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Lower Limits for Reporting High-Sensitivity Cardiac Troponin Assays and Impact of Analytical Performance on Patient Misclassification

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Guest: Dr. Kristin M Aakre from Haukeland University Hospital and the University of Bergen, Norway.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett. Cardiovascular disease is the most common cause of death worldwide and as a result patients with suspected acute coronary syndromes are often admitted to a hospital for further evaluation. Fortunately, only 1 in 10 patients admitted for investigation is formally diagnosed with myocardial infarction. Regarding the other 9 patients, some are diagnosed with other conditions but most receive some degree of unnecessary care.

To address this, several rapid rule-out pathways using cardiac troponin have been developed. For these pathways to be effective, however, assays must reliably detect very small changes across serial samples collected from the same patient. Often this requires accuracy at very low concentrations, presenting new challenges for manufacturers and laboratories.

A review article appearing in the March 2024 issue of *Clinical Chemistry* shares recommendations from the IFCC committee on the clinical application of cardiac biomarkers, specifically how assay performance characteristics impact efforts to rapidly evaluate acute coronary syndromes.

In this podcast, we are pleased to speak with the review article's lead author. Kristin M. Aakre is a medical doctor, consultant in clinical chemistry at Haukeland University Hospital, and professor at the University of Bergen in Norway. She has been working with cardiac troponin for more than 10 years and is the current chair of the IFCC committee on the clinical application of cardiac biomarkers.

Dr. Aakre, the upper reference limit for cardiac troponin assays is the 99th percentile of values from a healthy reference population. Is this the cutoff you refer to when talking about lower limits for reporting high sensitivity troponin concentrations and misclassification of patients?

Kristin Aakre: Actually not. Of course, the 99th percentile is a very, very important cutoff. It's included in the universal definition of myocardial infarction, and we use that to diagnose myocardial infarction. But what we are talking about in this article are actually even lower values than the 99th percentile. We are talking around like the limit of detection of the assay, the limit of quantification, and particularly many of those cutoffs which are used to rule out patients. They are around the limit of quantification, which would be where we have a 20% CV of the assay. So, it's much lower concentrations, actually, that we are dealing with here.

Bob Barrett: It seems that you are primarily focused on concentrations below the upper reference limit, and you suggest that we should monitor the analytical performance even as low as the limit of detection. Why is this so important? What's the clinical utility of the assays at such low concentrations?

Kristin Aakre: Well, I think this is important if you are going to do risk stratifications, for instance in the emergency department. If you want to use troponins to determine very early after admittance to the emergency department if this is a high or low risk to patients regarding myocardial infarction. So, if you want to diagnose myocardial infarction, if you only use troponins for that, you can monitor the analytical quality around the 99th percentils and probably a little bit lower but around that concentration.

But if you actually use troponins in the emergency department and you measure the patients when they arrive, and if they have very low concentrations, then you say this patient has a very low risk of myocardial infarction, he or she doesn't need to go to the cardiac ward. This patient can go either home, or he or she can go to another ward and be investigated for some other condition.

Well, if you use very low concentrations in that way, then you actually have to monitor the analytical quality around that concentration. So, it's all about what is the clinical use of the assay. If you use it to risk-stratify patients already in the emergency department, then you need to know the analytical quality around that concentration and those are going to be very, very low. Much lower than the 99th percentile. That is, if you use the 0/1-hour or 0/2-hours algorithms that are now recommended like in Europe, in the United States, and also in large parts of the world use those guidelines. And then the low concentrations are clinically important and well then as a lab, we need to monitor those concentrations. That's kind of our part of the obligations here.

Bob Barrett: When we talk about analytical performance, we mean both bias and imprecision in the context of 1-hour algorithms,

which of these are the most important to monitor and why is that?

Kristin Aakre: Yeah, a good question. Well, of course if you do serial samplings with 1-hour apart, and you calculate the delta value between the admittance sample and the 1-hour sample, of course, you need to have a very good analytical precision to be able to calculate those delta values. So, of course, the daily work, the daily practice, the precision is extremely important. If you have a very high position here, you cannot calculate delta values. However, most of the assays that are available, the high sensitivity assays that are available, those will have a very good precision also at low concentrations. That's why they are called high-sensitive.

But of course, the precision is important, but I think the other thing which is very often overlooked is the bias. We don't think so much about it because bias is usually present when we change the lot, then you can have a shift in the level of the assay going from one lot to another. We don't change the lot so often, a few times a year. We don't think so much about this but imagine you have a cutoff which is 5 nanograms per liter and everybody who is below 5 nanograms per litter they are determined as low risk of myocardial infarction. That would be maybe 50, 60, even higher percentage of your population or the population you measure.

And of course if the level of your assay then changes from 5 nanograms per liter to 6 nanograms per liter, a large proportion of those patients who were measured below 5, all those who had 4.9 or 4.8 or whatever, they're going to shift 1 nanogram up and they're going to be measured like 5.9, 5.4, and if that is large proportions of your population, then even a lot shift of only 1 nanogram per liter is going to affect very much the efficiency of the algorithm you use.

So, I think that's why we need to also pay some attention to bias when it comes to troponins and to the low concentration because it doesn't affect the safety so much, but because there are very, very, very few patients with myocardial infarction who will be measured around 5 or 6 nanometers when they arrive, very few. But all those people who are low risk, they're going to be around that concentration. And then if you shift a little bit one way or the other, this will change tremendously how many patients are below or above the cutoff. So, well, both are important, but in different ways.

Bob Barrett: Okay, fair enough. How do you think clinical laboratories will react to these suggestions? Is it feasible to monitor the analytical quality of troponin assays at such low concentrations?

Kristin Aakre: Yeah, how will they react? Well, I don't know but is it feasible? Yes, it is feasible. But you have to do some work yourself. A laboratory has to do some things on their own because it's not easy to buy commercial like internal quality assessment materials at those low concentrations. I don't think they are very much available at those low concentrations. But what we do, and many other laboratories also do, is we make our own internal quality assessment materials.

So, we will pool low concentration serum and make large pool, if you allocate those and freeze them and you can take up one allocation each day, and measure. So, you actually make your own material for internal quality assessment. And that is quite, it's a little bit work and you need a freezer, but it's doable for most laboratories, and that is for measuring the analytical CV. Of course, if you do this for many years, with the same materials, you will also be able to measure the loss variations when using that kind of system.

If you want to have a little bit more control of the bias, and we do that as well. We have several pools at different concentrations and every time we change like the reagent or the calibrator lot or anything else, we will take up a series of pools and we will measure all of them starting from very low concentrations and going higher so that we can each time see how much difference there is between the lot for different concentrations. It gives you a very, very good overview of how much your assay will vary throughout the years. If the producer says plus minus 10%, your lots are going to vary approximately plus or minus 10%. It's a little bit of work, but it's feasible.

Bob Barrett: Well, finally doctor, let's look ahead. Do you think the clinical use of troponins may change in a way that affects measurement considerations at the very low end of the measurement range?

Kristin Aakre: Well yes, I think so. The first publication came out maybe 15 years ago showing that troponins are very strong predictors of long-term cardiovascular risk.

And since that it has been discussed back and forth how can we use troponin for long-term risk prediction. And there are actually now some guideline recommendations, suggesting that we should use troponins and also neuropeptides to monitor the risk in certain populations like diabetes patients and so on. But it's not used like in general for large patient groups or for like in the general population, but this can change in the future because they are very strong risk predictors. They also tell you something about the overall status of your heart in a way.

And if we are going to use this clinically for individual patients, well, you know, what this large studies show is that the difference between the low and the high-risk population. The troponin values are not so different. If you have a little variation of 4 or 6 nanograms per liter, you can actually shift the patient from low to high risk because the upper quartile in these studies could be around 10, right? And the lower could be around 4 or even 5. So, the magnitude of the difference between the high and low risk patients is quite small and this is going to be long-term monitoring. So, lot variation is going to be important here.

So, this is a message to the manufacturers that if you want your assay to be used for long-term risk prediction, you have to pay very close attention to the lot variation and how much its total variation of your assay. It's not going to be useful if you just based on analytical variations. You can shift the patients from low risk to high risk or from intermediate risk to high risk for instance.

Yeah, if this changes in the future, both we as laboratories will need to monitor this closely, and also the manufacturers are going to need to step up and really take care of this and make sure the assays are stable over long periods.

Bob Barrett: That was Dr. Kristin Aakre from Haukeland University Hospital and the University of Bergen, Norway. She served as lead author of a review article in the March 2024 issue of *Clinical Chemistry* describing performance characteristics of cardiac troponin assays to support rapid rule-out pathways and she has been our guest in this podcast on that topic. I'm Bob Barrett, thanks for listening.