Towards novel theranostic approaches in cardiac transplantation medicine

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This editorial refers to 'MicroRNAs as non-invasive biomarkers of heart transplant rejection', by J.-P.D. Van Huyen et al., on page 3192.

Patients’ life expectancy after heart transplant (HTX) has increased tremendously since the first human transplantation in 1967.¹ This has been achieved by refined surgical techniques, advances in organ preservation and dramatic improvements in immunosuppressive therapies, delivered as a collective effort by multiple clinical and scientific disciplines. One of the most important areas that nowadays require further improvement in the optimization of clinical outcome is patient care following transplantation.² In general, the aim of post-operative care is to deliver adequate immunosuppression, prevent graft injury and rejection, and minimise the side effects and toxicity of drugs, guided by systematic monitoring of organ functions, clinical symptoms, therapeutic drug concentrations and biopsy results. Acute cardiac allograft rejection (ACAR) occurs in approximately 30% of cardiac transplant patients during the first year and at a rate of 1–7% per year thereafter.³,⁴ Optimization of the therapy is challenging; none of the existing diagnostic tools are able to predict clinical outcomes that could reliably guide therapeutic decisions. To overcome this, novel biomarkers are being developed that have adequate predictive potential; these biomarkers could complement or replace current diagnostic tools. Although it is invasive and costly, and false negative results are not uncommon, the ‘gold standard’ tool is still endomyocardial biopsy (EMB). Clinical indicators such as electrocardiogram (ECG) and imaging techniques (echocardiography, MRI) have been relatively unsuccessful in providing additional diagnostic or prognostic benefits. Biochemical markers of cardiomyocyte injury and stress, as well as inflammation, have also been assessed; these biomarkers have been measured mostly in blood samples to detect graft rejection. Cell-specific molecules such as troponin-I/T (TnI/T) or B-natriuretic peptide (BNP) (and NT-proBNP) are released from cardiomyocytes and are the ‘gold standard’ for ischaemic injury.⁵ These markers are increased during cardiac rejection but are rather non-specific.⁶ Tissue inflammation is part of the immunological process after transplantation; C-reactive protein (CRP) is an inflammatory marker highly associated with cardiac rejection; however, CRP elevation is rather non-specific and often does not indicate graft rejection.⁷ A key component in ACAR is the activation of cell-mediated immunity, which can be assessed by the US Food and Drug Administration (FDA)-approved Cyclex® ImmuKnow™ (which is approved by the FDA and bears the CE mark), an innovative approach that tests CD4+ T-Cell adenosine triphosphate (ATP) content after phytohemagglutinin-L stimulation.⁸ Low scores in the test appear to predict infectious risk in heart transplant patients; however, the association between high scores and rejection risk is so far inconclusive.⁹ The detection of single or multiple genetic markers of ACAR in the patient’s blood by gene expression profiling is complex and technically challenging. Out of the large number of different genetic markers so far studied, one approach, Allomap® (XDx, Inc., Brisbane, Australia) is approved by the FDA and available in Europe. The test uses 20 genes of relevant pathways from circulating mononuclear cells, reflecting the host response after transplantation. This test has been validated through the Cardiac Allograft Rejection Gene Expression Observational (CARGO) and the subsequent CARGO II studies and the test proved to be useful for ruling out.¹⁰,¹¹ The more recent Invasive Monitoring Attenuation by Gene Expression Profiling (IMAGE) study demonstrated that Allomap® testing not only indicates the probability of acute cellular rejection by a single ordinal gene-expression profiling test score but, in individual patients, the variability of a gene expression profiling test score over time may provide additional prognostic utility.¹² Another innovative marker of graft cellular injury is the appearance of circulating donor-specific cell-free deoxyribonucleic acid (Dscf-DNA). Although costs and the complexity of genotyping limited its usefulness a recent study, using a cost-effective targeted quantitative genotyping approach, demonstrated that the percentage of Dscf-DNA was increased in patients with rejection and can rule out ACAR with high accuracy.¹³

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The underlying molecular mechanism of acute graft rejection begins well before the appearance of pathogenic biomarkers, or of histological features. A novel approach in biomarker discovery focuses on circulating non-coding RNAs, such as microRNAs, or long non-coding RNAs from blood samples. MicroRNAs are key regulators of protein synthesis at the post-transcriptional level. These short, approximately 22 nucleotide, RNA molecules regulate multiple gene networks and they play a particularly important role in disease development. MicroRNAs are surprisingly stable in multiple gene networks and they play a particularly important role. These short, approximately 22 nucleotide, RNA molecules regulate regulators of protein synthesis at the post-transcriptional level. In recent years, circulating microRNAs as potential biomarkers were also analysed in solid organ transplantation studies, including small, pilot studies in cardiac transplantations. In a study of seven patients, miR-133a, miR-133b and miR-208a showed dynamic changes, similar to TnI, early after transplantation. Circulating miR-133b outperformed cTnI by predicting graft dysfunction. Despite the limitations of the study, circulating miR-133b could be one of the new, sensitive markers for monitoring and forecasting myocardial injury and recovery after heart transplantation. Another pilot study with 10 patients identified circulating miR-326 and miR-142-3p as having significant discrimination power to distinguish between normal and pathological histologies of the EMB samples.

A major study by Dong Van Huyen et al., published elsewhere in this journal, tested circulating miRNAs as potential biomarkers for acute allograft rejection. Blood samples were collected at the time of biopsies taken during the post-operative follow-up over a one-year period. Quantitative real-time PCR (qPCR) analysis of the cardiac biopsies showed that seven microRNAs were differentially expressed between normal and rejecting hearts. Most importantly, four microRNAs out of the seven (miR-10a, miR-31, miR-92a and miR-155) were also differentially detectable in blood samples, and correlated well with tissue expression. With the impressive number of 113 included heart transplant recipients, the study was well powered. All four microRNAs had significant discriminating power in the set cohort, which was further confirmed in additional samples from the validation cohort. As the authors pointed out, further studies are needed to confirm their findings in even larger, unselected prospective cohorts. Further stratification of patients may yield additional diagnostic benefits for patient subgroups, particularly for paediatric transplant patients. The study is based on a single blood collection; evidently more data is needed to explore the nature of circulating microRNA kinetics. Biomarker discovery studies commonly employ two different approaches: large-scale screening of candidates, whose data are then analysed for association with disease pathology, or a pathogenesis-driven strategy based on pre-selecting potentially relevant candidates and testing their performance. In this study, the authors chose the latter approach and selected 14 microRNAs relevant to cardiac allograft rejection, endothelial activation, injury and vascular inflammation. The obvious advantage of this approach was, arguably, the simpler study design and faster validation. On the other hand, it would be valuable to screen samples from the author’s large cohorts for novel microRNAs or other non-coding RNAs, such as lncRNAs or circular RNAs (circRNAs), which could provide not only additional biomarker candidates but also valued scientific information on the pathomechanism of allograft rejection. The usefulness of biomarkers depends on an accurate and easy-to-perform assay. In this study qPCR, the current state-of-the-art method for microRNA quantification, was used. Although qPCR is the mainstream technology, several other technology platforms are currently emerging. There are several major obstacles, such as low presence of microRNAs or other non-coding RNAs, normalization, issue interlab variability, and inconsistent sample processing standards, that need to be overcome before microRNA detection assays can be used in a clinical setting.

Improving cardiac transplant patients’ long-term management and quality of life continues to be a major challenge. In addition to postoperative care, another area where improvement is needed is in evaluation, monitoring and decision-making regarding the listing of patients, especially children. Current policies on the selection of candidates and the allocation of hearts for transplantation give priority to patients who are at greatest risk if they do not receive a transplant. Further refinement of allocation will be possible, based on solid evidence from large-scale, longitudinal, randomized clinical trials using validated biomarkers. On the other hand, large, randomized clinical trials cannot help with individualization of post-transplantation immunotherapies that are sufficient to avoid ACAR and adapted to individual changes in immune status. Personalized or ‘stratified’ medicine is a novel concept and holds enormous potential to increase the efficacy, safety and cost-effectiveness of treatments. New, validated biomarkers—quite possibly the above-mentioned circulating microRNA—may also be the cornerstones of patient stratification and theranostic approaches. These markers are needed to identify subgroups that can benefit from specific treatment options and that are at higher risk of toxicity or adverse effects, in order to improve organ—recipient matching and to predict outcomes. The study by Dong Van Huyen et al. is a major step in the right direction. Innovative future studies are emerging that employ novel biotargets, including circulating miRNAs, and may help to develop future diagnostic/predictive biosays in cardiac transplantation medicine.

References


