How viral infections fit into myocarditis

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When Rene Rodriguez, MD, makes the diagnosis of myocarditis, he's frequently sitting at the microscope with the cardiologist next to him. That's because diagnosing myocarditis on an endomyocardial biopsy specimen "has a lot to do with communication between pathologist and cardiologist," says Dr. Rodriguez, staff pathologist in the Laboratory of Cardiovascular Pathology at the Cleveland Clinic Foundation. The cardiologist provides the clinical signs and symptoms, while the pathologist acquires evidence of inflammation of myocardial tissue.

In a major advance in the diagnosis of myocarditis, this collaboration may soon become triangular, with essential data on the presence of viral infection of heart muscle coming from molecular tests performed by the virology laboratory on the endomyocardial biopsy, or EMB, specimen.

Unfortunately, collaboration between pathologist and cardiologist is more the exception now than the rule in diagnosing patients in whom myocarditis, particularly of viral origin, is suspected as the cause of idiopathic dilated cardiomyopathy, or DCM. For there is a sort of cultural divide between these two specialties. Cardiologists are from Rome—practical, action-oriented clinicians. The typical cardiologist asks, "Why should I order an EMB or a PCR for viruses if it is not going to change my management or treatment plan? "Most clinicians are convinced that the procedure does not provide adequate benefit relative to its complexity and invasiveness," says Eloisa Arbustini, MD, director of the transplant research area and chief of molecular diagnostic cardiovascular and transplant pathology at the Research and Care Hospital San Matteo in Pavia, Italy.

Pathologists, on the other hand, tend to be from Greece, wanting to satisfy their intellectual curiosity about the patient's illness and to understand its cause. "When you read the literature from the best centers in the U.S. and Europe," Dr. Rodriguez says, "where the cardiologists are familiar with [EMB] and do it right, about 20 to 30 percent of biopsies turn out positive for something you can identify. So I see the glass as 30 percent full rather than 70 percent empty."

With regard to viral myocarditis specifically, "Identifying the virus at this point doesn't really help direct treatment," says Debra Kearney, MD, assistant professor of pathology, pediatrics, and medicine at Baylor College of Medicine and Texas Children's Hospital, Houston. Still, she says, biopsy does provide more information about the pathophysiology of myocarditis. "As more research studies are done trying to develop strategies for treating viral infections, if we are able to identify a specific viral etiology, that can help direct future work," she says.

For those who are trying to prove that a substantial fraction of myocarditis has a viral etiology, this attitudinal dichotomy has created the very epitome and model par excellence of a Catch-22. Most clinicians don't want to do endomyocardial biopsies unless there is a treatment for whatever is discovered. But without obtaining biopsy tissue on which to do polymerase chain reaction assays for viruses, it has been difficult to prove that viral myocarditis is as important a cause of DCM as many people believe it is. For the same reason, clarifying the clinical course of viral myocarditis and defining which patient population could benefit most from antiviral treatment has also proved challenging. In the absence of this information, conducting meaningful therapeutic trials has remained out of the question.

Fortunately, over the past 10 to 15 years a few groups in the U.S. and Europe have put substantial efforts into resolving these questions by performing EMBs on thousands of children and adults (either DCM patients or heart transplant recipients) and assaying the biopsies for viruses by PCR. From these efforts evidence is emerging that, as Dr. Arbustini says, "viral etiology is very important in myocarditis." Evidence of viral infection of myocardium, along with inflammation, can be found in some cases of nonischemic, non-familial DCM. In addition, persistence of viral infection has been associated with chronic heart damage. "It's pretty clear-cut, in our view, that having viral genome in the myocardium is bad for you," says cardiologist Jeffrey Towbin, MD, professor of pediatrics and director of the molecular cardiology laboratory at Texas Children's Hospital and Baylor College of Medicine, Houston, who has led much of this work. "It portends a series of things requiring therapy that may turn into a chronic long-term disease process."

Based on these findings, a German group has conducted small phase two trials treating (with interferon-beta) patients with viral persistence in the myocardium as detected by PCR. In most patients, this therapy has enabled the host immune system to eliminate the virus (negative PCR), with a resultant improvement in cardiac function. A worldwide phase three trial of IFN-β is now being planned.

Trying to determine the incidence and impact of viral myocarditis has not been easy. Even clinical research teams willing to do biopsies and having the laboratory capability to detect relevant viruses by molecular methods have first had to answer a riddle: What does the clinical picture of myocarditis look like? It turns out that myocarditis is like the Sphinx: It clinically looks like a lot of different things. "Myocarditis is insidious, with a varied clinical phenotype and no specific cardinal symptoms," says Antonello Gavazzi, MD, ESC, director of cardiology, Cardiovascular Department, Ospedali Riuniti, Bergamo, Italy. Myocarditis often presents with a flu-like syndrome, but many patients are asymptomatic except for ECG abnormalities. At the other extreme are patients with signs and symptoms due to heart failure, some-

times even fulminant heart failure with severe left ventricular dysfunction.

Dr. Gavazzi also notes at least seven publications on myocarditis mimicking acute myocardial infarction. In one recent paper, 24 consecutive patients presenting as AMI with ECG abnormalities and elevated troponin T levels but with normal coronary angiograms were biopsied. Viruses were found in 71 percent of the biopsies, mostly echoviruses, parvovirus B19, and adenovirus (Kuhl U, et al. *Circulation*. 2003;108:945–950). He says, "We should suspect myocarditis in young people with clinical and ECG evidence of AMI when risk factors are absent and coronary angiography is normal."

In Dr. Rodriguez's view, "Any patient who comes down with heart failure of unexplained causes, particularly younger persons, if there is no evidence for coronary disease or anything in the valves that explains the condition, I think biopsy is warranted." Even though there is not yet a specific therapy for myocarditis, demonstrating its presence can rule out familial cardiomyopathy, which presents with similar symptoms. "In many cases patients have family members who died young and suddenly or had heart failure at an early age—20 to 50 years," he says. "And they did not have a reason for it based on coronary arteries or valves. If you have a biopsy that shows the patient has an inflammatory process, that by itself tells the cardiologist what the problem is, and they can tell the patient and family that it's unlikely that there are hereditary implications."

It is the prevailing opinion that viral myocarditis can proceed to cardiac dysfunction through three phases—infection, immune reaction, and DCM (Liu PP, Mason JW. *Circulation*. 2001;104: 1076–1082). "If the patient lives through the initial infection, he can end up with heart failure or dilated cardiomyopathy," says John Veinot, MD, cardiac pathologist at Ottawa Hospital and professor of pathology at the University of Ottawa. "We think some patients don't clear the virus and have ongoing damage. We also think that, even if the initial infection is cleared, you can get a type of secondary autoimmune response provoked by the virus that leads to cardiac damage." Many articles document autoantibodies in the sera of patients with inflammatory heart muscle disease, such as a cross-reaction between viral proteins and myosin. Dr. Veinot notes that distinguishing viral persistence from immune activation may become important, since the first might require an antiviral drug and the latter may benefit from immunotherapy. Endomyocardial biopsy is the obvious way to discriminate these two etiologies.

However, even when EMB is done, interpreting the specimen may not be straightforward. Basically the pathologist is looking for signs of inflammation of heart muscle. A commonly accepted system for making this determination is the Dallas criteria (Aretz HT, et al. *Am J Cardiovasc Pathol.* 1987;1:3–14). However, Dr. Gavazzi notes that this system has problems. He points out that, in the article in which 71 percent of 24 biopsies from patients with apparent AMI were positive for virus, only one of the 24 biopsies was positive for inflammation by the Dallas criteria.

In another study that Dr. Gavazzi says shows the inadequacy of the Dallas criteria, investigators studied 84 patients with DCM who were positive on EMB for an indicator of immune upregulation (HLA-DR), suggesting inflammation. Only seven (eight percent) of these patients were positive for myocarditis by the Dallas criteria. Fully 73 percent were negative, while 16 (19 percent) showed borderline myocarditis (Wojnicz R, et al. *Circulation*. 2001;104:39–45).

Dr. Arbustini says the Dallas criteria are "still relevant but need a critical approach."

"I have said that an active myocarditis can be diagnosed by any experienced pathologist," she notes.

Dr. Kearney believes that the Dallas criteria, which require the presence of myocardial inflammation and an effect of that inflammation on myocytes, are the current standard for diagnosing myocarditis. Myocyte degeneration and possible necrosis are the typical indicators of inflammatory damage. "Often the lymphocytes are in close apposition to the edge of individual myocytes, which may have a moth-eaten edge," Dr. Kearney says. "Also, lymphocytes overlie the myocytes. And you may see splitting and fraying of myocytes with lymphocytes seeming to lie between frayed segments."

The positivity rate of biopsies can be increased by taking an adequate number of samples and paying attention to the timing. In a 1989 autopsy study of 38 cases of known lymphomyocarditis, using a bioptome to take 10 slices per case from each ventricle, William Edwards, MD, and colleagues of the Mayo Clinic found a 55 percent positive rate for the left ventricle and a 63 percent positive rate for the right ventricle (Hauck AJ, et al. *Mayo Clin Proc.* 1989;64:1235–1245). Only about 20 percent of all slices were positive.

Sampling errors are a real problem, Dr. Rodriguez agrees, since myocarditis is a focal entity. In the Mayo study, he says, after the fifth slice there was not much difference in the statistical likelihood of getting a positive result. Even so, he has found, "some cardiologists take a lot more. At one place where I worked, the cardiologists took biopsies with 10 samples and occasionally I saw a biopsy with only one of 10 samples having inflammation, though that was the exception." It is generally accepted that even one slice with inflammation defines a positive result.

Dr. Arbustini says the number of samples per biopsy must be at least five; she likes to see eight.

An editorial on the value of EMB findings was published earlier this year by two cardiologists from the Cleveland Clinic. Titled "Endomyocardial Biopsy: A Procedure in Search of an Indication," it has already become well known among cardiovascular pathologists. The authors calculated Bayesian probabilities for results of EMB using accepted true- and false-positive rates. The study supports two conclusions. First, if the pretest probability of myocarditis is greater than 60 percent, a negative biopsy cannot exclude the clinically likely diagnosis. (More strictly, it cannot decrease the likely diag-

nosis to less than 50 percent.) Second, if an 85 percent posttest likelihood that the diagnosis is correct is accepted as a reasonable threshold for certainty, then a positive biopsy cannot achieve this criterion unless the pretest likelihood of myocarditis is at least 30 percent. That is, a positive biopsy does not establish a clinically unlikely diagnosis (Mills RM, Lauer MS. *Am Heart J*. 2004;147:759–760).

As for the timing of EMBs, the earlier the better. In one case of a patient with acute Coxsackie B virus-related myocarditis and DCM, Dr. Arbustini received five EMBs from day two to day 63 from onset. (The patient, who was maintained on a left ventricular assist device, died of cerebral hemorrhage on day 64.) Acute inflammation and early healing were seen in biopsies from days two to 36. In the biopsies from days 50 and 64, active inflammation and myocyte necrosis were absent. "Already by day 36 there was healing," Dr. Arbustini says. She concludes, "Two months from onset is clinically early, but not histologically early."

In addition to conventional histopathology, diagnosis of myocarditis is aided by immunohistology. Dr. Arbustini uses "a mini-panel of immunophenotyping and activating markers," including CD20, CD45RO, CD68, T and B lymphocytes, macrophages, and HLA DR. She advises caution with this last stain. "It frequently gives overinterpretation," she warns.

Now that virus infection has been demonstrated to be a major cause of myocarditis, Dr. Arbustini has added real-time PCR for viruses to her menu of analytical procedures. "It gives both the presence and quantitation of viruses," she says.

Even though EMB offers considerable valuable information, many cardiologists are reluctant to do it because of a perceived danger of perforation or tamponade. They wonder whether the benefit is worth the risk. "Cardiologists without extensive experience walk away from that dilemma," Dr. Rodriguez says. "But I have worked in centers where cardiologists are well trained to assess the entire patient, know the risk, and are experienced at doing biopsy. And in those places morbidity and mortality related to biopsy are very, very low."

Dr. Towbin has been at the forefront of research showing the presence and importance of viral myocarditis based on PCR analysis of EMBs. "Our biopsies are done by the people in our interventional catheterization group or our transplant group," he says. "That's key, especially in pediatrics. I can't remember the last time we had a perforation."

Another cardiologist who has also greatly advanced this area, Peter Schultheiss, MD, of Berlin, agrees with this assessment. "During the last 10 years we have done 300,000 biopsies, and we have had no complications except some effusion," says Dr. Schultheiss, who is director of the Medicine Clinic II, Department of Cardiology, University Hospital Benjamin Franklin.

Unwillingness to do EMB may have complicated and confounded interpretation of immunosuppression trials for myocarditis (Frustaci A, et al. *Circulation*. 2003;107:857–863). If immunosuppression is beneficial for nonviral myocarditis due to autoimmune diseases, for instance, but harmful for viral myocarditis, then EMB needs to be done to select appropriate patients. "Trials looking at immunosuppression of myocarditis have been largely negative," Dr. Veinot says. "However, patients in those trials have been quite a mixed bag. Some probably had active or persistent viral infections and some probably had immune activation. There previously has been little attempt to make a distinction between groups, so the overall trial comes out negative." As a result, he adds, "That is what clinicians 'know'—you can't do anything for myocarditis, except supportive measures, so why biopsy?" Even some ongoing contemporary trials have this flaw, he notes.

Endomyocardial biopsy is acquiring new importance with accumulating evidence that viral myocarditis is common and prognostically important and that it can be accurately detected by molecular assays. Some of the earliest work in this area was done by Neil Bowles, PhD, for his doctoral thesis at the Charing Cross and Westminster Medical School, London. Dr. Bowles is now assistant professor of pediatrics and associate director of cardiac genetics research at Baylor College of Medicine, Houston. When Dr. Bowles entered the field, extant data on the viral relation to myocarditis was based on serology. With many of the viruses implicated in myocarditis being common pathogens, such as the enterovirus Coxsackie B, simply finding antibodies to the virus in the blood of a person with myocarditis wasn't convincing.

"We investigated the role of Coxsackie B virus in myocarditis at the advent of molecular biology," says Dr. Bowles. Because such tests were not routine, a major part of his doctoral project was to clone a fragment of the Coxsackie B virus genome. Then he used the fragment to screen for the genome in EMB samples from patients with myocarditis and DCM. "We found evidence for the Coxsackie B virus genome in a significant proportion of patients, between 20 percent and 40 percent," Dr. Bowles says. In fact, as he points out, since the probe he used is found in the genome of several enteroviruses, this work only showed the presence of enteroviruses in myocardium (Bowles NE, et al. *Lancet*. 1986;1:1120–1123; Archard LC, et al. *Biochem Soc Symp*. 1987;53:51–62).

A few years later Dr. Bowles found himself working with Dr. Towbin, and their studies showed adenovirus to be more common than enteroviruses in EMB samples from myocarditis patients. "Our results met a certain amount of skepticism," Dr. Bowles says. "So we collaborated with other groups." Working with Dr. Schultheiss' group, they found PCR evidence for adenovirus in 12 of 94 EMBs from patients with idiopathic left ventricular dysfunction; enterovirus RNA was found in another 12 samples. None of the 14 control samples showed evidence of either virus (Pauschinger M, et al. *Circulation*. 1999;99:1348–1354).

"Now most groups also find adenovirus in a significant proportion of cases," Dr. Bowles says. The hypothesis that adenovirus was important was reinforced in the mid- to late-1990s when the same receptor was found to be used by Coxsackie B virus and adenovirus. It is now called the Coxsackie B virus-adenovirus receptor and has been found on myocytes, where it binds both viruses and mediates uptake into the myocardium.

Based on their findings, Drs. Towbin and Bowles set up a panel of viruses for screening—adenovirus, cytomegalovirus, Epstein-Barr virus, parvovirus, influenza virus A, respiratory syncytial virus, and herpesviruses 1 and 2. Initial results verified that enteroviruses (Coxsackie and ECHO) and adenovirus are a common cause of myocarditis. "Over the next seven to eight years we used this panel to screen EMBs from about 600 patients, mostly children but many adults," Dr. Bowles says (Bowles NE. *J Am Coll Cardiol*. 2003;42: 466–472). Results continued to be positive for enteroviruses and adenovirus but largely negative for other viruses.

Finding virus genomes in EMBs from myocarditis patients raised an obvious question, Dr. Towbin says: How do you know if there is a cause-and-effect relationship? One good approach to answering that question is to look at a large number of control samples, chiefly autopsy and surgical samples from people without myocarditis. "We found essentially that never do you see viral genome in the heart of a control patient," Dr. Towbin says. "In the occasional patient that we found virus in who was uncertain clinically, it turned into myocardial disease at a shortly later date."

On the basis of that information, Dr. Towbin decided to look not only at patients with presumed viral myocarditis, but also to extend their work to patients with DCM. "There was speculation that some cases of dilated cardiomyopathy resulted from burnt-out myocarditis that did not resolve successfully," he says. They found that about 20 percent of DCM cases had virus in the myocardium.

"If you were a reasonable reviewer," Dr. Towbin continues, "at that point you would say, 'Prove it to me.' We tried to figure out a way to demonstrate a cause-and-effect relationship in specific individuals." Their strategy was to look at heart transplant recipients. They could study transplant recipients in a serial way, since such patients get biopsies on a routine basis for surveillance to make sure they are not rejecting. "If you are a cardiovascular pathologist and you look under the microscope at a biopsy sample, you can't tell the difference between viral infection and chronic rejection," Dr. Towbin says. So the pathologist called rejection and the laboratory called virus infection. Results showed that, if the patient had virus in the myocardium by PCR, the pathologist diagnosed rejection and the patient had shortened survival. "If you look at a five-year Kaplan-Meier survival curve," Dr. Towbin says, "patients who were never positive for viral infection had actuarial five-year survival of about 96 percent. That is pretty darned good." Patients who had a positive PCR result at any time had about 65 percent five-year survival, a highly significant difference (Shirali GS, et al. *N Engl J Med*. 2001;344: 1498–1503).

"Everyone talks about cardiac rejection," Dr. Towbin says, "but it is not all that frequent. What kills you, at least in the pediatric heart transplant program, is that you develop transplant coronary artery disease. And at least in pediatric patients, transplant coronary disease is a result of viral infection. We believe that is true in adults as well." Work on viruses as a cause of adult rejection is underway.

Over the past 10 years data show a change in the common viruses causing myocardial disease. "Today other viruses are as frequent as Coxsackie B or adenovirus that were not important players five years ago," Dr. Towbin says. "We know that because we were doing the same studies five years ago using the same viral primers and these viruses were not there five years ago." For example, parvovirus B19 has become common, both in adults and in children and in the U.S. and Europe. Cytomegalovirus and Epstein-Barr virus are also occasionally found. Recent research has provided a logical basis for finding EBV in myocarditis: A group in Italy has just found that EBV is capable of infecting cardiomyocytes, so it may not merely be a passenger in white blood cells circulating through the heart.

At this point, Dr. Bowles says, "We didn't think we would get much more information from viral screening alone as a research tool, so in December 2001 we set it up on a fee-for-service basis offered by the John Welsh Cardiovascular Diagnostic Laboratory" (directed by Karla Bowles, PhD). Screening is done mostly for heart transplant patients and for those with myocarditis and DCM. They also receive samples from obstetrics. "A few years ago we found evidence of viral infection in fetuses who had heart disease," Dr. Bowles says. "Now a number of obstetrician-gynecologists send us samples for screening from fetuses whose hearts look abnormal on ultrasound, for instance." Samples are run weekly on Friday with results sent out the following Tuesday. "Assuming business continues to increase, we will probably introduce twice-weekly runs during the next year or so," Dr. Bowles says. "We are also going to start doing real-time PCR to get an idea of how many viral genomes we are detecting." Adds Dr. Towbin, "We think viral screening is an important part of the diagnostic and potentially the therapeutic process. Certainly if you do a biopsy and don't do PCR you're not getting all the answers."

In Germany, Dr. Schultheiss and his colleagues were working along a parallel track. They wanted to know not only whether enteroviruses could be detected in EMBs from patients with left ventricular dysfunction and clinically suspected myocarditis, but also whether these viruses were actively replicating. Active replication is marked by the presence of so-called minus-strand RNA, that is, RNA that is complementary to the plus-strand genome carried within the virus. In EMBs from 45 patients, they

found plus-strand enteroviral RNA in 18 (40 percent). Ten of these 18 biopsies contained minus-strand RNA as well, showing that a significant fraction—22 percent—of patients with left ventricular dysfunction and clinically suspected myocarditis had active enteroviral RNA replication in their myocardium, while another subset appeared to have latent persistent infection (Pauschinger M, et al. *Circulation*. 1999;99:889–895).

Dr. Schultheiss emphasizes that the presence of virus in the myocardium is an independent prognostic factor. In an early article from Dr. Bowles and his British colleagues, enteroviral RNA sequences were detected in 41 (34 percent) of 120 patients with left ventricular dysfunction. After a mean of 25 months' followup, these patients had a 25 percent mortality rate, compared with only four percent for clinically similar patients without enteroviral sequences (Why HJ, et al. *Circulation*. 1994;89:2582–2589).

Dr. Schultheiss' own data also prove that virus infection of the myocardium is an independent prognostic factor. Among 134 patients in whom a baseline EMB and PCR showed viral myocarditis caused by either enteroviruses or parvovirus, he and his colleagues performed a second EMB and viral assay six months later. In the majority of patients who had viral persistence, there was decreased left ventricular ejection fraction (LVEF), while those who spontaneously eliminated virus had improved LVEF.

This finding provided the rationale for use of the immunomodulator IFN-β as antiviral therapy in patients with viral inflammatory DCM who were persistently virus positive and who had left ventricular dysfunction in spite of the use of conventional heart failure medications. In the first pilot study, Dr. Schultheiss and his coworkers performed baseline biopsy to identify patients with chronic enteroviral or adenoviral heart disease. At six months patients were re-biopsied and the 22 patients who had viral persistence were started on IFN-β therapy, 18 million IU/week over six months. A third EMB was done after six months of treatment. All patients had eliminated virus at this six-month followup. Moreover, 14 patients had improved left ventricular ejection fraction, while the others were stable. Overall, LVEF improved from 44.7 percent to 53.1 percent. Dr. Schultheiss calls this a "slight but significant improvement." Among 36 contemporaneous controls, none improved and 20 deteriorated (Kuhl U, et al. *Circulation*. 2003;107: 2793–2798).

No patient who improved has relapsed so far. Among patients who eliminated virus on IFN-β treatment but did not improve LVEF at six months, many did improve at 18 months. "So elimination of virus and recovery [of cardiac function] is an ongoing process," Dr. Schultheiss says. In a second pilot study, similar results were found with patients who had persistent parvovirus B19 myocarditis and left ventricular dysfunction.

A European multicenter phase two study of IFN-β treatment is underway and should be complete at the end of 2004. If results confirm the pilot studies, a worldwide phase three trial will be organized, Dr. Schultheiss says.

If the phase three trial substantiates the positive results of the phase two studies, EMB will be poised to become more routinely indicated in cases of DCM suspected to be due to viral myocarditis. As the cardiologists from the Cleveland Clinic wrote in their editorial: "When clinicians can differentiate autoimmune processes and specific antiviral inflammatory responses, these disorders will certainly require different therapeutic approaches. As technology advances and specific antiviral agents become available, the role of endomyocardial biopsy in the diagnostic evaluation of suspected myocarditis will continue to evolve."

Dr. Veinot also sees an increasing role for molecular assays for viruses in this context. "We do not currently have this methodology widely routinely available," he says. "I think it is still mainly a research tool in most laboratories." However, he predicts, it will become important in a few years when the technology becomes more available and the users demand it.

"I think it is definitely going to happen. We will have to have a team approach," he says. "Anatomic pathologists will be interpreting the biopsies, while the virology laboratory will be doing PCR on them." □

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