Non-invasive approaches for the diagnosis of acute cardiac allograft rejection

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ABSTRACT

Despite modern immunosuppressive regimes, acute rejection remains a leading cause of morbidity and mortality in heart transplant recipients. Clinical features are unreliable, and therefore, screening is performed in order to detect rejection, and hence, augment immunosuppressive therapy, at an early stage, with the aim of reducing short- and long-term sequelae. Histological analysis of right ventricular myocardial tissue obtained at endomyocardial biopsy remains the ‘gold standard’ surveillance technique; however ‘biopsy-negative’ rejection occurs in up to 20% of patients, the procedure is associated with uncommon but potentially serious complications and it is expensive. Non-invasive screening would, conceivably, be safer, more tolerable and cheaper, and could potentially allow more comprehensive monitoring. The evidence for non-invasive methods of diagnosing acute rejection, including assessment of myocardial deformation, myocardial tissue characterisation, electrophysiological monitoring, visualisation of cellular and molecular components of rejection and peripheral monitoring of immune activation, is reviewed.

INTRODUCTION

Despite advances in immunosuppressive therapy, acute cardiac allograft rejection (ACAR) remains common during the first year post-transplantation, with an incidence of over 40%, and represents a leading cause of mortality during this period, responsible for approximately 12% of fatalities.1 Moreover, an episode of ACAR occurring during the first year, even when apparently successfully treated, confers higher two-year and four-year mortalities in those surviving beyond the first year, and is an independent risk factor for developing allograft vasculopathy.1

ACAR results from a recipient alloimmune-dependent process directed against donor major histocompatibility complex (MHC) antigens, or donor MHC-derived peptides presented by dendritic cells, via recipient MHC molecules.2,3 This process induces allospecific T-cell reactivity and, ultimately, graft cell loss. The cellular and molecular orchestration of events are complex, involving the early recruitment of innate cells, such as macrophages and eosinophils, in response to endogenous signals expressed and secreted by the graft consequent to myocardial stress and injury.4 These early inflammatory processes induce the chemotaxis and diapedesis of CD4+ and CD8+ T-cells, which generate allospecific immune responses. The entire process requires the redundancy and compensation of pro- and anti-inflammatory cells, modulated by regulatory phenotypes, including regulatory T-cells, interleukin-10-producing dendritic cells and natural killer and invariant natural killer T-cells.

For clinical diagnostic purposes, ACAR is categorised into acute cellular and antibody-mediated rejection (table 1A). Acute cellular rejection is well characterised. Its severity is graded according to established histological criteria and immunosuppressive therapy is generally augmented at grade 2R or higher (range 0–3R, figure 1A–D).5 Antibody-mediated rejection is less well characterised, although its incidence appears to be increasing and it carries a greater risk of haemodynamic compromise, graft dysfunction and death.6,7 Its diagnosis is based on histological and/or immunopathologic features (table 1B, figure 1E–H), although an absence of standardised diagnostic criteria means that considerable heterogeneity exists. Indeed many centres do not routinely perform immunopathological assessment.6 Consequently, the decision to treat is usually based on clinical manifestations of graft failure in the absence of cellular rejection.5 Mixed cellular and antibody-mediated rejection is also recognised, and ACAR often does not affect the myocardium uniformly.

Clinical features of ACAR are unreliable, with patients often remaining asymptomatic until haemodynamic complications ensue. Routine screening is therefore performed in order to detect ACAR, and hence augment immunosuppressive therapy, at an earlier stage, with the aim of preventing progression to more severe disease, and potentially reducing the risk of long-term complications. Histological analysis of right ventricular myocardial tissue obtained at endomyocardial biopsy (EMB) remains the ‘gold standard’ technique for ACAR surveillance, and patients undergo frequent biopsies (10–15) during the first postoperative year. However, due to sampling error related to the patchy nature of ACAR, variability in the interpretation of histological findings and non-routine screening for antibody-mediated rejection, ‘biopsy-negative’ ACAR (haemodynamic features suggestive of significant ACAR but apparently normal EMB) is reported to occur in up to 20% of patients.8 As a result, significant myocardial injury can occur before immunosuppressive therapy is intensified. Furthermore, EMB is invasive, with an associated complication rate of approximately 0.5–1.5% (including myocardial perforation, pericardial tamponade, arrhythmia, access-site complications and significant tricuspid regurgitation), is expensive and is disliked by patients, factors which prevent more frequent monitoring and, thus, limit optimal titration of immunosuppressive therapy.10,11
Non-invasive screening is highly desirable. It would conceivably be safer, more tolerable and cheaper, and therefore, could potentially allow more comprehensive ACAR monitoring. Furthermore, alternative approaches to diagnosing ACAR and evaluating its impact, such as assessment of myocardial function, myocardial tissue characterisation and peripheral monitoring of immune activation may, theoretically, overcome some of the other shortcomings associated with EMB, and better guide immunosuppressive therapy.

**IMAGING TECHNIQUES**

**Echocardiography**

Left ventricular (LV) size, wall thickness, mass and ejection fraction (EF) are insensitive markers of ACAR, as is the presence of a pericardial effusion, which is seen in approximately two-thirds of patients at 3 months post-transplant, and a quarter at 6 months, regardless of ACAR status.\(^{12-14}\) Nevertheless, echocardiography is commonly used clinically to assess LV systolic function when there is a high suspicion of ACAR despite negative EMB findings, and to monitor EF during confirmed episodes.

Doppler indices of mitral inflow are the most widely investigated imaging parameters for detecting ACAR; however, none have shown sufficient accuracy for clinical adoption, at least in part, because they are significantly affected by other factors, such as age, heart rate and loading conditions.\(^{15}\) Pulmonary venous flow indices are also non-discriminatory.\(^{13\,16\,17}\)

Myocardial performance index (MPI, sum of isovolumetric contraction time and isovolumetric relaxation time divided by aortic ejection time) was found to be significantly increased in patients with grade 2 and 3 ACAR compared with those with grade 0 in a small study by Leonard et al,\(^{18}\) however, as demonstrated in this and other studies, MPI is increased in transplant recipients in the absence of ACAR, and as such, its specificity for detecting ACAR is low (61% in this study, positive predictive value 57%).\(^{19}\)

Conflicting data exists with regards to the utility of pulsed-wave tissue Doppler imaging (PW-TDI). In a relatively large study (including nearly 400 biopsies) using PW-TDI obtained at the basal LV inferolateral wall, significant ACAR (≥grade 2) was associated with a significantly reduced peak early diastolic wall motion velocity (Em, also called Ea), and a significantly prolonged early diastolic time (TEm, time from onset of the second heart sound to peak of the early diastolic wave, Em) compared with when ACAR was absent (grade 0).\(^{20}\) Furthermore, during serial examination of 161 patients, a >10% reduction in Em had positive and negative predictive values (PPV, NPV) of 87% and 95%, respectively, for detecting ‘clinically significant’ ACAR (≥grade 2, or symptomatic grade 1) and a >10% prolongation of TEm had a PPV and NPV of 92% and 96%, respectively. The thresholds used were determined retrospectively, based on the reproducibility of these measurements. Changes in Em and TEm resolved completely within 66±24 h of starting antirejection therapy in 92% of patients. Systolic parameters were less discriminatory. Three smaller studies have also found Ea to be

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**Table 1** (A) International Society for Heart and Lung Transplantation (ISHLT) standardised cardiac biopsy grading for acute cellular rejection (1990 classification and 2005 revision);\(^{a}\) (B) ISHLT 2005 recommendations for grading of antibody-mediated rejection and 2011 working formulation for the pathological diagnosis of antibody-mediated rejection\(^{b}\)

### (A) Acute cellular rejection

<table>
<thead>
<tr>
<th>2005</th>
<th>1990</th>
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<tbody>
<tr>
<td>Grade OR</td>
<td>No acute cellular rejection</td>
</tr>
<tr>
<td>Grade 1R, Mild</td>
<td>Interstitial and/or perivascular infiltrate with up to one focus of myocyte damage</td>
</tr>
<tr>
<td>Grade 2R, Moderate</td>
<td>Two or more foci of infiltrate with associated myocyte damage</td>
</tr>
<tr>
<td>Grade 3R, Severe</td>
<td>Diffuse infiltrate with multifocal myocyte damage, oedema, haemorrhage, vasculitis</td>
</tr>
<tr>
<td>Grade 0</td>
<td>No rejection</td>
</tr>
<tr>
<td>Grade 1, Mild</td>
<td>Focal perivascular and/or interstitial infiltrate without myocyte damage</td>
</tr>
<tr>
<td>Grade 2, Moderate (focal)</td>
<td>Diffuse infiltrate without myocyte damage</td>
</tr>
<tr>
<td>Grade 3A, Moderate, Focal</td>
<td>One focus of infiltrate with associated myocyte damage</td>
</tr>
<tr>
<td>Grade 3B, Moderate, Diffuse</td>
<td>Multifocal infiltrate with myocyte damage</td>
</tr>
<tr>
<td>Grade 4, Severe</td>
<td>Diffuse, polymorphous infiltrate with extensive myocyte damage, oedema, haemorrhage, vasculitis</td>
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### (B) Antibody-mediated rejection

<table>
<thead>
<tr>
<th>2011</th>
<th>2005</th>
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<tr>
<td>pAMR0</td>
<td>Negative for pathologic AMR—both histologic and immunopathologic studies are negative</td>
</tr>
<tr>
<td>pAMR 1 (H+)</td>
<td>Histopathologic AMR alone—histologic findings present* and immunopathologic findings negative</td>
</tr>
<tr>
<td>pAMR 1 (I+)</td>
<td>Immunopathologic AMR alone—histopathological findings negative and immunopathologic findings positive†</td>
</tr>
<tr>
<td>pAMR 2</td>
<td>Pathologic AMR—both histological and immunopathologic findings are present</td>
</tr>
<tr>
<td>pAMR 3</td>
<td>Severe pathologic AMR‡</td>
</tr>
<tr>
<td>AMR 0, Negative</td>
<td>No histologic or immunopathologic features of AMR</td>
</tr>
<tr>
<td>AMR 1, Positive</td>
<td>Histologic features of AMR. Positive immunofluorescence or immunoperoxidase staining for AMR (positive CD68, C4d)</td>
</tr>
</tbody>
</table>

*Endothelial cell swelling, intravascular macrophage accumulation; pAMR, pathologic antibody-mediated rejection.
†Including immunohistopathologic evidence of immunoglobulin (IgG, IgM, and/or IgA) and complement (C3d, C4d and/or C1q) deposition in capillaries and/or CD68 staining of intravascular macrophages and/or C4d staining of capillaries.
‡Mixed inflammatory infiltrates, interstitial haemorrhage, oedema, endothelial cell pyknosis and/or karyorrhexis, capillary fragmentation, intravascular thrombi. (Part A adapted with permission from Stewart et al. Revision of the 1990 working formulation for the standardisation of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant. 2005;24:1710–20.)
significantly reduced in ACAR, although in two of these studies a cut-off of ≥ grade 3 ACAR was used to dichotomise, despite immunosuppression usually being augmented when ACAR is less severe. In light of these findings, some centres report that TDI has become an integral part of their post-transplant monitoring, used routinely to guide the timing of biopsies, which has led to a decline in biopsy frequency. Other studies have found no association between ACAR and Ea, TEm has not been widely investigated. The apparent limited value of TDI across these studies may be related to the exaggerated overall motion of the transplanted heart, which markedly influences TDI values, and the reproducibility of TDI measurements, which as demonstrated in other clinical situations, is often low. TDI parameters are also affected by other factors such as systemic infection. As such, TDI does not form part of ACAR surveillance in most centres.
Data regarding the utility of myocardial deformation for detecting ACAR is more promising, but remains heterogeneous. In all three studies assessing TDI-derived strain and strain rate, ACAR was associated with a significant reduction in peak systolic longitudinal strain. Kato et al. found a retrospectively determined threshold of $-27.4\%$ to have a sensitivity and specificity of 82% for detecting $\geq$grade 1B ACAR, although mean values in both non-rejecting (grade 0 or 1A; $-32.6\pm6.3\%$), and rejecting (grade $\geq$1B ACAR; $-20.7\pm8.0\%$) groups were higher than published normal reference ranges for this parameter. Demonstrating more physiological values, Marciniak et al. found lateral wall peak systolic longitudinal strain to be $-21\pm6\%$ in grade $\leq$1B ACAR and $-13\pm5\%$ in grade $>1B$ ACAR ($p<0.05$ for the difference), and Roshanali et al. found lateral wall and septal peak systolic longitudinal strain to be $15.2\pm4.7\%$ and $15.9\pm4.4\%$, respectively, in grade $<3A$, and $12.5\pm3.0\%$ and $11.8\pm4.8\%$, respectively, in grade $\geq3A$ ACAR ($p=0.030$ and 0.003, respectively). TDI-derived peak systolic radial strain, assessed in a single study, was also found to be significantly reduced in ACAR ($\geq$grade 1B), with a retrospectively determined cut-off $\leq30\%$ having a sensitivity and specificity of 85% and 90%, respectively. Using speckle-tracking echocardiography, a potentially more robust and reproducible method of assessing myocardial deformation, Pieper et al. also found global radial strain was significantly reduced in ACAR using a rodent transplant model, although only when ACAR was severe (grade 3B). Global circumferential strain was unchanged. Eleid et al., who performed serial echocardiograms in 51 patients post-transplant, found speckle-tracking-derived global peak systolic longitudinal, radial and circumferential strain measurements were not significantly different in those with ACAR compared with those without, with strain measurements remaining markedly impaired throughout the first two postoperative years independent of rejection status. Peak radial systolic strain rate was significantly reduced in ACAR ($\geq$grade 1B) in the study by Marciniak et al. and peak systolic and early diastolic radial and circumferential strain rates were reduced in severe ACAR (grade 3B) in the study by Pieper et al. Results regarding peak longitudinal systolic strain rate are inconsistent. Following on from early invasive work that found ACAR was associated with altered LV twist mechanics, a recent study using speckle-tracking echocardiography found apical rotation, basal rotation, twist and twist normalised for LV length were all significantly reduced in patients with $\geq$grade 2 ACAR compared with those with grade $\leq$1, with the onset of untwisting occurring later than normal, after aortic valve closure (figure 2). A prospectively determined 25% reduction in baseline twist had a sensitivity and specificity for detecting $\geq$grade 2 ACAR of 74% and 95%, respectively. However, contrary to the early invasive work which found that the early untwist rate was markedly reduced in ACAR possibly secondary to oedema-related changes in the elastic properties of the myocardium, untwist rate was unaffected in this study.

In a study that included 52 patients undergoing 220 biopsies, Angermann et al. found mean posterior wall end-diastolic two-dimensional integrated backscatter (2D-IB), an echocardiographic technique that utilises myocardial acoustic properties to characterise myocardial tissue, to be significantly higher in grade 1B or 2 ACAR, and in grade 3 ACAR, compared with grade 0. Furthermore, in patients experiencing multiple grades of ACAR, 2D-IB was significantly higher in grade 3 than in grade 1B/2. Posterior wall measurements were more discriminatory than septal. Receiver operating characteristic curve analysis revealed a 1.5 dB increase in posterior wall 2D-IB measurement had a sensitivity and specificity of 88% and 89%, respectively, for detecting $\geq$grade 1B ACAR, and 5.5 dB change had a sensitivity and specificity of 92% and 90%, respectively, for detecting $\geq$grade 3 ACAR. However, this technique is technically demanding, susceptible to poor image quality (20% of those screened in the study by Angermann et al. were excluded because of image quality) and artefact, and as a result, often has low reproducibility. Its utility in larger multicentre studies has not been assessed.

**Cardiovascular magnetic resonance**

T2-relaxation time is the most widely investigated cardiovascular magnetic resonance (CMR) parameter for detecting ACAR. T2-relaxation occurs due to interaction between hydrogen nuclei and its exponential decay time-constant, T2-relaxation time, is directly proportional to myocardial water content. Multiple studies using animal transplant models have shown a significant positive correlation between T2-relaxation time and histological severity of ACAR, and ex vivo myocardial water content. Furthermore, the increase in T2-relaxation time appears to be abolished with immunosuppressive therapy.
In the largest human study which included 68 patients undergoing 123 CMR scans, T2-relaxation time was significantly higher in grade 2 ACAR (57±5 ms) compared with grade 0 or 1 (50±5 ms and 51±8 ms, respectively); and in grade 3 (65±8 ms) compared with grade 2.\(^4\)\(^3\)\(^4\)\(^2\) A T2-relaxation time of ≥56 ms, determined retrospectively, had a high NPV (97%) for detecting significant ACAR (≥grade 2), although a low PPV (35%). Interestingly, 80% of patients who did not have significant ACAR on their baseline biopsy, but who developed significant ACAR during the subsequent month, had a baseline T2-relaxation time of ≥56 ms, possibly suggesting that initial biopsy results in these patients were falsely negative, and the actual PPV was underestimated. However, the study specifically recruited patients suspected of having ACAR, and as such, the true accuracy of this technique is unknown. Wisenberg et al\(^4\)\(^0\) found T2-relaxation time was elevated in all patients during the initial 25 days post-transplant irrespective of ACAR status, possibly due to ischaemia-reperfusion injury and cardioprotective solutions used perioperatively, and it only became predictive of ACAR after this period. Of note, there have been no studies published in the last decade regarding the utility of T2-relaxation time for detecting ACAR, despite huge technological advances in scanner hardware and sequence design (figure 3).

Evidence regarding the value of myocardial signal intensity analysis using T2-weighted imaging to detect ACAR is inconsistent, although the majority of studies have been performed using technology inferior to current standards, which may be particularly important given the low contrast-to-noise nature of these sequences. In a recent study using a T2-weighted short inversion-time inversion recovery sequence in 50 patients undergoing 68 CMR scans (mean 3 years post-transplant), there was no significant difference in relative myocardial signal intensity (ratio of myocardial to skeletal muscle signal intensity) in patients with grade 2 ACAR compared with those with grade 0 or 1.\(^4\)\(^1\)

Gadolinium contrast-enhanced CMR has not been widely investigated in the context of ACAR. Relative myocardial signal intensity in the early phase post-contrast, a proposed marker of inflammatory hyperaemia, was significantly higher in patients with grade 2 ACAR (4.1±0.3) compared with those with grades 0 and 1 (2.8±0.2; \(p=0.001\)) in a small study by Taylor et al.\(^4\)\(^3\) although larger studies are required to confirm these findings. Patchy mid-wall, ‘infarct-atypical’ late gadolinium enhancement (LGE) has been observed in two studies including 44 patients 3 years post-transplant, and 41 patients 5–12 years post-transplant, respectively.\(^4\)\(^1\)\(^4\)\(^2\) The prevalence of LGE was approximately 10% and 50%, respectively, but the presence of LGE was independent of current biopsy grade in both studies. It appears that the sensitivity of the LGE technique is insufficient to detect the microscopic and diffuse nature of myocarditis necrosis that occurs during ACAR episodes, but cumulative ACAR insults may result in patchy myocardial fibrosis that is visible. Prognostic data is not available. Two studies using adenosine stress perfusion CMR have shown myocardial blood flow to be reduced in patients with a prior history of ACAR compared with those without, which is in keeping with the findings of invasive studies, but this has not been assessed prospectively.\(^4\)\(^3\)\(^4\)\(^5\)\(^4\)

The risk of nephrogenic systemic fibrosis, an extremely rare but serious complication of gadolinium contrast administration in patients with advanced chronic kidney disease or severe acute renal failure, may limit the potential utility of contrast-enhanced CMR techniques. Cardiac devices also represent a potential limitation to CMR, although increasingly CMR-compatible devices are being manufactured.

**Molecular imaging**

Molecular imaging techniques show considerable potential for non-invasive ACAR diagnosis, although human translation is currently limited.

When dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) particles (15–30 nm) are injected intravenously, they are phagocytosed by macrophages, particularly ED1\(^+\). USPIO particles cause local perturbation in magnetic field, hence, macrophage accumulation can be detected as hypointense regions on T2\(^-\)-weighted CMR imaging. The use of USPIO particles in animal transplant models, together with larger micrometre-sized paramagnetic iron oxide particles that allow single cell in vivo imaging, has provided insight into the pathophysiology of ACAR, with macrophage infiltration seen to be highly spatially heterogeneous even in severe rejection, accounting for the sampling error associated with EMB.\(^4\)\(^6\)\(^4\)\(^7\) Furthermore, macrophage accumulation appears to occur subendocardially initially, with subsequent migration towards the endocardium as ACAR progresses.\(^4\)\(^6\) A number of studies have shown that the degree of signal loss on T2\(^-\)-weighted imaging increases with ACAR severity, and is modulated by immunosuppressive therapy.\(^4\)\(^6\)\(^4\)\(^8\)\(^5\)\(^1\)\(^4\)\(^9\) More recently, Wu et al.\(^4\)\(^7\) using CMR tagging to assess circumferential myocardial strain in conjunction with USPIO imaging, showed that regional loss of function corresponded to areas of macrophage infiltration. Current USPIO preparations are safe for human administration, but the utility of USPIO imaging to detect ACAR in humans has not been assessed.\(^3\)\(^2\)

A range of macromolecular components of ACAR have been targeted with radionuclide scintigraphy, including \(^11\)Indium-labelled antibodies targeted to myosin,\(^4\)\(^3\)\(^5\)\(^4\)\(^4\) released following cellular membrane disruption, \(^99\)technetium-labelled annexin-V\(^5\)\(^5\)\(^4\)\(^5\)\(^5\) a marker of apoptotic cell death, \(^11\)Indium-labelled lymphocytes\(^5\)\(^7\)\(^5\)\(^8\) and \(^99\)technetium-labelled oligonucleotides complementary to mRNA of interleukin-2.\(^4\)\(^9\) However, all such studies have been small, often with conflicting results, and the radiation burden associated with repeated radionuclide investigations may mean this technique is not clinically feasible.

Bioluminescence imaging has been used to visualise migration and proliferation of CD5\(^+\) leukocytes in an ACAR murine model; however, clinical translation of this technique is unlikely to be possible, in part, because of the limited light penetration and the requirement for large amounts of potentially immunogenic substrates.\(^6\)\(^0\) More recently Christian et al.\(^6\)\(^1\) used magnetofluorescent nanoparticles in conjunction with fluorescence molecular tomography and CMR to characterise macrophage activity in a murine model, demonstrating a close correlation between macrophage response and degree of ACAR. Fluorescence molecular tomography may be achievable in humans albeit with an intravascular fluorescence-sensing catheter, and therefore, its potential role may be to guide biopsies and reduce sampling error. Weller et al\(^6\)\(^2\) were able to detect fluorescent ACAR in rodents using ultrasound techniques in conjunction with microbubbles targeted to intercellular adhesion molecule-1, upregulated during ACAR, although in vivo capacity to detect less severe ACAR has not been reported.

**ELECTROPHYSIOLOGICAL MONITORING**

Although advanced ACAR may be associated with a reduction in QRS amplitude, thought to reflect a decrease in functional myocardial cell mass as a result of severe myocardial injury, and interventricular conduction delay, such surface ECG parameters are insensitive to the degrees of ACAR that are seen with
contemporary immunosuppression, as are increased QT interval dispersion and loss of heart rate variability. Arrhythmia occurrence is also an unreliable indicator of ACAR, although new-onset atrial and ventricular tachyarrhythmias, and high-degree atrioventricular block, have been observed in ACAR, and often prompt EMB in clinical practice.

The value of signal-averaged ECG (SAECG) for ACAR surveillance remains to be established. Severe ACAR was associated with a significant decrease in both peak and root-mean-square QRS complex amplitude in two studies, including 45 patients undergoing over 300 biopsies, although these parameters were insensitive to less severe ACAR. Data regarding other SAECG measurements, including the root-mean-square amplitude of the terminal 40 ms of the QRS complex, QRS duration and the presence of late potentials, are discordant, as are the results of studies assessing frequency domain analysis.

The utility of pacemaker-induced intramyocardial electrograms (IEGM) for ACAR monitoring has been assessed in two small multicentre studies. Two fractionally coated leads (leads covered with iridium particles resulting in a complex spatial, or fractal, surface arrangement, which increases the electrochemically active surface area at the lead-tissue interface leading to improved detection performance) are implanted epicardially at the time of transplantation, most commonly on the lateral LV wall and right ventricular outflow tract, although other arrangements have been described, and are connected to a subcutaneously positioned telemetric pacemaker. Ventricular-evoked response (VER) signals are recorded during pacing at a rate of between 100 and 130 bpm, depending on spontaneous heart rate, with the maximum negative slope of the descending part of the repolarisation phase (VER T-slew) determined to be the most sensitive VER parameter. Considerable variation exists between individuals, therefore, patients act as their own reference, with new measurements compared with averaged previous measurements.

In a prospective blinded study including 345 biopsies from 44 patients in four European centres, Grasser et al. found that a reduction in VER T-slew below a retrospectively determined 98% threshold had a NPV of 97% for significant ACAR (≥2 grade). PPV was only 11%. However, only 16 patients exhibited both significant and non-significant ACAR, with only 25 cases of significant ACAR recorded in total, and as such, this threshold was determined from a relatively small number of patients. Bourge et al. in a retrospective blinded study including 329 biopsies from 30 patients in five US centres, also found VER T-slew to have a high NPV for significant ACAR (a threshold of 93% had a NPV of 98%). PPV was only 15%. However, similar to the study by Grasser, there were only 18 cases of significant ACAR in total (per-patient data were not presented). Furthermore, there was marked variation in the definition of significant ACAR between centres; indeed the diagnosis of significant ACAR in three out of the 18 cases was made on clinical features alone, without biopsy data. False positive reductions in VER T-slew occurred in both studies due to infection and non-ACAR-associated heart failure. The authors of both studies suggested that VER monitoring could lead to an approximate 50% reduction in biopsy frequency; however, without more comprehensive data, this technique has not become widely adopted, particularly given that the majority of transplant recipients do not have a bradyarrhythmic indication for pacing, and the high associated complication rate seen in this population (2% and 3% of patients, respectively, in the studies by Grasser et al. and Bourge et al. required pacemaker explantation due to severe infection with pacemaker involvement).

**PERIPHERAL BLOOD MARKERS**

There has been considerable interest in peripheral markers of ACAR; however, given the multifaceted and heterogeneous nature of the disease, a single corresponding gene or protein biomarker with appropriate sensitivity and specificity to the process is unlikely. This is reflected in the number of studies reporting low or contradictory diagnostic accuracy of single targets, such as brain natriuretic peptide, the troponins, inflammatory cytokines, soluble adhesion molecules and soluble CD30.

A more realistic approach is via a multiparameter assessment of gene or protein biomarkers, and gene expression profiling of peripheral blood mononuclear cells has generated particular interest. Using microarray analysis, Horwitz et al. analysed blood samples for a total of 22 215 gene transcripts from patients before, during and after an ACAR episode. Ninety-one transcripts were identified as being differentially expressed in ACAR compared with control (seven genes were overexpressed and 84 were underexpressed), with 98% returning towards control levels, although not fully normalising, following immunosuppressive therapy. Cluster analysis of the 40 transcripts that showed at least a 25% change in expression demonstrated good discrimination between control and ACAR samples, and verified the intermediate expression profile seen following treatment, thus suggesting that gene expression profiling could be used as a tool for monitoring ACAR. Similarly, Schoels et al. demonstrated differential expression of cytokine genes in ACAR using real-time PCR.
Subsequently, Deng et al\(^7\) developed an algorithm based on the expression of a core group of 11 informative genes (involved in T-cell priming, platelet activation, erythrocyte proliferation and mobilisation and steroid response) and nine controls, which weighed the contribution of each gene to give a score that ranged from 0 to 40. A score below 30 had a negative predictive value of 99.6% for moderate to severe rejection (grade ≥3A/2R) in patients at least 1 year post-transplant. Performance was significantly affected by time since transplantation, being lower closer to time of transplant. Following on from this work, in the only clinical trial to date to assess a non-invasive method of ACAR surveillance with respect to patient outcome, Pham et al\(^7\) randomly assigned 602 transplant recipients to ACAR surveillance with either the gene expression profiling algorithm (available commercially as AlloMap) or with EMB. Encouragingly, during a median follow-up period of 19-months, gene expression profiling-guided management was non-inferior to traditional management with respect to a composite outcome of ACAR with hemodynamic compromise, graft dysfunction due to other causes, death or retransplantation, and resulted in significantly fewer biopsies. However, less than 20% of those eligible were enrolled, patients less than 6 months post-transplant were excluded, and those less than 1 year post-transplant made up only 15% of the study population (patients 6–12 months post-transplant were only included following a protocol amendment to facilitate recruitment). As such, the study population was selected as being at lower risk of ACAR, and possibly at lower risk of adverse outcomes due to undetected ACAR. A wide non-inferiority margin was used, and the primary endpoint was likely to have included events unrelated to ACAR, reducing the power of the trial. Furthermore, there were significantly less episodes of treated ACAR in the gene expression profiling group, primarily due to fewer observed episodes of asymptomatic ACAR. In addition, only six of the 34 (18%) treated episodes of ACAR in this group were detected in the asymptomatic phase, with the others manifesting as overt heart failure and/or a reduction in LV EF on echocardiography. By contrast, 22 of the 47 (47%) treated ACAR episodes in the EMB group were detected at the asymptomatic stage. Given that there was no difference in outcome between surveillance strategies, these observations suggest that there may be no benefit to routine screening for the early detection of ACAR in the longer-term post-transplantation, possibly supporting the practice of those centres no longer performing routine EMB after the first year post-transplantation, although a longer follow-up period would be required to assess for late consequences of untreated ‘late-treated’ ACAR.\(^8\)

**GUIDELINES**

The 2010 International Society of Heart and Lung Transplantation Guidelines for the Care of Heart Transplant Recipients assign gene expression profiling (AlloMap) a Class IIa recommendation, Level of Evidence B; stating that it ‘can be used to rule out the presence of ≥ grade 2R ACAR in appropriate low-risk patients, between 6 months and 5 years after transplantation.’\(^9\) VER monitoring and IEGM recorded non-invasively with telemetric pacemakers are assigned a Class IIa recommendation, Level of Evidence C; for use ‘in centres with proven expertise, in patients at low risk for rejection’. Echocardiography, CMR and biomarkers, such as brain natriuretic peptide, the troponins and C-reactive protein are not recommended as alternatives to EMB (Class III, Level of Evidence C). Molecular imaging is not included in the guidelines. Periodic EMB is described as the ‘standard of care’ for ACAR surveillance during the first six to twelve postoperative months (Class IIa recommendation, Level of Evidence C), but only in those at higher risk for ACAR thereafter.

**FUTURE DIRECTIONS**

Contemporary CMR techniques that allow comprehensive characterisation of acute myocardial injury, such as T1 and T2-relaxation time measurement (‘mapping’), have been shown to accurately identify other inflammatory myocardial conditions, and as such, are attractive for application in ACAR.\(^5\) CMR may also allow more reliable detection of subtle alterations in myocardial mechanics, and USPIO imaging which has been successfully used to demonstrate inflammation in conditions such as aortic aneurysm in humans.\(^6\) requires evaluation in human ACAR. In addition, mathematical modelling of immune cell interactions could be potentially used to phenotypically characterise the augmentation in immune response to alloantigen peripherally.

Clinically meaningful investigation of all such methods ultimately requires assessment in multicentre, outcome-based studies, with prospective recruitment of patients in the early phase (ie, within 1 month) post-transplantation and independent analysis.

**CONCLUSION**

Despite the clear potential benefits that non-invasive approaches to ACAR surveillance could offer, there remains a paucity of evidence for their use. The evaluation of non-invasive techniques is made more challenging by the imperfect nature of the current ‘gold standard’ screening technique, and by the relatively small size of transplant populations, indeed, the literature is dominated by small, single-centre studies, that often include patients who are outside the time period when the early detection of ACAR is likely to be most useful. The investigation of non-invasive methods has contributed to a greater understanding of ACAR, and some techniques have been adopted into clinical guidelines, albeit for appropriate subgroups of patients; however, further work, in multicentre trials with outcome-based endpoints, is required.

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