, pruritus, or other adverse events at the injection site were reported in 48 percent of vaccine recipients as compared with 17 percent of placebo recipients. In the same subgroup, serious adverse events were reported in 1.9 percent of vaccine recipients, as compared with 1.3 percent of placebo recipients, and the difference was statistically significant (P=0.03). Furthermore, although the Oka/Merck VZV vaccine can, on rare occasions, unmask a childhood immunodeficiency disorder, no cases of dissemination of the virus were reported that might have been attributed to the zoster vaccine in a person with an undiagnosed disorder such as lymphoma or leukemia.

Should zoster vaccine be recommended for every VZV-seropositive, middle-aged adult who is healthy (i.e., without immunocompromise due to cancer, drug therapy, or AIDS) and who has not had zoster? Two main factors affect the answer to this question. First is the future risk of zoster among middle-aged adults who were vaccinated in childhood as compared with the current risk. By 2047, most, although not all, middle-aged Americans will have received VZV vaccine in childhood. Like the wild-type VZV, the Oka/Merck vaccine virus becomes latent in ganglia.[9] If the viral burden is less in the ganglia of adults who were vaccinated in infancy, then the incidence of zoster may be reduced in those adults as compared with those who as children had naturally occurring chickenpox. Alternatively, the cell-mediated immunity to VZV in vaccine recipients may be reduced by their exposure to fewer cases of varicella, which would normally boost immune responsiveness, supporting the need to vaccinate middle-aged adults.

Second, the cost-effectiveness of vaccination must be considered before such a recommendation can be made. The Shingles Prevention Study used a disease-specific measure, the burden of illness due to herpes zoster,[10] to assess severity of illness among the study participants. Herpes-zoster burden-of-illness scores correlated with the number of quality-adjusted life-years gained,[10] the standard denominator of cost-effectiveness.[11] As with any new therapy, the cost-effectiveness of the vaccine will depend on its price. To nonindigent recipients of the currently used childhood VZV vaccine (Varivax), the price of vaccination is between $50 and $100 (the sum of the cost of vaccine plus the visit or facility fee). An adult vaccine might cost more, given its greater potency. Nevertheless, the zoster vaccine appears to have been highly cost-effective in the Shingles Prevention Study (i.e., in the range of $2,000 per quality-adjusted life-year gained, even assuming a vaccine cost of $500). This admittedly rough estimate does not include the cost of complications related to vaccination or the provider's time required to administer it. However, $2,000 per quality-adjusted life-year gained is probably an underestimation of the vaccine's cost-effectiveness, since gains in the quality of life among vaccine recipients are likely to persist beyond the three years of the study period. Thus, the vaccine should fall well within the traditional cutoff point of $50,000 per quality-adjusted life-year used to evaluate new therapies, and it would appear to be at least as cost-effective as other currently used vaccines.[12] Zoster vaccine may even be cost-saving.

If the FDA were to require another large multi-institutional study before zoster vaccine is licensed, it will be another decade until the findings of Ox-

man et al. can be confirmed or repudiated. Since zoster and its attendant neurologic complication of postherpetic neuralgia are common and serious, it seems prudent to market the zoster vaccine, but only if a large number of those vaccinated are followed closely, particularly those over 85 years of age, among whom the cell-mediated immune response to VZV is likely to be less robust than among those 20 to 25 years younger. This follow-up would allow not only a further determination of possible risk to vaccine recipients but also an estimation of the vaccine's effectiveness in the population of the oldest old. The Census Bureau projects that by the year 2040, there will be 8 million to 13 million Americans 85 years of age or older.[8] It is hoped that the use of a zoster vaccine will reduce or eradicate zoster and its related complications, just as measles vaccine has wiped out not only measles but also measles-associated postinfectious encephalomyelitis and subacute sclerosing panencephalitis in regions where the vaccine is used.

Even though my episode of zoster was brief, I would gladly have paid to receive the zoster vaccine, knowing that the risk of the development of zoster might have been 50 percent less. Grown-ups should welcome the zoster vaccine. We may need it more than children do.

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TAC — A New Standard in Adjuvant Therapy for Breast Cancer?

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Chemotherapy, an integral component of adjuvant treatment for women with resected node-positive breast cancer, improves disease-free and overall survival in many patients. Older regimens include such combinations as cyclophosphamide, methotrexate, and fluorouracil; doxorubicin and cyclophosphamide; fluorouracil, doxorubicin, and cyclophosphamide given in various doses and according to various schedules; and fluorouracil, epirubicin, and cyclophosphamide, among others. Standards of care continue to evolve, owing to the availability of new drugs and data from innovative clinical trials that have tested different combinations of existing and new agents.

The taxanes (docetaxel and paclitaxel) have emerged as potent agents for the adjuvant treatment of early breast cancer; studies involving more than 20,000 patients have been reported or are ongoing. In this issue of the *Journal,* Martin and colleagues give an account of one such study, the Breast Cancer International Research Group 001 trial, which represents an advance in the treatment of breast cancer.[1] The trial was a phase 3 randomized comparison of six cycles of docetaxel, doxorubicin, and cyclophosphamide (TAC) with six cycles of fluorouracil, doxorubicin, and cyclophosphamide (FAC). It is important to examine carefully whether the data from this study justify the consideration of TAC as a new standard — or even the new standard — of adjuvant therapy for resected node-positive breast cancer. Factors to consider include the clinical relevance of the differences in efficacy between TAC and FAC, the tolerability of the two regimens, the suitability of the control group in terms of the

CORRESPONDENCE

Electronic Alerts to Prevent Venous Thromboembolism

TO THE EDITOR: Computer reminders such as the one described by Kucher and colleagues (March 10 issue)[1] can draw attention to important situations, since medical errors frequently occur when physicians are busy, tired, or distracted.[2,3] The real challenge, however, is in developing programs that streamline care for physicians. If use of the program consumes time that would be better allocated elsewhere, it may simply shift the focus of a busy physician from one important area to another and thus simply shift the point at which errors are made. Therefore, more information on the program's ease of use would help readers determine its applicability and utility; such information might include a screen shot of the user interface, additional feedback from test subjects, statistics on the time required to use the program, and flow diagrams outlining where the program was used during a patient's admission and care process, who (e.g., interns, residents, or nurses) received alerts, and how many other kinds of alerts appeared on the Brigham and Women's Hospital computer system. Such information would help readers better ascertain how the program might affect a physician's workflow and whether it is a boon or a burden.

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TO THE EDITOR: Kucher et al. analyzed the effect of a computer-based reminder system designed to improve in-hospital thromboprophylaxis. The 90-day incidence of symptomatic venous thromboembolic events was high (4.0 percent, excluding events in the arms), and surprisingly, prophylaxis did not significantly reduce the incidence of venous thromboembolism. Among high-risk patients who have undergone hip arthroplasty and are given a week of prophylaxis, the 90-day incidence is 3.3 percent, and extending prophylaxis for 4 to 6 weeks is needed to lower the incidence significantly.[1,2]

In the study by Kucher et al., what was the mean length of hospitalization and the mean duration of in-hospital prophylaxis? Also, how did the authors adjust for or deal with the effect of rehospitalization (approximately 40 percent of the patients were rehospitalized) during the follow-up period?

Simply identifying high-risk medical patients and ordering subcutaneous heparin for a few days may not be effective. We need validated risk-stratification tools as well as clinical trials designed to determine whether short-duration, in-hospital prophylaxis reduces the incidence of symptomatic ve-

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nous thromboembolism. The results of two studies indirectly suggest that a longer duration of thromboprophylaxis may be necessary in high-risk medical patients.[3,4]

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THE AUTHORS REPLY: Drs. Lee and Chen and Dr. White and colleagues can rest assured that electronic alerts are a boon to physicians and to patients, not a burden. Only 30 alerts were issued for every deep-vein thrombosis or pulmonary embolism that was prevented. The alerts were straightforward and user-friendly. The fear of increasing physicians' workload is not justified.

We caution Dr. White and colleagues about the pitfalls in comparing other populations composed of patients undergoing orthopedic surgery with our high-risk population, which consisted mostly of patients undergoing medical therapy (83 percent of the entire cohort). Our protocol was simple and yielded a powerful result: a 41 percent reduction in the three-month incidence of venous thromboembolism. Future studies should fine-tune the types of prophylaxis that are most beneficial and should clarify the optimal duration of prophylaxis.

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Treatment of Brain Tumors

TO THE EDITOR: In the report by Rutkowski et al. (March 10 issue)[1] regarding treatment of early childhood medulloblastoma with postoperative chemotherapy alone, 20 of 43 children under the age of three years (46.5 percent) had desmoplastic medulloblastoma, and these 20 children had a five-year progression-free survival rate of 85 percent. The desmoplastic variant is considered to be less aggressive than classic medulloblastoma and occurs mainly in adolescents and adults.[2,3] As far as we know, desmoplastic histologic features are not found in a large proportion of children who are younger than three years of age. Could the somewhat higher-than-expected proportion of children with the desmoplastic variant in the study by Rutkowski et al. be secondary to the exclusion of 19 of the 62 patients originally registered in the study? The authors report a worse outcome for children less than two years of age, but they do not mention how many children were in this age group.

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TO THE EDITOR: The well-documented relation between intrathecal methotrexate and cognitive damage[1] is not adequately examined by Rutkowski et al.

Only 14 children in their study underwent neuropsychological assessments that tested for nonverbal tasks and that were not highly dependent on language.

A significant correlation between the leukoencephalopathy grade and the cumulative intraventricular methotrexate dose was noted, but the use of intraventricular methotrexate was not identified as an independent prognostic factor — so why use it?

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TO THE EDITOR: Hegi et al. (March 10 issue)[1] report on *MGMT* (O^6-methylguanine–DNA methyltransferase) gene silencing in patients with glioblastoma who were treated with radiotherapy with or without temozolomide. The clinical study, reported by Stupp et al. in the same issue,[2] showed that patients who received temozolomide plus radiotherapy had improved progression-free survival and overall survival. Patients with the methylated *MGMT* promoter who received temozolomide had the longest survival. However, irrespective of the treatment group, *MGMT* promoter methylation was an independent predictor of favorable overall survival. Progression-free survival was similar (and inferior) in all groups except patients with the methylated *MGMT* promoter who received temozolomide.

These apparently conflicting results can be resolved if one considers the salvage therapy that patients received. It seems likely that patients who did not receive temozolomide during the study received it after the study was completed. It is also likely that these patients and the other patients subsequently received treatment with regimens based on alkylating agents, whose activity is also affected by *MGMT* status.[3,4] Therefore, the improved survival of patients with a methylated *MGMT* promoter who did not receive temozolomide in the current study could be the result of a response to temozolomide or other alkylating agents given as salvage therapy.

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Dr. Seiter reports having received grant support from Schering-Plough.

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TO THE EDITOR: The trial of temozolomide reported by Stupp et al. demonstrates advancement in the treatment of glioblastoma multiforme. However, the authors fail to mention treatment with a biodegradable wafer containing carmustine (Gliadel Wafer, Guilford) approved in 2003 for primary grade III and IV gliomas. In the initial surgery setting, the median survival benefit of 2.3 months with the use of the carmustine wafer is similar to the 2.5-month survival benefit in the temozolomide trial.

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Dr. Ashby reports having received consulting fees and lecture fees from Guilford and is a registered lecturer for Schering-Plough. Dr. LaRocca reports having received consulting fees, lecture fees, and a research grant from Guilford, as well as lecture fees from Schering-Plough. Dr. Ryken reports having received consulting fees and lecture fees from Guilford and lecture fees from Schering-Plough. In addition, Dr. Ryken has served as a consultant and on advisory panels for Spinal Concepts and Medtronic and has received research grant support from Spinal Concepts and Xenova Biomedix.

TO THE EDITOR: I am surprised that Dr. DeAngelis (March 10 issue)[1] chose "A New Beginning" as the subtitle of her editorial accompanying the reports by Rutkowski et al., Stupp et al., and Hegi et al. I would have chosen the more sanguine expression "Is That All?" It has been 27 years since Walker et al.[2] reported a medial survival of 10 months; now we have Stupp et al. reporting a median survival of 14.6 months. This is not a very auspicious "begin-

ning." Although there are subgroups that have significantly prolonged survival, as reported by Hegi et al., glioma remains a dismal disease. Despite recent revolutions in brain imaging, surgery, and radiation delivery, there has not been a commensurate increase in survival. Furthermore, long-term survival can be accompanied by significant impairment in the quality of life.[3] It should be apparent that we have been barking up the wrong trees.

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DR. RUTKOWSKI AND COLLEAGUES REPLY: We agree with Drs. Paulino and Teh that desmoplastic medulloblastoma occurs more frequently in adolescents and adults than in children. However, to our knowledge there are no population-based studies on the incidence of medulloblastoma variants in very young children.[1-3] About 90 percent of children in Germany with a diagnosis of medulloblastoma were registered in our study during the study period. Among the 11 patients with medulloblastoma who were excluded from analysis, 3 children had the desmoplastic subtype, and 8 had medulloblastoma without further classification. Our data suggest that desmoplastic medulloblastoma may have a double-peaked age distribution, and the biology may differ between age groups. Twenty-three of the 43 children were younger than two years of age at diagnosis.

In our study, 20 of 27 patients (74 percent) who were alive 3.9 years or more after diagnosis underwent neurocognitive assessment (14 patients after chemotherapy alone, and 6 patients who received radiotherapy after chemotherapy). Parents of seven of the patients did not answer our request for testing or did not agree to it. The children were also tested for learning abilities, including vocabulary, motor abilities, simple reaction time, and an attention task. The complete neurocognitive data are being analyzed, and a 10-year follow-up is under way. There is evidence that deficits in attention may be more relevant for learning problems than deficits in verbal skills.[4] The majority of the 14 children (12) treated with chemotherapy alone are successfully attending a standard regular school; 1 child is attending a school for hearing-impaired children, and 1 a school for physically handicapped children.

Our study was not designed to compare treatment with and without intraventricular methotrexate. The multivariate analysis concerning intraventricular methotrexate as a prognostic factor refers to children receiving different cumulative doses. As compared with the results of other studies using systemic methotrexate, repeated doses of intraventricular methotrexate may have pharmacokinetic advantages.[5] Leukoencephalopathy was a clinically asymptomatic finding. In our opinion, the efficacy of our regimen outweighs its toxicity, but the contribution of intraventricular methotrexate should be further assessed.

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DRS. STUPP AND HEGI REPLY: Unlike Dr. Aiken, we truly believe that there is a new beginning for treatment and research in malignant glioma. Considering only median survival, an increase of five months since the report by Walker et al. may seem modest, but it represents an increase of 50 percent. Moreover, improved knowledge of the biology of gliomas allows us to identify tumors likely to respond to alkylating agents. If patients are selected on the basis of *MGMT* promoter methylation and receive temozolomide chemotherapy, two-year survival reaches 46 percent. The important issue of the quality of life,

emphasized by Dr. Aiken, was addressed as a secondary end point in our trial. The analysis showed that the addition of chemotherapy to standard radiation therapy had no significant detrimental effect on the quality of life.[1]

Dr. Seiter suggests that salvage therapy could explain why there was no difference in progression-free survival in the two groups we studied. Indeed, 72 percent of the patients initially treated with radiation therapy alone received salvage chemotherapy with an alkylating agent. Actually, more than 60 percent of patients received temozolomide after progression.

Dr. Ashby and colleagues believe that carmustine-impregnated wafers should be recognized as an alternative treatment. In our opinion, the results with carmustine wafers were disappointing, particularly because all patients had to undergo debulking surgery to implant the wafer to the resection cavity.[2] Patients with glioblastoma receiving carmustine wafers had a median survival of 13.5 months, as compared with 11.4 months with placebo, with no significant difference in survival beyond 18 months. In the European Organization for Research and Treatment of Cancer and National Cancer Institute of Canada trial, patients who underwent surgery had a median survival of 12.9 months with initial radiation therapy, as compared with 15.8 months for patients who received treatment with temozolomide during and after radiation therapy ($P<0.001$). More important, our trial shows an improvement in the two-year survival rate, which we consider meaningful. Malignant glioma is a disease of the brain beyond the visible local extension. Thus, treatments that target only local disease have inherent limitations. There may be a rationale for future trials that combine treatment with carmustine wafers and temozolomide with radiation therapy. The local administration of carmustine could be a strategy to exhaust the *MGMT* reservoir of the tumor.

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DR. DEANGELIS REPLIES: In the study by Stupp et al., the addition of temozolomide to radiotherapy prolonged the median survival by 2.5 months and increased the 2-year survival rate from 10.4 percent to 26.5 percent. Dr. Aiken says this is old news and an insignificant improvement. However, recent data indicate that many patients with glioblastoma in the United States receive suboptimal standard care; only 54 percent of patients receive adjuvant chemotherapy, regardless of whether they are treated in the community or at an academic institution.[1] Temozolomide is easy to add, has acceptable side effects, and could benefit, although not cure, thousands of patients. Many have called for a new way to treat glioblastoma, but to date, signal-transduction inhibitors, small molecules, and immunotherapy have been disappointing and have not yet led to even the incremental change observed with the addition of the relatively nontoxic temozolomide.[2,3] The study by Stupp et al. and the companion tissue analysis by Hegi et al. suggest that glioblastoma is a tractable problem. This will stimulate further research as investigators realize that we are starting to see the forest — and not just the trees.

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Euthanasia in Severely Ill Newborns

TO THE EDITOR: Verhagen and Sauer (March 10 issue)[1] emphasize that euthanasia is becoming acceptable medical practice for infants in the Netherlands in whom hopeless and unbearable suffering is present. Doctors are not all-knowing, but pediatric palliative care is a dynamic process that remedi-

ates suffering in children through careful assessment and treatment of all symptoms; the quality of life is enhanced, and families are supported.[2]

Access to pediatric palliation is poor, even in countries with first-class medical systems. A study in the Netherlands[3] revealed that the youngest patient receiving palliative care between March 2001 and February 2002 was seven years old. Verhagen and Sauer's conviction that life-ending measures can be acceptable in newborns conflicts with the recommendations Sauer made on behalf of the Confederation of European Specialists in Paediatrics. He and his colleagues invoked the doctrine of double effect and stated that every form of intentional killing should be rejected in pediatrics.[4] Perhaps if he and his patients had better access to palliative care, he might return to his ethical stance of 2001.

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TO THE EDITOR: Verhagen and Sauer observe that all reported cases of euthanasia in newborns in the Netherlands involved infants with severe forms of spina bifida. Mandatory folic acid fortification of flour would have prevented the development of spina bifida in most of these infants. The failure of the Dutch government and that of many other countries to require folic acid fortification remains a tragic policy error.[1] When will European and other governments require this simple, safe, and inexpensive action? Folic acid fortification has been shown in several countries not only to prevent spina bifida, but also virtually to eliminate folate-deficiency anemia and to reduce serum concentrations of homocysteine, with likely reductions in deaths from strokes and heart attacks.[2-4] I encourage all physicians to advocate forcefully for their governments to require folic acid fortification, using the emergency powers and expedited, short review process provided for in public health regulations. These regulations should be invoked to prevent the severe disease and disability that will continue to occur unnecessarily until mandatory folic acid fortification is implemented.

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TO THE EDITOR: When my cousin Jay was born, the doctors said, in so many words, that his diagnosis and prognosis were certain: severe spina bifida, a very poor quality of life, and no hope of improvement.[1] Jay did suffer. He suffered 26 surgeries and all of the indignities that follow from paralysis, incontinence, and bodily disfigurement. Moreover, like the rest of us, Jay never became fully self-sufficient.

Yet Jay bore his suffering with irrepressible hope and good humor that inspired and encouraged innumerable people who had the privilege of knowing him. When he died three days before his 14th birthday, 2000 people attended the funeral to celebrate Jay's uncommonly rich life. A passerby commented, "Someone important must have died."

With different parents, Jay could have qualified for the Groningen protocol. Doctors might have "performed a deliberate life-ending procedure"[1] in Jay after making claims no mortal can sustain[2] — that his prognosis was "certain," and his suffering was "hopeless and unbearable."[1] Those of us who knew Jay are glad there was no such opportunity.

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1. Verhagen E, Sauer PJJ. The Groningen protocol — euthanasia in severely ill newborns. N Engl J Med 2005;352:959-62.
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DRS. VERHAGEN AND SAUER REPLY: We agree with Oakley that folic acid fortification is important. However, it cannot prevent all abnormalities in newborns that cause unbearable suffering.

We cannot comment on Jay's case, described by Curlin, because we did not know him. He suffered, but according to Curlin, the suffering was acceptable. As we noted in our Perspective article, the role of the parents is paramount. Clearly, these parents were supportive, but the question is whether, without these parents, would the suffering have been bearable?

Murphy and Pritchard raise the issue that pediatric palliative care is not always accessible or adequate. They suggest that improvement in palliative care services could lead to a situation in which euthanasia in sick newborns would no longer be practiced. We agree that patients will certainly profit from improved access to palliative care. At the same time, we are convinced that euthanasia in patients with a hopeless prognosis and severe and sustained suffering, waiting for the "ideal" standard of care, can be acceptable. The Groningen protocol was designed to motivate physicians to adhere to the highest standards of decision making and to reduce hidden euthanasia by facilitating reporting. The protocol requires that all possible palliative measures be exhausted before euthanasia is performed. This requirement might do more in mobilizing the availability of palliative care services than the current situation of unreported practice.

The recommendations that Murphy and Pritchard refer to are a consensus statement of pediatricians in Europe.[1] Sauer's personal view is that active life-ending procedures can be acceptable.

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Medical Mystery — The Answer

TO THE EDITOR: The medical mystery in the April 7 issue[1] involved a radiograph (Fig. 1) in a patient who had undergone four lifesaving procedures between 1949 and 2002. The radiograph shows remnants of a therapeutic pneumothorax for pulmonary tuberculosis, a coronary-artery bypass graft, a stent repair of a type B aortic dissection, and a dual-chamber pacemaker for complete atrioventricular block.

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Editor's note: We received 729 responses to this medical mystery, from 68 countries. This is an underestimate of the actual number of people participating, since many responses represent a collaborative effort; for example, one response represented the collective effort of the University of Alabama internal-medicine residents, from their morning report.

Thirty-nine percent of the respondents correctly identified the old right-sided lung collapse used as a treatment for tuberculosis, 64 percent identified median sternotomy for a coronary-artery bypass graft, 77 percent identified the placement of a descending aortic stent, and 93 percent identified the placement of a pacemaker. The group from the University of Alabama was among the 25 percent of respondents who correctly identified all four procedures. Another 40 percent identified three of the four procedures correctly. Other suggested procedures included mastectomy, aortoaxillary bifemoral graft, esophageal repair, and lung transplantation.

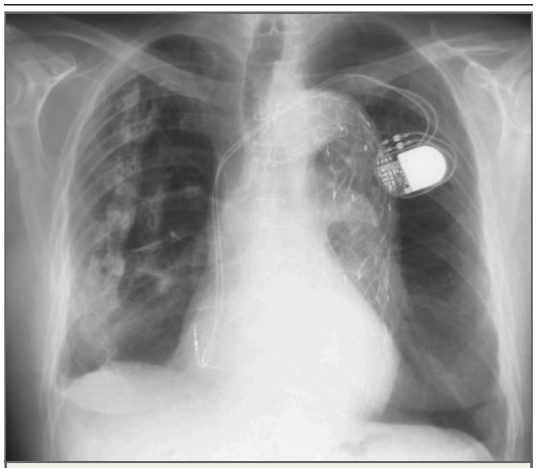

Figure 1. Radiograph in a Patient Who Underwent Four Lifesaving Procedures between 1949 and 2002.

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Transmission of Systemic Transthyretin Amyloidosis by Means of Domino Liver Transplantation

TO THE EDITOR: Familial amyloidotic polyneuropathy is a fatal autosomal dominant disease caused by amyloidogenic genetic variants of transthyretin. The liver is the predominant source of circulating transthyretin, and liver transplantation is the only treatment available for the disease.[1] Livers explanted from patients with familial amyloidotic polyneuropathy contain only microscopic amyloid deposits in hilar vessels and nerves and are otherwise uninvolved. Since 1995, more than 300 such livers removed at transplantation have been used sequentially as donor grafts for recipients with liver cancer or end-stage liver disease, in so-called domino liver transplantation.[2] We report here a case of systemic transthyretin amyloidosis in a 55-year-old man who had received a liver graft eight years earlier by means of domino transplantation from a 32-year-old patient with familial amyloidotic polyneuropathy who had the Val30Met variant of transthyretin.

The recipient had hepatitis C virus cirrhosis complicated by a 9-cm hepatocellular carcinoma, which precluded conventional cadaveric liver transplantation.[3] In 1996, he consented to domino liver transplantation; preexisting amyloid and potentially amyloidogenic disorders were ruled out before surgery.[4] He remained free from tumor recurrence but at eight years reported symptoms of dysesthesias in the lower extremities. Specimens from nerve and rectal biopsies contained transthyretin amyloid deposits as indicated by Congo red dichroism and immunohistochemical staining. In the ensuing six months, overt progressive peripheral neuropathy developed in the recipient, and according to provisions made in our initial counseling, he was listed for and subsequently underwent liver retransplantation in April 2005 with a standard graft from the cadaveric list. He was discharged home two weeks after transplantation and has had a smooth recovery with no complications.

Clinical disease is never present in systemic amyloidosis in the absence of amyloid deposits, and transformation of soluble proteins into amyloid fibrils in vivo is pivotal, but the precise molecular mechanisms are poorly understood. The penetrance of disease varies substantially, and amyloid deposition and symptoms occur in affected persons only in adulthood. Amyloid deposition may also depend on unknown age-related mechanisms that promote amyloid fibrillogenesis. In the present case, the rates at which amyloid appeared and the disease progressed in the recipient were accelerated as compared with the rates in the donor; it is possible that this is related to the recipient's older age or to inoculation with preformed amyloid fibrils that were present in the donor liver, which may be capable of triggering or promoting conversion of the acquired transthyretin variant into the pathogenic conformation of amyloid fibrils.

Subclinical cutaneous transthyretin amyloid deposits were recently reported in five other recipients of domino liver grafts.[5] Although liver donors with familial amyloidotic polyneuropathy remain a valuable resource, our data reinforce the need for additional caution and vigilant long-term monitoring of recipients.

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Correction of Factor XI Deficiency by Liver Transplantation

TO THE EDITOR: Liver transplantation is considered to be an appropriate therapeutic option for hemophilia complicated by cirrhosis.[1] Correction of deficiencies of factor VIII and factor IX by liver transplantation is well known.[2] Factor XI deficiency is a less common form of hemophilia that is associated with prolonged bleeding during surgery, rather than with spontaneous hemorrhage, as is seen in patients with hemophilia A or B. Correction of factor XI deficiency by liver transplantation has not been reported, to our knowledge.

Recently, we performed a liver transplantation in a 48-year-old man with hepatitis C virus (HCV) cirrhosis and hepatocellular carcinoma who had previously been found to have factor XI deficiency. His only known risk factor for HCV infection was a blood transfusion he received as treatment for unexpected bleeding after an inguinal hernia repair 28 years earlier. In the intervening years, he was found to have a prolonged partial-thromboplastin time. Investigation revealed an isolated factor XI deficiency (plasma factor XI level, 0.27 U per milliliter; normal range, 0.5 to 1.5). The levels of factors VII, VIII, and IX were normal. The patient was not of Ashkenazi Jewish ancestry.

In 2002, he was given a diagnosis of HCV cirrhosis. Hepatocellular carcinoma was diagnosed during the preparation for liver transplantation. In April 2004, his liver was removed, and the right side of a split-liver graft from a cadaveric donor was orthotopically transplanted. The procedure was complicated by intraoperative bleeding at the cut margin of the liver, which was brought under control by local measures. Fresh-frozen plasma was used to correct his coagulopathy during surgery. Postoperatively, he had a smooth recovery, and he has subsequently remained well. Factor XI levels returned to the normal range by day 7 after transplantation and have remained normal since that time (Table 1).

Transfer of factor XI deficiency from a donor to the recipient of an orthotopic liver transplant has been described in the *Journal*[3] and confirmed elsewhere.[4] That observation strongly suggests that factor XI production is confined to the liver. However, transfer of a clone of lymphocytes producing factor XI inhibitor with the transplantation was not ruled out.[3,4] Such a mechanism was recently shown to explain the development of acquired hemophilia A in a liver-transplant recipient.[5] Our observation of the correction of factor XI deficiency by liver transplantation supports the hypothesis that this coagulation factor is produced predominantly in the liver.

Table 1. Coagulation Values in a Patient with Factor XI Hemophilia Who Received a Liver Transplant for HCV Cirrhosis and Hepatocellular Carcinoma.*

Index	Normal Range	Value before Transplantation	Value after Transplantation			
			Day 3	Day 10	Day 17	Month 9
Factor VIII (U/ml)	0.5–1.5	1.84	—	—	—	2.3
Factor IX (U/ml)	0.5–1.5	0.56	—	—	—	1.12
Factor XI (U/ml)	0.5–1.5	0.27	0.55	1.17	1.25	1.65
Factor XII (U/ml)	0.5–1.5	0.8	—	—	—	1.63
Activated partial-thromboplastin time	22.4–38.8	42	34	28	30	25.5
International normalized ratio	0.8–1.2	1.4	1.3	1.0	1.0	0.8

* The dashes indicate that the value was not measured.

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INSTRUCTIONS FOR LETTERS TO THE EDITOR

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

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BOOK REVIEWS

PULMONARY CIRCULATION: DISEASES AND THEIR TREATMENT

Second edition. Edited by Andrew J. Peacock and Lewis J. Rubin. 614 pp., illustrated. London, Arnold, 2004. $198.50. ISBN 0-340-80782-2.

THIS SECOND EDITION OF *PULMONARY Circulation* is timely. Nine years have elapsed since its first edition in 1996, years in which we have witnessed such major scientific advances as the description of the human genome (2001) and, directly relevant to the pulmonary circulation, alteration of the gene for bone morphogenetic protein receptor type 2 (*BMPR2*) on chromosome 2q33. Two groups of investigators identified *BMPR2* mutations in about 50 percent of familial cases and 26 percent of sporadic cases of idiopathic pulmonary arterial hypertension at the midpoint (2000) between publication of the first and second editions of *Pulmonary Circulation*. Other notable events that occurred during the period between editions were the development of a new classification of pulmonary hypertension under the sponsorship of the World Health Organization, a revised classification, and new treatments that emerged from large multicenter controlled trials involving patients with pulmonary arterial hypertension — a disease that, just two decades ago, had a mean survival period of less than three years.

The book is a combined effort of investigators heavily invested in the study of the pulmonary circulation from the United States, many parts of Europe, Mexico, Canada, and Australia (the list of contributors is a who's who of pulmonary-hypertension experts in the world). These authors collectively have contributed to a better understanding of the pathogenesis of pulmonary hypertension and to the development of new drug treatments, which lend a "front stage" feeling to the book for the casual reader as well as the expert in the field.

The book's nine sections, with a total of 52 chapters, cover most known aspects of the pathology and physiology of the pulmonary circulation. The orientation is heavily tilted in favor of the diagnosis and treatment of pulmonary arterial hypertension (covered in parts 3, 4, and 6, which represent more than 350 pages), a prototypical disease of the pulmonary circulation and the most common syndrome affecting the pulmonary circulation. The various causes of pulmonary hypertension receive generous and comprehensive coverage. One excellent and concise chapter deals with the genetics of pulmonary hypertension, and the topic is further covered in other chapters. The section on pulmonary arterial hypertension is capped by a masterly overview of the currently available treatments that have emerged from recent large clinical trials, promising emerging drugs, and a rationale for future therapeutic research. This section is a comprehensive and important one for the expert in pulmonary hypertension as well as the general practitioner.

Part 5 elucidates the diagnosis and treatment of chronic (in addition to acute) thromboembolic disease and pulmonary vascular tumors. Both of these conditions, despite their rarity, are deservedly emphasized because there is a surgical treatment that favorably alters the prognosis. The chapters are written by investigators with some of the broadest expertise in these challenging disorders.

Several practical points make the reading of this book enjoyable: the extensive index and reference material, the stand-alone chapter structure, the explicit pathological slides and photographs, and the inventory of key points summarized at the end of each chapter. Up-to-date and practical guidelines are provided for each topic.

This book is at the vanguard of its type in the field of the pulmonary circulation, and it achieves the editors' ambitious goal of providing a balance between scientific review and clinically relevant, comprehensive guidelines for the busy practicing physician. One deficiency is the absence of a historical perspective on pulmonary circulation, the study of which dates back to Ibn Nafis (1210–1288), William Harvey (1578–1657), and more recently, David Dresdale, Peter Harris, and Paul Hamilton Wood, among many others. The late John ("Jack") Reeves (who died unexpectedly this year after a bicycle accident, just after participating in a conference on pulmonary circulation) would have been aptly suited to this task. Short of this, in the foreword to this second edition of *Pulmonary Circulation*, Reeves gives

us an insightful perspective on the breadth of the book and its raison d'être.

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DIFFUSE LUNG DISEASE: A PRACTICAL APPROACH

Edited by Robert P. Baughman, Roland M. du Bois, Joseph P. Lynch, and Athol U. Wells. 294 pp., illustrated. London, Arnold, 2004. $125. ISBN 0-340-81014-9.

THE LUNG IS EXCEPTIONAL IN THAT pathologic changes can be seen clearly in radiographs because of the air–tissue contrast. When bilateral diffuse, ill-defined shadows are seen, one begins to speculate on the cause — infection, inflammation, or neoplasm? This process is one of the most exciting by which chest physicians approach a diagnosis. As a practical guide, *Diffuse Lung Disease* provides current and approved approaches to diffuse lung diseases caused by complex inflammatory mechanisms. The book has two parts — one on general considerations and one on specific diseases, which includes 11 case presentations.

During the creation of a consensus statement on idiopathic pulmonary fibrosis in the late 1990s, the notion was established that the diagnosis of diffuse lung diseases should be collectively interpreted according to the clinical findings (crucial in the recording of patient history), radiologic results (obtained with the use of high-resolution computed tomography [CT]), and pathological findings (from biopsy specimens obtained during thoracoscopic surgery). Consistent with this notion, the book offers plenty of typical high-resolution CT scans and beautiful photomicrographs of pulmonary-biopsy specimens.

In contrast to these diagnostic procedures, the reader may find that the therapeutic approaches to diffuse lung diseases are limited. Conventional immunosuppressive agents are the principal treatments for pulmonary fibrosis, granulomas, or vasculitis, although the effects are not always satisfactory. However, macrolides have been found to be effective in chronic bronchiolitis, and rapamycin may target lymphangioleiomyomatosis. New monoclonal antibodies are promising agents for future clinical trials.

The implications with regard to the causes of diffuse lung diseases are surprisingly broad. For example, the common use of down clothing or bedding should be considered as a possible cause of hypersensitivity pneumonitis and the use of supplements such as vitamins as a possible cause of organizing pneumonia. Although it is not covered in this book, a recent example is the relatively high mortality associated with interstitial lung disease in patients taking gefitinib, a drug used in the treatment of non–small-cell lung cancer.

For a better understanding of diffuse lung diseases, a clarification of the causes, in molecular terms, is needed. One example in this book involves pulmonary alveolar proteinosis. An autoantibody against granulocyte–macrophage colony-stimulating factor has been implicated in the causation of idiopathic pulmonary alveolar proteinosis, and treatment for B-cell diseases thus could be considered for this condition. For the global search for genes related to diffuse lung diseases, precise family histories are indispensable. For this purpose, high-resolution CT scanning in asymptomatic siblings of patients with diffuse or destructive lung diseases could provide crucial information.

This book concisely summarizes our current understanding of diffuse lung diseases and will be a valuable guide to physicians in the examination of bilateral diffuse pulmonary shadows in chest radiographs.

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HANDBOOK OF THE VULNERABLE PLAQUE

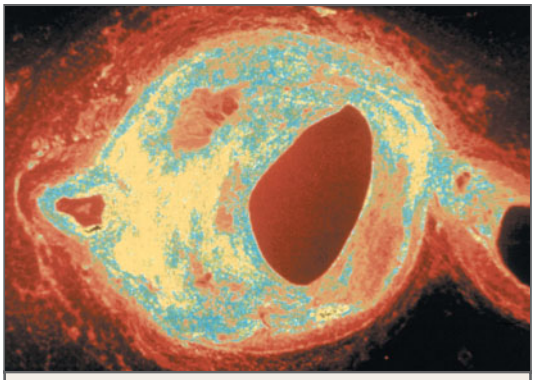
Edited by Ron Waksman and Patrick W. Serruys. 418 pp., illustrated. London, Taylor & Francis, 2004. $69.95. ISBN 1-84184-323-7.

ANGIOGRAPHIC OBSERVATIONS IN THE early 1980s confirmed that acute coronary thrombosis was the proximate cause of acute myocardial infarction — seminal studies that led to revolutionary treatments for the recanalization of oc-

cluded vessels. However, during the subsequent decades, the results of basic and clinical investigation have shown that the coronary clot is predominantly a secondary phenomenon; the true culprit in unstable ischemic heart disease is rupture of the underlying vulnerable atherosclerotic plaque.

Handbook of the Vulnerable Plaque was edited by two distinguished leaders in interventional cardiology, Patrick Serruys and Ron Waksman. They assembled a who's who of authors in the field to write 24 chapters covering the pathology, pathophysiology, detection, and treatment of vulnerable plaque in the coronary vasculature. The introductory chapter, which reviews concepts and definitions, is followed by a superb contribution on the role of platelets in plaque vulnerability. An outstanding chapter on pathology provides the anatomical basis for an understanding of the data from noninvasive and invasive imaging techniques (which constitute the primary focus of the book). There is a very helpful chapter on animal models, and the chapter on genetic determinants is an authoritative and scholarly review of the most fundamental underpinnings of the science. The latter chapter, together with one on treatment paradigms from molecules in peripheral blood, clearly heralds the future of the field, which is destined to continue to evolve from a focus on the vascular lumen and wall to one that is founded on genetic and molecular medicine.

Most of the book is devoted to both noninvasive and invasive techniques for the detection of vulnerable plaque. There are chapters on the established invasive imaging tools of selective coronary angiography and intravascular ultrasonography. New catheter-based techniques (including thermography, optical coherence tomography, near-infrared spectroscopy, and elastography) are considered in separate chapters. Two excellent chapters delve into the noninvasive imaging of vulnerable plaque. The discussion of magnetic resonance imaging is a definitive review of the topic. In a similar vein, the chapter on multislice computed tomographic angiography is an excellent review of the coronary-imaging potential of this noninvasive technique. Given that the vast majority of patients who are at risk for coronary disease or who have clinical symptoms will never make it into a catheterization laboratory for invasive evaluation, the chapters on noninvasive procedures nicely complement those on invasive procedures.



Color-Enhanced Photomicrograph of an Artery with Plaque.

Wellcome Photo Library.

Three chapters consider the treatment of vulnerable plaque, including a fine review of pharmacologic interventions. Two chapters on catheter-based therapies discuss the role of drug-eluting stents (as a "preemptive strike" against plaques that are vulnerable but not flow-limiting) and photodynamic therapy.

My chief criticism of this book is that the chapters on new catheter-based imaging techniques are redundant and that these techniques perhaps receive a disproportionate emphasis. For example, four chapters are devoted to thermography and three others to optical coherence tomography; a future edition (and I hope there is one) might consider one comprehensive chapter on each tool. Additional items I would like to see in a second edition include a comprehensive overview of the pathophysiology of plaque vulnerability and a discussion of vulnerable plaque outside the coronary circulation and its relationship with the instability of coronary plaque.

These minor criticisms aside, *Handbook of the Vulnerable Plaque* is the most comprehensive assessment available in the field. Small in size, it is printed on fine paper, is easy to read, and contains numerous illustrations of imaging scans and pathological specimens that are of the finest quality.

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THE KIDNEY AND HYPERTENSION

Edited by George L. Bakris. 234 pp., illustrated. London, Martin Dunitz, 2004. $125. ISBN 1-84184-270-2.

THIS BOOK PAYS TRIBUTE TO THE ADAGE "Hypertension goes with the kidney" by examining the relationship between renal function and blood pressure, from a perspective that is primarily clinical and therapeutic but is also grounded in epidemiology and pathophysiology.

The first of the book's three sections discusses such topics as the methodology of blood-pressure measurement, the differential diagnosis and prognostic significance of microalbuminuria in patients with and without diabetes mellitus, and isolated systolic hypertension, which is an increasingly important clinical problem in societies with rapidly growing elderly populations. One chapter is devoted to a discussion about how and when to evaluate patients who have elevated blood pressure for secondary hypertension. Since renal-artery stenosis is by far the most common cause of secondary hypertension, it receives more attention than endocrine-based causes of the disorder.

The second section of the book focuses on the treatment of hypertension in the general context of cardiovascular risk factors in patients with chronic renal disease. Angiotensin-converting–enzyme (ACE) inhibitors and angiotensin-receptor blockers are particularly important in this context because of their well-documented potential to reduce cardiovascular risk and afford nephroprotection by diminishing glomerular hyperfiltration. The pharmacologic actions of these drugs are presented in detail. The treatment of dyslipidemia in patients with kidney disease is discussed in a separate chapter, followed by chapters on general considerations of drug dosage in renal failure and on the treatment of hypertensive "urgencies" (which the authors differentiate from true hypertensive emergencies requiring immediate intervention).

The third section of the book takes up the treatment of hypertension in certain populations. Since diabetes mellitus is an important cardiovascular and renal risk factor, the disease is discussed in considerable detail, especially in connection with the use of ACE inhibitors and angiotensin-receptor blockers. The rationale for the preferential use of these drugs in patients with diabetes is clearly presented, with an overview of the recent literature. Other chapters specifically address the problems of hypertension in black and Asian patients and during pregnancy. In view of the complexity of the latter topic, including considerations of the placental barrier in antihypertensive-drug treatment, the authors succeed in giving a very informative overview.

The Kidney and Hypertension is well referenced, and though there is some inevitable redundancy of information in a multiauthored work, it does not distract from the book's usefulness. Internists and general practitioners who treat patients with renal and cardiovascular diseases will find the book highly informative. For medical students, the information may be somewhat too specialized. Nephrologists, however, might wish for specific topics to be discussed in greater depth. The book may be particularly useful for house staff in internal medicine and fellows in renal and cardiovascular studies because it provides an up-to-date review of a very important topic.

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CORRECTIONS

Methicillin-Resistant *Staphylococcus aureus* Disease in Three Communities (April 7, 2005;352:1436-44). In the Abstract on page 1436, the Methods and Results sections should have referred to "community-associated" infection, rather than "community-acquired" infection, as printed. We regret the error.

Prevention of Hepatitis B with the Hepatitis B Vaccine (December 30, 2004;351:2832-8). The brand name Pediarix (rather than Pediatrix) should have appeared in the right-hand column of page 2833, in lines 3 to 4 under the heading Hepatitis B Vaccine, and on page 2834 in the third footnote to Table 1. In the same footnote, the dosing schedule should have read "2, 4, and 6 months," rather than "0, 1, and 6 months," as printed. Also, on page 2837, line 12 of the right-hand column should have referenced Table 2, rather than Table 1, as printed.

Modification of Human Hearing Loss by Plasma-Membrane Calcium Pump PMCA2 (April 14, 2005;352:1557-64). On page 1557, lines 7 through 9 of the Summary should have read, "V586M was detected in two unrelated persons with increased sensorineural hearing loss, caused by a mutation in *MY06* (which encodes myosin VI) in one and by noise exposure in the other . . .," rather than "V586M was detected in two unrelated persons with increased sensorineural hearing loss, in the other caused by a mutation in *MY06* (which encodes myosin VI) in one and by noise exposure . . .," as printed. We regret the error.

Outcomes Associated with a Trial of Labor after Prior Cesarean Delivery (April 21, 2005;352:1718-20). In the letter by Smith, lines 18 through 20 of the first paragraph should have read, ". . . the numbers of infants who had a five-minute Apgar score of less than 4 and survived the neonatal period," rather than "the numbers of infants who died," as printed. We regret the error.