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Editorials

Troponin Autoantibodies: From Assay Interferent to Mediator of Cardiotoxicity

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Twenty years ago in *Clinical Chemistry*, cardiac troponin I (cTnI)[3] autoantibodies to the stable portion of cTnI near the carboxyterminal end of the molecule were first identified as an etiology for false-negative cTnI results *(1)*. Since then, there has been a continuing evolution of our understanding of cardiac troponin autoantibodies as both assay interferents as well as mediators of cardiac pathogenesis. Of particular clinical significance, there is an emerging link between cTnI autoantibodies and cardiotoxicity perhaps related to immune-checkpoint inhibition [i.e., anti–cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and anti–programmed cell death protein 1 cancer immunotherapy (PD-1)] *(2, 3)*.

Cardiac troponins are the biomarkers of choice for the assessment of myocardial injury, including myocardial infarction. Clinical assays for cardiac troponins are sandwich immunoassays directed to stable portions of cardiac cTnI and cTnT. As with all such assays, antibody interference can occur. This issue of *Clinical Chemistry* contains an article in which the authors have evaluated the impact of such antibodies for the various cardiac troponin fragments and complexes in a series of carefully done studies *(4)*. By gel filtration, cTnI autoantibodies were demonstrated to bind to cTnI when cTnI was associated with troponins T and C (the I–T–C ternary complex). However, they did not bind to either free cTnI or when cTnI was associated with C (the I–C binary complex). The authors thus suggest that the negative interference by troponin autoantibodies in cTnI immunoassays is a result of interference with circulating complexes of I-T-C. Importantly, they suggest that a similar effect can be seen with cTnT assays as well as with cTnI assays, given that the I-T-C complex is commonly detected with the cTnT assay as well. Based on the specificity of cTnI autoantibodies for the I-T-C ternary complex, the authors propose a model where, early after acute myocardial infarction, circulating cTnI is a mixture of I-C binary and I-T-C ternary complexes. By 10–20 h after infarction, most of the circulating cTnI is the I-C complex. This study then presents a new model for circulating troponins based on this in-depth evaluation of this interfering autoantibody.

The significance of negative interference by cTnI autoantibodies may be underestimated in clinical practice. These antibodies are prevalent in both healthy and disease populations. In one survey, 12.7% of blood donors had detectable cTnI autoantibodies *(5)*. The same study demonstrated that patients positive for other biomarkers had a similar prevalence: cTnI positive = 10.4%; B-type natriuretic-peptide positive = 10.5%; hepatitis C virus antibody positive = 13.5%. Patients positive for rheumatoid factor had an even higher cTnI autoantibody prevalence of 20.4%, although the autoantibody detection format was designed to avoid the typical false-positive interference from rheumatoid factor (autoantibodies directed to the Fc portion of antibodies). In a separate survey of patients with suspected myocardial infarction, 9.2% overall had troponin autoantibodies with similar prevalence in patients with or without myocardial infarction *(6)*.

Most commercial cTnI assays use antibodies to the stable midfragment of cTnI and thus are susceptible to troponin autoantibodies *(7)*. Strategies to mitigate the effects of cTnI autoantibodies on assays include the use of multiple capture antibodies with only one that targets the midfragment *(7)*. Unfortunately, the traditional strategies used by clinical laboratories to investigate immunoassay interference (dilution, analyte spike-in with recovery analysis or removal/extraction of interfering substances) are typically targeted to falsely positive values and are cumbersome and not very effective in identifying reduced, falsely negative values. Post-test surveys of archived material for cTnI autoantibodies may be useful investigations for laboratories; however, they are unlikely to benefit specific patients with cTnI autoantibodies who have already been evaluated in the setting of an acute coronary event. Fortunately, although these autoantibodies reduce cTnI values in some patients, they rarely change one from having an increased value to one that is normal. These data now clarify why this is the case. The antibodies target only part of the complex mix of fragments elaborated. One easy way to unmask this situation is with the use of a carboxyterminal assay for cTnI *(8)*.

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[3] Nonstandard abbreviations: cTnI, cardiac troponin I; PD-1, programed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte associated protein 4.

However, because of degradation of the carboxyterminal region and thus early loss of signal compared to conventional assays, this assay has not been used clinically.

An intriguing aspect of troponin autoantibodies is their evolving link to the pathogenesis of myocarditis and cardiomyopathy *(9)*. An early report suggested that cTnI (but not cTnT) could induce severe myocarditis *(10)*. In contrast, a more recent in vitro study examined peripheral blood white blood cells from patients with idiopathic dilated cardiomyopathy compared to controls *(11)*. In the white blood cells from patients with idiopathic dilated cardiomyopathy, there was a heightened response of the antiinflammatory cytokine interleukin-10 to cTnI. Furthermore, the increased interleukin-10 in response to cTnI was associated with lower high-sensitivity C-reactive protein and less advanced diastolic dysfunction *(11)*. A separate study proposed autoantibodies to cTnI as part of a model of general cardiac autoimmunity in the setting of type 1 diabetes *(12)*. Because the clinical outcomes of myocardial infarction are known to be worse in patients with type 1 diabetes compared to patients without diabetes, enhanced inflammation in the setting post myocardial infarction was examined as a possible etiology *(12)*. In this study, severe destructive inflammatory cardiac infiltrates were observed in the post myocardial infarction diabetic mice but not in control mice. The authors of this study also created a panel of cardiac autoantibody assays to characterize post–myocardial infarction patients with type 1 diabetes. Overall, autoantibodies to one or more cardiac antigens (cTnI, α-myosin heavy chain, β-myosin heavy chain, or α-actin 2) were identified in 83% of diabetic patients with post myocardial infarction; in comparison, only 15% of patients with type 2 diabetes and 4% of healthy controls had cardiac autoantibodies *(12)*. These findings suggest that cTnI may be one of many cardiac-specific antigens that may potentiate cardiac-specific autoimmunity in the context of myocardial infarction. This may be in part related to reports that patients with acute myocardial infarction who harbor anti-cTnI antibodies have lower ejection fractions and more adverse outcomes compared to those who lack these autoantibodies *(13)*.

Finally, in the era of immune-checkpoint targeted therapy, troponin autoantibodies may be both an important biomarker and mediator of pathogenesis. In 2001, mice deficient in the immune checkpoint receptor programed cell death protein 1 were observed to spontaneously develop autoimmune dilated cardiomyopathy and suddenly die from congestive heart failure *(14)*. Subsequently, the same research group discovered that cTnI is the major target antigen for the autoantibody in these mice *(3)*. The pathogenic potential of cTnI autoantibodies was then demonstrated by infusing cTnI monoclonal antibodies into wild-type mice and inducing cardiac dilation and dysfunction *(3)*. Programed cell death protein 1 has become well known in the medical literature and popular press as programed cell death protein 1 (PD-1). PD-1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) are major targets for cancer immunotherapy. Recently, a series of 8 patients with cardiotoxicity associated with anti-PD-1 or anti-CTLA-4 therapy was reported *(2)*. Although cardiotoxicity has been reported in mouse models for deficiency of PD-1 *(3, 14)* and CTLA-4 *(15)*, cardiotoxicity remains a rare side effect in humans treated with either anti-PD-1 or anti-CTLA-4 therapy. The 8 patients in this series were studied in collaboration between multiple institutions, and until this patient series there were few cases reported. Although troponin autoantibodies were not studied in these patients, the potential link between immune checkpoint inhibition and induction of cardiac-specific autoimmunity is intriguing. Furthermore, there may be a role for monitoring troponin autoantibodies in patients with cardiac disease who undergo immune checkpoint inhibition therapy.

The role of cardiac-specific antigens in cardiac disease is still in an early phase of understanding. Over the past 20 years there has been success in the characterization cTnI autoantibodies for improved laboratory analysis, which has been furthered now by the investigation of Vylegzhanina and colleagues *(4)*. In the next 20 years, there will likely be an enhanced understanding of the role of troponin autoantibodies in the pathogenesis of cardiac disease as well. Is the I-T-C ternary complex the key?

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