Supplementary material

Dorniak K, Stopczyńska I, van Heeswijk RB, et al. Cardiac magnetic resonance imaging with T2 mapping for the monitoring of acute heart transplant rejection in patients with problematic endomyocardial biopsy: in anticipation of new recommendations. Kardiol Pol. 2021; 79: 339-343. doi:10.33963/KP.15852

Table S1. T1 and T2 relaxation times measured as an average value of a global region of interest in a mid-ventricular short axis slice (with careful avoidance of artefacts and areas of scar where applicable) in clinically stable HTx patients (without ACAR) vs healthy volunteers.

	Healthy volunteers N= 22	HTx pts ACAR (-) N=12	*P*
T1 relaxation time [ms] mean (SD)	993 (21)	1019 (38)	*P* = 0.004*
T2 relaxation time [ms] median (Q1-Q3)	46 (44-47)	50 (48-52)	*P* < 0.001**

*) Student T-test, **) Mann-Whitney U-test;

ACAR, *acute cardiac allograft rejection*; HTx, *heart transplantation;* (Q1-Q3), *interquartile range;*

Table S2. T1 and T2 relaxation times along with T2STIR ratio and ECV in consecutive HTx patients with (N = 5) vs without (N = 12) ongoing ACAR. The measurements were performed in a global region of interest in a mid-ventricular short axis slice (In the T2STIR images, SI of the myocardium was subsequently divided by the skeletal muscle SI for T2STIR ratio) .
*) reduced number of cases due to a non-analysable or unavailable image for the respective variable; **) Mann-Whitney U-test was used for all comparisons; ACAR, *acute cardiac allograft rejection*; HTx, *heart transplantation;* ECV, *extracellular volume fraction;* Q1-Q3, *interquartile range;* SI, *signal intensity*; T2 STIR , *short-tau inversion recovery T2-weighted sequence*.

	HTx pts ACAR (+) N = 5	HTx pts ACAR (-) N = 12	*P***
T1 relaxation time [ms mean (SD)	N = 4* 1123 (64)	N = 12 1019 (38)	*P* = 0.013
T2 relaxation time [ms] median (Q1-Q3)	N = 5 58 (53-62)	N = 12 50 (48-52)	*P* = 0.009
T2-STIR SI ratio mean (SD)	N = 4* 2.3 (0.2)	N = 11* 1.8 (0.2)	*P* = 0.001
ECV [%] median (Q1-Q3)	N = 4* 43.0 (42-44)	N = 9* 27 (24-28)	*P* = 0.003

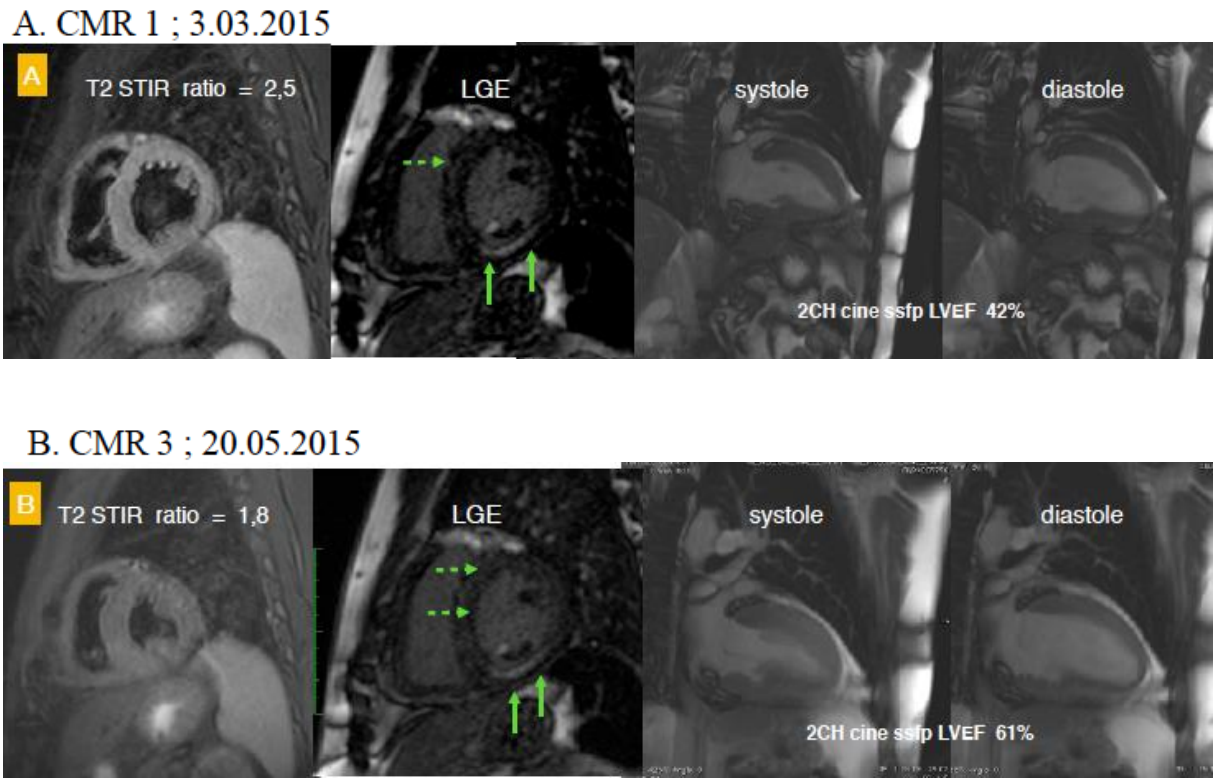

Figure S1.

A. Baseline CMR. Left to right: basal LV T2 STIR image showing edema with high SI ratio (myocardium/skeletal muscle) of 2.5; LGE image showing subendocardial scar in the basal inferolateral segment and disperse intramural fibrosis of the septum; sytolic and diastolic stillframes from 2-chamber cine series, with reduced LVEF (SAX) of 42%.

B. Follow-up cardiac magnetic resonance at two months post ACAR treatment. Left to right: basal LV T2 STIR image showing borderline edema with SI ratio (myocardium/skeletal muscle) of 2.1; LGE image showing greater extent of enhancement in the septum con-

sistent with progressive intramural fibrosis and subendocardial scar in the basal posterolateral segment; sytolic and diastolic stillframes from 2-chamber cine series, with improved LVEF (SAX) of 61%

ACAR, *acute cardiac allograft rejection*; CMR, *cardiac magnetic resonance;* LGE, *late gadolinium enhancement*; LVEF, *left ventricular ejection fraction*; SAX, *short axis cine stack*; SI, *signal intensity*; T2 STIR , *short-tau inversion recovery T2-weighted sequence.*

Suppl. material S1: Endomyocardial biopsy

According to the current guidelines, endomyocardial biopsy (EMB) remains the gold standard for the diagnosis of ACAR. The International Society of Heart and Lung Transplantation (ISHLT) recommends performing periodic EMB during the first 6 to 12 post-operative months for surveillance of HTx rejection, and after the first post-operative year, EMB surveillance for an extended period of time (e.g. every 4 – 6 months) in the case of higher risk for late acute rejection, in order to reduce the risk for rejection with hemodynamic compromise [1]. After 5 years EMB is optional in HTx recipients and should depend on clinical judgment and the risk for late allograft rejection [1]. Recent data suggest acute asymptomatic rejection to be rare after the second post-transplant year, and depicts routine surveillance studies beyond the second post-transplant year as not cost-effective [3]. Although the rates of routine EMB complications are relatively low, they may be clinically significant [4].

Suppl. material S2: Cardiac parametric mapping

With the advent of quantitative parametric techniques, per-pixel measurement ("mapping") of the basic magnetic properties of the myocardium became possible. These include T1 and T2 myocardial relaxation times and a derived value of extracellular volume fraction [ECV] calculated from pre-and post-contrast T1 maps and blood hematocrit. Both the T1 and T2 myocardial relaxation times increase with increased water content (edema) that, in the absence of late gadolinium enhancement (LGE), points to acute and potentially reversible myocardial injury.

Thus, the multiparametric tissue characterization aspects of cardiac MR (CMR) evolved from a predominantly qualitative to a quantitative exam. In addition to accurate quantitation of cardiac volumes, function and amount of fibrosis/scar, it now offers quantitative tissue characterization that shows acute injury/edema, ECV expansion due to interstitial fibrosis or amyloid, as well as several other pathologies [6].

The etiology of these alterations can be determined from or confirmed with other findings, including segmental or transmural distribution of changes, and from clinical and laboratory data.

Suppl. material S3: Statistical analysis.

Continuous variables were presented as mean (SD) or median (Q1-Q3), depending on data distribution. Categorical variables were presented as percentages. Student's t-test and Mann-Whitney U-test were used depending on data distribution and subgroup size, with the non-parametric test used whenever large difference in subgroup sizes and/or small numbers were noted. *P* values < 0.05 were considered statistically significant. The IBM SPSS Statistics (Version 26) was used for the analysis.

Suppl. material S4: Index ACAR and follow-up

To illustrate the utility of CMR we summarize serial findings in a 33 y.o. male at four years post HTx due to non-ischemic cardiomyopathy. This patient had a history of four prior ACAR episodes (confirmed as 2R or 3R cellular rejection on EMB according to the International Society of Heart and Lung Transplantation [ISHLT]) [1] and recent left anterior descending (LAD) coronary artery stenting due to graft vasculopathy. Prior to the index admission, the patient underwent 13 EMB procedures.

In May 2015, 15 months after his fourth ACAR episode, new RBBB and new regional LV systolic dysfunction were noted during a routine outpatient check-up, which prompted emergency hospitalization. Upon admission, recurrent nsVT were observed. The EMB was deferred due to concurrent dual antiplatelet therapy post-stenting. Features of global acute myocardial injury and moderately reduced LVEF of 42% were noted, with disperse late enhancement in the septum (suggestive of fibrosis and/or necrosis) and small subendocardial areas of scar in RCA and LAD territories (Figure S1A). Nevertheless, the patient remained subjectively asymptomatic. Even though the deferred EMB revealed only mild (grade 1R) cellular rejection with Quilty effect, and anti-donor heart antibodies were negative, methylprednisolone treatment was initiated. The index ACAR episode was previously described in [5]. The decision was based on the above findings, in line with current recommendations [1]. The ISHLT recommends that in patients with symptomatic ACAR, irrespective of ISHLT grade EMB result, a high-dose of iv corticosteroids (CS) should be the first-line therapy, (just as in asymptomatic ISHLT 3R rejection, whereas moderate acute cellular rejection [ISHLT 2R] can be treated with either IV or oral CS) [1]. On the follow-up CMR scans 18 days and 2 months later, gradual improvement of volumes and function as well as regression of features of acute injury were found (Figure S1B). Despite the lack

of recommendations concerning the role of CMR in the management of HTx patients, subsequent monitoring with CMR was undertaken as a decision making aid due to poor yield of the EMB.

With this approach, reappearance of edema and slight LV enlargement on a follow up CMR at 9 months of the index admission while the patient remained entirely asymptomatic and continued vigorous physical activity, suggested an imminent new ACAR episode. However, given the lack of any other features of ACAR and the excellent exercise tolerance at that time, EMB was again deferred. However, early follow-up CMR two months later showed deteriorated LV function and sustained edema features. This was parallelled by reduced exercise tolerance. These findings were confirmed by subsequent EMB, where grade 2R ACAR was found. However, the myocardium was present in only one of the 10 biopsy samples, with the remaining 9 samples containing predominantly fibrous tissue, rendering future use of EMB problematic.

Suppl. material S5: Parametric mapping - reference values

Of note, any proposed threshold should be regarded as a scanner specific value rather than universal guidance, as, ideally, reference values or cut-offs should be determined for each MR scanner individually, i.e. are scanner-specific rather than technique- or field strength-specific (however they do differ between techniques and magnetic field strengths) . This was recently confirmed in a meta-analysis by Snel and co-authors that included studies in HTx recipients [13].

Suppl. material S6: Study limitations

This preliminary work has some limitations. Firstly, the number of patients is fairly low. Secondly, male predominance in the patient group can be noted. Hence, the parametric mapping results might to some extent be affected by the small but significant gender-related differences seen in the published reference ranges at 1,5T scanners, which were not taken into account in our preliminary study in that the healthy controls had more equal gender proportions. However, this seems acceptable from the perspective of the published consensus paper on cardiac parametric mapping, where the acute myocardial injury was among the pathologies specified as large-magnitude biological changes, apparently imposing less strict requirements regarding the reference values (a control group of 15 -20 healthy individuals was considered sufficient) [6]. On the other hand, women are underrepresented in our consecutive patient group. Hence, even though females are known to have higher parametric mapping values than males, the significant influence of sex-related differences on our results seems unlikely, as these values were significantly higher in our patient group despite male predominance.