That set the stage for the Boston breast cancer conference, which was held during the last week of April. Although NBSS researchers did not present their data there, Kopans and Feig were freely detailing what they see as the study's unforgivable flaws. Meanwhile, Associated Press reporter Daniel Haney quoted Samuel Shapiro, a professor emeritus at Johns Hopkins University School of Hygiene and Public Health and an NBSS scientific adviser, as saying that the study had found a "differential in that direction [higher mortality]." And finally, the American College of Radiology topped things off by issuing its press release. The result: The story broke into the U.S. press in a big way, with numerous stories raising the issue of mammography's safety.

No one was more shocked by the news accounts than Miller and Baines, who say that in the year between the Cambridge and Boston meetings, they and their colleagues had collected and analyzed additional data, which made it clear that mortality of the younger women who had mammograms was not significantly higher than that of the controls. They also didn't find any benefit of mammography in women older than 50, however—a surprising finding in view of the several previous studies showing that it does save lives in that age group. Feig, for one, says that this proves that the Canadian study is flawed.

Although Miller isn't conceding any flaws in the NBSS design, he does say that the study probably doesn't give the definitive word on mammography's potential value to women under 50. "No study has found a benefit in younger women within a follow-up period of up to 10 years, and that's as far as we've been able to go," he explains, adding that the women might have to be followed for 15 to 20 years to see an effect.

Since Miller and Baines are now in the final stages of preparing the manuscript of the paper describing the NBSS results for submission to the *Canadian Medical Association Journal*, it's unlikely to appear before the end of the year. It will be eagerly awaited. "NCI is champing at the bit to evaluate the Canadians' final results," says Edward Sondik, deputy director of the institute's division of cancer prevention and control.

And while it's still too early to say whether the paper will change any minds about recommendations for mammograms in the younger age group, it might if researchers find the results persuasive. Indeed, Kopan's fears that the NCI will backtrack on its recommendations may not be unfounded. The institute will be "evaluating all available data," Sondik says. "But when you have that many women in a study, it will certainly weigh heavily."

–Malorye Allison

Malorye Allison is a free-lance writer based in Boston.

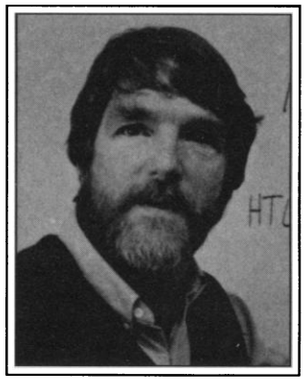
AIDS

CDC Closes the Case of The Florida Dentist

For almost 2 years, it has been a medical horror story that has captivated the nation—even the world. Nearly everyone who reads newspapers figured they knew the perpetrator: Florida dentist David Acer, who had apparently infected Kimberly Bergalis and four other patients with the AIDS virus, and one after another they were coming down with the disease. But, while there has been strong epidemiological evidence supporting that conclusion, proving it—either to the satisfaction of the scientific community or to a jury—has been extraordinarily difficult.

"The evidence strongly suggests that Acer was the proximal source for each of the [five patients'] infections."

–Gerald Myers



Now, scientists at the Centers for Disease Control (CDC) in Atlanta, where the case has been dissected, say the proof is there. Two papers, one in this issue of *Science* (p. 1165) and another that appeared last week in the *Annals of Internal Medicine* (15 May, p. 798) present a full accounting of the evidence that leads to the ineluctable conclusion that Acer infected Bergalis and the other four patients.

CDC researchers conducted a rigorous epidemiological study of the apparent transmission of the virus in Acer's dental practice and they employed the latest techniques of molecular analysis to nail down the proof. Along the way, the evidence was challenged in a law suit and became the focus of a bitter scientific dispute. And even now, some researchers are not entirely convinced that CDC has a watertight case. "We're not trying to say in any way that these guys' answer isn't correct," says physicist-turned-molecular biologist Temple Smith of Boston University, who along with mathematician Michael Waterman of the University of Southern California in Los Angeles has written a Perspective on the CDC paper in this issue of *Science* (see page 1155). "But probably correct is not the kind of statement that should end up in a courtroom."

Moreover, even as CDC closes the book on one aspect of the Acer case, another remains unresolved: How did Acer infect his patients? That mystery may never be solved. Yet, frightening as that may seem, the Acer case has, ironically, brought some reassurance about the risks of doctor-to-patient transmission of HIV. Since the case first broke, CDC has been conducting a major study of patients known to have been treated by HIV-infected health care workers. The results, published just last week in CDC's *Morbidity and Mortality Weekly Report (MMWR)*, show that of 15,795 patients in 32 practices, only 84 patients were HIV positive, and there was not a single confirmed case of health care worker to patient transmission.

CDC began investigating the dental practice of David Acer in 1990, when Florida health officials reported to CDC the case of Kimberly Bergalis, a young woman in her 20s with AIDS who had no identified risk factors for the disease. Based on epidemiological evidence and sketchy molecular analysis, CDC published an article in *MMWR* on 27 July 1990 raising the possibility that Acer had infected Bergalis. It was a shocking suggestion, for doctor-to-patient transmission of HIV had never been seen before. CDC, bowing to public panic, announced it would try to compile a list of procedures health care workers should not perform. (No such list has appeared, although revised guidelines about what precautions infected health care workers should take when treating patients are pending.) And there was worse to come: Seven of Acer's other patients were subsequently found to be HIV positive. Although CDC epidemiologists determined that three of them had engaged in behaviors that would put them at risk for contracting AIDS from sources other than Acer, the dentist seemed the most likely source of the virus that infected the other four. All had visited Acer on more than one occasion after the dentist had been diagnosed with AIDS, and all had had invasive procedures.

A clear case of poor hygiene in the dentist's office? Apparently not: CDC could identify no specific lapse in the dentist's procedures that would suggest how he had infected his

patients. Acer and his staff had been routinely wearing a mask and gloves when treating patients since 1987, and instruments were either autoclaved or dipped in antiseptic solution after use—both techniques that effectively kill the virus.

The mystery convinced CDC to bring state-of-the-art molecular analysis to bear on the individual viruses infecting Acer and his patients, to see whether they were really the same strain. At that point, CDC researchers were still far from convinced that Acer had infected his patients: "The laboratory work was done with the thought of disproving the epidemiologic suggestion," says Harold Jaffe, deputy director of the division of HIV/AIDS at CDC.

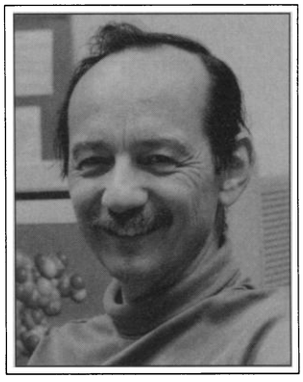
Molecular sleuthing. With the help of Gerald Myers, who has assembled an HIV sequence database at Los Alamos, CDC scientists began 2 years ago to examine the virus from Acer, his patients, and 35 AIDS patients who attended two HIV clinics located within 90 miles of Acer's practice. They looked at the DNA sequences of the gene that codes for the outer envelope virus. In particular, they used the polymerase chain reaction (PCR) to amplify a section of the envelope gene knows as V3, a highly variable region thought to be crucial for immune recognition.

First, using multiple clones of the patients' and dentist's virus, they showed that the viruses infecting the five patients, including Bergalis, who had no identifiable risk factors, were more closely related to each other than to viruses isolated from two of the three other infected patients (the third patient was discovered too late to be included in the analysis) and from all 35 local controls. And finally, using techniques developed by Myers, the CDC-Los Alamos team did what they call a signature analysis of the viral sequences: They compared the amino acid sequences in the V3 region among all the viruses they collected, once again showing that virus from the dentist and his five patients seemed to group together, separate from the rest of the viral pool.

When CDC published the preliminary results of this analysis in *MMWR* on 14 June last year, the evidence appeared overwhelming. "We were fully expecting that the sequences we got from [Bergalis] would be different from the dentist. We were amazed and disturbed that they were so similar," says the CDC's Jaffe.

Similar, yes. But was the likeness sufficiently strong to prove beyond reasonable doubt that Acer had infected his patients? A lawsuit brought by Richard Driskill, the third patient from Acer's practice found to be infected with HIV, quickly thrust scientific doubts about CDC's elaborate molecular techniques into a bruising public debate. Driskill sued Acer's insurers for $15 million in damages.

CIGNA Dental Health of Florida, the company that provided Acer's services, hired Lionel Resnick, chief of the retrovirology laboratory at Mount Sinai Hospital in Miami, to conduct his own analysis of the molecular evidence. In addition to collecting samples from some three dozen additional controls, Resnick used the Freedom of Information Act to compel CDC to turn over the sequence information it had already collected on Acer and his patients. Resnick then recruited Lawrence G. Abele, dean of arts and sciences at Florida State University, whose research focuses on molecular evolution, to help him with the project. Abele says he was not aware that Resnick's association was with CIGNA. Based on their analysis, Abele concluded that the CDC-Los Alamos team was not looking at a representative population when it reached its conclusions (*Science*, 24 January, p. 392).



Myers, who has seen the data set on which Resnick and Abele based their analysis, claims it is riddled with errors. Moreover, he points out that 2 days after he submitted his critique of the Resnick data to the lawyers in the case, CIGNA agreed to settle out of court for an undisclosed sum. Abele says that their analysis of the local control population is valid, and he is planning to submit it for publication.

"Probably correct is not the kind of statement that should end up in a courtroom."

–Temple Smith

Myers and his colleagues have since refined their analysis and the data are published in detail on page 1165 of this issue for the first time. "The evidence strongly suggests that Acer was the proximal source for each of the [five patients'] infections," says Myers, pointing in particular to the fact that all the patients had at least one virus with a signature pattern that perfectly matched the dentist's—and in some cases shared an unusual pattern rarely seen among HIV variants. Taking the molecular and epidemiological data together, Jaffe says CDC is now convinced that the dentist is the most likely source of the patients' infection.

Few researchers would disagree with these carefully worded statements. But, as the perspective on page 1155 indicates, some critics are not yet convinced that, even after 2 years of study, Myers and his colleagues have completely nailed down the case against Acer. They are particularly critical of the way control viruses were sampled.

Every patient infected with the AIDS virus does not have a single virus in his or her body, but rather a "swarm" of viruses, differing from each other by at least a few DNA base pairs. The best way to characterize the variability in an individual is to take an infected person's white blood cells, remove the DNA, and then put the DNA into multiple clones, hoping that each clone will represent one of the viral variants. That's what the CDC-Los Alamos researchers did for Acer and his patients. But in the case of the local controls, they skipped the cloning step, and did PCR directly on the DNA isolated from the white blood cells. And that, say critics, is a problem—potentially a big one.

"It's not well understood whether PCR gives you an amplification of a single virus," says Boston University's Smith, "or whether it gives you an amplification of some statistical average over some subset of the virus." According to Smith, this means it's not at all certain that the comparisons between patients and controls are representative.

Steven Wolinsky of Northwestern University agrees. Wolinsky, who has been studying HIV variation in mother-infant pairs, has shown that only through multiple cloning can you get an adequate picture of the background variability of the virus within an individual. Both Wolinsky and Smith say they are impressed by the signature analysis of the amino acid sequences from the viral envelope that Myers conducted for CDC, but both worry that without a better picture of the control population, the conclusions based on that analysis are limited.

Wolinsky and Smith also argue that the DNA fragments Myers and his colleagues analyzed were too short to contain sufficient variable sites to construct a robust phylogenetic tree. Myers agrees that longer sequences would be far more informative, but he says the analysis would have taken more time and energy than was practical.

Like the scientific criticisms of DNA fingerprinting, these critiques may simply reflect problems in employing a complicated new technique. Indeed, Myers predicts that in a few years, when the variation of HIV in the general population grows, it will be easier to tell whether one individual transmitted his or her characteristic viral "swarm" to someone else.

For now, however, CDC is satisfied it knows what happened in Acer's practice—even if it doesn't exactly know how.

–Joseph Palca