Quantitative myocardial blush grade for the detection of cardiac allograft vasculopathy
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Background  Cardiac allograft vasculopathy (CAV) progressively compromises microvascular perfusion and function in heart transplantation (HTx)-recipients. The aim of our study was to investigate the ability of quantitative myocardial blush grade (MBG) to detect CAV.

Methods  In consecutive HTx-recipients (n = 72) who underwent surveillance cardiac catheterization, MBG was assessed visually and quantitatively, by analyzing the time course of contrast agent intensity rise. Hereby, the parameter \( G_{\text{max}}/T_{\text{max}} \) was calculated as the plateau of grey-level intensity (\( G_{\text{max}} \)) divided by the time-to-peak intensity (\( T_{\text{max}} \)). HTx-recipients and 18 healthy volunteers underwent cardiac magnetic resonance, to assess diastolic strain rates and myocardial perfusion reserve during pharmacologic hyperemia.

Results  Significant correlations were observed between \( G_{\text{max}}/T_{\text{max}} \) with perfusion reserve and with mean diastolic strain rates (\( r^2 = 0.68 \) and \( r^2 = 0.58 \), \( P < .001 \) for both). Visual and quantitative MBG using a cutoff value of \( G_{\text{max}}/T_{\text{max}} = 2.7/\text{s} \) yielded significantly higher accuracy than stenosis severity on coronary angiograms for the detection of impaired microvascular integrity as a surrogate marker for CAV (AUC = 0.78, SE = 0.06, 95% CI = 0.66-0.87 for lumen narrowing versus AUC = 0.91, SE = 0.03, 95% CI = 0.84-0.97 for \( G_{\text{max}}/T_{\text{max}} \) \( P < .01 \)). Furthermore, quantitative MBG provided more robust prediction of survival (\( \chi^2 = 14.0, P < .001 \)), compared to visually estimated blush (\( \chi^2 = 5.4, P = .02 \)) and to coronary lumen narrowing assessment, (\( \chi^2 = 4.8, P = .04 \)).

Conclusions  Quantification of MBG can be performed on coronary angiograms of HTx-recipients, and may help with the identification of early CAV in patients with impaired perfusion reserve but without angiographically evident atherosclerosis. (Am Heart J 2010;159:643-651.e2.)

Despite advances in immunosuppressive drugs with improved treatment for rejection, long-term graft survival after heart transplantation (HTx) is still limited by the development of cardiac allograft vasculopathy (CAV).\(^{1,2,3}\) This particular type of atherosclerosis progressively compromises microvascular perfusion, ultimately causing congestive heart failure.\(^{2,3}\)

Currently, surveillance coronary angiography is used to detect CAV. However, assessment of lumen narrowing on angiograms provides little information on the microvascular integrity of the myocardium\(^{3}\) and may therefore yield low sensitivity for the detection of CAV.\(^{3}\) Intravascular ultrasound, on the other hand, has been proposed as a highly sensitive method to detect CAV.\(^{2,3}\) However, this technique is invasive potentially involving added risk for the patients, while it cannot evaluate intramural vessels and, thus, the presence of stenotic microvasculopathy.\(^{3}\)

Recently, we and others suggested that quantitative myocardial blush grade (MBG) provides an insight into pathophysiologic aberrations of the microvasculature in patients with acute ischemic syndromes.\(^{6,8}\)

In the present study, we sought to test the ability of quantitative MBG to detect impaired microvascular integrity in HTx-recipients. The obtained results were compared to diastolic strain rate and to myocardial perfusion reserve assessed by cardiac magnetic resonance (CMR), which is a surrogate marker of CAV.\(^{9}\)

Methods  Our study was supported by an institutional grant, provided by the Landesstiftung foundation Baden-Württemberg (H.D. and G.K.). The authors are solely responsible for the design and
conducted this study; all study analyses, the drafting and editing of the paper and its final contents.

Study population

We studied 78 consecutive HTX-recipients who underwent cardiac catheterization during a 21-month period. Six patients were excluded because MBG quantification was not feasible in at least one coronary territory due to technical reasons (diaphragmal movement, which confounded the alignment between diastolic frames). Thus, our final patient population consisted of 72 patients, including 45 men and 27 women with a mean age of 56 ± 11 years (range 23-76 years). Cardiac magnetic resonance was assessed in all patients within 4 weeks from cardiac catheterization. At the time of the studies, all HTX-recipients were in stable condition, and without clinical signs of heart failure or unstable angina. Cellular rejection (International Society for Heart Transplantation class >IA) was excluded by histological analysis of their myocardial biopsy at the time of the study. All patients were followed for at least 6 months and mortality data were obtained. No patients were lost during the follow-up period.

Eighteen healthy volunteers matched for HTx organ age underwent CMR, in order to acquire normal values for perfusion reserve and left ventricular function. All procedures complied with the Declaration of Helsinki, were approved by our local ethic committee and all patients gave written informed consent.

Cardiac catheterization and definition of patients groups

Coronary angiograms were performed in a standardized fashion, and at least two orthogonal views of every major coronary vessel and its side branches were obtained. All patients received 200 μg of intracoronary nitroglycerin before intravenous contrast injection, and vessels were classified using a semiquantitative grading system as follows: grade I, normal lumen; grade II, irregularities with diameter reduction <50%; grade III, irregularities with diameter reduction ≥50%; and grade IV, diameter reduction ≥50% and/or diffuse narrowing of small vessels. For analysis by patients, HTX-recipients were divided in 3 groups (A, B, C): group A included patients with normal vessels without focal stenoses or lumen irregularities (grade I); group B, patients with vessels irregularities and diameter reduction <50% (grade ≤ III); and group C, patients with severe CAV who had diameter reduction ≥50% (grade IV).

Visual assessment of myocardial blush grade.

For the assessment of myocardial blush, predefined projections were used. Myocardial blush grade was graded by 2 experienced investigators who were blinded to clinical and CMR data, on 25-Hz cine-loops, as follows: 0 = absent; 1 = strongly reduced; 2 = moderate myocardial blush but less than that obtained during angiography of a contra- or ipsilateral coronary vessel; and 3 = normal contrast density, comparable with the contra- or ipsilateral coronary artery.

Quantification of myocardial blush grade.

A computer-assisted procedure was used to quantify myocardial tissue level perfusion. Technical details with this procedure are described elsewhere. Briefly, the time course of MBG intensity rise was analyzed using electrocardiogram-gated angiograms. Regions of interest were placed in the distal perfusion territory of each coronary vessel to estimate the plateau of mean grey level pixel intensity (Gmax measured on a standard gray scale of 0-255) and the time to maximal intensity rise (Tmax measured in s). The ratio Gmax/Tmax was computed for each coronary territory. Furthermore, based on the temporal distribution of myocardial blush in epicardial vessels, arterioles and capillaries parametric quantification was applied according to the timing of maximal gray values. To allow for quantification of MBG, frames had to be long enough to allow filling of the venous coronary system, and images were acquired during breath hold to avoid potential artefacts due to diaphragmal shift.

Cardiac magnetic resonance examination

Heart transplantation recipients and healthy volunteers were examined in a clinical 1.5-T whole-body magnetic resonance (MR) scanner Achieva system (Philips Medical Systems, Best, The Netherlands). A standardized protocol was followed, aiming at (1) the assessment of left ventricular function using cine imaging, (2) the quantification of myocardial strain using strain-encoded magnetic resonance imaging (SENC), and (3) the assessment of the myocardial perfusion reserve.

Cine imaging.

A steady-state-free precession sequence was used to obtain the cine images of the 3 long-axis and 7 to 9 short-axis views, 8 mm in thickness. Typical parameters were: flip angle (FA) = 60°, repetition time/echo time (TR/TE) = 2.8/1.3 ms and acquired voxel size = 2.2 × 2.2 × 8 mm3. Planimetry of short-axis slices from the apex to the base was assessed using View Forum software (Philips Medical Systems, Best, The Netherlands) to determine end-systolic, end-diastolic volume (mL) and left ventricular (LV) ejection fraction (%).

Strain-encoded MRI.

The SENC pulse sequence is a modified spatial modulation of magnetization tagging pulse sequence. Using SENC color-coded functional images are generated, where the maximum of contraction is illustrated red, and lack of contraction is illustrated white. Three short-axis planes were acquired with 10-mm slice thickness. Typical parameters were: FA = 50°, TR/TE = 25/0.9 ms and acquired voxel size = 4.4 × 4.4 × 10 mm3. Quantification of myocardial strain and strain rate on SENC images was conducted using Diagnosoft SENC (Version 1.06, Diagnosoft Inc, Palo Alto, CA).

For each segment, the temporal course of regional myocardial strain was registered throughout the cardiac cycle to calculate (1) the peak systolic strain (S expressed in %) and (2) the mean diastolic strain rate over the duration from peak systole to 50% of the diastole (mean early diastolic SR, respectively expressed in 1/s).

Myocardial perfusion imaging.

For perfusion imaging, a 3-slice turbo field echo-echo-planar imaging sequence (FA = 50°, TR/TE = 2.8/1.3 ms and acquired voxel size = 2.5 × 2.5 × 8 mm3) was used. Stress was performed using a continuous intravenous infusion of 140-μg/kg per minute adenosine. After initiation of the sequence a bolus of 0.04 mmol/kg body weight gadolinium-DTPA (Magnevist, Bayer Schering, Germany) was injected at a rate of 5 mL/s. Quantification of myocardial perfusion was conducted in 3 short-axis slices using View Forum software. Manual contouring of endo- and epimyocardial borders was assessed and an automated algorithm was used to divide the myocardium into 6 equiangular segments per slice, and spatially averaged signal intensity (SI) values were used to plot SI curves over time. The mean SI before contrast agent
injection was subtracted from all post-contrast data, and the upslope of the resulting SI time curves was determined by using a linear fit, based on the least-squares regression line. By this approach, a myocardial perfusion reserve index was calculated by dividing the corrected upslope at pharmacologic hyperemia through that during rest, which served as a semi-quantitative estimate of the perfusion reserve in each segment.18 Normal values for myocardial perfusion reserve were assessed in healthy volunteers, and the presence of a perfusion reserve of <2 SD from the mean perfusion reserve was considered as a marker of impaired microvascular function and indicative for CAV.9

Assignment of myocardial strain and perfusion reserve to coronary vessels. Analysis of myocardial perfusion and strain was performed blinded to clinical data, and to the results from the invasive measurements in the three main coronary territories (left anterior descending, left circumflex, and right coronary artery. Segments were assigned to coronary vessels according to American Heart Association guidelines39 and strain, strain rate, and perfusion reserve values were calculated in each perfusion territory. For analysis by patients, the mean values of all 3 perfusion territories in each patient were considered.

Statistical analysis

Analysis was performed using commercially available software (SPSS, version 15.0 for Windows; SPSS, Chicago, IL) and data are presented as mean ± one SD. Gmax/Tmax was compared to perfusion reserve and to myocardial strain using linear regression analysis. Differences in perfusion reserve and diastolic function between volunteers and HTx recipients were assessed using one-way analysis of variance with Bonferroni’s correction for multiple comparisons. Intra- and interobserver variability for quantification analysis of strain, perfusion reserve and Gmax/Tmax were calculated by repeated analysis of 40 representative cases. Receiver operating characteristics were used to determine the diagnostic value of stenosis severity and MBG for the prediction of abnormal perfusion reserve as a surrogate marker for CAV in HTx recipients. The association of baseline and angiographic findings with mortality was investigated using Cox proportional hazards analysis of 40 representative cases. Receiver operating characteristic analysis for calculation of area under the curve (AUC), and its 95% CI was calculated using a linear fit, based on the least-squares regression line. By this approach, a myocardial perfusion reserve index was calculated by dividing the corrected upslope at pharmacologic hyperemia through that during rest, which served as a semi-quantitative estimate of the perfusion reserve in each segment.18 Normal values for myocardial perfusion reserve were assessed in healthy volunteers, and the presence of a perfusion reserve of <2 SD from the mean perfusion reserve was considered as a marker of impaired microvascular function and indicative for CAV.9

Results

Demographic parameters and relation of myocardial blush grade to CMR parameters

Data of HTx-recipients (n = 72) and of the healthy volunteers (n = 18) are illustrated in Table 1. Volunteers were younger than HTx recipients (P < .05), but the organ age was not significantly different (P = NS).

Significant correlations were observed between Gmax/Tmax and perfusion reserve both by coronary territory (Figure 1, A-C) and by patients (Figure 1, D). Further, correlations were observed between Gmax/Tmax and mean diastolic strain rates (Supplemental Figure 1, Appendix online), and with peak systolic strain (Supplemental Figure 2, Appendix online).

Results of invasive angiography and association with perfusion reserve and diastolic strain rate

Of 72 HTx-recipients, 36 showed normal coronary vessels (Group A), 22 had <50% stenosis in at least one coronary vessel (group B) and the remaining 14 had severe CAV with at least 1 ≥50% diameter lesion (group C). The distribution of MBG among the 3 groups is illustrated in Figure 2 analyzed by vessels (A) and by patients (B). Thus, reduced MBG of 0 or 1 was not only present in 14 patients with severe CAV (≥50% lesions), but also in 11 (19%) of 58 of patients with either <50% lesions (n = 6) or with entirely normal vessels (n = 5).

Myocardial perfusion reserve and mean diastolic strain rate were strongly reduced in group C with severe CAV compared to groups A and B and to healthy volunteers (Figure 3, A and C). Interestingly, further differentiation of Groups A and B depending on MBG, showed that those 11 patients with reduced MBG 0 or 1 and vessels with stenosis ≥50% (n = 6) or entirely normal vessels (n = 5) also had markedly diminished

### Table 1. Demographic, baseline MR parameters and hemodynamic data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy volunteers</th>
<th>HTx-recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of subjects</td>
<td>n = 18</td>
<td>n = 72</td>
</tr>
<tr>
<td>Age (y)</td>
<td>38 ± 9</td>
<td>56 ± 11†</td>
</tr>
<tr>
<td>Male sex</td>
<td>67% (12/18)</td>
<td>63% (45/72)</td>
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<tr>
<td>HTx organ age (y)</td>
<td>(N/A)</td>
<td>35 ± 13†</td>
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<tr>
<td>Yrs. after HTx</td>
<td>(N/A)</td>
<td>2.6 ± 3.4</td>
</tr>
<tr>
<td>Cold ischemia time (min)</td>
<td>(N/A)</td>
<td>160 ± 33</td>
</tr>
<tr>
<td><strong>Atherogenic risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>0% (0/18)</td>
<td>65% (47/72)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0% (0/18)</td>
<td>60% (43/72)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0% (0/18)</td>
<td>35% (25/72)</td>
</tr>
<tr>
<td>Smoker</td>
<td>0% (0/18)</td>
<td>4% (3/72)</td>
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<tr>
<td><strong>Baseline MR parameters</strong></td>
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<tr>
<td>Septal wall thickness</td>
<td>7.8 ± 1.8</td>
<td>11.3 ± 1.8*</td>
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<td>(basal segment, mm)</td>
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<tr>
<td>Lateral wall thickness</td>
<td>7.2 ± 1.6</td>
<td>7.9 ± 1.5</td>
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<tr>
<td>(basal segment, mm)</td>
<td></td>
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<tr>
<td>LV end-diastolic diameter (mm)</td>
<td>49 ± 4</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>LV end-systolic diameter (mm)</td>
<td>31 ± 4</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>LV end-diastolic volume (mL)</td>
<td>129 ± 25</td>
<td>125 ± 28</td>
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<tr>
<td>LV end-systolic volume (mL)</td>
<td>44 ± 14</td>
<td>43 ± 17</td>
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<tr>
<td>Baseline ejection fraction (%)</td>
<td>66 ± 6</td>
<td>66 ± 8</td>
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<tr>
<td><strong>Baseline hemodynamics</strong></td>
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<td></td>
</tr>
<tr>
<td>Heart rate (1/min)</td>
<td>67 ± 11</td>
<td>78 ± 11*</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>92 ± 6</td>
<td>92 ± 15</td>
</tr>
<tr>
<td>Double product (mm Hg/min)</td>
<td>8339 ± 1833</td>
<td>9865 ± 2213</td>
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<tr>
<td>Peak stress hemodynamics</td>
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<tr>
<td>Heart rate (1/min)</td>
<td>95 ± 9</td>
<td>91 ± 18</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>89 ± 12</td>
<td>89 ± 20</td>
</tr>
<tr>
<td>Double product (mm Hg/min)</td>
<td>11438 ± 3275</td>
<td>11373 ± 5745</td>
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</tbody>
</table>

Data presented as number of patients or as mean ± 1 SD; N/A indicates not applicable. * P < .05 for HTx-recipients versus control subjects. † P = NS for HTx-recipients organ age versus the age of control subjects.
perfusion reserve and mean diastolic strain rates, equaling group C. Conversely, those with MBG of 2 or 3 had significantly higher perfusion reserve and diastolic strain rates (Figure 3, B and D).

Prediction of impaired myocardial perfusion reserve by stenosis severity, visual and quantitative MBG

Visual and quantitative MBG using a cut-off value of $G_{\text{max}}/T_{\text{max}} = 2.7/s$ yielded significantly higher accuracy than stenosis severity on coronary angiograms for the detection of impaired microvascular integrity as a surrogate marker for CAV (Figure 4, A and B and Table II).

Figure 5 shows representative examples of 3 HTx-recipients, assigned to predefined groups (A, B or C). Hereby, besides conventional angiograms parametric images are provided (B, F and J), where epicardial vessels (yellow shades) and myocardial capillaries (blue shades) can be appreciated. In a patient with normal coronary vessels and MBG of 3 (strong blue shades in the parametric image), (Group A), perfusion reserve was high (Movie A, Figure 5, A-D). In another HTx-recipient, reduced MBG (weaker blue shades in the parametric image) was observed (Group B), which was associated with reduced perfusion reserve despite the presence of angiographically entirely normal coronary vessels (Movie B, Figure 5, E-H). Finally, in a patient with severe CAV
Survival analysis

At a mean follow-up of 1.6 ± 0.5 years, 10 patients (14%) died. Seven deaths were attributed to cardiac reasons, 2 were related to sepsis and 1 to cancer. Using a Cox proportional hazards model, quantitative MBG provided more robust prediction of survival ($\chi^2 = 14.0, P < .001$), compared to visually estimated blush ($\chi^2 = 5.4, P = .02$) and to the presence of epicardial coronary artery disease, ($\chi^2 = 4.8, P = .04$), (Table III). Interestingly, 2 of 3 patients with non-cardiac death had increased $G_{\text{max}}/T_{\text{max}} > 2.7$/s, while 6 of 7 patients with cardiac related death had reduced $G_{\text{max}}/T_{\text{max}} < 2.7$/s.

Observer agreement and time spent

The inter- and intra-observer agreements for the visual assessment of MBG were 82% and 89% respectively. Quantification of $G_{\text{max}}/T_{\text{max}}$ yielded intra- and interobserver variabilities of 10.8% and 13.7%, within a time-duration of 1.64 ± 0.52 minutes per vessel. Intra- and interobserver variabilities of 8.9% and 12.7% for perfusion reserve, 7.4% and 11.3% for peak systolic strain and 8.7% and 12.1% for mean diastolic strain rate were calculated.

Discussion

The results of our study indicate that (1) Quantification of MBG can be performed on coronary angiograms of HTx-recipients and is related to myocardial perfusion reserve and to regional and global diastolic function, and (2) Myocardial blush is reduced in HTx-recipients, not only in territories with $\geq 50\%$ lesions (group C), but also in $\sim 20\%$ of patients with $<50\%$ lesions or with entirely normal angiograms (groups A and B). These patients exhibit microvascular in the absence of significant epicardial disease, as implicated by impaired microvascular reserve and reduced diastolic function by CMR. Thus, MBG is possibly an early marker of CAV, which is indicative of microvasculopathy prior to the development of epicardial disease.

Pathophysiology of CAV and previous studies

Early CAV is clinically silent, and ischemia is usually not evident until the disease is far advanced.\textsuperscript{20} Experimental data suggested stiffness of the transplanted heart due to several $\geq 50\%$ lesions) (group C), MBG and perfusion reserve were reduced (Movie C, Figure 5, I-I).
cardiomyocyte hypertrophy and endocardial fibrosis as a principal mechanism for the development of CAV. Additional immunologic mechanisms operating in a milieu of non-immunologic risk factors cause vascular injury resulting to impaired microvascular integrity due to stenotic microvasculopathy. All these mechanisms may account for microvascular dysfunction, in the absence of critical epicardial lesions, so that the evaluation of lumen narrowing on coronary angiograms may miss early stages of the disease. In this regard, previous studies investigated the impact of TIMI frame counts and visually assessed MBG on clinical outcomes, elegantly demonstrating that abnormal microvascular function is present even in transplant recipients with normal coronary vessels and that both indexes are associated with mortality. Further recent data from histopathologic studies support the presence of microvasculopathy in the absence of epicardial disease, and its impact on mortality. All these data are in agreement with the results of our study, where in addition to stenosis severity and to visually assessed MBG, a computer assisted procedure was used to assess transplant microvasculopathy.

Technical aspects and results of the present study

From a technical point of view, CMR is a versatile noninvasive clinical tool for the quantification of myocardial perfusion and regional function, which was shown to detect impaired myocardial perfusion reserve in HTx-recipients with high accuracy. Nevertheless, although the versatility of CMR allows for the evaluation of myocardial function and perfusion with high reproducibility, this technique is associated with high costs and limited availability. Therefore, a simple angiographic parameter that could be used as a surrogate marker of microvascular integrity may be preferable. In our study, we demonstrated for the first time in the current literature that quantification of MBG is feasible with coronary angiograms, and offers an insight into alterations of microvascular function in HTx-recipients. Thus, $G_{\text{max}}/T_{\text{max}}$ was associated with myocardial perfusion and diastolic strain. Furthermore, this same parameter was an independent predictor of outcome. In agreement with previous studies, we found that impaired microvascular and diastolic function is not only present in vessels with $\geq50\%$ lesions (group C), but also in vessels which appear angiographically completely normal or show $<50\%$ stenosis, in the presence of low MBG (Group A and B). Conversely, vessels with high MBG of 2 or 3 yielded perfusion reserves and diastolic strain values, close to those measured in healthy volunteers. In addition, MBG allowed for detection of impaired myocardial perfusion reserve by CMR, as a surrogate marker for CAV. Hereby, it should be noted that the cutoff value selected in the present study ($G_{\text{max}}/T_{\text{max}} = 2.7/s$) closely resembles that selected in previous studies ($G_{\text{max}}/T_{\text{max}} = 3.1$) to identify territories with impaired microvascular integrity and to predict mortality in patients with acute ischemic syndromes.

Limitations

Intravascular ultrasound was not used to evaluate intimal thickness in angiographically “normal” coronary with reduced MBG. However, noninvasive measurements were conducted in all patients for the evaluation of microvascular integrity, which was previously shown to be associated with early CAV in HTx-recipients. Furthermore, the computerized analysis of myocardial blush grade used in our study is not commercially available and is therefore not yet implemented in the routine clinical work. This currently limits the applicability of blush quantification in the clinical realm. In addition, TIMI frame counts were not systematically measured, which is a limitation. However, TIMI frame count represents a measure of epicardial blood flow and may therefore be less sensitive for the detection of impaired microvascular

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**Figure 4**

![Prediction of CAV by angiography and visual MBG](image)

**A**

Visual (A) and quantitative MBG (B) using a cut-off value of $G_{\text{max}}/T_{\text{max}} = 2.7/s$ yielded significantly higher accuracy than stenosis severity on coronary angiograms for the detection of impaired microvascular integrity as a surrogate marker for CAV.

![Prediction of CAV by $G_{\text{max}}/T_{\text{max}}$](image)

**B**

$G_{\text{max}}/T_{\text{max}} = 2.7/s$ yielded significantly higher accuracy than stenosis severity on coronary angiograms for the detection of impaired microvascular integrity as a surrogate marker for CAV.
integrity, compared to myocardial blush. Humoral rejection on the other hand, an antibody induced and complement-mediated activation of endothelial cells and macrophages, may also have a significant impact both on myocardial perfusion and on outcomes. Improved methods to detect such pathology are crucial, since conventional immunofluorescence techniques may be negative in such patients, while the number of humoral rejections is expected to rise in the era of ventricular assist device implants and multiply transfused recipients. In our study, the presence of antibody-mediated rejection was not systematically evaluated, which is a limitation, particularly in light of its potential impact on microvascular function. Future studies, should account for alterations of myocardial perfusion.

Table II. Detection of impaired myocardial perfusion reserve as a surrogate marker for CAV

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cutoffs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC</th>
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<tbody>
<tr>
<td>Coronary angiography</td>
<td>Stenosis ≥50%</td>
<td>67%</td>
<td>73%</td>
<td>78%</td>
<td>61%</td>
<td>0.78</td>
</tr>
<tr>
<td>Visual MBG</td>
<td>MBG ≤1</td>
<td>98%</td>
<td>80%</td>
<td>87%</td>
<td>96%</td>
<td>0.91*</td>
</tr>
<tr>
<td>Gmax/Tmax</td>
<td>2.7/s</td>
<td>91%</td>
<td>90%</td>
<td>93%</td>
<td>87%</td>
<td>0.93*</td>
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Data are presented as percentages. PPV, positive predictive value; NPV, negative predictive value.
* P < .01 for coronary angiography versus MBG.

Table III. Variables for the prediction of mortality in HTx recipients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hazard ratios</th>
<th>95% CI</th>
<th>Total χ²</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>1.1</td>
<td>1.0-1.1</td>
<td>5.6</td>
<td>.02</td>
</tr>
<tr>
<td>Male gender</td>
<td>3.9</td>
<td>0.8-19.0</td>
<td>3.2</td>
<td>NS</td>
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<tr>
<td>Years elapsed after HTx</td>
<td>1.2</td>
<td>1.1-1.4</td>
<td>13.7</td>
<td>.001</td>
</tr>
<tr>
<td>Baseline ejection fraction (%)</td>
<td>0.99</td>
<td>0.9-1.1</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Stenosis severity by angiography (≥50% stenosis in at least 1 vessel)</td>
<td>4.2</td>
<td>1.0-16.8</td>
<td>4.8</td>
<td>.04</td>
</tr>
<tr>
<td>Visual MBG (0/1 versus 2/3)</td>
<td>5.4</td>
<td>1.3-21.3</td>
<td>5.4</td>
<td>.02</td>
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<tr>
<td>Gmax/Tmax (&lt;2.7/s)</td>
<td>10.5</td>
<td>3.6-58.7</td>
<td>14.0</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note: Data are presented as percentages. PPV, positive predictive value; NPV, negative predictive value.

Figure 5

Representative images of (A) a patient with normal coronary vessels (A), MBG (B-C) and myocardial perfusion (D). B) a patient normal coronary vessels (E) but reduced MBG (F-G) and perfusion (H), and C) a patient with manifest CAV (I) and concordantly reduced MBG (J-K) and perfusion reserve (L).
by this specific type of rejection. Simultaneously the ability of myocardial blush grade to detect perfusion abnormalities caused by humoral rejection remains to be elucidated by systematically considering C4d deposition and the presence of CD68 positive macrophages in allograft biopsies. Finally, follow-up CMR and blush data were not available in patients with normal coronary arteries and reduced MBG. Thus, the potential subsequent development of CAV in normal vessels with reduced MBG and its impact on clinical outcome merits further investigation in future longitudinal trials.

Conclusion

Quantification of myocardial blush grade is feasible in HTx-recipients and is related to myocardial perfusion reserve and to markers of diastolic function, assessed by CMR. Reduced MBG is present in ∼20% patients who have normal vessels by angiography, but simultaneously exhibit impaired microvascular and diastolic myocardial function, and may therefore be an early marker of angiographically silent CAV. Because MBG is easy to acquire and quick to quantify, the translation of these findings to the clinical realm appears promising, particularly in light of the absence of widely available and practical techniques, which can be used as reliable surrogate markers for the identification of early stenotic microvasculopathy.

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Disclosures

No conflicts of interest to declare for any authors.

References


Moderate correlations were observed between $G_{\text{max}}/T_{\text{max}}$ and early diastolic strain rate by coronary territories (A-C) and by patients (D).
Relatively weak, albeit significant correlations were observed between $G_{\text{max}}/T_{\text{max}}$ and peak systolic strain by coronary territories (A-C) and by patients (D).