A CME/CE-CERTIFIED ACTIVITY

FACULTY TRAINING TRANSCRIPT

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John Friedewald, MD: We’re going to start with a case presentation. This is a case of a 66-year-old male with type 2 diabetes mellitus for 16 years, hypertension for 20 years; has been on dialysis for four years, is morbidly obese with a BMI of 36; has single-vessel coronary disease; and had a percutaneous intervention and a stent two years ago; had a mitral valve replacement and is on warfarin anticoagulation; is non-sensitized with a calculated PRA of 0%. He has Medicaid insurance and smokes a half-pack of cigarettes per day, currently.

A deceased donor offer becomes available for our patient. The donor is a 62-year-old with hypertension, died of a cerebrovascular accident, had a terminal creatinine of 1.4 mg/dL, and that gave that donor a KDPI of 93%. In terms of HLA matching, the donor-recipients were a 1B and a 1DR match, so a four-antigen mismatch.

The polling question is, “Based on the characteristics of the patient and the kidney donor available, what’s the expected one-year graft survival based on the KDPI?” And this is directly derived from the next slide.

You can see that circle there is a KDPI of 93%, would portend a one-year graft survival of 82%. The correct answer to the previous question was “C – 82%.” You can talk about both the one- and two-year graft survival and the predictive ability of KDPI.

The polling question is, “Which of the following risk factors are not accounted for in the SRTR one-year graft survival models?” The purpose here is to point out things that are and are not included in risk adjustment to allow centers to take calculated risks with their patient transplant decisions. And the answer here is, “A” and “B.” “Coronary disease and candidate tobacco use.”

How do we assess risk and effects on program outcomes?

Now we’re going to move into, sort of, how programs are graded on their outcomes. We provide an SRTR program-specific report, or PSR. These are provided to all programs every six months, and it provides a program summary, wait list information, transplant information, and living donor follow-up summaries if the program, performs
living donor transplants, and it’s all shown here for an actual center that has been blinded.

**Slide 12 - SRTR Timelines for Evaluation**
The SRTR timelines for evaluation are important to point out so that centers understand, what they’re being graded on. And the SRTR examines a two-and-a-half-year cohort for outcomes and determines the number of observed events, how many actual graft and patient losses a center may have versus the number of expected events, and the expect events is where the risk adjustment comes in. And it’s important to remember the reports lag behind real time for up to a year to allow those one-year events – a graft or patient loss – to occur, and so it’s really old news in most cases. And the example’s given in the table here. The fall PSR cohort for one-year graft survival is based on transplants done between July 1, 2014 and the end of December 2016. Just to give everyone an idea of what we’re being graded on and how.

**Slide 13 – How Does SRTR Risk Adjust Outcomes?**
How do they risk-adjust? And this is a critical factor when you’re considering, recipient selection for transplant or even donor selection, as we’ll get to in a minute. This is the one-year deceased owner graft survival risk adjustment factors. And I point out in the blue box that noticeably absent are things like cardiovascular disease, other than the one thing we pointed to is peripheral vascular disease, which is there. And socioeconomic factors are mainly excluded except for recipients’ insurance. All of those factors have been shown to affect patient and particularly graft survival, but are not included in the models for a variety of reasons.

**Slide 14 - 1-Year Patient Survival Deceased Donor-Recipient Factors**
We look at, instead of graft survival, the one-year patient survival, for deceased donor factors that are included in risk adjustment, and they’re not the same list as for graft survival. So, again, depending on what you’re looking at, you’re being graded on a different number of factors. And, again, it’s important when your program is looking at their outcomes and how they’re doing, if the outcomes aren’t as expected, you might want to look and see, “Are all these things being accounted for?” and, “Do we have unaccounted-for risk factors that we can use as a mitigating factor, if we get flagged by either CMS or the Membership and Professional Standards Committee at UNOS MPSC?”

**Slide 15 - 1-Year Graft Survival and Patient Survival**
A group of donor factors, both for graft survival and patient survival on left and right there. Again, it’s good to become familiar with these different factors that go into risk adjustment so you can understand what you’re, again, being graded upon and what you’re not, more importantly, if you decide to take a calculated risk with a donor-recipient combination for a transplant.
Slide 16 - Criteria Used by MPSC to Identify Transplant Programs that Require Further Scrutiny
We get into the specifics of how each individual center’s graded by the MPSC at UNOS, to identify transplant programs that require further scrutiny, and it’s broken up based on the size of the center. Believe it or not, a “large center” is defined as a program that does more than ten transplants in a two-and-a-half-year period. And there are a number of centers that do not qualify for that and are actually small centers that do less than or equal to nine transplants in that two-and-a-half-year period. For small centers, any event – a graft or a patient loss – immediately triggers a review by the Membership and Professional Standards Committee, to see if they need further, help in terms of improving their outcomes.

For larger centers, we use what’s called a Bayesian Model to determine whether their outcomes are beyond what’s expected, and so that’s done by looking at the hazard ratio of the observed to expected. If you have the same number of observed events and expected events, your hazard ratio will be one. And the more of the observed events you have, compared to expected, your hazard ratio goes up.

And the way the Scientific Registry for Transplant Recipients – or SRTR – decides if a program’s hazard ratio is too high, they look at this probability that the hazard ratio is greater than a certain value. If there’s more than a 75% chance that the hazard ratio is greater than 1.2 or more than a 10% chance that the hazard ratio as greater than 2.5, then that large center would be flagged for further review by the MPSC. All centers get this report, from the SRTR every six months with this hazard ratio and with these probabilities, so you can look at your own center’s reports on the SRTR website.

Slide 17 – Bayesian Methods for Assessing Transplant Program Performance
This just shows a little more detail about that Bayesian Model used for assessing transplant programs. It shows the credible interval there – the 95% credible interval, and the dark line with the number is the actual hazard ratio for a Program A on the left or Program B on the right. The Program A on the right is a large program in this example, with 299 transplants in that two-and-a-half-year cohort that’s being examined by the SRTR. Their hazard ratio is 1.67, so that means their death rate, for instance, this is for patient survival – was 67% higher than expected.

That is higher than expected, but it falls within that confidence interval; the SRTR, then, would not flag this program. And, likewise, for a smaller program, on the right, this is one that did six transplants in a two-and-a-half-year cohort. There was one patient death in one year, with a hazard ratio of 1.37. Again, just to get familiar with how these look and how the SRTR decides if, a hazard ratio is beyond what’s expected.

Slide 18 - Cumulative Sum – CUSUM Reports
Now, we talked about how the reports from the SRTR, or the program-specific reports, are sometimes old news, that events that have happened may be two-and-a-half or more years ago, and so how do you get more up-to-date information about how your program’s doing? That’s through CUSUM reports, or cumulative sum reports.
CUSUM reports are better suited to ongoing quality improvement, for instance, in your QAPI process at your program, because they show actual events and the time they occurred rather than an average that can mask periods of high or low events. CUSUM reports look at a three-year period as opposed to a two-and-a-half-year period, and they’re much more recent, up to two months prior to their release date. So fairly recent data, and they’re more relevant to a program’s current practice and quality efforts. You may have had a bad run of events, a number of years ago, maybe a year and a half ago, but have corrected that, but that wouldn’t be reflected, necessarily, in your program-specific report, but it would in a CUSUM report.

On the right, you see that, in Panel A, a period of lower-than-expected event rates, where the line is moving downward, each tick downward is an improvement, and each tick upward is an actual event in real time. And across the bottom, is the three-year timeline, and you can see that actual graft or patient losses here – in this case, this is graft survival, so these would be graft losses – each tick up is a graft loss on the right-hand side there, and you can see there’s a very steep period where the line goes up, almost vertically; that would be a chance for a program to immediately look at root-cause analysis of those graft losses and see if there is some common denominator that they can correct to improve their patient outcomes. CUSUM reports are very helpful for an ongoing quality program to assess outcomes in more real time.

Slide 19 – Quality Improvement Methods and Strategies
What quality improvement methods and strategies does CMS expect? Medicare CMS expects all programs to have a robust quality process. Well, what does that mean? This is looking at quality assessment and performance improvement, or QAPI. This is from the CMS website, so as an effective transplant quality assurance and performance improvement program, or QAPI program, is ongoing – ‘cause you can’t have it just once; it has to be comprehensive, dealing with the full range of services offered by the transplant program, including things like patient safety, clinical care, quality of life, etc., all shown there.

The hospital leadership and governing body must be clearly engaged in the quality oversight, so that’s important, as well, to show that the hospital leadership is behind the transplant program and engaged with their QAPI process. But the transplant program must have systems in place to monitor care and look at their data drawing from multiple sources. The QAPI program must use methodologic approaches to determine when in-depth analyses are needed for further improvement, when to take a deep dive, for instance, on that last slide, where you saw a rapid period with a lot of graft losses, for instance.

And a transplant QAPI program must define, implement, and evaluate performance improvement interventions with the objective of improving quality care, and these can be ongoing quality projects within your program to improve certain aspects of your program.
Slide 20 – Case Presentation
Moving on we continue with the case presentation. From our previous case, our patient accepts the kidney offer that was mentioned. The patient received basiliximab for induction therapy, followed by tacrolimus, mycophenolate, and prednisone for maintenance immunosuppression.

Our patient experienced delayed graft function for ten days, but, by then, the serum creatinine declined to 1.8-to-2.1 mg/dL baseline range. Blood pressure was reasonably well controlled on metoprolol and amlodipine. But, by week four, unfortunately, BK virus is detected in the blood, with a viral load in the serum of 11,300 copies. In response to this, mycophenolate was discontinued.

Tacrolimus was continued, but the dose was reduced to a target level of 6 to 8 ng/mL. By week eight, the BK viral load is decreased to 2800 copies. There’s new HLA donor-specific antibodies, to HLA B3, with a mean fluorescence intensity of 1800, so relatively low level MFI, but positive by most cut-offs.

Slide 21 – Case Presentation
Further case presentation – by week 12, the BK viral load is now down to 975 copies. Serum creatinine is roughly the same at 2.1. Tacrolimus level is 3.3, on the lower side, and the patient admits to missing his nighttime dose. By week 15, the serum creatinine has now risen to 2.4. Tacrolimus level is at 5.1.

Blood pressure is 142/86, and the HLA B3 donor-specific antibody is now up to 2600 MFI. And you’ll also note, the detection of urine protein that’s now with a urine protein creatinine ratio of 0.3. By week 16, the creatinine’s up to 2.5. Tac level’s still at 5.2. Blood pressure is shown. Patient remains on warfarin for the mitral valve, metoprolol, and amlodipine. An ultrasound of the kidney’s done and is fairly unremarkable, except of elevated resistive indices.

Slide 22 – Polling Question
The question is “What’s the cause of the patient’s increasing serum creatinine? Is it T cell-mediated rejection, polyomavirus, antibody-mediated rejection, etc.?” And the answer is, “You cannot tell based on the available information – F.” But it’s good to walk through those different options for the audience.

Slide 23 – Subclinical Injury and Graft Loss
This is data from the publication, as shown, that talks about the progression of subclinical injury and eventual leading to graft loss and the question, “How can we intervene along the way?” What we’ve done is highlight our patient’s known risk factors here, which are circled in red. The patient had delayed graft function, as shown, also had inadequate immunosuppression, not through, necessarily, non-adherence – although the patient does mention missing a nighttime dose – but also through intentional minimization because we’ve lowered the immunosuppression in response to the polyomavirus infection.
We also know the patient developed the de novo donor-specific antibodies, as shown there with the red circle, as well. A number of risk factors for ongoing subclinical injury, which can eventually lead to more clinical injury to the kidney and eventual graft loss. Our patient we consider a fairly high risk for ongoing graft loss and graft injury and, eventually, graft loss.

**Slide 24 – Polling Question**

Then the question is, “In the light of the serum creatinine increase, what would you decide to do next?” “A” is hold warfarin, admit for IV heparin infusion, biopsy once anticoagulation is adequately reversed; “B” is empirically treat with plasmapheresis and IVIG for presumed AMR; “C” is measure donor-derived cell-free DNA; empirically treat with antithymocyte globulin for T cell-mediated rejection; or do nothing and continue to follow bloodwork. There’s no real right answer to this question; however, the most commonly answered one is “A,” doing a biopsy, which is considered the current standard of care for patients to diagnose rejection, particularly a patient who may have a combination of both cellular- and antibody-mediated rejection.

**Slide 25 – Graft Survival in DeKAF**

This shows some data from the DeKAF Trial, which is a trial looking at, “delayed trouble graft,” as they called it. These are patients that are further out from transplant, who have renal dysfunction, had to have an increase in creatinine greater than 25% with a baseline creatinine less than 2. The point of this is that ongoing inflammation and injury is probably more concerning in terms of graft risk than calcineurin inhibitor toxicity. The two lines there show the biopsies that showed primary, secondary calcineurin toxicity versus no CNI toxicity. Although there are a lot of concerns about how calcineurin inhibitor nephrotoxicity may contribute to ongoing graft loss, I think what’s been shown by DeKAF and other investigators is that ongoing alloimmune injury to the kidney probably is a greater risk.

**Slide 26 - Subclinical Acute Rejection**

Subclinical acute rejection, which is different grades of rejection, but subclinical really is defined as a histologic acute rejection; it could be cellular- or antibody-mediate, but stable renal allograft function. If you were just monitoring a patient the serum creatinine, you wouldn’t be able to detect this. It’s currently diagnosed only with a protocol or surveillance biopsy, and about half of centers in the Country do not routinely perform surveillance biopsies for a number of reasons, including logistics, costs, and the risk of biopsy, as the most frequently reported reasons why centers don’t perform protocol biopsies.

**Slide 27 - Currently Used Biomarkers in Kidney Transplantation**

Currently used biomarkers in kidney transplantation, the gold standard still is monitoring serum creatinine. We know that serum creatinine is a lagging indicator of graft injury and, really, a marker of function of the kidney rather than injury, but it still remains the gold standard for monitoring allograft.
We also monitor urine protein creatinine ratios, and increasing proteinuria can signal a variety of different problems with a graft, as well as immunosuppressive drug levels which are mostly considered monitoring for safety and efficacy rather than, really being able to predict rejection. But it still remains the gold standard for monitoring. And then, of course, indication kidney biopsies where an elevation of serum creatinine or an increase in urine protein would lead to a biopsy for cause.

Commonly used adjuncts beyond the gold standard include viral monitoring, which usually are a marker of over-immunosuppression, things like BK virus, cytomegalovirus, and Epstein-Barr virus, as well as monitoring for donor-specific HLA antibodies and protocol kidney biopsies at various time points. And, there are a number of different protocols out there in terms of when to do surveillance protocol biopsies to detect subclinical injury.

And then, of course, there are a number of developing biomarkers. There’s donor-derived cell-free DNA; blood gene expression profile tests; and then urine biomarker tests, both gene expression and protein-based tests, and we’ll discuss all of these in the subsequent slides.

Slide 28 - Need for Improved Detection of Under- and Over-Immunosuppression and Differential Diagnosis

We talk about the need for improved detection of over- and under-immunosuppression and differential diagnosis, and this really is the heart of personalized medicine, which we have not been able to achieve yet in the field of transplantation. We kind of take a “one size fits all” approach.

But the ideal biomarker, if we were looking for one, would be non-invasive, inexpensive, and provide a diagnosis with higher accuracy in a subclinical setting before real injury occurs and the creatinine goes up. This, clearly, doesn’t exist in medicine, but development moves towards better biomarkers, and that’s what we’re going to talk about next.

Serum creatinine, again, is not as sensitive or specific to renal allograft injury and immune injury, or the cause. It’s really a lagging indicator of injury, and we need to do better with improved biomarkers.

Slide 29 - Biomarkers in Development

We’re going to start to talk about biomarkers of development.

Slide 30 - Donor-Derived Cell-Free DNA as a Biomarker in Transplantation

We review donor-derived cell-free DNA as a biomarker in transplantation. Cell-free DNA consists of fragments of DNA in the blood that originate from cells undergoing cell injury to death. The DNA gets degraded very rapidly into nucleosomal units consisting of about 166 bases. Cell-free DNA is cleared from the blood by the liver and kidney and has a very short half-life of about 30 minutes but can be detected.
The idea here is that, if you can detect the difference between recipient- and donor-derived cell-free DNA, which is in the blood, you’re able to look at the percentage of donor-derived cell-free DNA, and if that percentage increases, that can raise the suspicion of injury of the allograft since that’s the only source of that donor-derived, rather than recipient-derived, cell-free DNA in the blood. And so the panel on the bottom-left shows that, for a variety of organs – heart, lung, kidneys, and liver – increases in donor-derived cell-free DNA in the plasma can suggest ongoing injury, whether from rejection or other causes. And the top-right panel shows the increase in cell-free DNA percentage, as well.

**Slide 31 - dd-cfDNA Is Very Sensitive But Not Specific for ABMR in Patients with Graft Dysfunction**

Key results from peer-review publications show donor-derived cell-free DNA is very low in stable transplant recipients elevate at the time of rejection, and decreases following treatment in a variety of different publications from different organs, including heart, lung and kidney. Donor-derived cell-free DNA is very sensitive but not specific for ABMR in patients with graft dysfunction. Again, not stable with patients who have an elevation of creatinine. If you run a donor-derived cell-free DNA, it is very sensitive, as you can see on the left-hand panel, for antibody-mediated rejection.

**Slide 32 - Low dd-cfDNA (<1%) in Patients with Graft Dysfunction has a Very Low Probability to be ABMR**

Low donor-derived cell-free DNA – less than 1% in patients with graft dysfunction – has a very low probability to be ABMR. The cell-free DNA test may be evolving as a way to differentiate between causes of graft injury. It seems to be particularly elevated, with ongoing antibody-mediated rejection.

**Slide 33 - Molecular Classifiers for Acute Kidney Transplant Rejection**

This is now gene expression profiles in the blood, so looking at messenger RNA rather than DNA in the previous test. And this is a publication from a group in 2014 looking at, again, peripheral blood gene expression profiles that are able to distinguish between patients with normal biopsies called TX, from acute rejection, and also those who have acute dysfunction without rejection, and that’s important to provide a differential diagnosis if creatinine’s elevated, to be able to determine between a cause of alloimmune injury or rejection from those other causes such as acute tubular necrosis.

**Slide 34 - A Peripheral Blood Diagnostic Test for Acute Rejection in Renal Transplantation – kSORT**

Again, very similar – the work, from Minnie Sarwal’s group showing, the same thing; this is a gene expression profile in the blood. And the idea here is that, as these tests become refined and developed, that there are clearly signals in the peripheral blood looking at using messenger RNA, that can be used as effective biomarkers. And, again, this is a similar trial looking at acute rejection versus normal and being able to differentiate those very well using this peripheral blood biomarker, so a non-invasive biomarker.
Slide 35 - Urinary-cell mRNA Profile and Acute Cellular Rejection: Kidney Allografts
Out of the blood, into the urine, these are the results of the CTOT-4 Trial, which were published in 2013, in *The New England Journal*. This is the work of Dr. Suthanthiran and colleagues and shows that, looking at gene expression profiles in urine – urinary cells can also distinguish rejection from normal transplants. And, the four panels show, in Panel A, this gene expression profile, in the urine that is below a certain threshold before biopsy and, on day zero, when the biopsy's done, showing rejection, you can see that the levels of these gene transcripts go up, prior to rejection.

Also, in the bottom left-hand corner in Panel C, you can see a patient that didn’t have rejection and a group that didn’t have rejection to those that did, and those that did have an elevation of this biomarker panel up to almost 30 days prior to the rejection. Possibly a test to, certainly, diagnose rejection less than, basically, but also to potentially monitor patients and predict when a rejection may occur, before it happens. Again, this raises the hope for seromonitoring of patients.

Slide 36 - Multicenter Validation of Urinary CXCL9 as a Risk-Stratifying Biomarker for Kidney Transplant Injury
This is now a protein in the urine. This is the results of the CTOT-1 Trial, looking at urinary CXCL9 as a risk-stratifying biomarker for kidney transplant injury. And a fairly busy slide, but you can see in Panel C, on the bottom left-hand corner, the patients that had acute rejection had much higher levels of this CXCL9 in the urine compared to those, with the blue lines that did not have rejection. And so, again, CXCL9 seems to be fairly specific once you've excluded a urinary tract infection, a very specific measure of acute rejection in kidney transplant, compared to those that did not have rejection.

In sum, we see that there are a number of non-invasive biomarkers, both in the blood and urine, that are in development, with the hope of, again, being able to diagnose earlier, less invasively, and more specifically ongoing allograft injury, to prevent further graft loss, as we saw it from the timeline in the earlier panel.

With that, I'll hand it over to Dr. Bloom, who will take us through the rest of the case presentation and the following slides. Thanks.

Slide 37 - Case Presentation, continued
Roy D. Bloom, MD: OK, the biopsy is performed, shows a Banff 1A acute a cellular rejection and no features of antibody-mediated rejection. SV40 is negative, so no evident of BK. The patient's treated with pulse methylprednisolone as the donor-derived cell-free DNA level comes back at 0.21, which is below the diagnostic threshold, for a major injury. Tacrolimus dose is increased to a target level of 6.8. The MMF is restarted at a reduced dose.

At week 20, the BK viral load is undetectable. The serum creatinine is now 2.3. The tac level is 8.9. At week 24, the BK viral load is still undetectable. The DSA to his HLA B3 is now 2800 MFI. Serum creatinine's stable at 2.3. Tac level is back down to 2.9. The patient admits to frequently forgetting his nighttime dose.
Slide 38 – Polling Question
Just to make the point that there’s no absolutely correct answer; this is more a polling question.

Slide 39 - Adjusted Rate of Allograft Failure in the US
Adjusted rates of allograft failure in the United States, showing overall all-cause graft failure. But what it shows, in the green curve is that, graft failure, because of patients returning to dialysis, requiring retransplant, has actually declined and now approximates patients whose grafts fail because of death with function. In the most recent, timeframe of collecting this data, you can see death with function is actually now slowly surpassing, death-censored graft loss as the reason for allograft failure.

Slide 40 - Why Do Kidneys Fail? Mayo Experience
The next slide looks at why kidneys fail. This is the experience from the Mayo that was published in *AJT*. In this study, they looked at data collected from 1996 to 2006. There were a total of 1,317 kidney transplant patients, of whom 330 had a graft loss mean of 50 months post transplant. Approximately, one-third or just over were due to death. 153 patients died, had primary graft failure, and they had biopsies done, a mean of 4.7 months prior to graft loss.

And, basically, what the figure shows is they adjudicated what the likely cause of graft loss was in these 153 patients, so med/surg causes such as sepsis and AKI accounted for about 16%, acute rejection – 12%. But about a third of the patients lost their grafts because – where the diagnosis was IF/TA, of whom a quarter had a history of acute rejection. And then about another third of patients lost their kidneys from glomerular disease, of which about 40% were transplant glomerulopathy, histologically. Rejection’s still being a major contributor to death-censored graft loss. Importantly, only one graft loss was attributed solely to CNI toxicity, underscoring that, while CNIs may be nephrotoxic, they seldom are the actual cause of allograft failure.

Slide 41 - Antibody-mediated Rejection and Non-adherence
The next slide looks at antibody-mediated rejection and non-adherence. This is a study, of about 315 patients who had indication biopsies, 60 progressed to failure in follow-up, a medium of about two to three years after biopsies. What you see is, again, the figure demonstrating that most of the patients lost their kidneys from either mixed rejection, probably ABMR, or ABMR, and then, when they correlated that with the history of adherence in patients, most patients, who lost their grafts from antibody mediated rejection have a history of being, non-adherent. Again, strongly associating antibody-mediated rejection with non-adherence.

Slide 42 - Non-adherence Predicts DSA Development
The next slide looks at patients according to the presence or absence of donor-specific antibodies. This is data from the Canadian group. You can see that 47 patients, or 15% of the cohort, developed de novo DSA. Among adherent patients, only 8% of patients developed de novo DSA. Among non-adherent patients, just under half of the
patients developed a DSA. And, as indicated with the Kaplan-Meier in future follow-up, graft survival was significantly worse in the non-adherent patients.

**Slide 43 - Healthcare Non-adherence and Graft Survival**

The next slide I’ve put in because it’s kind of an interesting, and contemporary study which looks at VA data. VA data’s a closed system, so, one can track medication possession ratios and clinical attendance, and or non-attendance quite robustly. And, basically, what these investigators did is they performed a longitudinal cohort study, that looked at the impact of clinic non-adherence and medication possession ratios on grafting patient survival and showing that again, just having an appointment no-show is associated with better graft survival than if you have an appointment no-show more often than 12% of the time, a medication possession ratio less 80% being progressively worse outcomes, and the combination of no-show more than 12% of the time and a medication possession ratio of less than 80% having the worst outcomes. Not only is it related to medication on adherence, but clinic no-shows, in combination have an additive effect.

**Slide 44 - Late Graft Loss: A Changing Paradigm**

Late graft losses are a changing paradigm. We’ve now see from the foregoing data that chronic rejection is the most frequent cause of death-censored graft loss. Chronic rejection is commonly due to insufficient immunosuppression, and this can occur in one of two major settings – one could be inappropriate prescription, essentially, minimizing or avoiding strategies that result in underexposure to immunosuppression, and this is the result of usually provider-based issues; the second being patient non-adherence, which is obviously primarily and centrally related to the patient.

**Slide 45 - Risk Factors for Non-adherence**

This is taken from a really nice recent review, looking at non-adherence in kidney transplantation. And, basically, there are five large buckets that you can look at reasons underscoring or leading to non-adherence, one being at the level of the health system or health provider, and these factors include: insurance status; access to care; provider-patient communications; transitioning, from pediatric to adult programs.

The next bucket, going from left to right, would be condition-related factors, so: how long since the transplant; transplant from a living donor; perceived health and physical limitations. Then there are treatment-related factors including: the number of medications; the side effect profile; and the dosing frequency. Then the patient-related psychosocial factors, as you see, include: the non-adherence, low literacy levels, psychological distress, low self-efficacy, etc. And then, finally, sociodemographic factors, including: minorities; ethnicities; low socioeconomic status; and, issues like adolescent and young adult problems.

**Slide 46 – Strategies to Improve Adherence**

If we look at strategies to improve adherence, the first way of trying to approach this is at the provider level; this obviously comes by avoiding underdosing. Remember, there is little margin for missing doses, so you should not err on the lower sides, because a
couple of missed doses can result in a catastrophic consequence for the patient. Ideally, use more simplified than complex regimens, as well.

**Slide 47 - Time in Therapeutic Range (TTR)**

The recent paper from *American Journal of Transplantation*. In 538 patients, this is data where the target levels were typically in the six to nine range for the first three months post transplant, and then, intended to be five to eight, between months four to 12. Although, almost all the patients received MMF; about half received rATG. And what they did, which was quite novel is they looked at time in the therapeutic range, in the first year.

And, based on similar type of data from dosing with Coumadin and INR management, TTRs of less than 60% are considered high risk. They looked at TTRs in the first year, the expected range was to be between 5 and 10 throughout that period, and you can see that, for de novo DSA, rejection, cell-free survival and graft-free survival, there were increases, in all these outcomes, in patients who had, a lower time in the therapeutic range, so again, suggesting that the variability, can be an important contributor to a bad outcome, related to excessive periods of being under-immunosuppressed.

**Slide 48 – dnDSA Development is Preceded by Drop in Tacrolimus Levels**

This is data again, from the Edmonton Group where they looked at 492 patients. 96% of the patients were first transplants, mainly low PRA, less than 10% who were sensitized. They had a follow-up of 87 months, and they had a mean of 97 tac levels per patient, so that’s quite robust data. When they looked at tacrolimus coefficient of variation, patients who developed a DSA had a higher CV than patients that did not develop DSA.

If you look at the figure, on the horizontal axis, they are looking at the different levels. The vertical axis or the mean tac trough levels, and then they looked by epoch, going from left to right. They looked at all months post transplant and one year, two years, etc., going from left to right. The blue bars are patients who were more than six months, who had looked at levels, in the more than six months prior to developing de novo DSA, red being less than six months prior to DSA.

What they showed is that, if you look at high-risk patients, patients who developed a DSA, in the preceding six months to the DSA tended to have lower tac levels than patient who did not develop DSA, had. The green patients were patients who did not develop DSA, and you can see that their levels were maintained, whereas those who developed DSAs, had lower levels compared to how they’d been, prior to the six months before the most recent tac level.

**Slide 49 – SYMPHONY: “Low Dose” Tacrolimus 4-7 ng/mL**

This is data from the Symphony study looking at the largest multi-center trial of immunosuppression in kidney transplant patients. This is here, really, to try and get some insight into what an optimal tac level should be for patients. Because we saw that, in the previous slide, when levels dropped off, there tended to be a high risk of
Best Practices in Care to Improve Outcomes for Transplant Recipients

developing de novo DSA. This is a four-arm study, essentially compared a standard
the low-dose tac target level was 4 to 7.

The actual achieved tac level in the top part of the figure shows that, at 12 months, it
was 6.4; at 36 months, it was 6.5, so, at the higher side of the target range. If you look
at GFR, tac, even at this “low dose,” was associated with the highest GFRs. The middle
figure, looking at rejection, shows lowest rejection rates in the patients with tac, as well.
Even when they extended, at 36 months follow-up, the low-dose tac group, still had the
best kidney function. Even at levels where the mean levels at 36 months are 6.5, they
still had better levels of kidney function than the other three groups.

Slide 50 - “Low-dose” Tacrolimus Results in More Rejection and DSA
A more recent study from Europe, looking at low-dose tac again. This was a
randomized controlled trial in 186, low-risk patients. All got basiliximab, tac, MPA, and
steroids of the first ten weeks. The target level during this time for tacrolimus was 8 to
12. At four months, arm one underwent a 50% reduction in the tacrolimus dose, so they
wanted to make sure that the level was not lower than three. Arm two – they continued
to dose tacrolimus to a level of 7 to 12.

The figure on the left shows the tacrolimus trough levels, and you can see that, over the
12 months following the randomization, you can see that they did get separation of the
levels that were significantly different. Of note, as shown in this figure on the right, there
was more rejection in the group, that underwent tacrolimus, dose reduction on
minimization. And there was also a higher proportion of patients who developed DSA in
arm one compared to arm two; although, there was no difference seen in EGFR. Again,
suggesting that lower levels put patients at a higher risk for a rejection.

Slide 51 - Target Tacrolimus Levels at 12-months Post-transplant
This essentially, just tabulates the the notable studies that have been randomized
controlled trials that have evaluated target tac levels at 12 months post transplant.
Symphony, as I’ve described, they achieved 12 months’ tac levels of 6.4. If you look at
the Opticept, study data, where there was one arm that was meant to be on, a reduced
dose, even on the patient the actual achieved tac levels were between 6 and 7.5.

And then showing other trials where they also aimed for, sort of, a reduced tac dose, but
still showing that the achieved levels at 12 months were over 6 – the Harmony Trial, the
Advance Trial. The De Graav Trial is a recent trial that compared patients to belatacept
and, compared tacrolimus to belatacept as a pilot study, but again showing that patients
in the tac arm had a higher level and still had better outcomes than the control group,
suggesting that in the absence of data, it’s probably better to not be too aggressive in
terms of titrating a tac dose below 6 during the first post-transplant year.

Slide 52 - Once-daily Formulations of Tacrolimus
What are the different tacrolimus formulations? The two new once-daily formulations;
ye’re not that new anymore. But the gold standard twice-daily tac is shown, in the
green curve, and then the, PK profile of Advagraf or Astagraf is shown in the orange curve. You can see they both are characterized by having an early peak around about, one and a half to two hours, and then decaying at similar rates. What’s the different PK profile is shown with, in Envarsus, which has a lower peak level and a lower time-to-peak level.

And the table on the right shows the comparisons of each of these once-daily preparations, when compared to immediate-release tac. They all have similar trough levels. The maximum peak is similar with the Astagraf and lower with Envarsus. Tmax similar with Astagraf, delayed with Envarsus. The overall exposure, if anything, is higher at the same dose, of the Envarsus, compared to the twice-daily, and lower with the Astagraf, compared to the twice-daily. When making conversions, you generally have to increase your dose by about 8%-10% when switching to Astagraf, and reduce the dose by about 15%-30% when, converting from, twice-daily tac to Envarsus.

Slide 53 - Extended-Release Tacrolimus vs Twice-Daily Tacrolimus RCTs
As far as the efficacy of the once-daily drugs, again, these are separate trials that are randomized controlled trials that compare the twice-daily to the extended-release and, in each case, showing that either, reflect that even graft loss or treatment failure outcomes are equivalent between the once-daily and the twice-daily, tac formulations.

Slide 54 - Persistence and Adherence in Kidney Recipients
Looking at the impact of these once-daily formulation on persistence and adherence, so one study that’s looked at this, it was a randomized controlled trial of stable transplant patients in the Netherlands. 219 patients randomized to once-daily or twice-daily tac. When they looked at persistence and adherence, they were both numerically and, in the case of adherence, also statistically better with once-daily formulation than twice-daily.

And, of note, the doses in the twice-daily were missed more frequently in the evening than in the morning. Once-daily, agents are generally given in the morning, and it seems to be associated with better adherence, at least in this one study.

Slide 55 - Terminology
Just to remind you of the terminology, adherence is the extent to which patients take medications as prescribed, in terms of timing, dosage, and frequency. Compliance is patients' passive following of providers' orders. And persistence reflects the duration of time that patients take the medication, from initiation to discontinuation of therapy.

Slide 56 – What Can Centers Do?
What can centers do, as far as non-adherence is concerned? This clearly requires a multidisciplinary approach. Different strategies that have been used include electronic monitoring and notification; personalized education programs.

It’s important that they are culturally and content-appropriate, and this obviously varies according to what the proportion of different, ethnic groups are in your own individual regions and programs. It’s important to reinforce but avoid confusing. Having more
face time is probably better than having less face time in terms of getting patients and their families engaged. And, to the extent possible, it’s good to try and track pharmacy refills.

**Slide 57 - Use of Electronic Monitoring to Predict Patterns of Early Medication Adherence in Renal Transplantation**

This is data from Minneapolis, where they used the MEMS – the Medication Event Monitoring System – which is a microprocessor that’s embedded in the cap of the bottle, and it records opening and closings of the cap. In this study, they actually looked, at opening and closings in the first two months, in this cohort of 195 patients and within the first two months post transplant, they identified about 22%-23% of patients, had decreased adherence by 7% or more in the first two months post transplant.

They then longitudinally followed these patients and looked at subsequent events, and you can see that patients who, had steady adherence in the early post-transplant period had better long-term survival; patients who had diminished adherence in the first two months had worse long-term, graft survival and higher risk of acute rejection.

**Slide 58 - Synergistic Effect of Epitope-Mismatch and Non-adherence on Graft Survival**

The same 195 patients, but, here, they looked at synergistic effect of non-adherence and epitope mismatch. And, interestingly enough, the sort of threshold with a risk from an epitope mismatch, increases at around 10 or 11, in patients whose epitope mismatch was less than 10, they didn’t appear to have significantly more rejection.

But, as the Kaplan-Meier indicates, both allograft survival with either, DR mismatch or DQ mismatch indicate that the combination of non-adherence as well as, epitope mismatch of either more than 10 for DR or more than 17 for DQ, is associated, with worse graft survival.

**Slide 59 - Eplet Mismatch Modulates Effect of Tacrolimus Trough Levels on the Development of dnDSA**

On the seam of eplet mismatching – over here, we’re going to orient you on the horizontal axis, looking at the various tacrolimus thresholds, going from less than three to less than 6, the Y-axis looks at the percentage of trough levels that were below threshold, and, the red being the high-risk patients who develop DSA, the blue being the high-risk patients who did not develop DSA.

But you can see, moving from right to left, is that, as the levels get to a lower and lower threshold, the more frequently, that the tac levels, are below that threshold, the higher the risk of DSA, development, especially in high alloimmune-risk patients.

**Slide 60 - Use of Electronic Monitoring with Reminders and Physician Notification**

An intervention study where patients were, given reminders and had physicians notified, when patients were non-adherent. This used the wireless pill bottle technology. It was a pilot study, where only the tac was monitored, and, we weren’t able to track adherence to other drugs, but just looking at bottle-measured tacrolimus adherence.
The flow diagram on the right shows you how the patients were randomized. 40 patients were given our standard of care; 40 patients got reminders; and 40 patients got reminders as well as notification of the physicians.

And you can see that, what’s really noticeable is that having the reminders alone does make a difference because there’s early divergence of the curves of the patients who were getting the reminders versus standard of care. Some were in the latter part of the trial, you can see that there was actually a divergence between the patients getting the reminders only, compared to those that got reminders and whose physicians were notified, suggesting that while this technology is a relatively effective intervention, there’s probably some kind of fatigue with tolerance to it so that when there’s no physician notification there’s kind of a decline in their adherence, as well.

**Slide 61 - Interventions for Medication Non-Adherence**

To summarize, interventions for medication on adherence – there are relatively few trials that have mainly been performed in adults. The effectiveness is probably quite limited. Multi-component interventions are more efficacious but not feasible on a large scale. Efficacy is evaluated primarily during the intervention period, so just because, you make some observation during the intervention does not guarantee that beyond the intervention period, this will be still in place.

Since there’s no permanent cure, intervention should ideally be given for as long as medical therapy is needed. Again, there are issues with feasibility of this. We know that non-adherence is an incurable disease in many patients. The most practical intervention, from a provide standpoint, is to modify the immunosuppression from twice a day to once a day, since there is some data that this does improve adherence. This would be part of the battle won.

**Slide 62 - Case Presentation, continued**

Getting back to the case, the patient switches to once-daily tac formulation. He immediately notices that it’s easier for him to adhere to this regimen. His tac levels consistently remain between 6 to 8 for the remainder of the first post-transplant year, 5 to 7 thereafter. His creatinine remains stable. There’s no significant proteinuria. BK viral load remains undetectable, and his low-level class 1 DSA persists.

**Slide 63 - Summary**

To summarize the activity from all of the different sections of this presentation, transplant centers should have effective quality assurance and performance improvement programs to optimize patient outcomes and to help achieve acceptable program-specific reports. Adoption of one of the newer approaches to better risk-stratify and mitigate chronic under-immunosuppression, due to provider under-dosing and patients’ non-adherence, may help improve allograft survival. And, finally, novel, non-invasive biomarkers to evaluate alloimmune injury are emerging and may represent a valuable adjunctive tool in the management of kidney recipients, going forward.

Thank you for your attention.