Relation of Low Serum Magnesium to Mortality and Cardiac Allograft Vasculopathy Following Heart Transplantation

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Hypomagnesemia is commonly observed in heart transplant (HT) recipients receiving calcineurin inhibitors. Since low serum magnesium (s-Mg) has been implicated in the progression of atherosclerosis, potentially leading to worsening coronary heart disease, arrhythmias and sudden death, we investigated the association between s-Mg and HT outcomes. Between 2002 and 2017, 150 HT patients assessed for s-Mg were divided into high (≥1.7 mg/dL) and low s-Mg groups according to the median value of all s-Mg levels recorded during the first 3 months post-HT. Endpoints included survival, cardiac allograft vasculopathy (CAV), any-treated rejection (ATR) and NF-MACE. Kaplan-Meier analysis showed that at 15 years after HT, both survival (76 vs 33%, log-rank p = 0.007) and freedom from CAV (75 vs 48%, log-rank p = 0.01) were higher in the high versus low s-Mg group. There were no significant differences in freedom from NF-MACE or ATR. Multivariate analyses consistently demonstrated that low s-Mg was independently associated with a significant 2.6-fold increased risk of mortality and 4-fold increased risk of CAV (95%CI 1.06 to 6.4, p = 0.04; 95%CI 1.12 to 14.42, p = 0.01, respectively). In conclusion, low s-Mg is independently associated with increased mortality and CAV in HT patients. Larger multi-center prospective studies are needed to confirm these findings and to examine the effect of Mg supplementation. **(Am J Cardiol 2020;125:1517−1523)**

Hypomagnesemia is commonly observed in heart transplant (HT) recipients and is invariably accompanied by myocardial magnesium depletion in the transplanted heart.[1] It has been reported that hypomagnesemia frequently develops within the first few weeks following transplantation, with a nadir in the s-Mg level in the second month post-transplantation.[1,2] An important contributory factor responsible for the hypomagnesemia observed after organ transplantation[3,4] is the administration of calcineurin inhibitors (CNIs), which induce Mg urinary wasting. The significance of these observations lies in the fact that magnesium plays an essential role in normal cardiac function.[5] Low serum magnesium (s-Mg) is associated with inflammation and disturbances in the regulation of vascular tone and endothelial function and has been implicated in the progression of atherosclerosis, potentially leading to worsening coronary heart disease and atrial and ventricular arrhythmias.[6−9] In addition, magnesium deficiency may cause myocardial fibrosis and platelet aggregation.[10,11] It was shown only recently that low s-Mg is also associated with an increased risk of coronary heart disease mortality and of sudden cardiac death.[7] Subsequently, it was shown that increased Mg intake provides protection against cardiovascular disease,[12] with possible implications for HT management. Studies in kidney transplants point to the negative impact of hypomagnesemia on post-transplant graft function and on cardiovascular risk, yet, the prognostic significance of s-Mg levels in HT patients is unknown.[5,13]

Methods

A retrospective cohort study was conducted on all consecutive patients ≥18 years of age who underwent primary HT and follow-up at our center from January 2002 to August 2017. The exclusion criteria were the absence of s-Mg measurements post-transplant and death within the first 3 months post-transplant. Data for each patient were systematically recorded upon intake and during each subsequent visit or medical contact. Donor data were obtained from the National Organ Transplantation Center and from the records of the hospitals at which the donors had died. Levels of s-Mg were measured with a Colorimetric Assay Kit (Xylidyl Blue-I Method) and levels of s-creatinine by the kinetic alkaline picrate (Jaffe's) method. For each patient, the average and median magnesium levels during the first 3 months following HT were determined, and the cohort was divided into low and high s-Mg groups according to the median magnesium value. The institutional protocol for initial post-transplant immunosuppression was consistent during the time period covered by the study and comprised a calcineurin inhibitor, a mycophenolate-based

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drug, a corticosteroid, and a polyclonal induction agent. The study was approved by our institutional review board.

The primary outcomes for this analysis were freedom from coronary artery vasculopathy (CAV) and survival. Secondary outcomes included freedom from any-treated rejection (ATR) and nonfatal major adverse cardiac events (NF-MACE), which included stroke, myocardial infarction, percutaneous coronary intervention, permanent pacemaker and peripheral vascular disease. CAV was diagnosed by coronary angiography and invasive hemodynamic assessment performed annually, along with clinical assessment and echocardiography, according to the recommended nomenclature for CAV of the International Society of Heart and Lung Transplantation (ISHLT) consensus statement.[14]

Rejections were diagnosed by routine or clinically indicated endomyocardial biopsy (EMB) and classified according to the revised ISHLT classification system for rejection.[15] Clinically significant any-treated rejection (ATR) was defined as an event that led to acute augmentation of immunosuppression in conjunction with an ISHLT ≥2R right ventricular endomyocardial biopsy result or noncellular rejection (biopsy-negative rejection) with hemodynamic compromise (decrease in LVEF by >25%).[16] For each patient, two rejection scores were calculated, as follows. (1) Total rejection score (TRS), as a measure of the severity of the rejection, was calculated according to the following weighting: 0R = 0, 1R = 1, 2R = 2, and 3R = 3. 2). Any rejection score (ARS), which reflected the total number of rejections, regardless of their severity, was calculated on the basis of 0R = 0, 1R = 1, 2R = 1, and 3R = 1. Each score for each particular patient was normalized by dividing it by the cumulative scores for the total number of biopsy specimens taken during the study period for that patient.[17]

The cohort was divided into 2 groups according to the median of the magnesium-average of the first 3 months after transplant, which was 1.7 mg/dL. Data are presented as means ± standard deviation if normally distributed, or as median and interquartile ranges. Continuous variables were tested with the Kolmogorov-Smirnov test for normal distribution. Categorical variables are expressed as frequencies and percentages. The groups were tested with the chi-square test for categorical variables and with a *t* test or Mann-Whitney-Wilcoxon test, as appropriate, for normal/non-normal distributed continuous variables. Analysis of 15-year mortality and CAV was presented using the Kaplan Meier curves and compared by the log-rank test. To explore the independent association of s-Mg and outcomes, Cox proportional hazards models for 15-year mortality and CAV were constructed. The Cox proportional hazards model for 15-year mortality included the following covariates: s-Mg (dichotomized above or below median), recipient age, gender, and creatinine-average of the first 3 months from transplant. Covariate selection was based on clinical judgment and covariates that significantly differed between the 2 groups (Table 1). The model for the CAV outcome was similarly constructed with the following covariates: s-Mg, recipient age, gender, creatinine-average of the first 3 months from transplant, and hypertension as a time-dependent covariate. Correlations between s-Mg and tacrolimus, and between s-Mg and cyclosporine were conducted with Pearson's correlation after log transformation. We took all the test results of all patients within 3 months from transplant.

Statistical analyses were conducted using R foundation (version 3.5.1).

Results

Of the original population of 166 consecutive patients, the 16 patients who died within the first 3 months were excluded from the analysis. Of the remaining 150 patients (mean age 47 ± 15 years) that constituted our study population, 113 were men and 37, women. The median s-Mg level of the cohort was 1.71 [1.60, 1.88] mg/dL. In accordance with the median value of all s-Mg levels recorded during the first 3 months post-HT, patients were divided into high (n = 75) and low (n = 75) s-Mg groups. The mean s-Mg was 2.0 ± 0.3 mg/dL and 1.6 ± 0.1 mg/dL for the high and low s-Mg groups, respectively ($p < 0.001$). Baseline clinical characteristics of patients in the 2 groups are presented in Table 1. Baseline patient and donor clinical and demographic characteristics were similar for the 2 groups, except higher creatinine levels in the high s-Mg group. CNIs therapies (cyclosporine vs tacrolimus) were distributed similarly in the 2 study groups.

A significant correlation was demonstrated toward greater decrease in s-Mg during the 3-month period in patients with higher cyclosporin and tacrolimus levels ($r = -0.13$, $p = 0.0015$; $r = -0.24$, $p = 0.0002$, respectively). Mean trough CNIs (cyclosporine or tacrolimus) were similar in both s-Mg groups**.** Similarly, rates of high trough cyclosporine (>200 micg/l) or tacrolimus (>10 micg/l) values (Table 1) were similar in the 2 s-Mg groups.

Kaplan-Meier survival analysis showed that at 15 years after HT survival was significantly higher for the high s-Mg group than for the low s-Mg group (Figure 1A). CAV as a cause of death was more frequent in the low s-Mg group (Supplementary Table 1). Multivariate analyses consistently demonstrated that low s-Mg was independently associated with a significant 2.6-fold increased risk of mortality (Figure 1B). Consistently, multivariate analysis using hypertension as a time-dependent covariate, also adjusted for age, gender and 3-months mean creatinine, also demonstrated low s-Mg to be associated with higher mortality [hazard ratio (HR) 2.63, 95% CI 1.08 to 6.39, $p = 0.04$].

Kaplan-Meier survival analysis showed that at 15 years of follow-up CAV-free survival was significantly higher in the high s-Mg group than in the low s-Mg group (Figure 2A). Multivariate analyses consistently demonstrated that low s-Mg was independently associated with a significant approximately 4-fold increased risk of CAV (Figure 2B). Consistently, multivariate analysis using hypertension as a time-dependent covariate, adjusted also for age, gender and 3-months mean creatinine, supported the finding that low s-Mg was associated with higher CAV (HR 4.01, 95% CI 1.12 to 14.31, $p = 0.04$). When s-Mg was introduced as a continuous variable, adjusted for age, gender and 3-month average creatinine values, similar association was observed (per 1-mg/dL increase, HR 0.54, 95% CI 0.37 to 0.78, $p = 0.002$).

Kaplan-Meier analyses showed that at 15 years following HT there were no significant differences in freedom

Table 1
Baseline characteristics of the high and low magnesium groups

Variable	Magnesium level		
	High(n = 75)	Low(n = 75)	p value
Recipient age (mean years ± standard deviation)	49 ± 15	45 ± 16	0.223
Donor age (mean years ±standard deviation)	26 ± 14	24 ± 13	0.249
Recipient gender (male)	55 (73%)	58 (77%)	0.705
Donor gender (male)	43 (66%)	57 (80%)	0.095
Etiology (ischemic)	34 (46%)	35 (47%)	1.000
Recipient body mass index (mean kg/m^2 ± standard deviation)	28 ± 33	24 ± 5	0.338
Donor body mass index (mean kg/m^2 ± standard deviation)	25 ± 5	25 ± 4	0.497
Diabetes mellitus	16 (21%)	14 (19%)	0.838
Hypertension	26 (35%)	25 (33%)	1.000
Dyslipidemia	31 (43%)	31 (41%)	1.000
Past smoker	27 (36%)	25 (33%)	0.863
Assist device	11 (15%)	20 (27%)	0.098
Status 1	48 (64%)	53 (71%)	0.486
Panel reactive antibody >30%	0 (0%)	3 (4%)	0.224
Recipient blood type (A/AB/B/O)	38%/14%/15%/3%	36%/7%/25%/3%	0.409
Average creatinine* (mg/dL)	1.4 ± 0.7	1.2 ± 0.4	0.012
Average magnesium* (mg/dL)	2 ± 0.3	1.6 ± 0.1	<0.001
Tacrolimus treatment	37 (49%)	48 (64%)	0.109
Tacrolimus* (mean micg/l ± standard deviation)	12.4 ± 2.7	12.5 ± 2.4	0.813
Tacrolimus* (>10 micg/l)	32 (86%)	43 (90%)	0.920
Cyclosporine treatment	23 (31%)	20 (27%)	0.718
Cyclosporine* (mean micg/l ± standard deviation)	280 ± 49	274 ± 38	0.630
Cyclosporine* (>200 micg/l)	23 (100%)	20 (100%)	1.000
Ischemic time (mean min ± standard deviation)	162 ± 41	161 ± 44	0.953
Mean pulmonary pressure (mean mmHg ± standard deviation)	32.6 ± 12.1	35.9 ± 13.5	0.158
Cardiac output (mean ± standard deviation)	3.6 ± 1.2	3.3 ± 1.2	0.129
Pulmonary vascular resistance (mean ± standard deviation)	2.9 ± 1.7	3.2 ± 2.2	0.441
Cytomegalovirus mismatch	23 (41%)	16 (33%)	0.542
Statins post-heart transplantation	67 (89%)	68 (92%)	0.799
Hypertension post-heart transplantation	35 (66%)	26 (48%)	0.094
Diabetes mellitus post-heart transplantation	30 (40%)	34 (46%)	0.570

* Average during the first 3 months after transplantation.

from NF-MACE (log-rank p value = 0.316), malignancy (log-rank p value = 0.129) or end stage renal disease (log-rank p value = 0.338) between the 2 groups. Additionally, there were no differences in rejection scores between the low and high s-Mg groups (Total rejection score: 0.30 ± 0.25 vs 0.30 ± 0.24, p = 0.990; any rejection score: 0.29 ± 0.24 vs 0.28 ± 0.22, p = 0.896, respectively), or in the freedom from ATR (log-rank p value = 0.321). There was a trend toward higher − albeit non-significant − rates of sudden cardiac death or pacemaker implantation in the low s-Mg group (12.2% vs 2.7%, p = 0.057).

Discussion

While the exact frequency of hypomagnesemia in HT populations is unknown, most patients in our cohort exhibited hypomagnesemia, hence the importance of investigating this subject with its potential profound adverse clinical implications and need for intervention. The results of our investigation suggest that low s-Mg is independently associated with a significant ~3-fold increased risk of mortality and a significant ~4-fold increased risk of CAV. To the best of our knowledge, this is the first study to investigate the implications of low s-Mg on HT outcomes.

The high prevalence of hypomagnesemia in HT recipients may be attributed to 5 main causes. First, immunosuppression medications, including CNIs, induce Mg urinary loss. Contributory mechanisms are known to be the down-regulation of renal expression of the epidermal growth factor and of the ion channel TRMP6 in the collecting tubule.[3−5] In addition, mTOR inhibitors induce hypomagnesemia through inhibition of Na-K-Cl co-transporter 2 expression in the thick ascending loop of Henle.[18] Second, other frequently used medications that contribute to low s-Mg include loop diuretic agents, which are frequently administered both pre- and postoperatively, and proton pump inhibitors, which are also frequently prescribed in HT patients due to concomitant steroid therapy.[19] Third, impaired gastro-intestinal absorption of Mg due to diarrhea is commonly seen in these patients. Fourth, volume expansion is also frequently seen in the post transplantation period. Finally, metabolic derangements, including metabolic acidosis and insulin resistance, are contributing factors to hypomagnesemia.

A particularly important consideration in the evaluation of our findings is that s-Mg constitutes only a minimal portion of the Mg present in the body and thus intracellular Mg may be a more accurate measure of the body's Mg status;

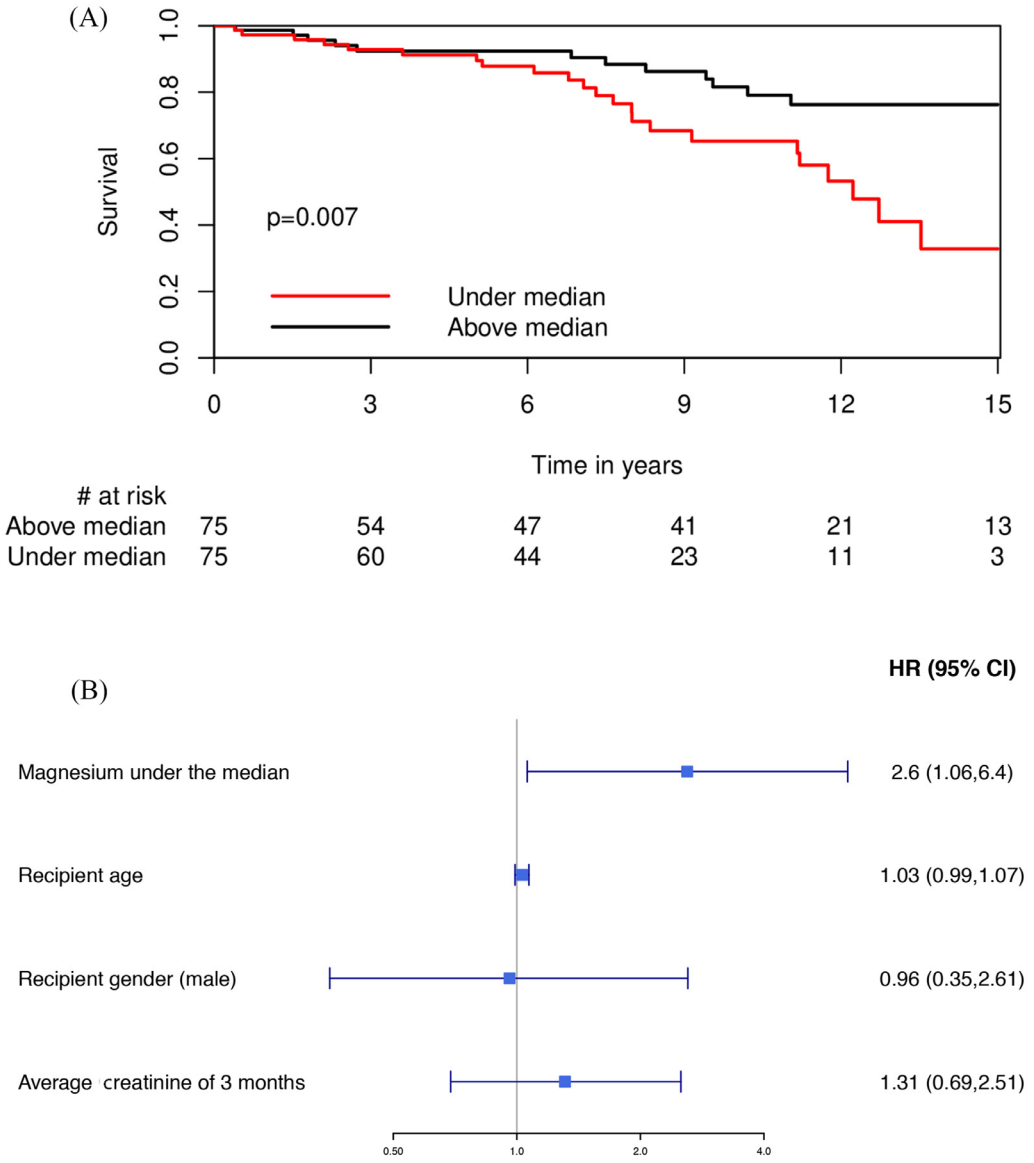

Figure 1. (A) Kaplan Meier curves for 15-year survival in high (above median) and low (below median) s-Mg groups. (B) Forest plot of Cox regression: Multivariate analysis - predictors for late mortality. CI = confidence interval; HR = hazard ratio.

however, methods for intracellular Mg measurements are not yet affordable for routine clinical practice.[20] A study investigating the interrelation between serum and myocardial magnesium showed that hypomagnesemia predated decreases in myocardial magnesium by 2 to 6 weeks and the degree of hypomagnesemia was matched by a similar depletion of myocardial magnesium.[1] Importantly, in that study significant myocardial magnesium depletion did not develop when serum levels were maintained within normal limits. Taking that study together with our findings, we may reach an understanding of the important clinical implications, as follows: The facts that myocardial magnesium depletion lags behind hypomagnesemia and that s-Mg levels within normal limits are not associated with myocardial Mg depletion combined with the association of low s-Mg with adverse primary endpoints indicate there is a window of opportunity to correct low s-Mg. Currently, however, there are no formal recommendations for s-Mg monitoring or Mg supplementation for HT recipients, although both are widely practiced.

Although survival after HT has steadily improved in the past 5 decades, there has been no significant improvement in the mortality rate beyond 1 year after HT in the past 2 decades, probably because the contributory factors to long-term mortality, particularly CAV, remain a challenge for detection and treatment.[21] Our study did not show any association of s-Mg with rejection rates or severity, and therefore we assume that the effect of low s-Mg on allograft vasculopathy is independent of rejections. This notion combined with the fact that long-term mortality including CAV has not changed dramatically in the past decades despite the decrease in rejections further support the importance of our findings regarding the proposed role of s-Mg in the etiology of CAV.

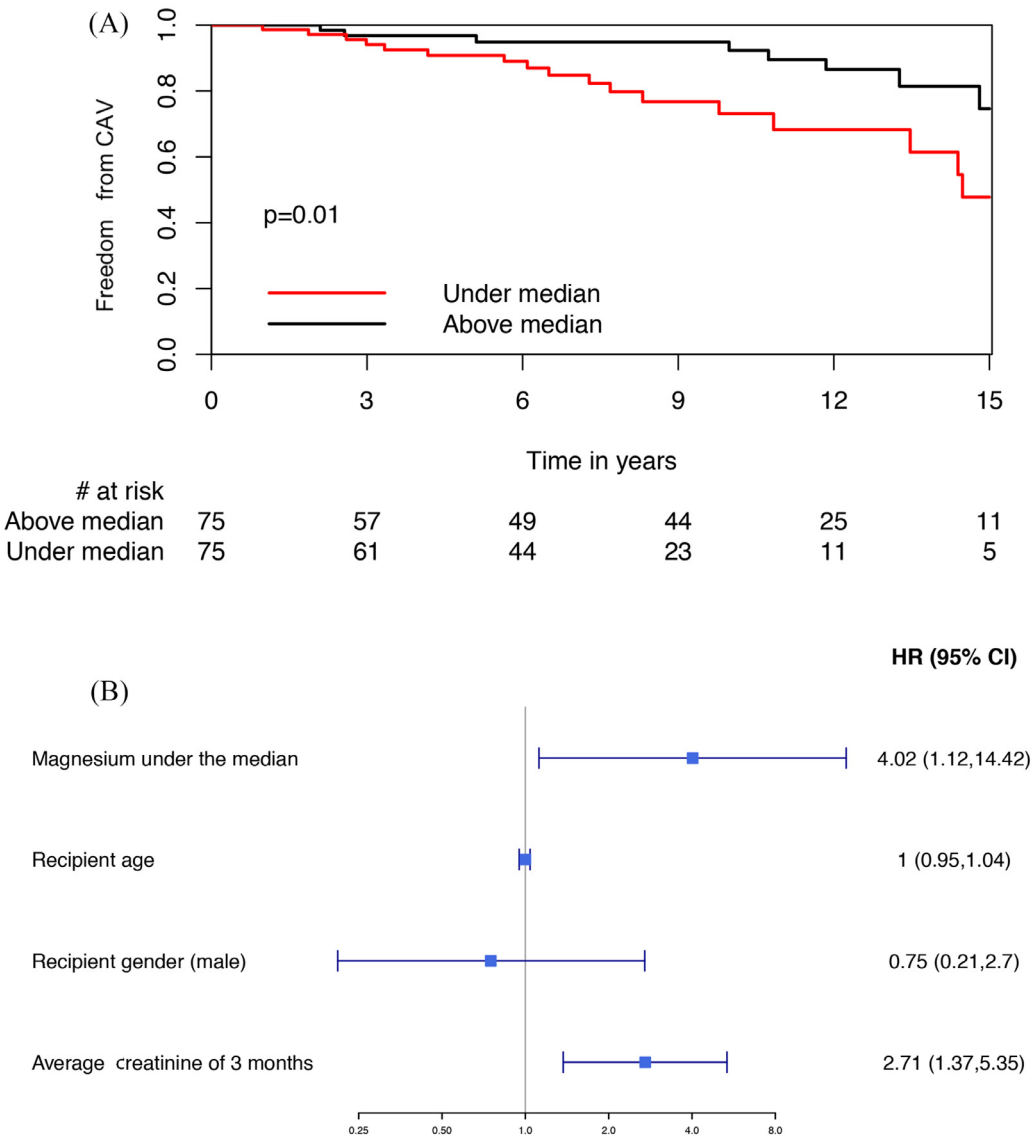

Figure 2. (A) Kaplan Meier curves for 15-year freedom from cardiac allograft vasculopathy (CAV) in high (above median) and low (below median) s-Mg groups. (B) Forest plot of Cox regression: Multivariate analysis - predictors for cardiac allograft vasculopathy (CAV). CI = confidence interval; HR = hazard ratio.

CAV remains the leading cause of death in cardiac transplant recipients.[14] The effect of magnesium deficiency may be integrated into the various stages in the pathogenesis of CAV known to us today, as follows (1) In terms of the pathogenesis of conventional risk factors, low s-Mg has been shown to play a role in endothelial dysfunction, dyslipidemia, inflammation, arterial hypertension, and diabetes.[22,23] In addition, it has long been known that low s-Mg is an independent risk factor for coronary heart disease, including accelerated atherosclerosis, vascular calcification and coronary spasm.[24] (2) Prolonged magnesium depletion has been shown to be associated with a rapidly progressive cardiomyopathy characterized by intracellular calcium deposition and mitochondrial disarray.[1,25,26] When intracellular levels of Mg drop, the reciprocal relation between myocardial Mg and Ca levels leads to secondary calcium overload and to the accumulation of calcium deposits within the myocytes. If magnesium depletion is prolonged, this process becomes irreversible. These calcium deposits, similarly to ischemic injury, result in myocyte necrosis and fibrosis, with subsequent appearance of arteriopathic features of small- and medium-sized coronary arteries that are similar to those seen in accelerated coronary disease and that compromises graft survival after HT.[1] (3) In terms of inflammation, hypomagnesemia is associated with increased C-reactive protein levels, leukocyte and macrophage activation, NFKB/cytokines activation and platelet aggregation.[27] On the other side of the balance, Mg supplementation has been shown to have favorable effects on left ventricular EF, small arterial compliance and endothelial function, and to be inversely associated with cardiovascular disease risk,[28–30] considerations that add further credence to our call for Mg monitoring and possibly supplementation in HT patients.

There are several limitations to our study. First, there is the inherent limitation of observational trials that uncovers

associations but precludes the determination of a cause-and-effect relationship. Second, our current practice does not include routine intravascular ultrasound assessment, which might be associated with underestimation of CAV when the visual angiography result is apparently normal. Third, this study was limited by being based on a single-center experience and not all possible confounders were recorded or adjusted for in this single-center study. Finally, measuring s-Mg and not intracellular magnesium levels may influence the assessment of the patients' magnesium status. The present results will therefore require confirmation in larger cohorts and preferably with a prospective study design.

In summary, our investigation demonstrated that low s-Mg levels are associated with an adverse prognosis and predict clinical worsening or death. Given the frequency of CAV and its profound importance on outcomes, correcting hypomagnesemia soon after HT could translate into a significant improvement in clinical cardiovascular morbidity and mortality. It remains to be determined whether these findings represent a clinical marker or suggest a potential therapeutic target. Our results call for prospective studies to evaluate the impact of low s-Mg correction after HT as well as the best therapeutic modality.

Disclosures

The authors have nothing to disclose.

Author Contributions

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Elad Maor: Investigation, supervision.

Elad Asher: Investigation, supervision.

Dov Freimark: Investigation, supervision.

Robert Klempfner: Investigation, formal analysis, supervision.

Yael Peled: Writing - Original draft, writing - review and editing, project administration, investigation, methodology, data curation.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2020.02.030.

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