On the track of new approaches to myocarditis

Amy Carpenter Aquino

May 2022—Studies show promise for new approaches to biomarkers for myocarditis diagnosis, one of which is circulating microRNA mmu-miR-721.

Another biomarker, sera soluble ST2 (sST2), which has been found to be clinically useful in predicting heart failure, could be added to existing biomarkers used to diagnose patients with myocarditis, interpreted according to sex and age.

And serial high-sensitivity troponin measurements might be another approach to diagnosing and monitoring myocarditis.

Authors of the microRNA study investigated the expression of novel miRNAs by Th17 cells, which play an important role in myocarditis and myocardial infarction, and they detected in mice and humans an miRNA (mmu-miR-721 in mice, hsa-miR-Chr8:96 in humans) that could be used to differentiate the two (Blanco-Domínguez R, et al. *N Engl J Med.* 2021;384[21]:2014–2027). Additional study is needed, and the miR-721 has not yet been evaluated in other cardiac disorders, such as dilated cardiomyopathy, from which myocarditis must be distinguished in the clinical setting, the authors write.

Dr. Li

The miRNA reported in the 2021 study dramatically increased clinical sensitivity and specificity for the diagnosis of myocarditis, says Jieli Shirley Li, MD, PhD, D(ABCC), assistant professor in the Department of Pathology, Ohio State University Wexner Medical Center, who was not involved in the research. Dr. Li highlighted the study in her virtual presentation at last year's AACC meeting and shared her view about this and more with CAP TODAY recently.

The challenges associated with invasive endomyocardial biopsy have been the impetus for studies on noninvasive methods for diagnosing myocarditis, Dr. Li says. Past studies on miRNA in humans or animals with myocarditis reported that the miRNAs were not specific for myocarditis, the pathophysiology of which is complex, she says. "The inflammation and/or necrosis of cardiac tissues could happen at a very close time or in a certain time order, or mixed, happening at the same time," Dr. Li explains. "We can see the similar trend of dynamic changes of troponin with the dynamic changes of inflammation biomarkers, but we are not able to confirm that troponin can differentiate myocarditis and myocardial infarction. The finding of novel microRNA"—the human homologue, designated as hsa-miR-Chr8:96—"can make it and fill the hole."

Myocarditis is an autoimmune inflammatory disorder post a viral infection, and the microRNA study showed that one immune pathway, interleukin-17 in type 17 helper T cells (Th17), is important in causing fibrosis. "And that's been shown to be important in what happens in some people as they go from myocarditis to dilated cardiomyopathy [DCM]," said DeLisa Fairweather, PhD, director of translational research, Department of Cardiovascular Diseases, Mayo Clinic, Jacksonville, Fla., and a coauthor of the study, in a recent interview.

Acute myocarditis is sometimes missed in the clinical setting and caught later as it progresses to dilated cardiomyopathy, she notes. Th17-type responses facilitate remodeling that leads to dilated cardiomyopathy—"it's kind of post-acute myocarditis," she explains. "The animal studies have shown that Th17 responses are important

in promoting the transition from myocarditis to DCM.

“So our collaborator in Spain looked at the presence of the miR in people,” she continues. “We also looked for the miR in our viral model, which helped to further establish that this was not just a fluke,” but instead present in patients and virally induced animal models of myocarditis. The collaborator then confirmed the finding of the human miR in several hospitals where they had groups of samples of patients.

All of this connects to soluble ST2, Dr. Fairweather, a coauthor of the ST2 study, says (Coronado MJ, et al. *J Am Heart Assoc.* 2019;8[2]:e008968). “They’re all in a linear progression,” she explains, referring to the pathogenesis of disease. TLR4 [Toll-like receptor 4] is early in the acute phase and leads to IL-1 beta production, and part of that family is the ST2 receptor. The cytokine that activates the ST2 receptor is IL-33. What follows next is IL-6 and Th17. “You start off with macrophages—TLR4, IL-1 and IL-6, IL-33 and ST2—and that progresses to the next phase of a Th17 response, where you go on to dilated cardiomyopathy, and then the Th17 response becomes really important,” Dr. Fairweather says.

In the ST2 study of myocarditis patients, Dr. Fairweather and her coauthors found that sera sST2 levels were higher in men and women with clinically suspected myocarditis and biopsy-confirmed myocarditis compared with healthy individuals who did not have cardiovascular disease. They found that elevated sST2 levels were associated with worse heart failure symptoms in patients with myocarditis based on New York Heart Association class, similar to the findings of other heart failure studies, but in their study the association was seen only in men (age 50 and under), not women. Myocarditis and DCM are known to occur more often in men than women.

“So the sST2 level,” Dr. Li said in her AACC presentation, “might be the potential diagnostic biomarker for myocarditis with age- and sex-specific criteria.”

“What we find with myocarditis,” Dr. Fairweather says, “as has been found also with other allergy or inflammatory conditions, is that sST2 is protective and IL-33 makes disease worse. We had published that before—that if you gave recombinant IL-33, it induced a type of eosinophilic myocarditis that is similar to giant cell myocarditis,” a more fatal form of myocarditis. Giving recombinant sST2, on the other hand, “inhibited that process, so that’s kind of the two working together.”

With that background, “we were really interested because sST2 has been shown for a number of other types of heart failure to be a biomarker in the sera,” says Dr. Fairweather, who is also associate professor of medicine, Mayo Clinic College of Medicine and Science.

Nothing had been published about the sex differences related to sST2, she says. “The whole pathway of TLR4/IL-1, and ST2/IL-33, and even Th17 and IL-6/TGFβ/IL-17—all pathways that are found to promote acute myocarditis and progress to dilated cardiomyopathy—are elevated in males and not in females in the animal model. And now as we’re doing translational studies looking at people, we’re finding the same thing.” Finding the age difference (under or over age 50) was also significant, she adds. “We’re trying to understand that better and pursuing studies in that area,” Dr. Fairweather says.

Awareness of myocarditis among the public is higher now because of its relationship with COVID-19, she says. “They didn’t know what it was before, and the cases are much more common and it’s spurred more interest and research.” Myocarditis was always known to be caused by viruses, she says, but the association with SARS-CoV-2 “has proven without any doubt such a direct relationship.”

All that she and others knew from their myocarditis research was seen in COVID-19 patients who had myocarditis, she says—“the type of immune response, involvement of complement and thrombotic responses, elevated cytokines/cytokine storm, and the increased occurrence in males compared with females. It’s the same mechanisms and the same pathway. So I think sST2, as far as the COVID myocarditis, would be the same as what we had been studying for the role of ST2 and other biomarkers.”

Myocarditis has an autoimmune component, she says, “so virus has always been speculated to cause autoimmune

disease." She has written extensively about how viruses can cause autoimmune disease, "but it's been difficult to prove." Here too COVID has helped to shed light: "It's really shown how viruses can cause autoimmune disease."

Dr. Fairweather

Opinions vary on how much SARS-CoV-2 has caused myocarditis, and the discussion is based on what is known as the Dallas criteria, developed in the 1980s. "It requires that if you take a biopsy, you see both inflammation and necrosis. But from our basic research studies, you don't need necrosis at all for the presence or development of cardiomyopathy and heart failure related to myocarditis," she says. "The chances of finding those two together in biopsies are low." That reduces the number of myocarditis diagnoses found with autopsy or in biopsies. "I think the rate of cardiac inflammation would be much higher post-COVID" without the necrosis requirement, she says.

Dr. Fairweather notes the importance of mitochondria and the role of the virus with mitochondria, which has to do with aging "and why myocarditis is such a beautiful example of this situation with aging." She is studying how mitochondria drives the sex difference in disease and myocarditis, she says, adding that publications to come will highlight how important it is.

Even though heart failure is the No. 1 killer of women, it happens later in life in women compared with men. "Women are really protected against heart failure by estrogen," Dr. Fairweather says.

"That's a big sex difference: Women have a big advantage as far as the heart goes. And we find that estrogen/estrogen receptor and estrogen-related receptor are important in protecting the heart after viral infection during myocarditis in women and women upregulate just about everything that can help the heart including healthy mitochondria." Testosterone increases the inflammatory response, she says, and estrogen dampens the response and offers protection.

Based on the most recent expert consensus published in 2020, recommended lab tests for suspected acute myocarditis include myocardial damage biomarkers, which are high-sensitivity troponins and CK-MB, Dr. Li said in her AACC presentation, though "there is weak correlation between troponin release and the severity of cardiac function" (Ammirati E, et al. *Circ Heart Fail.* 2020;13[11]:e007405). Others are CRP, ESR, CBC with differential, serological and virological tests ("rarely informative," she said), PCR, and autoantibodies. For SARS-CoV-2 vaccination-induced myocarditis, the CDC lists troponin level above normal limit as a laboratory evaluation criterion in its guidelines for diagnosis. "So troponin is a relatively more specific cardiac biomarker in the diagnosis of myocarditis, even in the course of COVID-19 vaccination," said Dr. Li, who is also co-director of the OSU clinical chemistry and toxicology laboratory.

In a 1997 study, troponin and CK-MB were measured in patients with biopsy-proven myocarditis (Smith SC, et al. *Circulation.* 1997;95[1]:163–168). "The troponin values were significantly greater in patients with myocarditis. In contrast, CK-MB levels were not significantly elevated," she said. "So troponin has a better sensitivity than CK-MB for the diagnosis of myocarditis."

A 2014 study that evaluated a panel of biomarkers to diagnose patients with suspected myocarditis looked at hs-TnT, N-terminal B-type natriuretic peptide (NT-proBNP), copeptin, and mid-regional pro-adrenomedullin (MR-proADM) (Ukena C, et al. *Clin Res Cardiol.* 2014;103[9]:743–751). "In patients with biopsy-proven acute myocarditis, high-sensitivity troponin concentration is dramatically higher compared with the chronic myocarditis and nonmyocarditis groups," Dr. Li said of the study findings. "However, none of the other biomarkers showed any

predictive value for the diagnosis of myocarditis."

In their study published in 1997, Smith, et al., also analyzed the troponin levels in 32 of the 53 patients with myocarditis who had information about the duration of their heart failure symptoms, Dr. Li said. "The troponin level was significantly higher in the 20 patients with symptoms lasting less than one month compared with the 12 patients with symptom duration greater than one month. And 11 of the 20 patients with symptoms for less than one month had the value of elevated troponin level, but only one of the 12 patients with the longer duration of symptoms had elevated troponin. So troponin might be an early indicator of heart failure caused by myocarditis."

In examining the relationship between the histopathologic pattern and severity of myocarditis to troponin elevation, the authors found that the mean troponin level was increased in the diffuse myocarditis group, but the difference did not achieve statistical significance, Dr. Li said. "So troponin elevations do not always correlate with histological severity of myocarditis."

Authors of another earlier study reported that when a 40-year-old patient diagnosed with acute myocarditis was prescribed prednisolone, her troponin level decreased from 10 ng/mL to 0.5 ng/mL (Quiroz R, et al. *J Heart Lung Transplant.* 2010;29[7]:820–822). "However, when prednisolone was stopped, the troponin level increased to 12 ng/mL," Dr. Li said, adding that troponin levels did not correlate well with BNP levels.

When the patient's prednisolone was restudied three weeks after discharge, her symptoms had improved and her troponin levels had declined to 0.5 ng/mL. "The troponin levels may not indicate the ongoing cardiomyocyte necrosis, but rather imply a poor prognosis," Dr. Li said.

In another example of the use of serial troponin and BNP levels to monitor myocarditis therapy, authors of a case report found the troponin and BNP levels of an acute myocarditis patient declined after conventional therapy for heart failure (Kadota S, et al. *J Cardiol.* 2008;52[2]:154–158). However, "six months later, the patient showed clinical symptoms—general fatigue and dyspnea—and was readmitted," she said. "The echocardiogram showed severe left ventricular dysfunction, and both troponin and BNP levels were significantly elevated again."

There was neither elevation of creatine kinase nor ST-T change on the EKG, she said, and the endomyocardial biopsy showed severe lymphocytic infiltration, which indicated active myocarditis. After treatment with immunosuppressant drugs, troponin levels decreased dramatically. BNP levels also decreased, though much more slowly. The second endomyocardial biopsy showed mild lymphocytic infiltration. "So the dynamic change of troponin and BNP in a patient following acute myocarditis might be the indicator for the patient's response to treatment," Dr. Li said.

The same finding was achieved in COVID-19 patients at OSU (Scarl RT, et al. *Ann Cardiol Vasc Med.* 2021;4[1]:1041). In 81 patients hospitalized for COVID-19, Dr. Li and colleagues observed the serial measured troponin levels were dramatically elevated in nonsurvivors compared with survivors. "Thus, this could be a good marker for severe myocarditis cases," Dr. Fairweather says. "However, less severe cases either recover or may progress to DCM and may not be captured by high-sensitivity troponin as a biomarker."

The OSU findings are consistent with those of others, Dr. Li said (Sandoval Y, et al. *J Am Coll Cardiol.* 2020;76[10]:1244–1258; Zhou F, et al. *Lancet.* 2020;395[10229]:1054–1062). Sandoval, et al., write in their review of cardiac troponin in assessing myocardial injury in COVID-19, "The structured use of serial cardiac troponin has the potential to facilitate risk stratification, help make decisions about when to use imaging, and inform stage categorization and disease phenotyping among hospitalized COVID-19 patients."

"So serial measured troponin levels might be a good approach for the diagnosis and prognosis of myocardial injury or myocarditis caused by the SARS-CoV-2 virus," Dr. Li said. "Theoretically," she tells CAP TODAY, "elevation of blood troponin levels indicates ongoing cardiomyocyte necrosis. It might be elevated with lymphocytic infiltration in cardiac tissues resulting from a virus attack, but it has not been confirmed yet."

In her group's study, Dr. Li says, the dynamic change of elevation of troponin with hospitalization days has a

similar trend with that of other inflammation biomarkers (IL-6, ferritin, PCT, and D-dimer), "indicating that the release of troponin in myocarditis/myocardial damage resulting from COVID-19 is closely associated with systemic inflammation."

In a 2019 study of 56 children with viral myocarditis, the serum concentration of CK-MB, TNFα, and high-sensitivity CRP were found to be statistically different in the acute stage, recovery stage, and the control group (Chen J, et al. *Open Life Sci.* 2019;14:38–42). "The levels gradually decreased as the disease progressed," Dr. Li said. "So the dynamic change in the inflammatory factors, like TNF-alpha and high-sensitivity CRP, might reflect the development of viral myocarditis in children. And they might be used as serology markers to identify viral myocarditis in the acute and recovery phases."

A study published in the 2021 *European Journal of International Medicine* confirmed for troponin a well-defined diagnostic role in myocarditis, Dr. Li said (Scicchitano P, et al. *Eur J Intern Med.* 2021;85:56–62). The aim of this multicenter, retrospective study of 104 patients with myocarditis was to provide the DAME (Diagnosis of Acute Myocarditis in Emergency) score for the fast identification of such patients in the emergency department.

The determinants of the score were fever, chest pain, ESR >20 mm/h, hs-CRP >3 mg/L, hs-troponin serum levels >3 ng/L, and left ventricular ejection fraction <50 percent. Each received a score ranging from zero to four, and a final score of four or greater was related to a 75 percent probability of myocarditis. A score between one and four was related to a 57 percent probability.

Pietro Scicchitano, MD, PhD, a coauthor of the study and professor, cardiovascular diseases section, Department of Emergency and Organ Transplantation, University of Bari in Italy, tells CAP TODAY: "The DAME score better pointed out the relevance of a comprehensive evaluation of patients when admitted to the emergency department for a chest complaint. High-sensitivity troponin I is included in the score and acquires a more reliable use for the overall assessment of patients admitted to the ED, beyond acute coronary syndromes." The study confers to troponins the role for excluding heart lesions, he says, "although they should be evaluated with further parameters."

Dr. Li says the DAME score might help prevent delay in diagnosing myocarditis, but routine use of the score, Dr. Scicchitano says, hasn't caught on in the scientific community. "We usually adopt it in our clinical practice," he says, "but we hope to implement it beyond our hospital."

Like the DAME score, Dr. Li says, machine learning can be of use in diagnosing or monitoring myocarditis, but here, too, as with serial troponin measurement, sample size is the challenge. "It is never easy to have a study on myocarditis with a large sample size," she says, adding, "All the published studies just contribute the best they can."

Mortality prediction in pediatric myocarditis was the subject of a study published last year in which the authors compared the performance of traditional logistic regression models with that of a machine-learning-based model. Logistic models were good at predicting patients who were likely to survive, they found, but performed poorly in predicting patients who were likely to die. "On the contrary," they write, "the ML model had a good balance between sensitivity and specificity, and would be much more useful in clinical settings where predicting mortality is more crucial than predicting survival."

The probability of mortality can be satisfactorily approximated when any of these variables are present, they say: mechanical ventilation, ECMO use, cardiac arrest, ventricular fibrillation, and acute kidney injury.

Dr. Chou

Fu-Sheng Chou, MD, PhD, a coauthor of the study and neonatologist at Kaiser Permanente in Riverside, Calif., says the ML model "exemplifies the power of data and data science."

"Causal interference and predictive modeling are now at our fingertips, thanks to the computing power and the emergence of the data science discipline," he tells CAP TODAY.

He and his coauthor reported in the study that it was "striking" to them that acute kidney injury carried considerable weight in putting patients at a significant risk for mortality. He says AKI in the context of pediatric myocarditis may be a result of poor vital organ perfusion because of poor contractility, or cardiogenic shock. "In the context of overwhelming sepsis, distributive shock may also contribute to acute kidney injury. We were surprised that its association with mortality has not been established in the literature despite it being a reasonable indicator of overall disease severity." They speculate, Dr. Chou says, that it's due to the rarity of the disease. Identifying AKI as an important predictor of mortality affirms the importance, he says, of having multicenter datasets and well-curated data in health care research.

He applauds the Healthcare Cost and Utilization Project for its efforts in publishing the Kids' Inpatient Database used in his study to investigate risk factors associated with myocarditis "and to turn the identified risk factors into a prediction tool." The database has its limitations, he says, but he hopes the findings of his study will serve as initial "observations" and prompt other retrospective and prospective studies with dedicated cohorts on the pathophysiology underlying the cause-effect link between the identified risk factors and mortality outcome.

"Pediatric myocarditis is a devastating yet understudied disease," Dr. Chou says.

Amy Carpenter Aquino is CAP TODAY *senior editor.*