[MUSIC PLAYING]

ADRIANA ZEEVI: It was very informative this morning, was the two talks about allocation. And one of the opinions there was that the question of the new allocation would impact a subgroup of patients who are the sensitized patient, what will happen to them in terms of the allocation.

And so I think it's very important to understand that how you define a sensitized patient. And that will be my task here, and how you approach that, a change over time, and improved. And that's one reason that many patients are referred here. And I think we have, now, a list of at least 20% to 25% of the sensitized patients that we take care. I think it's very telling about how the changes and improvements in the technology allow us to do that. And also, a great team of technicians and surgeons that want to do the help.

So what I would like to just first of all, I have no-- nothing to disclose for this talk. So I will talk a little bit about the characterization of the antibody, and how that change. And I think the previous speaker spoke very easily about titer, about MFI, about those things. So I'd like to tell you exactly what I mean by that. But in this institution, people really understand. And that is great.

Then I will also mention about how we approach and how we use that to make a difference. And then how we evaluate the sensitized patient, and then we also come to some kind of understanding who should be desensitized. Because not every patient who has any kind of antibody, you want to take that task.

So I would just jump right into a very nice illustration here to kind of bring together the type of testing that we have. So we are talking about, today, characterisation of HLA-specific antibodies. There are also non-HLA antibodies that come along now, and we have to start to pay attention. But today I will limit myself to the HLA antibodies class 1 with a single chain and the ABC, and the class 2-- understanding the complexity of class 2 is more of a challenge, with the TR, TQ, and TP. And the fact that the class 2 have two chains, and particularly the BQ and BP antibodies can come with a polymorphous, and the alpha chain and the beta chain combine. So now it's not so simple to say just one number. Now I have to have two or three numbers to describe the specific antibody. And that makes a very big difference to be precise with what you are looking.

So what do you want to know? We want to know if the patient is sensitized, if he has any antibody. And there was a very simple test with a bead, that kind of you put the-- they blotted a lot of class 1 or class 2, and you can just use that to screen yes/no. And that is the first Luminex-based screening.

I also want to say that we moved from cell-based to solid phase because that gives us the sensitivity and specificity that we did not have before. It comes with some penalties, too. But right now we are talking about the benefits. So this relatively cheap screening doesn't have much of a specificity, but can tell us yes or no HLA antibodies. Again, it will miss some, and we are not using that anymore clinically. It was a very nice cheap way to go through a large population to say is somebody sensitized or not.

The second source of beads are those that now they have a hole like a cell. So you remember, we were talking about a sensitized patient, using a panel of cells. I can take 50 cells and say, how many of those are positive? Use the serum of the patient, and come up with a percentage. That was not very correct. Because depending on my cells that I had in the panel, that could be skewed in one or another direction.

So now they use these beads that each bead has blotted on one cell, but only the class 1 or the class 2. So you have two A's, two B's, two C's-- that's one bead. And there are 100 beads that represent a population, that you can now have multiple phenotypes that are present. And then you have 100 beads for class 2. And we use that to come up with a better definition of somebody that has a high or a low paneled reactive antibody. And I'll show you examples.

And then the Cadillac is when you really want to know what is the specificity. So now we have 100 beads. Each bead has only one specific HLA molecule on it. And now you can very easily say, OK, positive or negative, what is the specificity. And that is when we use it for single-antigen bead to describe what the patient has.

OK, next slide. So here we have on the top, an illustration of a high PRA, or panel reactive. So as you can see here, this is the mean fluorescent intensity. Right here, we have the phenotype of each bead. And from the slope, you see that there are very few little cells, a few beads that are negative, and most of them are positive. So 96% of those beads responded to that sound, meaning that this is a high PRA. I cannot tell you the specificity based on that. But I can tell you this is highly sensitized. In this case, its class 1. I can do the same thing with a class 2. Here, I have a much lower PRA, so only these three cells-- three beads were positive. And they may have something unique in common. But again, to know the specificity, I need to go to the next step.

Now also I want to introduce the calculated PRA. Because people talk about the two things like this is one. So the PRA, looking with the PRA panel of this 100 beads gives you this relationship of sensitized/not sensitized. The calculated PRA has to take in consideration one most important thing-- what is the specificity of the antibody. And then the question is, what is the frequency of that antibody in the donor population? Talking about a large donor population. And then the number that comes out, it's combining the specificity and the frequency of that antigen. And it is much more correct to then refer to the calculated PRA. And I will show you examples of that.

So coming to the calculated PRA, as I mentioned, the frequency of-- it's based on the specificity, based on the single-antigen bead technology, and then the frequency of that antigen in a population of 12,000 donors that were typed. And then it's, again, a number that we can use, either what to avoid, how highly this patient is sensitized, what would be the chances to this patient to have a donor, should this patient be desensitized.

So this is the calculator and all the coordinators that take care of the pre-transplant. They know that. And they will choose to enter in each of these boxes the specificity that we declare on this patient. And then you hit the calculator, and you can do that only for class 1, only for class 2, all together.

And this is an example. So this is the specificity, the single-antigen bead assay, which tells us that the specificity is in that group of A2, A28, and B57, B58. These were the positive beads. In fact, this belongs to one family of the A2 [INAUDIBLE]. But the point is, we know that. Now we take this specificity, and we enter in the calculator, and we come up with the calculated PRA.

To give you an example in this next slide, if I would just choose to enter only one specificity, A2, the calculated PRA for that would be 47%. If I choose A3 only, it will be 22%. So in the past, if I had two cells that had A2, and two cells that have A3, these two patients will have exactly the same PRA, 20%. Out of 100, two cells responded. The truth is that A2 is much more prevalent in the population. That means more people carry that antigen. So if I have an antibody to A2, my donor pool goes down to 53%. This person that has this antibody, the donor pool is now higher, 78%.

Now if you combine with multiple specificities, and you see now what the difference. In fact, if you have an antibody that is very broad, many, many beads are positive. If you have this BW6 antibody or BW4, you have a very low chance to have a donor that doesn't carry that specificity. And these are the patients that we are sometimes in trouble in terms of having that kind of reactivity and finding a specific donor.

So repeating in terms of what we are doing today, this is the Luminex-based single-antigen bead assay that gives us the ability to identify the specificity. And again, we call the strengths of that specificity based on the mean fluorescent intensity. And anything that's below 1,000 is negative, over 1,000 is positive. But it's not blindly that. Because if we see a specificity even under 1,000, but the pattern is there, we keep that in mind. We follow that. Because the next time, it can be much higher, particularly post-transplant, when there is an indication that a new system is already revved up.

We don't have an assay that can go and detect the precursor to that, the B cells that activated. That would be great in the future to have that. We only detect the protein that is already out in the serum. But the cells may be there. Patients may have memory, and we have seen that with patients that had, for instance, a pregnancy, and they come to the transplant. We don't know the specific antibody. It's very low. We don't call it. And a week later, we have an AMR. So that is a possibility.

The next--

[CELL PHONE RINGING]

And I knew that. That's why I give you.

The next thing that is very important, and Cody used that so well, is the titer. And why this is important. So here we have an estimation of the level of the antibody cannot be done only based on MFI. Because you can have a very low MFI or high MFI, and as you dilute the sera, you can see that in some patients, in fact, the MFI increases, in other patients, it goes down. So there is a possibility that the MFI is hiding something. Because there is some inhibition, and particularly when there is a high-titer antibody.

So putting a neat serum and getting an MFI is a good start. It gives us a relative strength. But to tell the clinicians that you have a strong high-titer antibody, or an intermediate, or a week, we have to do like we do that in every infection. You take the serum, you dilute that, and you repeat the assay, and you see what the MFI is upon repetition and dilution. And in some cases, it dilutes down very nicely. So this is, in fact, a weak antibody, but started as an MFI of 10,000, versus another one that started at MFI of 2,000 and by dilution it goes up. And in fact, only at 1 to 1,000, you can see that the antibody is still at 10,000 MFI. So we learned that by experience. And now when we give the information to the clinicians, how to treat what you have in terms of potential pathogenesis, you need to know the titer.

And the last one that we also use in this center-- and I think, again, is depending how you use it and what's the clinical value is-- is to identify if an antibody has the ability to bind complement. Complement, knowing-- when we talk about acute antibody-mediated rejection, you're looking for deposition and complement in the biopsy, meaning the complement is a very important mediator of graft damage. If the antibody that binds cannot mediate that, you may have still a problem, but it won't be as acute. So when we do the Luminex assay, and now we have the whole realm of total antibody burden, we lost that ability to discriminate between complement binding and non-complement binding.

And so that is something that, again, was a simple solution by an assay that we can do. And we can define the C1q binding, which is the first complement, or there are others that do the C3B, and there was some publication of C4D-- either. It's the point that you try to capture, by looking with a few tests, the strengths, the titer, and the ability to bind complement. And things are related, and I will mention that as we go on.

So here is an example, whereby we are looking to antibody patterns, and how the risk assessment for the antibody level. If I ask a question about the given patient, and I see that by the Luminex single-antigen B, and this is the neat sera, undiluted, gives me a spectrum between 500 to maybe 2,000, I would say that's a weak antibody. But upon dilution, I can see now that in fact this anti-A2 anti-68 or 69, at 1 to 4, and the 1 to 16, up to 1 to 64. It goes up.

So we had what's called the prozone. But this is happening when you have a lot of antibody, and there is a competition because you cannot have a continuing increase as there is binding to that bead. And there is also a complement in the serum that can bind to that, and then interfere with the detection. So in this case, in fact what it looked like binding the weak antibody, by dilution, we find that even at 1 to 16 and 1 to 32, it's over 1,000.

If you were to cross this antibody, you have more problems immediately post-transplant than the antibody that is in the lower panel, which bind neat antibody. You can see they're all higher than 8,000. So you could, in the past two, we say strong, don't cross, it, this is it. And now, after dilution, we see that that antibody drops very quickly under 2,000. So it's only at 1 to 4. And if this patient needs that donor and that's the only thing you cross, we go for it.

And we also ask, immediately post, to look for anamnestic response. But again, we give more opportunity. That is part of how we-- and I'll show you-- evaluate the sensitized patients. So it's important to keep that in mind.

The next animation, the complements-- so when the antibody binds complement, clearly is a higher-titer antibody. And there were several studies from us, but those are from others. This is a study from Dr. Tambor. And it shows that at levels of-- for class 1-- 1 to 16, after 1 to 16, you see more of a binding. And for class 2, at least 1 to 32. So if you see complement binding, you know that there is a higher-level antibody.

Is that all? Well, I would say no. Because another aspect of complement binding is also the type of IgG subtype. Antibodies that are circulating have, in fact, four flavors-- IgG1, IgG2, IgG3, and IgG4. Among those, IgG1 and 3 are the complement binding-- strong complement binding is 3, followed by 1, 2, a little bit, and 4, almost none.

So now the composition of that DSA, this is called Donor-Specific Antibody, and not Donor Service Area, which was very, very unique this morning-- I learned. Wait, what? DSA doesn't mean always the same.

[LAUGHTER]

So this DSA, if its composition is mainly IgG1 and IgG3, it would be a very strong complement binding with the appropriate titer. So you need the two aspects. So when some centers will say, well, I don't need to do C1q because I have titer. I would say, I can see a high-titer IgG2 mainly, and you don't have complement binding.

So by having complement binding, there are two points there. You have a titer of minimal, plus you have that IgG composition that you may want to be very much aware because we don't do the IgG subtypes clinically. We do that in research, and we learn a lot from it, and I hope, in the future, the reagents will be good to do that also for clinic.

So now, this is a very nice research, somebody from the group from Dr. Reid. And it's kind of summarizing what I mentioned, in terms of not all antibodies are created equal. And we really need to understand, particularly when we evaluate patient pre, or we evaluate the patient for response to therapy like we showed with the AMR in carfilzomib, the response therapy was losing the C1q. In fact, their titer went down, and we have less problem in those patients.

So what do we do now to evaluate the patient? We determine the HLA antibody profile by single-antigen, and then the titer by dilution. We determine the complement binding. And then we start the discussion-- what is the allowable risk for a given patient? Because you may have a clinical condition that you don't want to take the patients to too much of treatment. So no risk comes out-- the fact that you have to take in consideration we need to do post-transplant. So if you cannot treat the patient maybe more aggressively, that's not a good patient to go for desensitization.

Again, the CPRA should be either 50 or higher. And then, as I mentioned, currently about 20% of our patients on the waiting list are in this category.

And here is a patient that was referred to us. And so they asked me, can you look through the results? And I said, no, I have to have to have the serum, and I have to do the testing here. Because the other institution didn't do what we do. So as we received, and this was the need, and based on the need, the CPRA was 50%. This is only looking to the class 1. And again, you see most of the responses are used for the CPRA over 2,000. And having all these blue dots, this was the specificity, and this is the CPRA.

But as soon as I diluted the sera down to 1 to 4, you can see that the CPRA goes down. And with 1 to 16, there were only three positive, and also not even 2,000. So overall, we started with a CPRA of 50%. But by dilution, I could show that this will not be a difficult antibody to treat.

Now this patient also has class 2 antibodies. And we did the same thing. My class 2 was 81%, just based on the distribution with all the antibodies that were positive over 2,000. Again, it went down to 66 and to 22. There was only two that were positive at 1 to 16. And in this patient, the combined CPRA class 1 and class 2 were 41%. But I used the very low cutoff of 1,000. So by 1 to 4, you can say that you can really show a decrease. And 1 to 4 would mean at least two weeks of plasmapheresis. And because every time it's a 1 to 2, 1-- a two-fold dilution. And with some other intervention, I could see an easy potential treatment for this patient to improve the odds to get a transplant that is not crossing a level of antibody.

So the other thing that we do once we have this information, we do what's called a virtual crossmatch. So now we know the antibody profile, we have a donor, and we know the donor typing. And then, in the middle of the night, we have the nice conversation-- to go or not to go, depending on what do you know about the most current HLA antibody profile. And that is very key to this discussion. Because if this patient has been done months ago, and we don't even always realize that a pack of red blood cells with a few leukocytes is a sensitizing event, or a platelet transfusion is a sensitizing event. It changes. So we really need to be as accurate as possible and as current as possible.

The other thing that is very important is to know the full range of typing. So previously, UNOS was not demanding to have the full typing-- A, B, C, BR, DQ, DP-- now, it is. So we have a much better information. Because we may know of a positive antibody, but we don't have the donor typing complete. We don't know if the donor carries or doesn't carry that antigen, that target. So that's very important. And then we decide yes or no based on the phases that Massimo was putting up there.

So as I mentioned, prediction of the active crossmatch result, and then again, what level of crossmatch. So there are very-- this complement-dependent cytotoxicity would be the crossmatch that we will do for the lung patients post-transplant. But I can even give you the higher sensitivity, the flow crossmatch.

So depending on the level of the antibody and the characteristics, we can predict if it is a flow or a CDC-positive. We want to avoid the CDC by all means. But we might transplant with a potential flow positive, yet with intervention at the time of transplant and following the transplant.

The next, as I mentioned, center, patient, and organ-specific, meaning you need to define the allowable for crossing an unacceptable HLA antibody. And clinical state of the other patient, the ability to undergo intervention of the antibody removal therapy, and so forth. And then the current information is key, and the comprehensive donor typing.

So I would like to go to a case that was right here. So again, a 53-year-old Caucasian female has systemic sclerosis, severe pulmonary hypertension, and this patient was sensitized, was listing CPRA of 90%. She underwent three times of desensitization using PLEX IVIg, and carfilzomib, which was so nicely introduced by the previous speaker. And then she was treated empirically twice post-transplant to prevent the rebound, because that's another very important consideration.

And here is looking to the single-antigen for class 1 and class 2. And the CPRA was 90% based on that. And then the C1q CPRA was half of that. So again, we've kind of focused to see that we can lose the C1q CPRA, so basically calculating only based on what was complement-binding.

And here is kind of the summary of this patient. So before the first carfilzomib, the CPRA using the 2,000 MFI cutoff, and the C1q CPRA. And then you see that the IgG CPRA kind of stayed the same until the time of transparent. However, the C1q CPRA, after the second carfilzomib diminished, it stayed negative, and this patient was transparent successfully. And this is at the time of transplant, where there is no complement-binding antibody, but there are still 79% CPRA based on the regular antibody. And there were some DSAs at the time of transplant.

So in summary, in this case of this highly sensitized patient, we used loss of C1q as kind of the potential to list the patient, and the appropriate donor was identified. The crossmatches were negative. The patient had the preform DSA. The crossmatch CDC was negative because C1q was negative. So I anticipated that. Post-lung transplant, DSA was kept low, and to the negative was the additional treatment. And again, the patient improved correlated with the lung function.

And this is a summary of all the patients that went through desensitization experience here. And the ones that carry the little star were the patients that were transplanted. So in this case, this first patient not only that it did not change by IgG, the C1q went higher, meaning that you almost tickle the system, and then the titers went up. And this patient was not taken further. This one had no C1a before, and so it was easy. After the first treatment, there were very low level antibodies.

And this case-- again, this is the case that I just mentioned. Here are two patients that received two rounds of desensitization. In this case, we did not achieve much of change, even though there were some drop in C1q. But this patient has not been transplanted. And here, we have a successful transplant.

So I wanted to bring this paper from the group in Toronto because they're talking about survival in sensitized lung transplant recipients with very operative desensitization. The thing that I like to make the difference between their approach and our approach, the patients come to transfer, they have a spectrum of antibody, they don't know exactly characteristically so like we do, just there is DSA or there is no DSA. And then these patients will receive different types of treatment that included PLEX, IVIg ATG, or just PLEX and IVIg, or just PLEX alone. And then they show if the DSA went away and so forth.

And yes, I think the success would be to identify what exactly responded and didn't responded in this group of patients. And these patients are not treated pre. And so you don't know how they will behave. Because as I showed you, some patients can rebound even more than they have. And those patients will not be as easily treated right after the transplant. But it's an approach to take every comer and try to cross some level of DSA.

I like to mention that this is what we are doing right now. So the very red one, AVOID, is the high-titer C1q-positive antibody. Higher than 1 to 16, and complement-binding. We are discussing the possible low-titer C1q negative. And I think the problem is not that you can have one. It's sometimes there is more than one, and then there is a synergy between them. And that's not something that can be easily defined. And then definitely accept anything that's negative.

And what we also can do to identify this potential synergy is surrogate crossmatch. So if I have a patient that we have the time, and there is some low-level antibodies, and I can take a donor that we have cells, identify these positive antigens, and see if the combination of expressing, on the same cell, two or three of these low-level antibodies makes it worse, then by surrogate crossmatch I can rule out or rule in that yes, we can cross that if time comes. So again, it's more work upfront, but it's really helping a lot at the time of the middle-of-night decision-making.

The other thing that the surrogate crossmatch can help is to identify really some type of antibody that we learned that exist, which are the denatured HLA antigens. So you call it antibody 2B8, or antibody 2, class 1 or other class 2. But in fact, as the beads are made, there could be something that is unmasked that is not in the natural confirmation what is on the cell. So by the testing, I call it positive, but by their function, it doesn't bind complement, it doesn't bind to the real cell.

So here I give you an example. The T-cell flow crossmatch, very easy to identify these type of antibodies. We have an antibody to A24, and we take a cell that has A24, and we have a positive crossmatch. And you see that, by dilution, the MFI dilutes down, and that fits very well.

On the other side, we have an antibody to B37. There is a very strong antibody, even by dilution doesn't go down well. And that's already an inkling as to know that this is something not so real. And when we put cells to look for positive binding in flow-- negative, even at that type of MFI. So this B37, it's not something that I should be concerned to cross, yet by all means it looked like I should be.

And we had a case of a pediatric heart, where we had this B8 antibody that looked like not natural-- not really to the confirmation of a truly B8 antibody. We did the surrogate crossmatch, we show that it's not positive, we remove that from the patient-- "unacceptable." And the patient was transplanted with a B8 donor, and is doing fine.

So again, we open the door where the door may be closed because something that the assay tells us. But we need to really assess that correctly, and identify the specific and the real antibodies.

So just to finish-- and I hope I'm in time-- I think we have to have this integrative and multiplexed assessment of DSA which puts together the puzzle of identifying the MFI, the titer. The future would be looking to the [INAUDIBLE] defining the HLA better, not only by A, B, C [INAUDIBLE] but exactly the amino acids that may be different between patient and donor, the IgG subclass, preform or de novo, C marker binding, and then come up with what we call risk prediction, graft failure, and response to therapy.

So I want to thank you. I always enjoy to be part of the team, and thank you very much.