Clinical Chemistry

Digital Droplet PCR for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury

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Guest:
Dr. Ekkehard Schütz is an Associate Professor of Molecular Diagnostics at the Georg-August University in Göttingen, Germany, and the Chief Technology Officer at Chronix Biomedical in San Jose.

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

Cell free DNA from transplanted organs in the circulation of transplant recipients is a potential biomarker of rejection. But most of these methods entail high costs, long turnaround times and the need for donor DNA.

The December 2013 issue of *Clinical Chemistry* describes a rapid and cost effective method using digital droplet PCR to quantify donor derived DNA in transplant recipients. The senior author of that paper was Professor Ekkehard Schütz and he joins us in today's podcast.

Dr. Schütz is an Associate Professor of Molecular Diagnostics at the Georg-August University in Göttingen, Germany. He is also the Chief Technology Officer at Chronix Biomedical in San Jose.

Dr. Schütz, what is the need for biomarkers in solid organ transplantation?

Dr. Ekkehard Schütz: Yeah, we have to just go back to some numbers first, so in the U.S. we are doing kind of about 30,000 solid organ transplantations per year, and about 200,000 patients are living with organ grafts. But on the other hand we have a 100,000 patients waiting for transplant, and this ratio of 1:3 is about the same worldwide.

So that makes the case that there still is the shortage of donor organs, and one major problem that we are facing is that if you, for instance, look at kidney transplantation which is by far the largest group of solid organ transplantations, we have over a period of 10 years, about 50% of organs that are lost over that period of time.

So the need of biomarkers here is set by that case. We have still a lot of solid organ rejection over that time, and we are

looking for a variety of biomarkers to catch these rejections early enough, so we can avoid chronic rejection that's going on over time. And that's more or less the basic need for biomarkers, really to avoid graft losses over the time and the patient seems to be very well.

Bob Barrett: What kind of biomarkers are of interest and what type are the most helpful in monitoring the post-transplant period?

Dr. Ekkehard Schütz: Yeah we right now have a whole variety of biomarkers and most of that are directed either to predict the immune status of the patient or the other large group of biomarkers is directed to the immunosuppressive drugs directly. So the most biomarkers that are done today on a regular basis is the therapeutic drug monitoring of immunosuppressive drugs, and this is one very helpful diagnostics here, so we can kind of avoid especially over-immunosuppression with all the consecutive problems like infections, side effects and the like.

And on the other hand we can also look at the lower level of the so called therapeutic range, so if patients are below that, then the risk to get a rejection dramatically increases. But all that is not really true on an individualized basis, so there are patients that are pretty fine with lower TDM of the immunosuppressive drugs, and others who need more. And so there are a variety of biomarkers that we are looking at right now that are directed exactly into this case. So cytokines, these are expression levels of immune activity markers, and the like.

So, but, none of these markers directly interrogates what's going on with the organ itself; it's more or less directives trying to predict something.

Bob Barrett: Doctor, what are the advantages of using cell free DNA and laboratory diagnostics, and in particular, as a biomarker of rejection?

Dr. Ekkehard Schütz: That's to understand the circulating free DNA is a way of directly interrogate what's going on with the transplanted organs. So the principle behind it is that that we are using the differences in the genetic make up between the graft which is the donor organ and the recipient to detect what is the amount of DNA that is circulating in the peripheral blood, and in this case in the plasma, and this really gives us direct answer--is something going on with the organ--so it's a direct marker of organ integrity so we can monitor that real time.

And as soon as the -- for instance rejection hits the organ, we can show that there is an immediately an increase of cell free DNA that is coming from the graft, so we have more or

less a real time measure of organ integrity and this is exactly what the difference is to all the indirect biomarkers.

Bob Barrett: What were the design goals for the graft derived DNA assay that you published in *Clinical Chemistry*?

Dr. Ekkehard Schütz: Yeah the design goal was that there were earlier publications about what's to be expected in terms of concentrations of graft DNA in the circulation, and so one of the design goal was that we needed to achieve a good precision and reliability at, let's say, around 1% graft DNA that is circulating, compared to the circulating DNA that is in the recipient. Everybody has circulating DNA in the plasma. So what we are trying to detect is a very low amount that is coming from the donor organ.

So that was one of our design goals to get an assay that is very precise at this low-end, around 1%, is I think what's needed. And the second design goal was that we are coming up with an assay that can be done within one day.

So, since that's more or less the clinical need, if a surgeon sees there is a rejection going on, they want to treat it normally with steroids, for instance, as soon as possible, and so that was our second design goal to come up with a system that has a one day turnaround time.

Bob Barrett: What are the technical advantages of using a digital PCR system?

Dr. Ekkehard Schütz: Yeah, the digital PCR overall has clear technical advantages and it's a very direct measure of molecules. It's more or less molecule counting.

So at that point you don't really need a calibrator here, you are just counting molecules and you are counting the amount of molecules that are coming from the graft, and compare that to the amount of molecules that are circulating from the recipient, and you can use that as a direct measure.

So there is no other steps involved that give you variability, it's just the straight molecule counting, and just giving the amount of DNA that we normally have in circulation, which is around 1000 to 2000 molecules or genomic copies per ml.

With this digital PCR, if we are at just say around 1%, then we are at numbers that are already having very, very good inherent precision. So that's more or less the whole background here, it's a very straightforward physical measurement of molecule counting.

Bob Barrett: Well finally, doctor, can the assay that your group developed help to detect subclinical rejection and help individualize immunosuppressive therapy?

Dr. Ekkehard Schütz: Yeah, giving out first results, we think we are really very positive that we can do that. We have seen a couple of rejections in our preliminary studies on liver transplantations already, and one interesting point was that the increase of graft DNA was kind of proceeding the increase of conventional biomarkers, like liver function test AST, or like by at least a day.

So it's very sensitive, and so we really think that we can see rejections before they really affect the organ in terms of going into a full blown rejection.

And the second part of the question, individualized immunotherapy; that really looks also very promising, since if you compare the circulating graft DNA to what you see in terms of therapeutic drug monitoring of immunosuppressive drugs, then we can truly see a correlation here that people or patients that have very low levels of Taq polymerase or cyclosporine that they overall can show higher graft DNA.

And as I said in one of the earliest statements, it's not always the case that a patient needs exactly the same amount of immunosuppressive drugs as another patient. This direct measure of organ integrity may really help us to individualize the immunosuppressive therapy.

Bob Barrett: Dr. Ekkehard Schütz is an Associate Professor of Molecular Diagnostics at the Georg-August University in Göttingen, Germany and also the Chief Technology Officer at Chronix Biomedical in San Jose. He has been our guest in today's podcast examining cell free DNA as a biomarker after organ transplantation.

I am Bob Barrett, thanks for listening!