Korean Society of Cardiology 2023

Abstract for Plenary 2 Lecture: The molecular diagnosis of rejection in heart transplantation

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Despite the overall success of heart transplantation as a definitive treatment for endstage heart failure, cardiac allograft rejection remains an important cause of morbidity and mortality. Endomyocardial biopsy (EMBx) has been the standard of care for rejection monitoring but is associated with several diagnostic limitations and serious procedural complications. The use of molecular diagnostics has emerged over the past decade as a tool to potentially circumvent some of these limitations. We present an update on novel molecular approaches to detecting transplant rejection, focusing on 3 categories: gene expression profiling, cell-free DNA and intragraft mRNA transcripts.

Gene expression profiling (GEP) of peripheral blood mononuclear cells can provide information regarding the recipient's alloimmune response to the donor heart. Commercially available GEP testing monitors the expression of eleven genes to identify cardiac allograft recipients who are at low risk for acute cellular rejection (ACR). A multicenter randomized clinical trial showed that patients who were monitored with GEP and those who underwent routine biopsies had similar 2-year cumulative rates of the composite primary outcome (rejection with hemodynamic compromise, graft dysfunction due to other causes, death, or re-transplantation). Patients who were monitored with the use of GEP underwent fewer biopsies per person-year of follow-up. A limitation of GEP is that it was developed and validated only for ACR but not antibody-mediated rejection (AMR).

Donor-derived cell-free DNA (ddcfDNA) can serve as a non-invasive biomarker for disease, infection, and tissue injury/rejection. In a study by De Vlaminck et al., ddcfDNA showed excellent agreement with clinical rejection and, importantly, serial measurement of ddcfDNA predicted clinically significant outcomes after treatment for rejection in these patients. In a multicenter, prospective study the investigators demonstrated that there was a significant increase in ddcfDNA for patients with AMR and ACR. The performance of ddcfDNA assessed against the endomyocardial biopsy had an AUC of 0.9. A limitation of using ddcfDNA is that it does not differentiate between AMR and ACR.

When biopsies are performed, the measurement of intragraft gene expression - mRNA transcripts - has significant potential to improve biopsy interpretation. These mRNA transcripts can be assessed using a variety of platforms, including RT-PCR, microarrays, RNA sequencing, Nano string, and others. For this testing, genes are grouped together into pathogenesis-based transcripts (PBT), for example endothelium associated transcripts, cytotoxic T-cell associated transcripts, interferon-gamma induced transcripts and macrophage associated transcripts to diagnose ACR and AMR. The molecular microscope (MMDx) is a central biopsy diagnostic system that compares the biopsy to a Reference Set, using ensembles of predefined machine-learning derived algorithms. MMDx correlates with histology, but often with discrepancies, at least part of which is expected given the interobserver disagreement in histologic diagnosis of rejection. The MMDx suggests mechanisms of rejection and is an adjunct to the histology read to better characterize the findings of the EMBx.

It is believed that the use of these molecular tests to detect rejection will offer a non-invasive means to detect rejection while the MMDx will more accurately detect the presence and type of rejection in the EMBx.

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Abstract for Heart Failure 2: **Xenotransplantation: The first pig heart to human experience**

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A surgical first was performed on January 7, 2022, when the first pig to human heart transplant was performed by the surgical team led by Dr. Bartley Griffith and Dr. Muhammad Mohiuddin at University of Maryland Medical Center. This xenotransplantation surgery was a pioneering achievement which was pursued after decades of experiments with lower species to species transplantation studies. The need for this important surgical breakthrough is underscored by the scarcity of donor hearts. Some 3,817 Americans received human donor hearts last year in 2021, more than ever before, but the potential demand is still higher with waitlist mortality around 9%. This bold move and surgical breakthrough could not have been possible without advances in technology and the brilliance of the research team. Several breakthrough innovations were accomplished to make this surgery a reality. A key problem is that antibodies produced by the recipient could recognize certain antigenic sugars on the surface of pig cells as being foreign. CRISPR-Cas9 genome editing was able to address this problem. Altogether, 10 genes were altered. Three genes were knocked out that enabled pigs to synthesize these antigens on the cell surfaces. Six human genes were edited into the pig which included two anti-inflammatory genes, two genes that promote normal blood coagulation (prevents blood vessel damage) and two regulatory protein genes that help suppress the antibody response. A final gene modification was done to prevent the pig heart from overgrowing. This phenomenon was observed in transplanting pig hearts into baboons. Another challenge was preparing the pig donor heart to be transported without deterioration. It was noted that when a pig heart was transplanted into a baboon, it began to fail unless the pig donor heart was infused with a nutrient solution before transplant. To

address this problem the team relied on a method developed by Lund University using an ex vivo device for storing and treating the donor heart after it is removed. The heart is submerged in a circulating broth that includes hormones such as adrenaline, cortisol and dissolved cocaine. Among the many important aspects of this first pig to human heart transplant was selection of adequate immunosuppression. An experimental antibody medication called KPL–404 made by Kiniksa Pharmaceuticals, Ltd was used. KPL-404 inhibits the production of anti-pig antibodies by binding to CD40, suppressing the activity of antibody-producing B cells, and inhibiting their crosstalk with T cells that coordinate the immune system's response. This medication works along with the 10 altered genes, but it is believed that the immunosuppression used was vital for success.

The patient was weaned from ECMO, and the xenograft functioned normally without apparent rejection. Sudden diastolic thickening and failure of the xenograft occurred on day 49 after transplantation, and life support was withdrawn on day 60. On autopsy, the xenograft was found to be edematous, having nearly doubled in weight. Histologic examination revealed scattered myocyte necrosis, interstitial edema, and red-cell extravasation, without evidence of microvascular thrombosis — findings that were not consistent with typical rejection. Studies are under way to identify the mechanisms responsible for these changes.

It is not clear as to what metrics will measure success in these initial endeavors. Is it survival at 30 days, 90 days or 1 year? Complications and quality of life measures will also be of paramount importance. We may indeed be at the beginning of a new era in solid organ transplantation.