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MicroRNAs in Idiopathic Childhood Nephrotic Syndrome

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Guests:
Drs. Johan Lorenzen and Thomas Thum are from the Hannover Medical School in Hannover, Germany.

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

MicroRNAs are present in body fluids and have the potential to serve as disease biomarkers. A study in the April 2013 issue of *Clinical Chemistry* explored the clinical value of microRNAs in serum and urine as biomarkers for idiopathic childhood nephrotic syndrome.

This paper was accompanied by an editorial by Johan Lorenzen and Thomas Thum, both from the Hannover Medical School in Hannover, Germany. They both join us today in this podcast.

First Dr. Thum, what are microRNAs and how are they transported in blood?

Dr. Thomas Thum: Well, microRNAs are small endogenous RNA molecules that are surprisingly stable in blood, because normally RNAs are degraded easily, but those RNAs, they are encapsulated in small vesicles and bound to certain proteins which prevent degradation, and those molecules, they are transported throughout the blood and can be detected easily.

Bob Barrett: It has been reported that microRNAs are surprisingly stable in blood and bodily fluids, why is that?

Dr. Thomas Thum: Yeah, that was an intriguing finding that people found that the RNAs are very stable, because normally, as I said, RNAs are degraded, but we now believe that due to the inclusion in small vesicles, the vesicles protects the RNA itself from, for example, RNAses that normally degrade RNAs, and also they are bound stably to certain proteins that also protect those microRNAs from degradation, thus they are relatively ideal candidates for biomarker and biomarker discoveries.

Bob Barrett: Dr. Lorenzen, in reporting results, microRNA results are usually normalized to an internal control. Why is that, and how is the normalization performed?

Dr. Johan Lorenzen: Yeah, so in cell culture models, so if you look at, let's say, kidney cells, for example, you would usually normalize your microRNAs to an internal control which is stable within the cell. This would be RNU6B, for example, or small nucleolar RNA 202, but in bodily fluids and often blood there is no normalizing control which can be found stable.

Some researchers have used miR-16, for example; others miR-1249, but we have found, for example, that miR-16 is not stable in the blood. So currently you have to somehow treat your samples with an external control, which you have to spike in, as we would say, into the sample during the RNA isolation process.

And what we do currently is we spike into the samples synthetic C. elegans microRNAs, which really need to be spiked to these samples. So this is actually really important.

Bob Barrett: Will analysis of circulating microRNAs enter clinical application soon?

Dr. Johan Lorenzen: So there is growing interest in the community and also more and more people that measure microRNAs as potentially biomarkers in various body fluids, it's not only serum, plasma, it's also in urine or cerebrospinal fluid or other body fluids. And we started with analyzing only, let's say, dozens of patients, but now we are studying and the other people are studying hundreds of patients and we have very good data that there are certain microRNAs patterns that are really helpful in the diagnosis of certain diseases.

So we really think that within the next five years or so there will be certain microRNAs patterns that will be very helpful in the clinics to decide, for example, for certain diagnostic decisions, but also the microRNAs might have prognostic potential.

Bob Barrett: What are the obstacles to clinical applications?

Dr. Johan Lorenzen: One of the obstacles we already shortly discussed is the difficulties with the normalization of the microRNAs. So we have to put great power to identify the, let's say, intra-assay variability, specificity, and sensitivity and so on of those PCR-based applications, and that we will need a lot of time, money, and also people in the lab that need to develop the tools that they are really ready then for clinical applications.

Bob Barrett: Finally, Dr. Lorenzen, what about point-of-care applications, is there or will there be a bedside test?

Dr. Johan Lorenzen: It's actually a very important question. As Dr. Thum just stated one problem is in the normalization procedure and

what's also currently a big obstacle is the fact that it is very time-consuming to analyze microRNAs within the circulation.

So you would need to obtain the sample, then you would need to direct the RNA, and you would need to do some reverse transcription, and finally you would do a real-time PCR to have an idea of the deregulation of the microRNAs in your blood or urine samples. This takes several hours.

And if you have a patient who, let's say, has a myocardial infarction, for example, or some other very critical disease, you would need to have an idea of your biomarker deregulation within optimally minutes or at least half an hour or so.

So right now there still needs to be some care taken or some research has to go into this field now to be able to have a bedside test of microRNA expression within, let's say, 30 minutes or so, so that you can really initiate therapeutic decision based on that. That's actually I would say a long way to go until we are ready to do that.

Bob Barrett: Doctors Johan Lorenzen and Thomas Thum are from the Hannover Medical School in Hannover, Germany. They have been our guests in this podcast from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.