The relation between hypomagnesaemia and vascular stiffness in renal transplant recipients

Steven Van Laecke1, Céline Maréchal2, Francis Verbeke1, Patrick Peeters1, Wim Van Biesen1, Olivier Devuyst2, Michel Jadoul2 and Raymond Vanholder1

1Renal Division, Department of Internal Medicine, Ghent University Hospital, Ghent, Belgium and 2Service de Pédiatrie Cliniques Universitaires St-Luc, Brussels, Belgium

Correspondence and offprint requests to: Steven Van Laecke; E-mail: steven.vanlaecke@ugent.be

Abstract

Background. Arterial stiffness is a strong predictor of outcome. Hypomagnesaemia, by its association with arterial hypertension, endothelial dysfunction, dyslipidaemia and inflammation, might affect vascular stiffness. As hypomagnesaemia is common in renal transplant recipients (RTR), we examined its potential association with arterial stiffness.

Methods. Cross-sectional analysis. Evaluation of vascular stiffness in 512 RTR from two university centres at a median of 72 months post-transplantation. Determination of carotid–femoral pulse wave velocity (PWV) (SphygmoCor). A multiple linear regression analysis was used to investigate the independent relationship between magnesium serum level and PWV with the following covariates: age, diabetes, smoking status, body mass index, blood pressure, heart rate (HR), C-reactive protein (CRP), high-density lipoprotein cholesterol, parathyroid hormone and use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, diuretics, calcium channel blockers, statins and calcineurin inhibitors next to their drug levels.

Results. Lower serum magnesium was independently associated with PWV (P = 0.018) in addition to age, CRP, HR, diabetes and mean arterial pressure (model R² = 0.45; P < 0.001). The relationship between magnesium and PWV was attenuated (P = 0.054) after adjustment for the use of sirolimus, which was associated with higher magnesium levels (P<0.001) and lower PWV (P = 0.013). In patients >55 years (median age), however (low), magnesium remained an independent predictor of PWV (P = 0.024) after accounting for the same covariates.

Conclusions. Serum magnesium is an independent predictor of arterial stiffness in RTR, especially in patients >55 years.

Keywords: hypomagnesaemia; pulse wave velocity; sirolimus

Introduction

Hypomagnesaemia plays a role in the pathogenesis of arterial hypertension, endothelial dysfunction, dyslipidaemia and inflammation [1]. Each of these factors has been associated with vascular stiffness [2–4]. Therefore, hypomagnesaemia may directly or indirectly affect vascular stiffness [5].

As hypomagnesaemia is common in renal transplant recipients (RTR), it seems relevant to compare vascular stiffness between hypomagnesaemic and normomagnesaemic RTR. We recently identified post-transplantation hypomagnesaemia as an independent predictor of new-onset diabetes after transplantation (NODAT) [6] which suggests a relationship with insulin resistance in analogy with findings in other study populations, such as obese children [7]. Insulin resistance in its turn is a major determinant of arterial stiffness, e.g. in obese children and middle-aged adults [8,9].

Pulse wave velocity (PWV), a direct non-invasive and reproducible measure of arterial stiffness, is a surrogate marker for cardiovascular outcome [10,11], a finding that has also been validated in the RTR population [12,13]. However, few studies have addressed this determinant of vascular stiffness in large cohorts of stable RTR. Despite improvement of indices of arterial function post-transplantation [14,15], PWV is still higher in RTR than in healthy controls [16].

The main objective of this study was to assess the possible relationship between serum magnesium (Mg) levels and PWV in RTR while accounting for the potential effect of age, blood pressure, diabetes or inflammatory characteristics.

Materials and methods

Patients

Patients eligible for the study were recruited from the outpatient clinics of two tertiary university hospitals. Recruitment took place between February 2004 and December 2006. All patients transplanted for at least 3 months and in a stable clinical condition were eligible for inclusion. Exclusion criteria were combined organ transplants and age <18 years. Diabetes was defined as antidiabetic drug treatment at the time of assessment. The study was approved by the Local Ethical Committees and written informed consent was obtained from each participant.

Protocol for assessment of central PWV

Studies were conducted after at least 10 min in the supine position. Blood pressure was recorded using a validated oscillometric device (Omron MI-
Laboratory measurements
Serum calcium (Ca), phosphorus (P), haemoglobin, Mg, total cholesterol, high-density lipoprotein (HDL) cholesterol and parathyroid hormone (PTH) were determined using standard methodology on samples obtained in the immediate period preceding the assessment of PWV. High-sensitivity C-reactive protein (CRP) was determined turbidimetrically and serum creatinine with a rate-blanked compensated Jaffe method, both on a Roche/Hitachi Modular P analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Mg was assessed using a xylidyl blue method (within-run CV: 0.7 and between-run CV: 1.1%). Drug levels were measured by a microparticle enzyme immunoassay measurement (AxSym and IMx; Abbott diagnostics) for respectively cyclosporine and tacrolimus levels at the time of assessment of PWV.

Statistical analysis
Descriptive statistics were expressed as means ± SDs and median (interquartile range) for normally and non-normally distributed variables, respectively. Deviation from normality was assessed by the Kolmogorov-Smirnov test. CRP was log-transformed because of its skewed distribution. The two-tailed Student's t-test or the Mann-Whitney U-test were used for comparisons between groups, depending on the distribution. Differences in frequency of categorical variables were assessed by the chi-square test or Fisher's exact test as appropriate.

Multiple linear regression analysis was used to evaluate which factors were independently associated with PWV using both backward elimination and forward selection of variables. Associations were first analysed without adjustments and then with adjustments for potential confounders. Predictor variables were selected based upon the correlation in the univariate analysis (P<0.1) or in the previous studies and included the following relevant demographic, anthropometric, haemodynamic and biochemical variables: age, gender, length, weight, body mass index (BMI), race, smoking status, diabetes, creatinine, glomerular filtration rate (GFR) according to the Modification of Diet in Renal Disease (MDRD) formula, time since transplantation, time spent on dialysis, haemoglobin, serum levels of Ca, P, Mg, total cholesterol, HDL cholesterol, MAP and heart rate (HR). Additionally, treatment with angiotensin antagonists, diuretics, calcium channel blockers, statins, antiplatelet agents, vitamin D substitution, Ca supplements, corticosteroids and calcineurin inhibitors (CNI), sirolimus were also included in the regression analysis to assess their potential confounding effect. Interactions known from the literature were also taken into account. Interactions known from the literature were also included in the regression analysis to assess their potential confounding effect. Interactions known from the literature were also included in the regression analysis to assess their potential confounding effect.

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previous time spent on dialysis, time since transplantation, haemoglobin, Ca, P, creatinine, GFR and use of beta-blockers, vitamin D, Ca supplements, corticosteroids or acetylsalicylic acid.

Univariate comparison of patients on sirolimus versus no sirolimus

PWV was lower in the sirolimus group (P = 0.013) and there was no difference in PWV between patients on cyclosporine versus tacrolimus. Patients on sirolimus at the time of clinical assessment had higher Mg levels (P < 0.001) and lower Ca levels, were more anaemic, and had higher cholesterol levels and lower diastolic blood pressure. They had been on dialysis for a longer time but were assessed earlier post-transplantation. Patients on sirolimus instead of the sirolimus group also received more Ca supplements (P = 0.04), higher doses of corticosteroids (P = 0.011) and more mycophenolate mofetil (MMF) (P = 0.009).

There was a trend towards a higher creatinine, a higher proportion of males and a lower BMI in the sirolimus group.

There was no difference in the use of statins nor in HDL cholesterol, InCRP, PTH, P, age, MDRD; intake of angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB), diuretics; intake of acetylsalicylic acid, smoking, MAP, HR and the prevalence of diabetes between both groups.

Univariate comparison of patients according to age (≤ Versus > 55 years) and quartiles of Mg levels

Patients ≤55 years (the median of the study population) with higher Mg levels had been transplanted earlier, had remained a shorter time on dialysis, had higher PTH and cholesterol levels, lower InCRP levels, took more vitamin D and Ca supplements, were less frequently taking CNI and were more frequently on sirolimus (Table 3). There was a trend towards higher tacrolimus drug levels in association with lower Mg levels (P = 0.068). No relationship between Mg levels and cyclosporine drug levels was observed.

Patients >55 years had decreasing PWV with increasing Mg levels (P = 0.008) (Figure 1). They were also less frequently on CNI with increasing Mg levels and had a lower blood pressure in the highest Mg quartile. There was no relationship between CNI drug levels and Mg levels.

In both age categories, there were no differences in use of corticosteroids, azathioprine or MMF according to Mg quartiles nor in the prevalence of diabetes apart from a trend towards more diabetes in the lowest Mg quartiles in the younger age group (P = 0.125).

Comparison of Mg levels according to diabetes, blood pressure and PWV in patients aged >55 Years

Mg levels were lower in the higher PWV group (more than the median of 8.06 m/s) irrespective of the presence or absence of higher MAP (more than the median of 98.1 mmHg). This was significant in non-diabetics (P = 0.015) and a trend for diabetics was observed (P = 0.052). The lowest Mg levels were measured in the diabetic subpopulation with both higher blood pressure and PWV (Figure 2).

Multivariate analysis of PWV as dependent variable

The independent predictors retained in the final regression model were Mg, age, diabetes, InCRP, HR and MAP. In contrast, HDL cholesterol, use of statins, BMI, use of diuretics, ACE inhibitors/ARBs, past and present smoking, PTH and use of vitamin D were eliminated. Backward elimination and forward selection procedures generated the same final model (Table 4, Model 1).

However, after adjustment for the use of sirolimus, the association with Mg was attenuated (P = 0.054), while the use of sirolimus (B of −0.592 and beta of −0.075 with a P-value of 0.035) itself was an independent predictor of PWV (Table 4, Model 2).
Stratification according to median age (55 years) revealed that the prognostic impact of low Mg levels was mainly driven by the older patient population and that the relation of Mg with PWV remained significant (P = 0.024) in this subgroup after adjustment for the same covariates as in the overall model (Table 4, Model 3).

A subanalysis of patients uniformly on CNI (n = 421) showed Mg to be an independent predictor of PWV in

Table 3. Patient characteristics of patients with age ≤ 55 years or >55 years according to quartiles of Mg

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1.2–1.77 mg/dL)</td>
<td>2 (1.78–1.91 mg/dL)</td>
<td>3 (1.92–2.04 mg/dL)</td>
<td>4 (2.04–2.72 mg/dL)</td>
<td></td>
</tr>
<tr>
<td>Age ≤ 55 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>7.56 ± 1.34</td>
<td>7.44 ± 1.52</td>
<td>7.31 ± 1.55</td>
<td>7.31 ± 1.51</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.57 ± 0.77</td>
<td>1.58 ± 0.50</td>
<td>1.54 ± 0.62</td>
<td>1.81 ± 0.95</td>
</tr>
<tr>
<td>Time since transplantation (months)</td>
<td>78.8 ± 60.8</td>
<td>81.0 ± 67.1</td>
<td>101.3 ± 73.1</td>
<td>114.2 ± 98.2</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
<td>30.2 ± 22.9</td>
<td>25.5 ± 28.5</td>
<td>39.2 ± 38.1</td>
<td>25.0 ± 18.0</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>60.0 ± 46.2</td>
<td>62.9 ± 69.7</td>
<td>65.0 ± 58.7</td>
<td>96.0 ± 126.2</td>
</tr>
<tr>
<td>lnCRP (mg/dL)</td>
<td>−1.32 ± 1.36</td>
<td>−1.84 ± 1.33</td>
<td>−2.09 ± 1.39</td>
<td>−1.66 ± 1.08</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.1 ± 8.8</td>
<td>40.7 ± 8.6</td>
<td>46.3 ± 8.5</td>
<td>43.4 ± 9.2</td>
</tr>
<tr>
<td>Intake of vitamin D</td>
<td>38.7%</td>
<td>27.6%</td>
<td>42.9%</td>
<td>51.7%</td>
</tr>
<tr>
<td>Intake of calcium supplements</td>
<td>45.3%</td>
<td>44.7%</td>
<td>42.9%</td>
<td>65.0%</td>
</tr>
<tr>
<td>Intake of cyclosporine</td>
<td>33.3%</td>
<td>50.0%</td>
<td>69.6%</td>
<td>36.7%</td>
</tr>
<tr>
<td>Intake of sirolimus</td>
<td>57.3%</td>
<td>46.1%</td>
<td>18.2%</td>
<td>11.7%</td>
</tr>
<tr>
<td>Intake of sirolimus</td>
<td>9.6%</td>
<td>2.6%</td>
<td>8.9%</td>
<td>28.3%</td>
</tr>
<tr>
<td>Age &gt; 55 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWV (m/s)</td>
<td>11.23 ± 3.25</td>
<td>9.8 ± 2.67</td>
<td>9.85 ± 2.52</td>
<td>9.63 ± 2.59</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.35 ± 0.60</td>
<td>1.30 ± 0.37</td>
<td>1.47 ± 0.60</td>
<td>1.57 ± 0.83</td>
</tr>
<tr>
<td>CMAP (mmHg)</td>
<td>101.5 ± 12.8</td>
<td>100.2 ± 10.8</td>
<td>103.8 ± 16.2</td>
<td>93.7 ± 11.9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.8 ± 5.1</td>
<td>64.5 ± 6.1</td>
<td>62.8 ± 5.8</td>
<td>65.3 ± 6.3</td>
</tr>
<tr>
<td>Intake of cyclosporine</td>
<td>26.4%</td>
<td>67.3%</td>
<td>50.0%</td>
<td>46.3%</td>
</tr>
<tr>
<td>Intake of tacrolimus</td>
<td>69.8%</td>
<td>19.6%</td>
<td>34.7%</td>
<td>17.9%</td>
</tr>
<tr>
<td>Intake of sirolimus</td>
<td>6.0%</td>
<td>15.4%</td>
<td>9.7%</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

To test for differences between patients with low versus high Mg levels according to the quartiles. Chi-square tests were applied for qualitative variables and analysis of variance for quantitative variables. Only those parameters for which a difference between patients with low versus high Mg levels with P < 0.05 are mentioned except for creatinine and PWV.

Fig. 1. Decreasing pulse wave with increasing magnesium (Mg) levels according to quartiles of Mg. This was statistically significant only in the population aged >55 years (n = 256) (dark grey) in both the second and third versus the first Mg quartile (with the lowest Mg levels) (*P < 0.05) and the fourth versus the first quartile (**P = 0.003). Analysis of variance was applied. Error bars represent 95% confidence interval.

Fig. 2. Magnesium serum levels were lower in the higher PWV group (more than the median of 8.06 m/s) irrespective of higher blood pressure (more than the median mean arterial pressure of 98.1 mmHg). This was significant in non-diabetics (*P = 0.019) and a trend for diabetics was observed (P = 0.052). The lowest Mg levels (1.84 ± 0.23 mg/dL) were measured in the diabetic subpopulation with both higher blood pressure and PWV. PWV, pulse wave velocity; DM, diabetes mellitus.
the older study population (P = 0.018) (Table 4, Model 4). Adjustment for CNI trough levels did not attenuate this relationship.

Discussion

The most important finding of our study is that a low Mg level is a predictor of PWV and thus of vascular stiffness independent of clinically relevant covariates and especially in older RTR.

Aortic stiffness has independent predictive value for total and cardiovascular mortality in hypertensive patients [17]. It has previously been suggested that magnesium deficiency plays a pathophysiological role in aortic elasticity in rats [18,19] and in the development of high PWV in hypertensive patients [20]. We thus performed an analysis in a large cohort of almost uniformly hypertensive RTR (Table 1) to assess the potential association between hypomagnesaemia and increased PWV. The choice for RTR as study subjects was inspired by the frequent occurrence of hypomagnesaemia in this population [6,21].

It has been shown in different models that Mg deficiency is implicated in altered vascular reactivity, tone, inflammation and structural remodelling, often together with higher intracellular Ca levels, and deregulation of Na/Mg exchangers and/or transcellular Mg transporters, such as transient receptor potential melastatin 7 (TRPM7) [1]. Vasodilatory properties of Mg have been recognized clinically already almost a century ago and may partially relate to its effects on the levels of vasoactive agents, such as endothelin-1 and angiotensin II [22]. Magnesium has been associated with endothelial function both in vitro [23,24] and in vivo [25,26]. Schechter et al. [27] showed in patients with coronary heart disease a relationship between Mg levels and endothelial function assessed by endothelium-dependent brachial artery flow-mediated vasodilatation that could be improved by Mg supplementation. Both endothelial dysfunction and oxidative stress, which is also associated with hypomagnesaemia [28], are pivotal processes in vascular remodelling [29] and consecutively atherosclerosis and vascular stiffening. Of note, a long-term moderate magnesium-deficient diet has recently been shown to enhance the age-related structural changes of the aortic wall (media thickness, increased collagen content and reduction in the elastin/collagen ratio) in older rats [18], which might be in line with our findings of an association between high PWV and hypomagnesaemia in older RTR.

### Table 4. Multivariate regression models with PWV as dependent variable

<table>
<thead>
<tr>
<th>Model</th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>P-value</th>
<th>CI for B</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Age</td>
<td>0.104</td>
<td>0.007</td>
<td>0.530</td>
<td>&lt;0.001</td>
<td>0.091–0.118</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>−0.904</td>
<td>0.381</td>
<td>−0.802</td>
<td>0.021</td>
<td>−1.653 to −0.155</td>
</tr>
<tr>
<td></td>
<td>lnCRP</td>
<td>0.198</td>
<td>0.031</td>
<td>0.103</td>
<td>0.005</td>
<td>0.062–0.335</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.052</td>
<td>0.007</td>
<td>0.274</td>
<td>&lt;0.001</td>
<td>0.039–0.065</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>0.019</td>
<td>0.008</td>
<td>0.083</td>
<td>0.022</td>
<td>0.003–0.035</td>
</tr>
<tr>
<td></td>
<td>Diabetes (yes/no)</td>
<td>0.560</td>
<td>0.250</td>
<td>0.081</td>
<td>0.025</td>
<td>0.070–1.050</td>
</tr>
<tr>
<td>Model 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Age</td>
<td>0.104</td>
<td>0.007</td>
<td>0.526</td>
<td>&lt;0.001</td>
<td>0.090–0.117</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>−0.751</td>
<td>0.389</td>
<td>−0.608</td>
<td>0.054</td>
<td>−1.515 to 0.013</td>
</tr>
<tr>
<td></td>
<td>lnCRP</td>
<td>0.204</td>
<td>0.070</td>
<td>0.105</td>
<td>0.004</td>
<td>0.066–0.341</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.052</td>
<td>0.007</td>
<td>0.274</td>
<td>&lt;0.001</td>
<td>0.039–0.065</td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>0.019</td>
<td>0.008</td>
<td>0.086</td>
<td>0.018</td>
<td>0.003–0.035</td>
</tr>
<tr>
<td></td>
<td>Diabetes (yes/no)</td>
<td>0.589</td>
<td>0.251</td>
<td>0.085</td>
<td>0.020</td>
<td>0.094–1.083</td>
</tr>
<tr>
<td></td>
<td>Sirolimus (yes/no)</td>
<td>−0.592</td>
<td>0.280</td>
<td>−0.075</td>
<td>0.035</td>
<td>−1.143 to −0.041</td>
</tr>
<tr>
<td>Model 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Age</td>
<td>0.142</td>
<td>0.027</td>
<td>0.312</td>
<td>&lt;0.001</td>
<td>0.088–0.195</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>−1.680</td>
<td>0.736</td>
<td>−0.138</td>
<td>0.024</td>
<td>−3.132 to −0.228</td>
</tr>
<tr>
<td></td>
<td>lnCRP</td>
<td>0.258</td>
<td>0.128</td>
<td>0.120</td>
<td>0.045</td>
<td>0.006–0.510</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.071</td>
<td>0.012</td>
<td>0.355</td>
<td>&lt;0.001</td>
<td>0.047–0.095</td>
</tr>
<tr>
<td></td>
<td>Diabetes (yes/no)</td>
<td>1.042</td>
<td>0.372</td>
<td>0.168</td>
<td>0.006</td>
<td>0.308–1.775</td>
</tr>
<tr>
<td></td>
<td>Sirolimus (yes/no)</td>
<td>−1.373</td>
<td>0.525</td>
<td>−0.158</td>
<td>0.009</td>
<td>−2.344 to −0.278</td>
</tr>
<tr>
<td>Model 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Age</td>
<td>0.140</td>
<td>0.031</td>
<td>0.299</td>
<td>&lt;0.001</td>
<td>0.079–0.201</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>−2.043</td>
<td>0.855</td>
<td>−0.156</td>
<td>0.018</td>
<td>−3.731 to −0.356</td>
</tr>
<tr>
<td></td>
<td>Diabetes (yes/no)</td>
<td>1.264</td>
<td>0.417</td>
<td>0.198</td>
<td>0.003</td>
<td>0.441–2.086</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>0.074</td>
<td>0.013</td>
<td>0.363</td>
<td>&lt;0.001</td>
<td>0.047–0.100</td>
</tr>
</tbody>
</table>

<sup>a</sup> CI, confidence interval; SE, standard error. Only those parameters with a P-value of <0.05 are mentioned in the model except Mg in Model 2. P<0.05 for statistical significance.

<sup>b</sup> Variables included in the model are age, Mg, lnCRP, MAP, HR, diabetes, PTH, vitamin D use, BMI, Ca channel blockers, ACE inhibitors/ARB, diuretics, statins, HDL cholesterol and smoking. Only the significant predictors are shown after both backward and forward modelling.

<sup>c</sup> Variables included in Model 1 and use of sirolimus.

<sup>d</sup> Variables included in Model 2 in patients >55 years (median of the study population).

<sup>e</sup> Variables included in the Model 1 in patients >55 years with CNI in their drug regimen (n = 421).
In our model, diabetes mellitus (covering both NODAT and pre-existing diabetes) was an independent predictor for PWV (Table 4, Models 1–4). Disturbances of glucose metabolism including type 2 diabetes mellitus were previously associated with increased arterial stiffness in a large population-based cohort [30]. In recent analyses in RTR, patients with NODAT also had a higher PWV versus non-NODAT patients [31,32].

We recently demonstrated that hypomagnesaemia is an independent predictor for NODAT [6]. Hypomagnesaemia might consequently be a common denominator linking NODAT, inflammation and vascular stiffness. Previous data anyhow have shown a relationship between inflammation (CRP) and vascular stiffness both in the general population [4] and in the RTR [16] that parallels the findings of our multivariate model (Table 4, Models 1–3). Also, magnesium depletion has been related to inflammation both prospectively in rodent models [33] and cross-sectionally in healthy humans [34]. The other way around, Mg supplementation in aldosterone-infused mice has been demonstrated to attenuate the pro-inflammatory and pro-fibrotic effects of aldosterone [35] confirming the established relationship between Mg and inflammation. Importantly, however, adjustment for both diabetes and inflammation (CRP) in our model did not significantly alter the relationship between PWV and Mg.

PWV was lower in patients on a sirolimus-based immunosuppressive regimen. It could be hypothesized that the observed differences in PWV in drug regimens containing CNI versus mammalian target of rapamycin (mTOR) inhibitors might partially relate to differences in Mg levels, which are substantially higher in patients on mTOR inhibitors (Table 4). The cross-sectional design of the study with the potential of hidden confounders and selection bias together with the fairly small cohort of patients on sirolimus, however, impede to draw preliminary conclusions from these findings. Moreover, the relationship persisted after multivariate adjustment for many covariates including Mg (Table 4, Model 2). A subanalysis of patients on CNI >55 years demonstrated that Mg remained an independent predictor of PWV (Table 4, Model 4). Adjustment for drug levels did not alter the relationship between Mg and PWV.

It has been suggested [36] that CNI induce changes in arterial compliance, increasing vascular risk with potential mechanisms of excess endothelin production, elastin loss and vascular collagen accumulation [37,38]. However, the information on possible differences in vascular stiffness between patients on CNI and mTOR inhibitors is scanty. It has been shown that everolimus inhibits the development of transplant vasculopathy and the increase in intimal thickness of heart transplant recipients [39]. In a recent prospective randomized controlled trial in RTR, PWV remained stable after switching from cyclosporine to everolimus, while it continued to rise in the group that remained on cyclosporine [40]. It can thus be speculated that not only differences in blood pressure but also a different impact on Mg levels might play a role in these findings. Unfortunately, data on Mg levels in the aforementioned studies to test this hypothesis are lacking.

The subanalysis of our patient cohort according to recipient age indicated that Mg as a determinant of vascular stiffness was mostly confined to the older recipient category (>55 years) (Tables 2–4; Models 3 and 4). These findings are reminiscent of data demonstrating that Mg administration exerted the most beneficial impact on the arterial stiffness of pressurized mesenteric arteries from older rats [41]. The 50- to 55-year age window was previously shown to be the threshold where pulse pressure begins to increase steeply as a consequence of increased aortic stiffness [42]. The Mg levels in patients aged >55 years were lower in the patients with higher PWV irrespective of blood pressure and diabetes at the time of assessment (Figure 2). The lowest Mg levels were, however, observed in the diabetic subpopulation with the highest blood pressure and PWV. Hypomagnesaemia as such could have prognostic impact in RTR. Low Mg levels in RTR were previously associated with glucose metabolism disorders [6] and hypertension [43].

A strength of the present study is the large number of observations, allowing the adjustment for a high number of important covariates and/or confounders. Also, hypomagnesaemia is highly prevalent in RTR [6,21] and as such its potential associations might be of great clinical importance.

Vascular calcification was not evaluated in our study as it was a post hoc analysis of a previously collected database. Considering data where lower Mg levels were associated with both increased intima media thickness [44] and cardiac valve calcifications in haemodialysis (HD) patients [45] together with an accelerated detrimental evolution of peripheral arterial calcifications in peritoneal dialysis (PD) patients [46], a relationship in RTR between Mg levels and artery calcification certainly merits further attention, considering the high prevalence of hypomagnesaemia in this patient population [6,21] and preferably in prospective observational or interventional trials. More recent data interestingly have generated a molecular basis for this phenomenon. Magnesium regulates vascular calcification by impacting on osteogenic differentiation of vascular smooth muscle cells next to an increased expression of anticalcification proteins, such as osteopontin and matrix Gla protein [47]. Supplementation of Mg has recently been demonstrated to counteract the development of vascular calcification in a mouse model [48]. It can be speculated that an association between Mg levels and vascular stiffness also might partially explain the accelerated development of vascular calcification in conditions of Mg deficit.

There are also other limitations to this study. Firstly, this is a cross-sectional study which implies that no conclusions can be drawn about a possible cause-and-effect relationship between Mg and vascular stiffness, even if hypomagnesaemia has been shown to enhance inflammation, endothelial dysfunction and oxidative stress. Secondly, pre-transplantation PWV measurements were unavailable. The determination of pre-transplantation PWV (and Mg), however, was beyond the scope of this study, where we essentially aimed to assess the potential relationship between PWV and Mg levels in a balanced outpatient clinic RTR population, with
potentially less disturbing confounders (such as fluid overload, anaemia and graft dysfunction) than that observed in the immediate post-transplant period. Moreover, in dialysis patients, there might be differences of PWV between PD and HD patients [49] and pre-transplantation Mg levels in HD patients highly depend on the time of assessment. Thirdly, urinary Mg levels are lacking. Such data could provide a mechanistic view on how hypomagnesaemia is linked to drug toxicity and possibly vascular stiffness. However, adjustment for CNI drug levels, which from a theoretical point of view might correlate with urinary Mg concentration, considering the CNI-induced TRMP6 inhibition at the level of the distal collecting tubule [50], did not attenuate the relationship between PWV and Mg. All in all, it is conceivable that hypomagnesaemia, if any, is rather a reflection of previous cumulative excretion than instant excretion at the moment of assessment of the plasma levels. Fourthly, hidden confounders such as dietary factors or selection bias might have had an unidentified impact on our data analysis. It cannot be excluded that other unknown factors also contributed to the observed differences. However, on the other hand, the relationship between PWV and Mg observed in the global analysis was validated in a more homogenous population of RTR on CNI.

Finally, it can be claimed that the determination of extracellular Mg levels (a minor fraction of the total body Mg content) is not representative of body Mg status. However, a fairly good correlation was previously observed between extracellular serum Mg levels and the intracellular levels measured by the gold standard technique of $^{31}$P nuclear magnetic resonance spectroscopy [51]. The superiority of intracellular Mg determination in leukocytes or erythrocytes over the classical extracellular measurements still remains controversial [52] and is largely unexplored in RTR rendering the use of alternative measurement tools of body Mg status at the present time a justifiable but not crucial option.

In conclusion, to our knowledge, this study is the first to demonstrate that Mg is an independent predictor of PWV in RTR, particularly in patients >55 years. Future studies should be directed at evaluating the potential beneficial effect of Mg supplementation on the long-term evolution of PWV, especially since this treatment is inexpensive and safe. Longitudinal studies will also need to provide further information on potential causal pathways linking hypomagnesaemia, NODAT and arterial stiffness.

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