The Molecular Microscope Diagnostic System: What Is It and How Are We Using It in Our Patients?

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In the current era of solid organ transplant, the community is experiencing an exciting time of technological innovation. One area of particular importance is in the application of genomics to clinical care. Over the course of the lifespan of a cardiac allograft, we take great care to balance preventing immunological damage to the heart while maintaining sufficient immune function to prevent infection. How do we know when that balance is correct? How do we know when there is rejection? Traditionally the endomyocardial biopsy (EMB) has served as the gold standard method to evaluate for rejection. Pathologists stain EMB tissue and examine it for lymphocytes and evidence of myocyte damage under a microscope. However, there is much more information present in a biopsy specimen than is visible under the microscope using a hematoxylin and eosin (H&E) stain. What if we had a way to see subtle signals of damage before it was visible to the eye?

One tool which may help is the Molecular Microscope ® Diagnostic System for Heart (MMDx-Heart)1,2. MMDx-Heart is a gene expression profiling system that takes EMB samples and analyzes their messenger RNA (mRNA) gene expression for evidence of rejection and cardiac injury. The test uses GeneChip™ Custom Microarrays to determine which genes are being expressed in the biopsy tissue. The general concept behind this process is that cells up- or down-regulate genes to make proteins depending upon their function/activity. In the case of transplanted organs, we can use these changes in gene expression, measured by the types and amounts of mRNA present, to give us insight into what sorts of activity is occurring on the cellular and molecular level. MMDx-Heart processes biopsy samples, quantifies the mRNA expression, and then categorizes the gene sets into the different molecular phenotypes. As part of the test, MMDx-Heart categorizes mRNAs into 15 gene sets and 4 overall phenotypes: (1) No rejection or injury, (2) Cellular Injury, (3) Antibody Mediated Rejection or (4) T-cell mediated rejection. The individual gene set results are reported as discrete data and as a visually plotted composite for a particular biopsy sample.

Traditional pathology evaluation might not be able to identify subtle injury under the microscope, but molecular diagnostics can provide some insight3. By combining the information from the traditional biopsy with the MMDx-Heart gene profile, one can get a more complete picture of what is happening at the cellular level. The traditional biopsy allows one to see what type of cells are in the sample (cardiac myocytes, inflammatory/immune cells) and what condition the cells are in (e.g. are the myocytes damaged or swollen). The MMDx-Heart gene profile provides insight into what cell programs are active and inactive (lymphocytes inducing damage, blood vessel cells responding to antibody mediated damage, cardiomyocytes recovering from injury) by quantifying mRNA expression patterns.

One might consider a 5-year-old heart transplant patient with a history of a positive CDC cross-match undergoing a per-protocol biopsy 3 weeks after heart transplant. This is an immunologically high-risk patient and it may be difficult to determine if the patient has symptoms of heart failure because they are still recovering from transplant surgery. In addition, some ischemia-reperfusion injury is expected to be present in the first few weeks after a heart transplant. Understanding the specific molecular patterns present in the biopsy tissue would be useful in this scenario.

Logistically, using MMDx-Heart requires a few adjustments to the biopsy process. It starts by requesting a free specimen kit, which contains the specimen sample tube containing RNAlaterTM, tracking barcodes, and a form for basic demographic and clinical information. Unlike the other new technologies used to monitor for rejection on peripheral blood samples, MMDx-Heart is a tissue diagnostic performed on the biopsy itself. During a cardiac catheterization, the cardiologist obtains 1-2 additional pieces of endomyocardium. Having two samples improves the reliability, and allows for quality control. Once the biopsy is obtained, the samples for MMDx-Heart evaluation are placed in the supplied specimen tube with the RNAlaterTM solution which helps to preserve the RNA fragments in the sample for analysis. For U.S. hospitals, the biopsy specimen is then shipped to the Kashi Lab in Oregon via an overnight currier for analysis. At the lab, the mRNA levels are measured against the Reference Set database, and the report is generated in approximately 2-3 business days. At our institution, this process is independent of the

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pathology lab. Our results are uploaded into the electronic medical record and complement with the results of the traditional biopsy. This data enriches our understanding of the traditional pathology when used in the correct clinical context.

So, which patients get an MMDx-Heart at our center? The answer: not everyone. We have found MMDx-Heart to be useful in the following scenarios:

- *Elevated filling pressures in the acute post-transplant period: The MMDx-Heart can be helpful in distinguishing between ischemia-reperfusion injury and antibody mediated rejection which have similar appearance by traditional histology, but different molecular profiles.*
- *Evaluation of transplant patients with suspected rejection due to acute heart failure symptoms and/or changes in cardiac function on echo.*
- *Patients with suspected rejection who have already received high dose steroids prior to biopsy. In this situation, high-dose steroids can result in rapid decrease in the number of lymphocytes in the myocardium however the molecular signature of rejection persists for a longer time.*
- *Patients with de novo DSA in whom we are trying to understand the significance – is this just a circulating antibody or is there antibody induced damage?*
- *Patients who are highly sensitized pre-transplant or those with a history of antibody mediated rejection (AMR).*
- *Patients with a history of non-adherence as it may give us insight into whether there is subclinical allograft injury or rejection.*

One of the defined phenotypes of the MMDx-Heart test is the "Injury Related" phenotype. While this can be seen in the context of rejection, some patients demonstrate this phenotype without rejection. It is the most common molecular phenotype that we have seen in our institutional experience. It is sometimes seen it in patients with elevated filling pressures, and it is unclear if this a persistent finding after prior rejection episodes or if it represents the allograft response to other types of injury. A study in the adult heart transplant population evaluated "for cause" endomyocardial biopsies assessed with MMDx-Heart at a single center. They found that of the patients with normal histology but abnormal MMDx-Heart showing cellular injury, 7 of the 10 patients identified experienced adverse outcomes (biopsy negative rejection with reduced function, donor specific antibodies and/or death) within 3 months of the biopsy4. This suggests that molecular phenotyping may be important at identifying higher risk patients. One of our future goals is to compare patients with an "Injury Related" phenotype to those with the "No rejection or injury" phenotype to determine if there are differences in patient outcomes over time.

At Boston Children's Hospital, we have sent 101 unique MMDx-Heart tests on 41 different patients since May 2019. Thirty-nine percent of those samples have resulted as abnormal with the most common phenotype being the least concrete diagnosis: "Injury related." There is still significant room for learning about what the MMDx-Heart means for an individual patient but we believe that there is value in being able to identify, treat and trend molecular markers as we care for our patients. We look forward to developing best practices for the use of the MMDx-Heart test along with the rest of the PHTS community.

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