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Sex-Specific 99th Percentile Upper Reference Limits for High Sensitivity Cardiac Troponin Assays Derived Using a Universal Sample Bank.

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Guest: Dr. Fred Apple is a professor of Laboratory Medicine and Pathology at the University of Minnesota and co-director of the Clinical and Forensic Toxicology Laboratory at the Hennepin County Medical Center in Minneapolis.

Bob Barrett:

This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

When it comes to measuring cardiac troponins, the 99th percentile is firmly established as an essential component for interpreting results. However, just how to select healthy reference subjects for deriving those 99th percentiles for cardiac troponin assays, still needs to be clarified.

A paper appearing in the March 2020 issue of *Clinical Chemistry* examined over 800 specimens from a Universal Sample Bank in a multi-center study that utilized several different high sensitivity assays for cardiac troponins to help address that very question. The lead author for that study is Dr. Fred Apple.

He is a professor of Laboratory Medicine and Pathology at the University of Minnesota and co-director of the Clinical and Forensic Toxicology Laboratory at the Hennepin County Medical Center in Minneapolis. He is our guest in this podcast. So Dr. Apple, please, remind us of the importance of the 99th percentile value of cardiac troponin I or troponin T assays and how it relates to the new generation of high-sensitivity troponin assays.

Dr. Fred Apple:

Cardiac troponin I and/or cardiac troponin T uses the 99th percentile concentration, that we refer to as the upper reference limit or URL. This is the value that has been designated as the medical decision cut off to determine a positive or abnormal result and used by both the Universal Definition of Myocardial Infarction and a recently published IFCC Committee on Clinical Applications of Biomarkers and the AACC Academy expert opinion manuscript that defines the 99th percentile as that diagnostic cut off.

It's been used since the year 2000 and most importantly, now, as we move into the era of high-sensitivity cardiac troponin assays in the United States, they've been used also globally for over 10 years. This is the line in the sand being used for the diagnosis in which a rising or falling value with at least one value above this 99th percentile needs to be increased in the

symptom picture of clinical indication for diagnosis of MI or determination of myocardial injury.

Bob Barrett: In this paper, you and your colleagues used the so-called Universal Sample Bank as the source of specimens. Tell us a bit about that and why that's unique.

Dr. Fred Apple: You know, a group of us got together in 2014, quite a ways ago, and we realized that many papers published in the literature, when we read these manuscripts in clinical laboratory medicine and in clinical medicine journals that people use the 99th percentile URL based on different populations. So it's very difficult to compare one assay to another to get any kind of uniformity to understand similarities or differences. So we worked with the AACC, and the AACC kindly funded a study where at the 2015 AACC national meeting in Atlanta, a group of laboratory and medical directors got together in collaboration with the CDC and put together a blood draw room and stations in which we had IRB informed consent institution review board system in place where we took volunteers and anyone participating at the national meeting to come into this room, sign a waiver or informed consent, and donate their blood to be appropriately handled by universal procedures to isolate serum and different types of plasma and freeze those away at minus 70 with a concept that the AACC would sell these "Universal Sample Bank" specimens to any manufacturer that would want to use them to primarily determine what their 99th percentiles are for their new high-sensitivity or contemporary assays.

The importance of this is that, it now has a group of over 400 men and 400 women that are normal that could then be put into practice to compare assays to assays. Our group extended it in the paper we're talking about today, that we were able, at the donation of the AACC to give us the Universal Sample Banks and the kind donation of the multiple manufacturers to provide assays to two core labs, described in the paper, so we could determine 99th percentiles for men and women in apparently healthy population so that we could get a comparative determination across all assays of high-sensitivities in the marketplace and some in research.

Bob Barrett: How did the results of the study compare with the current evidence-based literature on this topic?

Dr. Fred Apple: Well, the results of the study were quite, I think, unique for multiple ways. Compared to the evidence-based literature, as I have mentioned, it would be expected that every normal reference population studied by individual manufacturers' assays would have a defined overall cut off for 99th percentile-- in males, 99th percentile, in females, 99th percentile. And then if another company that uses their own population in their own region of the country or in the world, they would have another - their own 99th percentile. But to compare the 99th percentile

between assay, A used in let's say, Sweden and assay B determined in Southern California, we do know that the ethnic racial, gender, age differentials for populations in different parts of the world would be different. And that is confirmed that the 99th percentile is determined in this study are going to be different potentially from 99th percentile determined with a different group of apparently normal patients.

So this study was, I would say, quite unique in that we got to compare multiple assays for troponin T and multiple assays for troponin I within the same apparently healthy population of Universal Sample Bank. And then we get to look at those in comparison to what the individual assay population is and we compare and contrast how they differ and how they are similar.

Bob Barrett: Dr. Apple, tell us what you believe are the most important findings in this study and what are their implications for both clinicians and for the laboratory?

Dr. Fred Apple: I'd say there were three primary findings that I think were very important for the study. First, we got a better understanding of how to define normality. So our expert group from this Universal Sample Bank, instead of just taking all these normals from a hypothetical, that's a normal baseline to health questionnaire, we also implemented surrogate exclusionary biomarker criteria utilizing a natriuretic peptide to look for underlying myocardial heart failure potential, silent. We looked at hemoglobin A1c underlying diabetes or pre-diabetes, and we looked at estimated glomerular filtration rate and DFR, looking for silent or underlying renal insufficiency. Also, we eliminated patients that were on a known cardiovascular medication like statins. And inherently, most in a large number of reference range studies would not do that extra surrogate biomarker screening to eliminate maybe up to 10% or 15% of apparently healthy normals, especially in the aging population in which they may be silently increased but yet, abnormal. So we pared it down and that hasn't been done in the literature before to really get a clean set of normal. That was one thing that was interesting.

The second thing that I think was important is that we carefully looked at the three most common statistical methods that have been used to define what a 99th percentile value are: the Harrell-Davis method, the nonparametric method, and the robust method. The data shows there are differences for each of those statistical methods.

The third thing we showed clearly based on the Universal Sample Bank is that the assay used will have a great impact on what the 99th percentiles will be for men different than women. This is a very important finding because like most laboratory tests now with high sensitivity that we can measure the predominantly greater than 50% of apparent normal

individuals, we have statistically different male and female 99th percentile cutoffs. So those three things I think are the most important observations from our findings.

Bob Barrett: Why should laboratory medicine practitioners in clinical practice or in research use the sex-specific 99th percentile upper reference limits and how does a laboratory decide exactly what limits to use?

Dr. Fred Apple: The question of what laboratorians, what, let's say, cardiologists, emergency medicine physicians, or even those doing research, what they decide to use, whether they use the package insert, whether they use the findings in our study, or using something from the peer-review literature, is very important on predicating what results they will see. So for example, one of the things we showed in our study is, by definition, a high-sensitivity assay is defined on the 10% or less than precision at the 99th percentile. But more importantly, that the critique of having greater than 50% of a normal individual having a measurable rate above the limit of detection of the assay.

What we observed here compared to the literature and where investigators and laboratorians have to keep in mind is that the limit of detection of assay, let's say assay A, could vary between 1.1 and 2.0. So if you're looking to determine if that qualifies as a high-sensitivity assay, where you draw your lines to determine your LOD, even though they both could be published in the peer review literature, will have an impact at how many patients are measured. So what happens here is if someone has to choose what cutoff to use, a package insert for an assay might have cutoff of let's say 10, 20, and 30. But if you look at our paper because we use exclusionary surrogate marker cutoffs, the cutoff might be lower. It might be 5, 10, and 15. So the laboratorians are going to have to take the cutoff from population from a peer review literature or take the cutoffs from let's say our paper which are lower and make a decision. Are they going to they be going to explore, willing to pick up, more positives versus less number of positives at a higher concentration?

So the implications here are defining how many patients have an increased troponin that have an increased myocardial infarction or an increased detection of injury, and then predicate those observations on management and therapy. So it's a fine balance. The important thing here to keep consideration is that the laboratory needs to work with their clinical colleagues in determining what the best set of sex-specific cutoffs for men and women are going to be used. The most highlighted thing that's starting to show up more frequently is, with the sex-specific cutoff for women, they are now growing number of studies that shows you pick up more women with injury and more women with myocardial

infarctions. And then we have to decide how those patients will be managed differently compared to using an overall cutoff, which if it's a higher number, you pick up fewer number of women that would be positive.

Bob Barrett: Quite a number of individual specimens in this study had concentrations of troponin below the limit of quantitation for each method. Does this suggest that we are in need of even more sensitive methods for measuring the cardiac troponins?

Dr. Fred Apple: Well, if I were a listener of this podcast, I would refer them to our figure 2 which is a post-exclusion data for 99th percentiles for men and women and overall. And we also on the same schematic, we not only show the 99th percentile but we show what percent of measurable values are above the LOD. So in some of the assays that are determined high sensitivity per the manufacturer and sometimes in the evidence-based literature, some of the measurable values just dip below that 50th percentile. So it could be one of two things. As I mentioned, the LOD used to determine the numbers that are measurable will impact the percent of measurable values.

So for example there are three papers in the literature using let's say one assay's 99th percentile and ability to measure, and two out of the three shows that they measure 80 to 85% of normal. And then let's say another study shows that it only measures 48%. But if they vary the LOD and both have been published correctly anywhere from 1.1 in one study to 1.9, you can see there'd be a difference. However, in the assays that consistently show less than 50% measurable values, I think those are the assays that need to be worked on to improve their analytics so they could measure at lower concentrations, and this typically shows up in women. Women have just by physical standards smaller hearts, and therefore a smaller amount of natural turnover of cardiac troponin I and T, and therefore typically run lower 99th percentiles.

Bob Barrett: Your paper talks about a continuum of increased risk for adverse events with increasing concentrations of serum troponin. Should laboratories start reporting relative risk rather than a concentration in upper reference limit?

Dr. Fred Apple: So there's a couple of papers in the literature that have looked at the clinical adverse event risk stratification role of troponin while the concentrations are still within the reference interval below the let's say sex-specific URL. My own group published a paper in *Circulation* as a research letter back in 2019, which showed this very nicely that if you look at a population of patients that comes through the emergency department and go through the serial testing to rule in or rule out a heart attack, that their troponin concentrations never increase above the 99th percentile.

Therefore by definition, there is no myocardial injury, there is no myocardial infarction. The data shows that even within the reference interval below the 99th percentile upper reference limit, that there is a two to three fold gradient of risk of either major adverse cardiac survival or cardiac event even within that reference interval. And it's similar I'd say, in analogy to what we've observed early on with CRP using high-sensitive CRP. There's clinical information to be gleaned within the reference interval and not necessary for healthy people, but for patients who have normal troponins for being evaluated for maybe other indications. And how this could be clinically reported is going to have to be evolved.

I'm a believer down the road within the next two to three to four years that relative risks will be posted on our electronic health record to say if this female patient came in and we know the upper reference limit is 25 and they come in with a value of 20, this person is going to be at a risk of 2.35 of having an adverse event at 180 days. It could be a major adverse cardiac event or just all cause survival.

So my answer to sum it up is that I think there's going to be more reporting over the future instead of just a positive value, someone rules in or rules out for MI. But I think we're going to start looking at odds ratio or hazard ratios, or risk ratios as the evidence grows and the numbers and larger numbers of patients grow into the thousands to be able to feel comfortable reporting out this risk adverse number.

Bob Barrett: Well, finally Dr. Apple, were there any other surprising findings from your study and where do you go from here?

Dr. Fred Apple: I would say that the most interesting observation and finding -- and something I learned as we wrote this paper and had it reviewed -- was that we had an expert statistical reviewer pointing out to us that the robust statistical methodology was really not designed to be used to determine 99th percentile. The script that was written by a statistician named Horn that is referenced in the paper, this methodology was really derived to look at the central 95th percentile up to the 97.5th upper reference limit.

So, what we found because of the way the script was written, it defined the robust 99th percentile, we observed a lot of values that we weren't even seeing, able to calculate at 99th percentile because so many of the women's values were low. So what we have observed was that when we went through the literature that many studies have been using the robust method to determine and publish a 99th percentile. And my take-home message discussing it with now statisticians is that it's inappropriate how the current script is being used and you find it online. And the recommendation came across working with one of the Associate Editors of *Clinical Chemistry* that we should

be very clear in our discussion, that while we report the results of the robust method, we recommend that this robust method should not be used until the script is improved to work around the 99th percentile and just not around the 97.5th percentile. Because again, the numbers published, the numbers used by clinicians or laboratorians, will have a huge impact on how patients are classified. But we shouldn't be using 99th percentile upper reference limit on a methodology that might not be good enough to use to make patient decisions.

Bob Barrett:

That was Dr. Fred Apple from the University of Minnesota and the Hennepin County Medical Center in Minneapolis. He has been our guest in this podcast on upper reference limits for high-sensitivity cardiac troponin assays using a Universal Sample Bank. He is co-author of the paper describing that approach that appears in the March 2020 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.