Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications

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Guest: Dr. Jonathan Bromberg, from the Department of Surgery, Division of Transplantation at the University of Maryland School of Medicine.

Randye Kaye: Hello, and welcome to this edition of "JALM Talk" from *The Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye.

After a patient has undergone a kidney transplant, they are monitored closely for rejection by measurement of kidney transplant function, specifically by serum creatinine, which is used to estimate the glomerular filtration rate of the kidney and urinalysis for proteinuria.

However, these tests are not sensitive or specific. Donor-derived cell-free DNA measurement in the circulating blood of transplant recipients is thought to have utility for clinical monitoring of tissue injury and therefore transplant rejection in heart, lung, liver, and kidney transplants, as increased levels of this biomarker are seen during acute rejection.

However, the normal concentration of donor-derived cell-free DNA in successful kidney transplant patients has not been determined. An article titled "Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications" published in the November 2017 issue of JALM, determined the biological variation and reference intervals of this biomarker in this patient population to aid in discrimination between rejection versus no rejection in kidney transplant patients

The first author of this article is Dr. Jonathan Bromberg from the Department of Surgery, Division of Transplantation at the University of Maryland School of Medicine, and he is our guest for today's podcast. Welcome Dr. Bromberg. Let's start with this question. What is donor-derived cell-free DNA?

Jonathan Bromberg: Okay. So every cell and every tissue in our body can be undergoing changes and there can always be a few cells in every single tissue and in every single organ that are dying. And as some of these cells die, they release the DNA from their nucleus, and that DNA can be found as free DNA in the blood or in the serum or in the plasma. So, in somebody

who has received an organ transplant, such as a kidney, if there are a few cells in that kidney organ that are dying, you can actually find DNA that is derived from that donor kidney.

Now, what makes this particularly convenient for a test is that the DNA derived from that donor will be genetically different from the DNA that's derived from all of the other cells in the kidney recipient. And because that DNA is different at the level of the DNA sequence, you can use advance sequencing methodologies and advance statistical methods to determine how much of the DNA that's floating around in somebody's blood is derived from the donor and derived from the recipient. The next advantage we have discovered in our studies is that if an organ has inflammation or damage, there is increased amount of DNA coming from that organ or tissue, and therefore the amount of donor-derived cell-free DNA will be increased. And this increase in this magnitude can be used as a measure of either inflammation or damage within that organ.

So essentially, you have what is sometimes been called a liquid biopsy, is you obtain a blood sample from an individual and from that blood sample, you can find DNA from that donor tissue or donor organ. So instead of sticking a needle into the organ to get a biopsy which is what we often do in clinical transplantation, we just get a very simple blood sample and can find some of the same information just from processing that blood sample.

Randye Kaye: Wow! That's fascinating and a lot less painful for the patient as well. Now, I think you may have already answered this question, but I just want to make sure, you might have something to add, about why this biomarker is useful for the care of kidney transplant recipients. Anything to add about that?

Jonathan Bromberg: So I think there are several reasons that it's useful. The first is, instead of doing a biopsy which is potentially painful, potentially complex and complicated, with some complications that can occur to patients, which is a lot less convenient, which is a lot more expensive. We can actually just do a blood test and that might also be a very easily done blood so the patient doesn't actually have to come to the transplant center hospital, but perhaps can get the blood drawn at almost any phlebotomy center around the state, around the country.

So, the convenience to the patient, the patient's families, and to the physicians is dramatically increased. Also, biopsies can only be performed on an infrequent basis, again because of the complexity and cost of a biopsy, whereas this so-called liquid biopsies or cell-free DNA

potentially could be performed far more frequently in terms of following patients for a whole variety of issues to monitor the allograft.

There are many situations where we are not sure whether or not we should get a biopsy on patients for many different clinical reasons, because we're not exactly sure if something is going on in the graft, or if something bad is going on in the graft such as inflammation or rejection or infection.

Again, with a test like this, we might be able to tell with a far greater degree of accuracy whether there is a problem or not and whether we actually do need to get a biopsy or not. So for a whole host of reasons, this information can tell us a lot about patients, either for routine monitoring of patients who have normal graft function or monitoring of patients who have problems with their graft and monitoring of patients after we've treated them for a problem and perhaps making a much earlier and more accurate diagnosis if somebody has an early problem, early inflammation, or early rejection.

So the potential for using this is quite large and there could potentially be several different important clinical scenarios that we could bring this test to bear on.

Randye Kaye: Why is defining the biological variability of this new biomarker important to clinicians?

Jonathan Bromberg: I think this is important not only for this test but for any test we want to adopt clinically. You want to know that the test is sensitive and specific and accurate and highly reproducible and that there is not a huge range of variability. The more variability there is for a test, the less accurate it's going to be and the less useful it's going to be for anybody trying to manage a patient. The more accurate a test is with less variability, the easier that test will be to use, the more likely the result of that test will be meaningful, that they will give the practitioner a kind of black and white type of piece of information, yes, the kidney is okay or no, the kidney is not okay and I need to do something else.

As a clinician taking care of patients, that's the kind of information you want and that's also the kind of information that patients and families want. They want something definitive, they certainly don't like hearing bad news, but they would like something definitive so they don't have to worry about, is something going on or not going on? If nothing is going on, then everybody can rest freely and if there's something going on, yes, we do need to do this next step when we're quite sure we need to make the next step in order to treat you properly and get on with things.

Randye Kaye: So with that in mind, what were the results and the clinical implications of your study? For example, what have you established to be the reference ranges for DD and changes that maybe expected as part of normal biologic variation of cell-free DNA?

Jonathan Bromberg: So in our initial small studies we found that for well-functioning kidney transplants if there's nothing much going on the donor derives self-free DNA is usually less than 1% of the total cell-free DNA in a person's blood sample. And if the cell-free DNA is greater than 1%, there is a very good chance that there is something going on in this kidney that might be a problem, such as infection, rejection, a medication reaction, recurring disease, or something else that should then lead one to some additional tests on the patient. These additional tests could be blood tests, urine tests, perhaps an ultrasound of the kidney, or perhaps even a biopsy. But as we are learning more about the use of the test, if we couple the results of the cell-free DNA with other routine tests that we get on kidney patients, we can then make a decision about what a likely diagnosis or series of diagnoses might be, and then determine what the next important step might be.

We particularly found that if the cell-free DNA is greater than about 2% or 2.5%, the patient had a very high chance of having a type of rejection called antibody-mediated rejection. And this is very important because it's one of the more difficult diagnoses and one of the more difficult rejections to treat in patients. So, if we now have a test that can diagnose antibody-mediated rejection earlier and easier than our other tests, it might allow us to start treating patients earlier and in a more effective fashion.

As published in our paper, there are a number of other values in terms of the coefficient of variation of this test and some of the other cutoff points, the positive predictive value, negative predictive value, I won't go into those specifics here on the podcast, but suffice it to say that the performance of this test is very good and compares very well with other highly commonly used blood tests that we use for a whole variety of other purposes in clinical medicine.

Randye Kaye: Wonderful, sounds like an exciting new development. So, is this new test approved and available to renal transplantations?

Jonathan Bromberg: So the test has just become approved by Medicare within the last week.

And we're hoping people will start using it and getting comfortable with using it and figuring out what works best in the real clinical world, and whether this is going to be a better, easier, and more solicitous way for diagnosing issues or for assuring us the patients are doing well, and making it easier just to deliver care in the long term for the kidney transplant recipient.

Randye Kaye: Wonderful! And just one final question, is there anything else you'd like to add that we haven't covered?

Jonathan Bromberg: I think we've really covered everything. I thank you very much for publishing our report and giving us the opportunity to explain a little bit more about it.

Randye Kaye: That was Dr. Jonathan Bromberg from the University of Maryland School of Medicine talking about the JALM article "Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications" for this podcast. Thanks for tuning in for "JALM Talk." See you next time and don't forget to submit something for us to talk about.