

Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

The difficulty of accurately measuring hormones that circulate in low concentrations is well-known. A report published in the July issue of *Clinical Chemistry* showed that free thyroid hormone concentrations measured by liquid chromatography-tandem mass spectrometry or LC-MS/MS correlate to a greater degree with thyroid stimulating hormone values compared with concentrations measured by immunoassay.

The superior ability of LC-MS/MS to document the well-known thyroid hormone TSH relationships supports the use of this measurement technique in a variety of clinical situations.

We have as our guest today Dr. Steven Soldin, Professor of Pharmacology and Endocrinology at Georgetown University Medical Center and coauthor of the article.

Tell us Dr. Soldin, why did you suspect problems with the direct analog immunoassay methods for Free T4 and Free T3?

Dr. Steven Soldin: Well, the concerns date back at least one-and-a-half decade, and our endocrinologist at Children's National Medical Center had a lot of problems with our Free T4, Free T3 immunoassays and often would call me saying that the results do not agree with the TSHs that they were getting.

In fact, we instituted there a system which involved reflex testing. So when the result was less than the 2.5<sup>th</sup> percentile for Free T4, Free T3, or above the 97.5<sup>th</sup> percentile, we would automatically send the sample out for the gold standard, which at that time was equilibrium dialysis immunoassay. The results came back, 50% of the time, completely in the normal range.

This indicated of course that there were problems with the direct immunoassay method, and we continued this practice until we were able to introduce better technology. Now, so that was one of the reasons.

The CAP Proficiency Testing Program as well as the New York State Proficiency Testing Program have indicated that the equilibrium dialysis immunoassays agree with the equilibrium dialysis mass spectrometry assays and the ultrafiltration mass spectrometry assays. But the results of those sorts of definitive methods are very different from the results obtained by the direct analog immunoassay method.

So for both these reasons it was clear that there were problems with Free T4 and Free T3 measurement.

Host: Well, are there specific analytical problems with immunoassays for thyroid hormones in pregnancy?

Dr. Steven Soldin: Yes, there certainly are. The pregnant woman has a lot of nonspecific heterophilic antibodies, and these clearly affect the validity of the direct analog Total T4 and Total T3, as well as Free T4 and Free T3 measurements.

We did several studies which indicated that in a non-pregnant women, there was a fairly good agreement between Total T4 and the mass spectrometric methods for Total T4. But during pregnancy, the correlation between immunoassay and mass spectrometric results became poorer and the correlation coefficients in fact decreased with increased in gestational age. So that we may start off in a non-pregnant woman with an R value of 0.9, but that would drop to perhaps an R value of 0.5 late in pregnancy. So the correlation between immunoassay and mass spectrometry decreased with lengths of gestation.

Host: Well, with that in mind, have you compared the ultrafiltration tandem mass spectrometry methods with equilibrium dialysis immunoassay or tandem mass spectrometric methods?

Dr. Steven Soldin: Yes, we have compared our ultrafiltration tandem mass spectrometric method with the equilibrium dialysis immunoassay. We chose to compare it with the Nichols assay, which was the definitive gold standard at the time, and we in fact obtained a slope of one with excellent correlation, provided the ultrafiltration was carried out at 25 degrees centigrades. So if you did the ultrafiltration at 25 degrees centigrade, you got exactly the same results as the Nichols equilibrium dialysis immunoassay; the latter being performed though at 37 degrees.

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We have also done comparisons with other tandem mass spectrometric methods. In other words, we have compared ultrafiltration tandem mass spectrometry with equilibrium dialysis tandem mass spectrometry and found fairly good correlations, incidentally not quite as good as the one that we obtained with the Nichols procedure.

Host: In your opinion, should ultrafiltration be performed at 25° or 37°C?

Dr. Steven Soldin: That's an interesting question, and I think it really depends on the site and on what they choose to do. This method was

developed at Georgetown University and then introduced at Children's National Medical Center.

The endocrinologist at Children's National Medical Center wanted results that were, if possible, identical to the Nichols equilibrium dialysis immunoassay. So we had to use 25 degrees centigrade at Children's National Medical Center.

Having said that, there obviously are advantages to doing the ultrafiltration at 37, which is more physiological, and in fact, all the commercial labs or those providing Free T4, Free T3 measurements by equilibrium dialysis followed by mass spectrometry today, they are all using 37 degrees centigrade.

What is the difference here? We did a temperature study and the results at 37 for both Free T4 and Free T3 are 1.5 times higher than the results at 25 degrees centigrade. So it really shifts your reference intervals and/or your results by a factor of 1.5.

Host: Doctor, how have you validated the superiority of the tandem mass spectrometric method compared with the current immunoassay procedures?

Dr. Steven Soldin: Well, in our recent paper in *Clinical Chemistry*, we have done studies in four different population groups, and we compared each of these groups with the immunoassay results versus the log of a TSH, and we also compared the tandem mass spectrometry result versus the log of the TSH.

In all cases, we found that the tandem mass spectrometric procedure gave results that correlated far better with the log of the TSH than did the immunoassay. Again, emphasizing that tandem mass spectrometry essentially is a superior method clinically to the identification of both hyper and hypothyroidism.

I should point out that in some of our studies we have shown that the direct analog immunoassay method actually compares fairly well with ultrafiltration tandem mass spectrometry between 0 and the 10<sup>th</sup> percentile and between the 90<sup>th</sup> and the 100<sup>th</sup> percentile.

So the problem areas are in the patients that are at risk of being diagnosed as hyper or hypothyroid, and it's precisely there that immunoassays do not operate well.

Host: Well, what are the implications regarding cost per test and turnaround time?

Dr. Steven Soldin: We're now doing these tests routinely at Children's for at least two-and-a-half, three years. They've done over 30,000

patients this way. Immunoassays for Free T4, Free T3 are no longer available at Children's. The method by the way has also been introduced at Georgetown as well as where it was first developed of course and also at NMS Labs.

So there are three sites that are performing Free T4, Free T3 test routinely using the ultrafiltration tandem mass spectrometric method.

With regard to the cost per test and the turnaround time, basically all of these sites will do one batch of samples per day. So you get a 24 turnaround time, which for Free T4 and Free T3 is adequate. These are not STAT tests. I can tell you the cost implications. The equipment is fairly expensive; it needs a fairly high power tandem mass spectrometer.

We found at Children's that the instruments had been completely paid off within five months of us going live with that method. Immunoassays, as I mentioned for Free T4 and Free T3 are no longer available at Children's National Medical Center.

Host: Dr. Steven Soldin is a Professor of Pharmacology and Endocrinology at Georgetown University Medical Center, and he's been our guest in this podcast from *Clinical Chemistry*. I am Bob Barrett. Thank you for listening.

Total Duration: 10 Minutes