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Influence of Macrotroponin on the 99th Percentile Threshold in 2 High-Sensitivity Cardiac Troponin Assays

Article:
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Guest: Dr. Nina Strandkjær is a medical doctor and clinical researcher at Herlev University Hospital in Denmark.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Cardiac troponin measurement is an integral part of the evaluation of acute myocardial infarction [MI], with the fourth universal definition of MI requiring a rise or fall in cardiac troponin and at least one value above the assay-specific 99th percentile.

Given the consequences of a missed diagnosis, manufacturers perform rigorous studies involving hundreds of samples to define their assay-specific 99th percentile with a high degree of confidence. These reference interval studies recruit only individuals who meet strict inclusion and exclusion criteria, but the collected samples generally aren't evaluated for the presence of interfering substances.

One such substance is macrotroponin, troponin coupled with an endogenous antibody that prevents its clearance, resulting in artificially high measured concentrations. Do currently available assays detect macrotroponin and if so, how does it impact the 99th percentile? And what about subsequent medical decisions made on the basis of a troponin result?

A new research article, appearing in the August 2025 issue of *Clinical Chemistry,* evaluates the impact of macrotroponin on two commercially available high-sensitivity cardiac troponin assays and discusses the implications of these findings for assay manufacturers, laboratorians, physicians, and most importantly, patients. In this podcast, we welcome the article's author. Dr. Nina Strandkjær is a medical doctor and clinical researcher at Herlev University Hospital in Denmark. Her current work focuses on how macrotroponin interferes with cardiac troponin assays and challenges the current definition of myocardial infarction. So, doctor, let's start with the basics. What was the motivation behind this study and what exactly is macrotroponin?

Nina Strandkjær: Thank you for those questions and thank you for having me. Before we dive in, I'd just like to start by acknowledging the entire research group for their valuable contributions, especially the two senior authors, Dr. Iversen and Dr. Hammarsten. So to answer your question regarding the motivation behind this study, it actually started during a national rollout of new troponin thresholds in Denmark where we noticed something was odd.

The Siemens high-sensitivity troponin I assay was giving us really high 99th percentile values and these numbers just didn't match what we were expecting to see in healthy reference individuals, and that raised concerns about some kind of analytical interference. So that actually led us to suspect macrotroponin.

So to answer your second question, macrotroponin is a complex formed when cardiac troponin binds to immunoglobulin D. Essentially, the body's immune system is holding onto the troponin molecule in the bloodstream, and it does not appear to be harmful in itself, but it causes troponin levels to remain elevated for a long time, even when there's no ongoing myocardial injury. And, because most assays can't distinguish between free troponin and macrotroponin, this can lead to misleadingly high results.

So, in the clinical setting, you might see a patient who shows up with elevated troponin levels on the lab report, but they are completely stable and asymptomatic and not actually experiencing myocardial injury. And that creates a lot of diagnostic confusion and it can lead to unnecessary testing, misdiagnosis, or even delays in getting the right care.

But with this study, our goal was actually to take a step back and look at the bigger picture. We wanted to understand how common macrotroponin is in a healthy reference population and whether it might be skewing the diagnostic thresholds that we use every day in the clinical practice.

Bob Barrett: Okay. Now your findings suggest that the 99th percentile threshold might be artificially elevated in some assays. Could you explain how this happens and why it matters for clinical care?

Nina Strandkjær: Yes, absolutely and that's one of the more uncomfortable truths here because we often think of the 99th percentile as a fixed objective cutoff. You know, something rooted purely in biology. But in reality, that number depends entirely on who you include in the reference population and how the assay behaves in those individuals.

So if your reference population includes people with macrotroponin and you're using an assay that's sensitive to

it, yeah, then you'll end up with an inflated 99th percentile. That might sound like a small technical issue, but it can have very real consequences in the clinic.

We risk underdiagnosing myocardial injury especially the smaller infarctions or the early presenters, simply because the threshold has been distorted. So we need to acknowledge that the 99th percentile isn't purely biological. It's also very much shaped by how the assay works and that makes it vulnerable to certain types of interference.

Bob Barrett: Well, given the level of interference that you've uncovered, do you think manufacturers have done enough to address this and how should they respond, both technically and in terms of transparency?

Nina Strandkjær: To be honest, I don't think they've done enough. At least not yet. There has been growing awareness of macrotroponin interference, but so far, we haven't seen much in terms of systematic action from the manufacturers.

So ideally, they should revisit how their assays are built, especially when it comes to antibody selection and epitope targeting, in order to reduce the sensitivity to macrotroponin complexes. But realistically, a more immediate and practical step would be to offer the option to remove IgG from samples directly on the existing platforms, or even better to develop a simple routine compatible tool for immunodepletion that labs can actually use in daily practice.

I mean, at the very least, we need transparency and right now many assay inserts don't even mention macrotroponin. If one assay shows 76% interference rate among the highest 10% troponin values and another one shows none, that's not random variation. That's a systematic issue.

So, I really think labs and clinicians need to know about these limitations. They need to be able to respond to elevated results with confidence and avoid being misled by what looks like a real signal but really isn't.

Bob Barrett: Well, finally, doctor, do you think that your findings could affect current clinical guidelines?

Nina Strandkjær: I think they should, yes. Right now, guidelines treat the 99th percentile as a kind of a fixed truth, but we may need to revisit that. If a threshold is based on the top 1% of values in a reference population and we don't account for analytical interference like macrotroponin, then the foundation becomes a bit fragile. And with this study, we've shown that the 99th percentile can be influenced by these analytical artifacts like macrotroponin. And we must assume that has clinical

consequences, especially when we are using that threshold to rule in or rule out myocardial injury.

So, I do think future guidelines should at least recommend confirmation of elevated troponin values, particularly when the clinical picture doesn't fit. And ideally, they should incorporate assay-specific strategies to detect and to manage potential interference. And actually, the latest educational recommendation from the IFCC do touch on this. They acknowledge that biological interferences, including macrotroponin, can affect the 99th percentile, and they recommend excluding those samples from reference cohorts to avoid skewing results, and--which was actually what we did in this study.

So, it's really not about throwing out the tools we have, it's about refining them and making them more precise and better aligned with what's actually going on in the patients.

Bob Barrett: That was Dr. Nina Strandkjær from Herlev University Hospital in Denmark. She wrote a research article in the August 2025 issue of *Clinical Chemistry* describing the influence of macrotroponin on the 99th percentile threshold of two high-sensitivity troponin assays, and she's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.