Proton-Pump Inhibitor and Tacrolimus use is Associated with Hypomagnesemia in Connective Tissue Disease: A Potential Pathogenic Link with Renal Deterioration and Recurrent Infections

Hironari Hanaoka (✉ hanaoka@keio.jp)

Keio University School of Medicine <https://orcid.org/0000-0002-5172-1102>

Jun Kikuchi

Keio University School of Medicine

Yuko Kaneko

Keio University School of Medicine

Noriyasu Seki

Mitsubishi Tanabe Pharma Cooperation

Hideto Tsujimoto

Mitsubishi Tanabe Pharma Cooperation

Kenji Chiba

Mitsubishi Tanabe Pharma Cooperation

Tsutomu Takeuchi

Keio University School of Medicine

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Abstract

Background: Low levels of serum magnesium (Mg) perturb renal tubular cell function and, lymphocyte resulting in renal deterioration and an imbalance in mononuclear cells. Here, we investigated the influence of hypomagnesaemia in patients with connective tissue disease (CTD).

Methods: We retrospectively evaluated CTD patients who visited our hospital during 2019 with available serum Mg levels. Patients were divided into two groups, those with or without hypomagnesemia (<1.8 mg/dL) and compared by rate of hospitalization for severe infection and cumulative renal deterioration. They were also compared by fractions of lymphocytes, and natural killer (NK) and dendritic cell (DC) subsets as measured by fluorescence-activated cell sorting (FACS) analysis.

Results: Among 284 patients, hypomagnesemia was detected in 63 (22.2%). Multivariate analysis revealed that use of proton-pump inhibitors (PPIs) (OR 1.48, $p=0.01$) and tacrolimus (TAC) (OR 6.14, $p<0.01$) was independently associated with hypomagnesaemia. Renal deterioration rate was significantly higher in TAC and/or PPI users with hypomagnesemia ($p=0.01$). Hospitalization rate for severe infection was also higher in patients with hypomagnesaemia ($p=0.04$). FACS analysis showed lower counts for CD8+ T cells, CD19+ B cells, NK cells, and DC in hypomagnesaemia ($p=0.03$, $p=0.02$, $p=0.02$, and $p=0.03$, respectively).

Conclusions: Use of TAC and PPIs may be associated with hypomagnesaemia and lead to poor renal outcomes and severe infection in CTD.

Background

Magnesium (Mg) is an abundant intracellular cation which acts as a co-factor for more than 300 enzymes involved in a number of fundamental functions. Mg deficiency leads to many pathogenic conditions, including cardiovascular mortality, stroke, chronic kidney disease (CKD) progression, osteoporosis, and insulin resistance [1–6]. Hypomagnesemia is also associated with the development of recurrent infections due to its role as a second messenger in T cell activation and contribution to the cytotoxicity of natural killer (NK) and CD8 + T cells. [7–9].

Causes of hypomagnesemia are categorized into three groups: decreased dietary intake, impaired gastrointestinal absorption, and increased renal loss [10]. Several medications are known to influence serum Mg levels through these mechanisms. Proton-pump inhibitors (PPIs) inhibit pH-dependent active Mg absorption via transient receptor potential melastatin (TRPM) 6 and 7 channels in the intestine [11]. Calcineurin inhibitors (CNIs) are also associated with low serum Mg concentrations [12, 13]. In kidney transplantation recipients, CNIs decrease serum Mg concentrations rapidly and profoundly by wasting Mg through inhibition of TRPM6 in the renal tubule. CNIs interfere with TRPM6 in the renal tubule, but do not alter TRPM6 function in the intestine [14]. However, although both PPIs and CNIs, including tacrolimus (TAC), are frequently used in patients with connective tissue diseases (CTDs) [15–22], little is known about the influence of these drugs on serum levels of Mg in the management of CTDs.

The aim of this study was to investigate the prevalence of hypomagnesemia and its clinical impact on patients with CTD in relation to PPIs and TAC.

Material And Methods

Patients and evaluation of clinical data

We reviewed consecutive patients who visited Keio University Hospital from January 2019 to December 2019 and were diagnosed with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), Sjögren syndrome (SS), systemic sclerosis (SSc), mixed connective tissue disease (MCTD), anti-neutrophil cytoplasmic antibodies-related vasculitis and other rheumatic diseases according to the respective classification criteria [23–37]. Patients with available serum Mg levels were included in the study. We collected clinical characteristics, treatments administered for more than 3 years, laboratory data, estimated glomerular filtration rate (eGFR), and hospitalization due to infection from diagnosis to December 2019.

This study was approved by the Ethics Committee of Keio University School of Medicine. Blood samples for flow cytometric analysis were obtained after the subjects gave written informed consent, as approved by the Institutional Review Board.

Definition

Hypomagnesemia was defined as a serum Mg concentrations < 1.8 mg/dL [38]. Renal deterioration was defined as a greater than 30% decline in serum creatinine levels from baseline [39]. Fractional excretion of Mg (FEMg, %) was calculated with the following formula: $FE_{Mg} = (U_{Mg} * P_{Cr}) / (0.7 * P_{Mg} * U_{Cr}) * 100$ [40], where U and P refer to the urine and plasma concentrations of Mg and creatinine (Cr), respectively. Serum Mg concentration was multiplied by 0.7, as only about 70% of circulating magnesium is free and filtered across the glomerulus. Normal limit of FEMg is defined as less than 2% [40].

TAC measurement

TAC concentration in fresh whole blood samples collected 12 hours after the last TAC administration was measured by the TACR Flex Dimension immunoassay method using a Dimension EXL analyzer (Siemens Healthcare Diagnostics, Tokyo, Japan) [17].

Flow cytometry

Blood samples at the time of serum Mg measurement from our cell bank of RA patients treated with methotrexate (MTX) monotherapy were analyzed with fluorescence-activated cell sorting. Samples were stained with antibodies (BD Biosciences and BioLegend; Table S1) and fixed by Phosflow Lyse/Fix Buffer (BD Bioscience). Flow cytometric analysis was conducted on an LSRFortessa™ X-20 (Becton Dickinson) and analyzed by FlowJo ver.10 (FlowJo LLC). Phenotypes of immune cell subsets were defined based on the Human Immunology Project protocol (Table S2) [41]. Mean numbers of each immune cell phenotype were compared.

Statistical analysis

Continuous values are shown as median and interquartile range (IQR). Comparisons between two groups were performed with the Mann-Whitney *U*-test for continuous variables and the chi-squared test or Fisher's exact test for categorical variables. Four groups were compared by analysis of variance. Cumulative renal deterioration rates were analyzed by the Kaplan-Meier method with the log-rank test. Correlations of two continuous variables were analyzed with the Spearman rank correlation coefficient. To identify independent parameters, binary logistic regression analysis was used with variables having a P-value < 0.005 in a previous univariate analysis as covariates. A P-value < 0.05 was defined as statistically significant.

Results

Clinical characteristics

A total of 284 patients with CTD were included in the study. Median (IQR) age was 64.0 (48.0–73.0) years and 83.8% were female (Table 1). Underlying CTDs were RA in 108 patients (38.0%), SLE in 59 (20.8%), PM/DM in 20 (7.0%), SSc in 24 (8.5%) MCTD in 10 (3.5%), polymyalgia rheumatica in 10 (3.5%), microscopic polyangiitis in 8 (2.8%), IgG4-related disease in 9 (3.2%), SS in 6 (2.1%), adult Still's disease in 6 (2.1%), arthritis with palmoplantar pustulosis in 6 (2.1%), eosinophilic granulomatous polyangiitis in 4 (1.4%), psoriatic arthritis in 4 (1.4%), sarcoidosis in 2 (0.7%), Takayasu's arteritis in 2 (0.7%), granulomatous polyangiitis in 2 (0.8%), Behçet's disease in 2 (0.8%), diffuse fasciitis in 2 (0.8%), and familial Mediterranean fever in 1 (0.4%). Glucocorticoid was used by 41.5% of total patients and median (IQR) dose was 0.0 (0.0–4.0) mg/day. Among all patients, 141 (49.6%) used PPIs and 68 (23.9%) used TAC. Hospitalization for severe infection was seen in 25 (8.8%) patients. Hypomagnesemia was observed in 63 (22.2%) patients.

Table 1
Patients characteristics

	All (n = 284)	Normal Mg (n = 221)	Hypomagnesemia (n = 63)	p
Age, years	64.0 (48.0–73.0)	71.0 (51.0–74.0)	55.0 (38.0–69.0)	0.029
Male:Female	46:238	35:186	11:52	0.752
Underlying disease				
RA (%)	108 (38.0)	94 (42.5)	14 (22.2)	
SLE (%)	59 (20.8)	36 (16.3)	23 (36.5)	
PM/DM (%)	20 (7.0)	11 (4.9)	9 (14.2)	
SSc (%)	24 (8.5)	23 (10.4)	1 (1.6)	
MCTD (%)	10 (3.5)	9 (4.1)	1 (1.6)	
PMR (%)	10 (3.5)	7 (3.2)	3 (4.8)	
Others* (%)	53 (18.7)	41 (18.6)	12 (19.0)	
Serum electrolytes				
Mg, mg/dL	2.1 (1.9–2.2)	2.1 (2.0-2.2)	1.7 (1.7–1.8)	< 0.001
Na, mEq/L	140.9 (139.7-142.3)	141.2 (139.8-142.4)	140.5 (139.3-141.9)	0.163
K, mEq/L	4.2 (3.9–4.4)	4.2 (4.0-4.4)	4.1 (3.9–4.3)	0.087
CL, mEq/L	105.0 (104.0-107.0)	106.0 (104.0-107.0)	105.0 (103.0-106.0)	0.241
Ca, mEq/L	9.2 (8.9–9.4)	9.1 (8.9–9.4)	9.2 (8.9–9.4)	0.608
P, mEq/L	3.5 (3.2–3.9)	3.5 (3.2–3.9)	3.5 (3.1–3.9)	0.489

Results show median (interquartile range) unless otherwise indicated.

*Others include microscopic polyangiitis, IgG4-related disease, Sjogren syndrome, adult Still's disease, arthritis with palmoplantar pustulosis, eosinophilic granulomatous polyangiitis, psoriatic arthritis, sarcoidosis, Takayasu's arteritis, granulomatous polyangiitis, Behçet's disease, diffuse fasciitis, and familial Mediterranean fever.

Mg, magnesium; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; MCTD, mixed connective tissue disease; PMR, polymyalgia rheumatica; eGFR, estimated glomerular filtration rate; L-FABP, liver-type fatty acid binding protein; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; GC, glucocorticoid; PPIs, proton-pump inhibitors; TAC, tacrolimus; MMF, mycophenolate mofetil; MTX, methotrexate; AZA, azathioprine; HCQ, hydroxychloroquine.

	All (n = 284)	Normal Mg (n = 221)	Hypomagnesemia (n = 63)	p
eGFR, ml/min/1.73 m ²	68.0 (56.0–80.0)	63.0 (56.0–80.0)	70.0 (56.0–81.0)	0.462
Urine markers				
β2-microglobulin, ×10 ² μg/L	1.5 (0.9–2.9)	2.1 (0.9–3.2)	1.2 (0.7–1.9)	0.386
α1-microglobulin, mg/L	3.3 (1.6–6.5)	3.8 (1.6–6.9)	3.3 (1.6–5.8)	0.636
L-FABP, μg/g⊐Cre	2.4 (1.6–4.7)	3.1 (1.7–4.8)	2.2 (1.4–4.4)	0.164
NAG, IU/L	5.0 (2.5–8.2)	5.0 (2.4–8.1)	5.3 (3.4–9.6)	0.120
NGAL, μg/g⊐Cre	21.7 (14.0–38.5)	21.7 (15.1–37.1)	21.7 (12.9–46.0)	0.770
Medication				
GC, (%)	118 (41.5)	78 (35.3)	40 (63.4)	0.001
GC dose, median (IQR) mg/day	0 (0–4)	0 (0–3)	3 (0–5)	0.001
TAC (%)	68 (23.9)	34 (15.3)	34 (53.9)	0.001
MMF (%)	13 (4.6)	6 (2.7)	7 (11.1)	0.006
MTX (%)	81 (28.5)	71 (32.1)	10 (15.9)	0.001
AZA (%)	19 (6.7)	17 (7.6)	2 (3.2)	0.213
HCQ (%)	27 (9.5)	13 (5.9)	14 (22.2)	0.001
PPI (%)	141 (49.6)	93 (42.1)	48 (76.1)	0.001
Hospitalization due to infection	25 (8.8)	15 (6.7)	10 (15.8)	0.042
Results show median (interquartile range) unless otherwise indicated.				
*Others include microscopic polyangiitis, IgG4-related disease, Sjogren syndrome, adult Still's disease, arthritis with palmoplantar pustulosis, eosinophilic granulomatous polyangiitis, psoriatic arthritis, sarcoidosis, Takayasu's arteritis, granulomatous polyangiitis, Behçet's disease, diffuse fasciitis, and familial Mediterranean fever.				
Mg, magnesium; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; MCTD, mixed connective tissue disease; PMR, polymyalgia rheumatica; eGFR, estimated glomerular filtration rate; L-FABP, liver-type fatty acid binding protein; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; GC, glucocorticoid; PPIs, proton-pump inhibitors; TAC, tacrolimus; MMF, mycophenolate mofetil; MTX, methotrexate; AZA, azathioprine; HCQ, hydroxychloroquine.				

Factors associated with hypomagnesemia

The patients were divided into two groups according to the presence of hypomagnesemia. Clinical characteristics were compared between the normal Mg (n = 221) and hypomagnesemia groups (n = 63) (Table 1). Median age was significantly higher in the normal Mg group than in the hypomagnesemia group (71.0 years vs 55.0 years, $p = 0.029$, respectively). Renal function, electrolyte concentrations except for Mg, and urine markers were not different between the two groups. Rates of use of glucocorticoid, PPI, TAC, mycophenolate mofetil (MMF), and hydroxychloroquine (HCQ) were significantly lower in the normal Mg group than in the hypomagnesemia group (glucocorticoid, 35.3% vs 63.4%, $p = 0.001$; PPI, 42.1% vs 76.1%, $p = 0.001$; TAC, 15.3% vs 53.9%, $p = 0.001$; MMF, 2.7% vs 11.1%, $p = 0.006$; HCQ, 5.9% vs 22.2%, $p = 0.001$, respectively). Use of MTX was significantly higher in the normal Mg group (32.1% vs 15.9%, $p = 0.001$).

Hospitalization due to severe infection from the diagnosis of CTDs to December 2019 occurred significantly less frequently in the normal Mg group than in the hypomagnesemia group (6.7% vs 15.8%, $p = 0.042$).

Multiple logistic regression analysis identified the use of PPI (odds ratio 1.45, confidence interval 1.0-3.29, $p = 0.009$) and TAC (odds ratio 5.99, confidence interval 2.93–12.24, $p < 0.001$) as independent factors associated with hypomagnesemia (Table 2).

Table 2
Multivariate analysis for factors associated with hypomagnesemia

Factor	Odds ratio (95%CI)	p
Age	0.96 (0.95–1.05)	0.152
SLE	1.47 (0.54–3.97)	0.445
RA	0.84 (0.32–2.16)	0.727
GC use	1.14 (0.49–2.71)	0.753
PPI use	1.45 (1.01–3.29)	0.009
TAC use	5.99 (2.93–12.24)	< 0.001
MTX use	0.72 (0.27–1.95)	0.523
HCQ use	1.71 (0.53–5.52)	0.371
SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; GC, glucocorticoid; PPIs, proton-pump inhibitors; TAC, tacrolimus; MTX, methotrexate; HCQ, hydroxychloroquine.		

Association of drugs and Mg levels

To investigate the effect of TAC and PPI on serum Mg levels further, we divided all patients into 4 groups according to the use of TAC and PPI (Fig. 1A) and compared magnesium levels. Median (IQR) levels of serum Mg were 2.1 (2.0-2.2) mg/dL in patients without TAC and PPI, 2.1 (1.9–2.2) mg/dL in those with

only PPI, 1.9 (1.8–1.9) mg/dL in those with only TAC, and 1.8 (1.8–2.9) mg/dL in those with both TAC and PPI ($p < 0.0001$).

We then calculated FEMg in patients with hypomagnesemia ($n = 57$) (Fig. 1B). The FEMg was 1.7 (1.5–2.7)% in those without TAC and PPI, 2.2 (1.6–3.2)% in those with only PPI, 3.9 (2.6–4.9)% in those with only TAC, and 2.7 (1.3–4.7)% in those with both TAC and PPI ($p = 0.04$), reflecting the different mechanisms of causation of hypomagnesemia, namely that TAC inhibits reabsorption of Mg in the kidneys with a consequent increase in excretion of Mg in urine, and that PPI causes wasting of Mg in the intestine.

The relationship of serum TAC concentrations with serum Mg levels and FEMg corroborated the effect of the drugs. In patients who did not use PPI, TAC concentrations were negatively correlated with Mg levels ($r = -0.61$, $p < 0.01$, Fig. 2A) and positively with FEMg ($r = 0.38$, $p = 0.05$, Fig. 2B). In patients who used PPI, these correlations disappeared (Mg levels, $r = -0.25$, $p = 0.19$, Fig. 2C; FEMg, $r = -0.07$, $p = 0.73$, Fig. 2D). We further investigated 28 patients who had discontinued PPI at the attending physician's discretion (without tacrolimus, $n = 22$; with tacrolimus, $n = 6$): in patients who did not use TAC, serum Mg levels were significantly increased after PPI discontinuation from 2.0 (2.0–2.2) mg/dL to 2.2 (2.0–2.4) mg/dL, $p = 0.04$, Fig. 3A), while FEMg did not change (from 1.9 [1.4–2.7] % to 2.2 [1.9–2.7]%, $p = 0.16$, Fig. 3B); in patients treated with TAC, in contrast, serum Mg concentrations and FEMg were not changed (Mg, 1.8 [1.6–3.0] mg/dL to 2.0 [1.6–4.6] mg/dL, $p = 0.82$, Fig. 3C; FEMg, 2.3 [1.3–3.0]% to 2.1 [1.6–8]%, $p = 0.56$, Fig. 3D).

Relationship between hypomagnesemia and renal deterioration

As the use of TAC and PPI was the major cause of hypomagnesemia, we investigated the sequential renal function of patients treated with TAC and/or PPI ($n = 173$) from the initiation of these drugs until the last observation. When we divided the patients according to the presence of hypomagnesemia, the cumulative renal deterioration-free rates were significantly higher in patients with normal Mg ($n = 124$; 80.7%; observation period, 5.0 ± 2.9 years) than in those with hypomagnesemia ($n = 49$, 65.7%, observation period, 5.3 ± 3.4 years) ($p = 0.007$, Fig. 4). Of note, renal deterioration was not related with TAC use (renal deterioration-free at last observation, TAC users 84.5% and non-TAC users 90.1%, $p = 0.34$).

Effect of hypomagnesemia on immune cells

Among the 283 patients enrolled in this study, 17 patients who were also registered in another cohort of our university had information on peripheral blood mononuclear cells analyzed with FACS at the time Mg concentrations were measured. All 17 of these patients had RA and were treated with methotrexate alone. Six of these 17 patients had hypomagnesemia while the other 11 had normal Mg levels. These patients did not differ with regard to sex, disease duration, disease activity, or MTX dose (Table S3). In contrast, numbers of CD8 + T cells, CD19 + B cells, NK cells, and DC were significantly lower in the patients with hypomagnesemia than in the patients with normal Mg ($p = 0.03$, $p = 0.02$, $p = 0.02$, and $p = 0.03$,

respectively, Fig. 5). Hospitalization due to infection was observed in 1 patient with hypomagnesemia (16.6%) and 1 with normal Mg (9.1%) ($p = 0.64$).

Discussion

In our study, hypomagnesemia was observed in approximately one-fifth of patients with CTD and was associated with renal deterioration and hospitalization due to severe infections. The development of hypomagnesemia might have been caused by the use of TAC and PPIs.

Our study showed a high renal deterioration rate in patients with hypomagnesemia. This finding is consistent with previous studies which reported the association of hypomagnesemia with incident CKD [42], a decline in eGFR in CKD patients [43], and progression to end-stage renal disease in diabetic nephropathy [44]. Laecke et al investigated 1,650 patients with CKD with a median follow-up of 5.1 years and reported that a 1-mg/dL decrease in baseline serum Mg was associated with a yearly decrease in eGFR of 5.1%. Another report in Japanese patients with diabetic nephropathy ($n = 144$) showed that patients with hypomagnesemia were twice as likely to progress to end-stage renal disease compared to those with a normal range. The pathogenic mechanism of hypomagnesemia related to renal deterioration is not fully understood, but hypomagnesemia is considered to damage renal tubules. In one study, incubation of tubular epithelial cells in low-Mg medium increased the rate of apoptosis, whereas this effect was significantly suppressed when Mg concentration was increased [45].

In our study, 22.2% of patients with CTDs showed serum magnesium levels below 1.8 mg/dL. Low levels were associated with a high hospitalization rate due to severe infection. An association of hypomagnesemia and recurrent infection has been reported. Patients with X-linked XMEN, a hereditary immune deficiency syndrome in which dysfunction of the magnesium channel MAGT1 in T lymphocytes leads to a low intra-lymphocytic free magnesium concentration, suffer from recurrent infection [46]. Hypomagnesemic rats were shown to die earlier than control rats when injected with intravenous *Escherichia coli* endotoxin, whereas magnesium supplementation improve survival [47]. In a clinical report on kidney transplantation, low serum Mg was associated with an increased hazard of infection, and every 0.1 mg/dL reduction in serum magnesium below 2.0 mg/dL increased the hazard ratio by 15% [48]. Hypomagnesemia decreases T cell numbers, activation, and the cytotoxicity of CD8 + T cells and NK cells [49]. Although this study did not examine lymphocyte function, we did identify a decrease in the number of CD8 + T cells, CD19 + B cells, NK cells, and DC in RA patients with hypomagnesemia. Taken together, the decreased function and number of mononuclear cells caused by hypomagnesemia may be associated with the impaired immune function in hypomagnesemia.

Hypomagnesemia in patients with CTD was significantly associated with the use of TAC and PPIs in our study. These findings are consistent with the action of these drugs, namely with TAC's interference with Mg-reabsorption from urine and PPIs interference with Mg absorption from the intestines [11–13]. Of the two drugs, TAC's interference on systemic Mg transportation is much stronger than that of PPIs, given that the reabsorption of Mg in the kidneys can handle 20-fold greater amounts of dietary Mg than

absorption from the intestines [1]. In fact, our study showed that patients using TAC had lower Mg concentrations than those using PPIs, but that the combination use of PPIs and TAC did not show any additional lowering effect on Mg concentration than TAC use alone. In our study, the discontinuation of PPI increased Mg levels in patients without TAC, suggesting that hypomagnesemia caused by PPIs is reversible. We therefore recommend the monitoring of serum Mg levels in patients treated with PPIs, and consideration of the discontinuation when hypomagnesemia emerges. As for patients treated with TAC, Mg levels did not change when PPIs were stopped. We speculate that this is due to the far stronger effect of TAC than PPIs on lowering serum Mg levels. However, while we cannot conclude that serum Mg concentration would increase after TAC discontinuation because no patient discontinued TAC in our study, we can say that TAC dose should be reduced by monitoring TAC concentration to as low as possible to prevent hypomagnesemia, given our findings that TAC concentrations were negatively correlated with serum Mg concentrations.

Our study has several limitations. First, it is a retrospective, single-centered cohort with a small sample size. This could have caused a degree of selection bias. Second, serum magnesium levels were measured cross-sectionally. Changes in magnesium levels over the period of observation of renal function were therefore unclear, which weakened the discussion about the relationship between hypomagnesemia and renal deterioration. Third, PPI was discontinued at the discretion of the attending physicians, which may have resulted in a degree of selection bias. Confirmation of our findings will require a multi-center prospective study.

Conclusions

The use of TAC and PPIs was associated with hypomagnesaemia and led to poor renal outcomes and severe infection in patients with CTDs. The lowest possible dose of TAC should be prescribed in the management of CTDs, and the need for PPIs should be periodically reassessed.

List of abbreviations

Mg, magnesium; CTD, connective tissue disease; NK, natural killer; DC, dendritic cell; FACS, fluorescence-activated cell sorting; PPIs, proton-pump inhibitors; TAC, tacrolimus; CKD, chronic kidney disease; TRPM, transient receptor potential melastatin; CNIs, calcineurin inhibitors; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; PM/DM, polymyositis/dermatomyositis; eGFR; estimated glomerular filtration rate; FE, fractional excretion; Cr, creatinine; MTX, methotrexate; ESRD, end-stage renal disease.

Declarations

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Keio University School of Medicine. We obtained informed written consent from all subjects prior to collecting samples in accordance with the tenets of the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

None

Funding

None

Authors' contributions

H.H. performed the majority of research and analyzed and interpreted the data; N.S., K.T., and K.C. performed the FACS analysis; J.K. and Y.K. collected patient's data and samples; T.T. designed the research, interpreted the data and supervised and organized the study; all authors wrote the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Comparison of magnesium level and fraction excretion of magnesium by drug use. All patients were divided into 4 groups according to tacrolimus and proton pump inhibitor use. Comparison of serum Mg levels among the 4 groups is shown (A). Dotted line indicates the normal limit of magnesium level (1.8 mg/dL). Comparison of FEMg in patients with hypomagnesemia is shown (B). Dotted line indicates the normal limit of FEMg (2.0%). FEMg, fractional excretion of magnesium; TAC, tacrolimus; PPI, proton pump inhibitors.

Figure 2

Association between tacrolimus concentration and magnesium level or fractional excretion of magnesium by drug use. In patients without PPI, TAC concentration was significantly correlated with Mg level ($r = -0.61, p < 0.01$) (A) and FEMg ($r = 0.38, p = 0.05$) (B). In patients treated with both TAC and PPI, in contrast, no association was seen between TAC concentration and Mg level ($r = 0.25, p = 0.19$) (C) or FEMg ($r = -0.07, p = 0.73$) (D). FEMg, fractional excretion of magnesium; TAC, tacrolimus; PPI, proton pump inhibitor.

Figure 3

Serial change in magnesium level after discontinuation of proton pump inhibitor. Magnesium level was significantly increased after discontinuation of PPI in patients without TAC ($p=0.04$) (A), but no significant difference was seen in FEMg (B). In patients with TAC, no change in magnesium level or FEM was observed after PPI discontinuation (C, D). FEMg, fractional excretion of magnesium; TAC, tacrolimus; PPI, proton pump inhibitor.

Figure 4

Cumulative renal deterioration-free rate. A significantly lower renal deterioration-free rate was observed in patients with hypomagnesemia compared to patients with normal Mg ($p=0.007$). Mg, magnesium; TAC, tacrolimus; PPI, proton pump inhibitor.

Figure 5

Flow cytometric analysis in patients with and without hypomagnesemia. Lower counts for CD8+ T cells, CD19+ B cells, NK cells, and dendritic cells were observed in patients with hypomagnesaemia ($p=0.03$, $p=0.02$, $p=0.02$, and $p=0.03$, respectively).

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