Three-Dimensional Self-Navigated T₂ Mapping for the Detection of Acute Cellular Rejection After Orthotopic Heart Transplantation

Ruud B. van Heeswijk, PhD,¹ Davide Piccini, PhD,¹,² Piergiorgio Tozzi, MD,³ Samuel Rotman, MD,⁴ Philippe Meyer, MD,⁵ Juerg Schwitter, MD,⁶,⁷ Matthias Stuber, PhD,¹,⁸ and Roger Hullin, MD⁷

Background. T₂ mapping is a magnetic resonance imaging technique measuring T₂ relaxation time, which increases with the myocardial tissue water content. Myocardial edema is a component of acute cellular rejection (ACR) after heart transplantation. This pilot study compares in heart transplantation recipients a novel high resolution 3-dimensional (3D) T₂-mapping technique with standard 2-dimensional (2D) T₂-mapping for ACR detection. Methods. Consecutive asymptomatic patients (n = 26) underwent both 3D T₂ mapping and reference 2D T₂ mapping magnetic resonance imaging on the day of endomyocardial biopsy (EMB). 3D T₂ maps were obtained at an isotropic spatial resolution of 1.72 mm (voxel volume 5.1 mm³). 2D and 3D maps were matched anatomically, and maximum segmental T₂ values were compared blinded to EMB results. In addition, all 3D T₂ maps were rendered as 3D images and inspected for foci of T₂ elevation. Results. T₂ values of segments from 2D and reformatted 3D T₂ maps agreed (p > 0.5). The highest 2D segmental T₂ values were 49.9 ± 4.0 ms (no ACR = OR, n = 18), 48.9 ± 0.8 ms (mild ACR = 1R, n = 3), and 65.0 ms (moderate ACR = 2R). Rendered 3D T₂ maps of cases with 1R showed foci with significantly elevated T₂ signal (T₂ = 58.2 ± 3.6 ms); 5 cases (28%) in the OR group showed foci with increased T₂ values (>2 SD above adjacent tissue) that were not visible on the 2D T₂ maps. Conclusions. This pilot study in a small cohort suggests equivalency of standard segmental analysis between 3D and 2D T₂-mapping. 3D T₂ mapping provides a spatial resolution that permits detection of foci with elevated T₂ in patients with mild ACR.

The International Society of Heart and Lung Transplantation registry indicates that 25% of adult heart transplant (HTx) recipients have 1 or more episodes of acute cellular rejection (ACR) within the first postoperative year.¹

Received 19 September 2016. Revision requested 25 September 2016. Accepted 4 October 2016.

¹ Department of Radiology, Lausanne University Hospital (CHUV), University of Lausanne, Lausanne, Switzerland.
² Advanced Clinical Imaging Technology, Siemens Healthcare IM BM PI, Lausanne, Switzerland.
³ Department of Cardiac Surgery, Lausanne University Hospital (CHUV), University of Lausanne, Lausanne, Switzerland.
⁴ Institute of Pathology Lausanne University Hospital (CHUV), University of Lausanne, Lausanne, Switzerland.
⁵ Cardiology, Department of Medical Specialties, University Hospital Geneva, Geneva, Switzerland.
⁶ Center for Cardiac Magnetic Resonance (CRMC) Lausanne University Hospital (CHUV), University of Lausanne, Lausanne, Switzerland.
⁷ Cardiology, Lausanne University Hospital (CHUV), University of Lausanne, Lausanne, Switzerland.
⁸ Center for Biomedical Imaging (CIBM), Lausanne, Switzerland.

The authors declare not conflicts of interest.

Roger Hullin receives grant support from Novartis, the Swiss Heart foundation, the Emma Muschamp Foundation, and the Swiss National Science Foundation (320030_147121/1); Ruud van Heeswijk receives grant support from the Emma Muschamp Foundation and the Swiss National Science Foundation (PZOOP3_154719).

R.v.H. developed and applied the 3D self-navigated T₂ mapping HTx recipient data collection, statistical analysis, interpretation of the results, writing of the article. D.P. participated in the development of the 3D self-navigated T₂ mapping. P.T. participated in the interpretation of study results. S.R. participated in the pathohistological reading of the endomyocardial biopsies. M.P. participated in the interpretation of study results. M.S. participated in the co-development of the 3D self-navigated T₂ mapping; implication in study design. R.H. participated in the study design, data analysis, quality control of data, writing of the article.

Correspondence: Roger Hullin, MD, Cardiovascular Department, Centre Hospitalier Universitaire Vaudois (CHUV), University of Lausanne, Rue du Bugnon 46, 1011 Lausanne, Switzerland. (roger.hullin@chuv.ch).

Copyright © 2017 The Author(s). Transplantation Direct. Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

ISSN: 2373-8731
DOI: 10.1097/TXD.0000000000000635
series comparing results from histological grading of EMBs with autopsy findings. Furthermore, there is a specificity concern, because concordance of histological grading by different pathologists was only 71% in the 937 EMBs obtained by the cardiac allograft rejection gene expression observational study II trial.

In an animal model of heart transplant rejection, the T2 relaxation time, a physiological property of a given tissue in a magnetic field, increased with the severity of rejection and in linear relationship with the myocardial tissue water content. Compatible with these observations, the International Society of Heart and Lung Transplantation ACR grading recommendations require the presence of edema for diagnosing severe rejection (3R), but not in mild (1R) or moderate (2R) ACR. This does not exclude the presence of edema in human low-grade ACR but acknowledges that standard processing of EMB for histological reading does not permit reliable detection of minor quantities of interstitial edema.

At present, ACR detection in HTx recipients on the basis of fast breath-held 2-dimensional (2D) T2 mapping at a magnetic field strength of 1.5 T permits analysis of 3 slices of 10-mm thickness with a spatial resolution of $1.9 \times 2.5 \times 8 = 37 \text{ mm}^3$ or greater. This technique can be used to very accurately detect 2R or greater ACR, which histologically presents with multifocal or diffuse infiltration of the whole heart. Mild rejection with its patchy nature, however, requires whole-heart screening with high spatial resolution for reproducible discrimination of edematous from adjacent nonedematous tissue. Though the clinical relevance of 1R may be argued, its high incidence and the risk for progression to more severe ACR provide a strong argument for its noninvasive detection. Furthermore, absence of ACR when using a technique that is able to detect 1R will impact on the guidance of immunosuppression. This pilot study therefore aimed to validate a novel 3-dimensional (3D) self-navigated cardiac T2-mapping technique with high spatial resolution (1.72 x 1.72 x 1.72 = 5.1 mm$^3$) at 3 T throughout the whole heart by direct comparison with 2D T2-mapping at high resolution (1.25 x 1.25 x 5 = 7.8 mm$^3$) and EMB-based ACR detection.

**MATERIALS AND METHODS**

Approval from the local ethics committee was obtained (protocol 250/2013). All participants provided written informed consent. A total of 26 consecutive asymptomatic HTx recipients in stable phase after HTx (55–4275 days) were included (mean age, 52 ± 9 years; 3 women; mean donor age, 42 ± 12 years; time after HTx, 699 ± 674 days). Immunosuppression was always guided by EMB histology; coronary angiograms showed no relevant coronary vasculopathy.

Magnetic resonance imaging (MRI) was performed on the day of EMB procurement using a clinical magnetic resonance scanner with a magnetic field strength of 3 T (Magnetom Trio, Siemens Healthcare) and with a 32-channel radiofrequency coil. High-resolution navigator-gated radial 2D T2 maps were acquired in 3 short-axis slices as a reference (see Table 1 for magnetic resonance pulse sequence details). The self-navigated isotropic 3D radial whole-heart T2 map (voxel size 1.72 x 1.72 x 1.72 = 5.1 mm$^3$) was obtained during free breathing. Acquisition, processing and reading of the MRI results was performed with the observer blinded to EMB results.

Segments of 2D and 3D T2 maps were manually drawn in accordance with current AHA guidelines after reformatting and slice-thickness matching of the latter. Equivalency of 3D with reference 2D T2 mapping was tested comparing the highest segmental 2D and 3D T2 values in groups of HTx recipients without (0R), mild (1R), or moderate/severe ACR (≥2R). In addition, all 3D T2 maps were rendered as 3D images (Figure 1) and inspected for foci of T2 elevation.

All values are represented as mean ± SD T2 values were compared with a 2-sided paired Student’s t-test with Bonferroni correction for multiple comparisons, with P less than 0.05 considered significant. In case of a single value in a group, standard deviation or P value was not calculated.

**RESULTS**

Mild ACR was present in 3 patients, 1 patient had 2R; no EMB showed immunohistological signs of acute humoral rejection. Four 3D T2 maps were discarded due to insufficient image quality. Mean T2 values of segments from 2D and reformatted 3D T2 maps agreed well: the highest segmental 2D and 3D T2 values in groups of HTx recipients without (0R), mild (1R), or moderate/severe ACR (≥2R) were 49.9 ± 4.0 ms versus 49.1 ± 3.8 ms (0R), 48.9 ± 0.8 ms versus 49.2 ± 1.3 ms (1R), and 65.0 ms versus 66.1 ms (2R) (P > 0.51 for all comparisons). However, rendered 3D T2 maps of the 3 cases with 1R showed foci with significantly elevated T2 (T2 = 58.2 ± 3.6 ms) that were not visible on

| TABLE 1. An overview of the used MRI parameters |
|-----------------------------------------------|
| Parameter | 2D T2 maps | 3D T2 maps |
|-----------|-------------|------------|
| No. maps per scan | 3 | 1 |
| Pulse sequence basis | Radial GRE | Radial bSSFP |
| Echo time TE, ms | 1.9 | 1.33 |
| Repetition time TR, ms | 4.3 | 2.6 |
| Total radial readouts per image (−) | 310 | 5696 |
| T2 preparation durations, ms | 0/30/60 | 0/30/60 |
| Flip angle, degrees | 15 | 35/70 |
| Spatial resolution, mm$^3$ | $1.2 \times 1.2 \times 5 = 6.9$ | $1.72 \times 1.72 \times 1.72 = 5.1$ |
| Respiratory motion compensation readouts per heartbeat (−) | Lung-liver navigator | Self-navigation |
| Total acquisition duration | −3 × 5 min (depending on the respiration pattern and heart rate) | Fixed 178 heartbeat; −18 min |

GRE, gradient echo; bSSFP, balanced steady-state free precession.
respective 2D $T_2$ maps (Figures 1B, E). In addition, rendered $T_2$ maps from 5/18 patients (28%) without ACR in the EMB showed foci with increased $T_2$ values with greater than 2 standard deviations of difference when compared with adjacent tissue and similar to foci detected in patients with 1R ACR (Figure 1B, black arrow). The 3D $T_2$ map of the single 2R case showed elevated $T_2$ values throughout the left ventricle (LV) in a relatively heterogeneous pattern (Figure 1C).

**DISCUSSION**

This pilot study with 26 consecutive asymptomatic HTx recipients presenting for a scheduled control biopsy demonstrates corresponding segmental $T_2$ values in 2D and 3D $T_2$ maps of patients with 0R and 1R, indicating equivalency of the novel 3D $T_2$ mapping algorithm with the actual $T_2$ mapping standard. Furthermore, rendered 3D $T_2$ map showed foci with significantly increased $T_2$ values compatible with local ACR in all patients with EMB-proven ACR suggesting that this novel algorithm has the potential to detect ACR with a sensitivity that is noninferior to the criterion standard of ACR detection. Consistent with previous studies, the 2R case demonstrated throughout the whole LV elevated $T_2$ values that were several standard deviations above the 0R value.

Retrospective analysis of the 4 discarded maps showed that the main cause of insufficient image quality was most likely insufficient communication with the performing technologist, because in 3 of the 4 patients, only routine shimming was performed, whereas cardiac shimming is essential for balanced steady-state free precession–based cardiac pulse sequences at 3 T. This resulted in several dark-band artifacts (banded signal voids) through the heart, which in turn caused the self-navigation to perform suboptimally. In addition, in 2 of the 4 patients, the timing was most likely not set to the correct phase of the heart, resulting in too noisy and blurred data.

In this pilot study, rendered 3D $T_2$ maps showed foci with significantly elevated $T_2$ values in 28% of patients without histological signs of ACR in the EMB. Moreover, the respective 2D $T_2$ mapping segments in these study patients did not show elevated $T_2$ values compared to adjacent segments. This observation may suggest superior sensitivity of 3D $T_2$ mapping when compared with histological grading of EMBs or 2D $T_2$ mapping. In fact, both techniques have inherent major methodological limitations that decrease their sensitivity for mild ACR detection: in particular, sampling error related to EMB procurement and dilution of the $T_2$ values of increased intensity in a larger voxel volume. The results of this pilot study therefore encourage the investigation of the hypothesis that 3D $T_2$ mapping may allow for noninvasive detection of mild ACR. However, this hypothesis needs validation in a larger patient cohort and should use concomitant intragraft gene expression analysis to prove the presence of ACR in patients with foci of increased $T_2$ values but negative histology in the EMB.

The guidelines for the care of heart transplant patients recommend adjustments of maintenance immunosuppressive therapy only in HTx recipients with moderate or severe ACR, which may argue the prognostic benefit associated with the detection of mild ACR. However, intragraft gene expression analysis indicates that the gene expression profile of histological grade 1R ACR is close to the profile of 2R ACR in almost half of all cases. Because ACR $\geq$ 2R is associated with a decrease in survival after HTx, adjustment of the strength of ongoing maintenance immunosuppressive therapy in patients with 1R ACR might be beneficial, and 3D $T_2$ mapping might be a useful tool for noninvasive detection of mild ACR. However, before HTx recipients are exposed to the risks associated with increased strength of immunosuppression, a critical appraisal of the prognostic

**FIGURE 1.** 3D and 2D $T_2$ maps of patients with ACR 0R, 1R, and 2R. A-C, Examples of volume-rendered 3D $T_2$ maps that were segmented along the center of the endocardium of the LV. D-F, Corresponding basal 2D $T_2$ maps. The 3D $T_2$ maps of patients with 1R show patches with significantly elevated $T_2$ values (black arrow). The color bar indicates $T_2$ values in ms.
relevance of focally increased T2 values is mandatory in a longitudinal follow-up study of HTx patients with EMB-guided immunosuppression.

ACKNOWLEDGMENTS
The authors thank the research coordinator Nathalie Lauriers R.N. for her continued support.

REFERENCES
1. Lund LH, Edwards LB, Kucheryavaya AY, et al. International Society of Heart and Lung Transplantation The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report—2014; focus theme: retransplantation. J Heart Lung Transplant. 2014;33:996–1008.
2. Topalidis T, Warnecke H, Muller J, et al. Endomyocardial biopsies for diagnosis of rejection—the potential margin of error. Transplant Proc. 1990;22:1443.
3. Bhalodolia R, Cortese C, Graham M, et al. Fulminant acute cellular rejection with negative findings on endomyocardial biopsy. J Heart Lung Transplant. 2006;25:989.
4. Nakhlíeh RE, Jones J, Goswitz JJ, et al. Correlation of endomyocardial biopsy findings with autopsy findings in human cardiac allografts. J Heart Lung Transplant. 1992;11:479.
5. Crespo-Leiro MG, Zuckermann A, Bara C, et al. Concordance among pathologists in the second Cardiac Allograft Rejection Gene Expression Observational Study (CARGO II). Transplantation. 2012;94:1172.
6. Aherne T, Tscholakoff D, Finkbeiner W, et al. Magnetic resonance imaging of cardiac transplants: the evaluation of rejection of cardiac allografts with and without immunosuppression. Circulation. 1986;74:145.
7. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. J Heart Lung Transplant. 2005;24:1710.
8. Butler CR, Savu A, Bakal JA, et al. Correlation of cardiovascular magnetic resonance imaging findings and endomyocardial biopsy results in patients undergoing screening for heart transplant rejection. J Heart Lung Transplant. 2015;34:643.
9. Waisnuth R, Prothmann M, Utz W, et al. Variability and homogeneity of cardiovascular magnetic resonance myocardial T2-mapping in volunteers compared to patients with edema. J Cardiovasc Magn Reson. 2013;15:27.
10. Holweg CT, Potena L, Luikart H, et al. Identification and classification of acute cardiac rejection by intragraft transcriptional profiling. Circulation. 2011;123:2236–2243.
11. van Heeswijk RB, Piccini D, Feliciano H, et al. Self-navigated isotropic three-dimensional cardiac T2 mapping. Magn Reson Med. 2015;73:1549.
12. van Heeswijk RB, Feliciano H, Bongard C, et al. Free-breathing 3 T magnetic resonance T2-mapping of the heart. JACC Cardiovasc Imaging. 2012;5:1231.
13. Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation. 2002;105:539.
14. Bano W, Feliciano H, Coristine AJ, et al. On the accuracy and precision of cardiac magnetic resonance T2 mapping: A high-resolution radial study using adiabatic T2 preparation at 3 T. Magn Reson Med. 2017;77(1):159–169.
15. Söderlund C, Ohman J, Nilsson J, et al. Acute cellular rejection the first year after heart transplantation and its impact on survival: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. Transpl Int. 2014;27:482.
16. Lund LH, Edwards LB, Kucheryavaya AY, et al.; for the International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report-2013; Focus Theme: Age. J Heart Lung Transplant. 2013;10:951.