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The Proportion of Donor-Specific Cell-Free DNA in Blood as a Marker of Transplant Rejection: Not an Absolute

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Guest: Dr. Iwijn de Vlaminck of the Meinig School of Biomedical Engineering at Cornell University in Ithaca, New York.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Donor-derived, cell-free DNA has been reported to be a valuable tool as a marker of rejection following heart, kidney, liver, lung, and other organ transplantations. However, possible changes in donor-derived, cell-free DNA reference values over time have not been previously evaluated. But now, in the October 2020 issue of *Clinical Chemistry*, a group of workers based in Germany examined this question in a paper titled, "Time-Dependent Apparent Increase in Donor-Derived, Cell-Free DNA Percentage in Clinically Stable Patients Between One and Five Years Following Kidney Transplantation." That paper was accompanied by an editorial by Dr. Iwijn de Vlaminck of the Meinig School of Biomedical Engineering at Cornell University in Ithaca, New York, and we're pleased to have Dr. de Vlaminck here as our guest in this podcast. So, doctor, what is the current role for cell-free DNA liquid biopsies in transplantation medicine?

Iwijn de Vlaminck: Well, I would say it's an exciting time for cell-free DNA and liquid biopsies in general in transplant medicine, and this all started – this story started with Dennis Lo, who discovered the presence of donor-specific, cell-free DNA in the blood of transplant patients, and then the field stayed quiet, quite dormant, waiting for the right molecular tools to be developed, I suppose. It was the lab of of Steve Quake at Stanford, where I was lucky to be a postdoc, that played an important role about 15 years later, demonstrating the utility of cell-free DNA in both retrospective and prospective studies in heart and lung transplantation, the utility that donor-specific, cell-free DNA could be a marker of transplant rejection. And since then, the progress has been very impressive. Donor DNA has become – I think it's fair to say—one of the best investigated biomarkers in solid organ transplantation, and what is really impressive also is that it's used not only in heart and lung transplantation but across many different settings in solid organ transplantation, which is quite unique for transplant markers. These initial studies have then also been followed by analytical validation and

clinical validation studies and since then, in the last few years, donor-specific DNA has really started to impact the care of transplantations. Tens of thousands of patients have already benefitted from donor-specific, cell-free DNA tests.

Bob Barrett: What are some of the outstanding challenges in this field?

Iwijn de Vlaminck: Well, I think it's related to the fact that donor-specific, cell-free DNA, or donor DNA, is really a marker of injury, is really a marker of the total injury to tissues in the transplant organ. Now, this is actually a strength also because it makes it easy to interpret, it's quantifiable, and it can be trended over time. I think it explains the success of this technology also because it's something they can easily be communicated. But at the same time, the strength that it's a measure of total injury that can be obtained on it basically is also a weakness because it doesn't tell you what the source of this injury is. It doesn't communicate or doesn't provide information about the mechanism of that injury. And now, in transplantation obviously, you can assume that injury is related to rejection, but it could also be related to for example, infection.

Bob Barrett: What particular issues were raised in the recent publication by the Göttingen group et al in Clinical *Chemistry*?

Iwijn de Vlaminck: Well, the issue that was raised is that most studies to date have monitored the proportion of donor-specific, cell-free DNA in the blood, that is to say they've quantified the relative amount of donor DNA and that can easily be calculated as the amount of donor DNA divided by the sum of the amount of donor DNA and recipient DNA. And so, the greatest contributor to that denominator is actually the amount of recipient DNA, and so the issue that is related to that, and that's not something that was brought up the first time in this particular paper, the issue that was related to that is that the amount of recipient DNA is not necessarily stable. For example, there are factors such as exercise that are known to lead to significant increases in the total amount of cell-free DNA in the blood. So, the amount of recipient DNA might change due to factors that have nothing to do with transplant health. And so, that is a potential issue.

Bob Barrett: Doctor, you wrote an editorial on the work of Oellerich and his coworkers. What are the key contributions of their study?

Iwijn de Vlaminck: So, what this group of investigators have done is they have followed a group of a little bit more than 300 clinically-stable kidney transplant recipients. So, these were all patients that did well, that did not reject, and they followed these patients over time. They did a longitudinal study and

quantified the amount of donor DNA and recipient DNA and relative amount of donor DNA in more than 900 plasma samples. And when they did that, they made the remarkable observation that there was a significant decline in total amount of cell-free DNA and, therefore, presumably a significant decline in recipient DNA with time after transplantation, and a commensurate increase in the proportion of donor-specific DNA.

So, that relates to what I just mentioned, that the relative amount of donor DNA is of course strongly dependent on the amount of recipient DNA in the sample. And the authors reasonably suggest that this observed decline in total cell-free DNA with time after transplantation is due to a decrease in apoptosis rate, so the rate at which cells die for white blood cells as the immunosuppressant drug doses are tapered off. So, and this study therefore provides an interesting and timely perspective and really calls into question the singular focus on the relative amount of donor DNA as a relevant readout of transplant health. Now, I have to add to this that these authors, as I've mentioned before, they only looked at stable transplant recipients. And so, they have not shown decisively that the total amount of donor DNA, the concentration of donor-specific DNA in the blood, is a better marker of transplant injury. So, that is something for follow-up studies to investigate.

Bob Barrett: Well, finally, let's look ahead. What do you see is further work that needs to be done in the future, and where do you see this field five, or maybe ten years down the road?

Iwijn de Vlaminck: Yeah. That's a great question. That's a fun one. I would get back to that initial challenge that I raised, that donor-specific DNA is just a marker of total injury in all the tissues in the transplant organ, and what I think what will happen in the next few years is you'll see cell-free DNA-based measurements that can report more refined information of the cell types that are being injured in the organ, and this can for example be achieved by tracking epigenetic marks that are comprised within these molecules, and they carry information about the cell types of origin. These types of measurements have been developed and are being developed in other fields and I think what you will see is that some of this will leak over in the transplant field. So, what I'm hopeful for is that there will be liquid biopsies available in the near term, in the next five or ten years, that take advantage of cell-free DNA and that provide more informative readouts of transplant health than is currently available.

Bob Barrett: That was Dr. Iwijn de Vlaminck of the Meinig School of Biomedical Engineering at Cornell University in Ithaca, New York. He was discussing the role of donor-derived, cell-free

DNA as a marker of rejection following organ transplantations. His editorial, as well as an original scientific paper examining time-dependent increases in donor-derived, cell-free DNA percentage following kidney transplantation, appear in the October 2020 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.