Clinical Chemistry

Performance Criteria for Testosterone Measurements Based on Biological Variation in Adult Males: Recommendations from the Partnership for the Accurate Testing of Hormones

Article:
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Guest:
Dr. Robert Fitzgerald is the Director of the Toxicology Laboratory and Associate Director of the Clinical Chemistry Laboratory in the Department of Pathology at the University of California, San Diego.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I'm Bob Barrett.

Studies have shown that some testosterone assays have important limitations, particularly when used to measure this hormone in women and children. Some of these procedures can have errors of nearly 500%. This situation led to a consensus statement, endorsed by 11 scientific, medical, and laboratory organizations, with the stated purpose of improving "the quality of research, patient care, and public health through broad implementation of standardized testosterone results that are accurate, reliable and comparable over time."

In the December 2012 issue of *Clinical Chemistry*, the Partnership for the Accurate Testing of Hormones (PATH) published *Performance Criteria for Testosterone Measurements Based on Biological Variation in Adult Males: Recommendations from PATH*. The senior author of that report is Dr. Robert Fitzgerald from the Department of Pathology at the University of California, San Diego, where he is the Director of the Toxicology Laboratory and Associate Director of the Clinical Chemistry Laboratory.

Dr. Fitzgerald is our guest in this podcast. Doctor, from a clinical perspective, why is it important to measure testosterone?

Dr. Robert Fitzgerald: Testosterone really is responsible for the differences between males and females. So, on a very basic level, that's where it starts. Clinically there's a variety of conditions where testosterone is important, such as ambiguous genitalia in newborns that actually is critical,

because it determines, or at least has a major role in determining sex of that individual. It's also used to diagnose hypogonadism in males. In females it's important for diagnosis of polycystic ovarian syndrome, a syndrome that affects somewhere between 6% and 8% of all women. So it has a huge impact.

Bob Barrett: Well, let's look back just for a minute. Can you give us a brief history of testosterone measurements?

Dr. Robert Fitzgerald: Interestingly, testosterone was discovered not until the 1930s, I think, it was 1935 when scientists took several kilograms of bull testicles and isolated 10 milligrams of testosterone which he was able to characterize. So it's been less than 100 years that we even knew about testosterone and that was where the beginning of it.

The first assays were bioassays where they took extracts and they administered them to castrated roosters. The end point of measurements was how much the comb size had changed and they had actually come up with what they call a capon unit, a pretty crude measurement of testosterone activity and androgen activity. Sort of move forward with that in clinical specimen with the development of gas chromatography and in particular electron capture detectors where we had sufficient sensitivity to measure testosterone at low concentrations.

Gas chromatography required a lot of sample prep and with the development of radioimmunoassay in the '70s, we sort of switch to be more immunoassay-based. Immunoassays initially were radioimmunoassays with or without extraction and they provided our most reliable measurements at that time. In the early '80s and I guess into today, different immunoassay platforms have been developed that are direct immunoassays that are the most commonly ones used clinically.

Today, the two assays that are used would be immunoassay-based platforms and primarily mass spectrometry-based platforms.

Bob Barrett: When we usually think of measuring testosterone in adult men, why is it important to measure this hormone in women and children?

Dr. Robert Fitzgerald: As I mentioned previously, in children, for development of muscles and bone growth, it's important in the case of ambiguous genitalia. It's important in females. It's important if they have hirsutism, if they have problems with menstrual irregularities and things like that.

Bob Barrett: There have been a large number of reports on problems with using immunoassays to measure testosterone. Can you tell us some more about these issues?

Dr. Robert Fitzgerald: The problems with the immunoassays we've known about for 15 years and really, it relates to the fact that there are so many circulating steroids, molecules that have this very similar structure as testosterone that are at widely varying concentrations. In particular, it's most difficult to measure testosterone using an immunoassay for specimens from women and children. And again, that's because the concentration of testosterone in that patient population is quite low.

So, testosterone is present at low concentration in a milieu of high concentrations of other structurally related compound. So the antibody has difficulty just recognizing the testosterone. So in men, the immunoassays work reasonably well. In women and children, they can be off by as much as 500% so that would be a fivefold error in the measurement.

In fact, when we first become involved with this, we showed that the immunoassays didn't work very well for women and that was followed up subsequently by a fairly complete study in 10 different immunoassays, and we actually showed that you could guess better than some of the immunoassays that were FDA-approved, which is quite startling really to me.

Bob Barrett: It is. Let's go back and repeat that, you said that the FDA has approved assays that have errors of up to 500%. Why did these assays get approved and why is it taking so long to address these issues?

Dr. Robert Fitzgerald: So the assays get approved and it's really the whole, what is known as the 510(k) process for approving clinical diagnostics, and 510(k) is where a new assay is judged against current performance. So, if you have an assay that doesn't work very well and it was historically FDA-approved, it's hard to make improvements, because if your new assay is better, it's not going to agree with the old assay. So that's one of the challenges. I think there are ways to address that and hopefully we will address that.

Bob Barrett: Why is it taking so long to address the issues?

Dr. Robert Fitzgerald: So it's taking so long to address the issues, I think, science sometimes moves slow and one of the reasons is that we haven't had everyone at the table. We knew analytically that the assays didn't perform very well. It was well-established in the literature. It wasn't until we

get better dialogues with the endocrinologist, with the pediatricians, with the CDC, and the NIST to come up with validated reference methods that we could actually stand behind and say, “We all sort of believe this result as the true value.” And they can document that as well as the clinicians understanding and coming to realize that the assays that they were making decisions on weren’t very good.

Bob Barrett: Doctor, what exactly is the Partnership for the Accurate Testing of Hormones?

Dr. Robert Fitzgerald: So that is PATH and that’s a group and that’s really the change mechanism that I see that’s going to be successful in making major improvements in measurements of testosterone and other hormones. It’s probably the widest group of stakeholders that need to be involved. It involves national societies: The American Association for Clinical Chemistry, The Endocrine Society, The Pediatric Society, the pediatric endocrinologists. So it’s both the people that make the measurements as well as the people that use them clinically as well as the national societies and journals that are going to publish it, sort of reaching out to address it on all aspects.

Bob Barrett: And who funds the projects?

Dr. Robert Fitzgerald: PATH was initially funded, I think, through The Endocrine Society, and The Endocrine Society is clearly taking a leadership role to bring PATH together and to bring the various stakeholders together to address the issue. How that gets continued funding is a little bit unclear to me.

Bob Barrett: And can anyone join the partnership?

Dr. Robert Fitzgerald: Yes, that’s administered through The Endocrine Society and we’re actively looking for funding mechanisms as well as stakeholders that can have an impact in improving measurement and utility of androgen and other steroid measurement.

Bob Barrett: Well, finally, doctor, your manuscript proposes to set total error goal of plus or minus 16% for all testosterone assays. Is that realistic and are we making progress?

Dr. Robert Fitzgerald: We are making progress. If you think that we have errors right now of 200%, 300%, 400%, having our total error budget of 16%, we’ve made orders of magnitude or at least an order of magnitude improvement in the accuracy. It really sort of sets a stake in the ground and gives us a target to shoot for. Not only does it set realistic goals which are achievable, and the paper

actually shows that there are assays that can achieve these total errors of 16%, it gives everyone something to shoot for.

If you look at other major standardizations across platforms, probably the best example is hemoglobin A_{1c} measurements. Initially, the variability in those measurements was huge, and once there was a standardized reference method to define clearly an accurate A_{1c} measurement, it allowed people to standardize against that and the coefficients of variations and consequently, really sort of the bottom line is, the ability to take care of patients is dramatically improved.

Bob Barrett: Dr. Robert Fitzgerald is the Director of the Toxicology Laboratory and Associate Director of the Clinical Chemistry Laboratory in the Department of Pathology at the University of California, San Diego. He's been our guest in this podcast from *Clinical Chemistry*.

I'm Bob Barrett. Thanks for listening.