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Original Article

ORIGINAL ARTICLE

Ionized and total magnesium levels in patients with chronic kidney disease: associated factors and outcomes

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ABSTRACT

Background. The association between hypo- and/or hypermagnesaemia and cardiovascular (CV) outcomes or mortality has shown conflicting results in chronic kidney disease (CKD) and has been conducted on total magnesium (tMg) levels. Thus, the objectives of the present study were to (i) describe the serum ionized Mg (iMg) concentration in patients at various CKD stages, (ii) measure the correlation between iMg and tMg concentrations, (iii) identify their associated factors and (iv) determine whether serum tMg and/or iMg concentrations are associated with major adverse cardiovascular events (MACE) and mortality before kidney replacement therapy in CKD patients.

Methods. Chronic Kidney Disease–Renal Epidemiology and Information Network (CKD-REIN) is a prospective cohort of CKD patients with an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2. Baseline iMg and tMg serum concentrations were centrally measured. Adjusted cause-specific Cox proportional hazard models were used to estimate hazard ratios (HRs) for first MACE and for mortality.

Results. Of the 2419 included patients, median age was 68 years, and the mean eGFR was 34.8 mL/min/1.73 m^2. Concentrations of serum iMg and tMg were strongly correlated ($r = 0.89$, $P < .001$) and were independently associated with eGFR. The adjusted HR [95% confidence interval (CI)] for MACE associated with the baseline serum tMg level was 1.27 (0.95; 1.69) for patients in Tertile 1 and 1.56 (1.18; 2.06) for patients in Tertile 3, relative to patients in Tertile 2. The HR (95% CI) of death according to serum tMg concentration was increased in Tertile 3 [1.48 (1.11; 1.97)]. The adjusted risk for MACE and mortality (all-cause or CV) associated with the baseline serum iMg level was not significantly different between tertiles.

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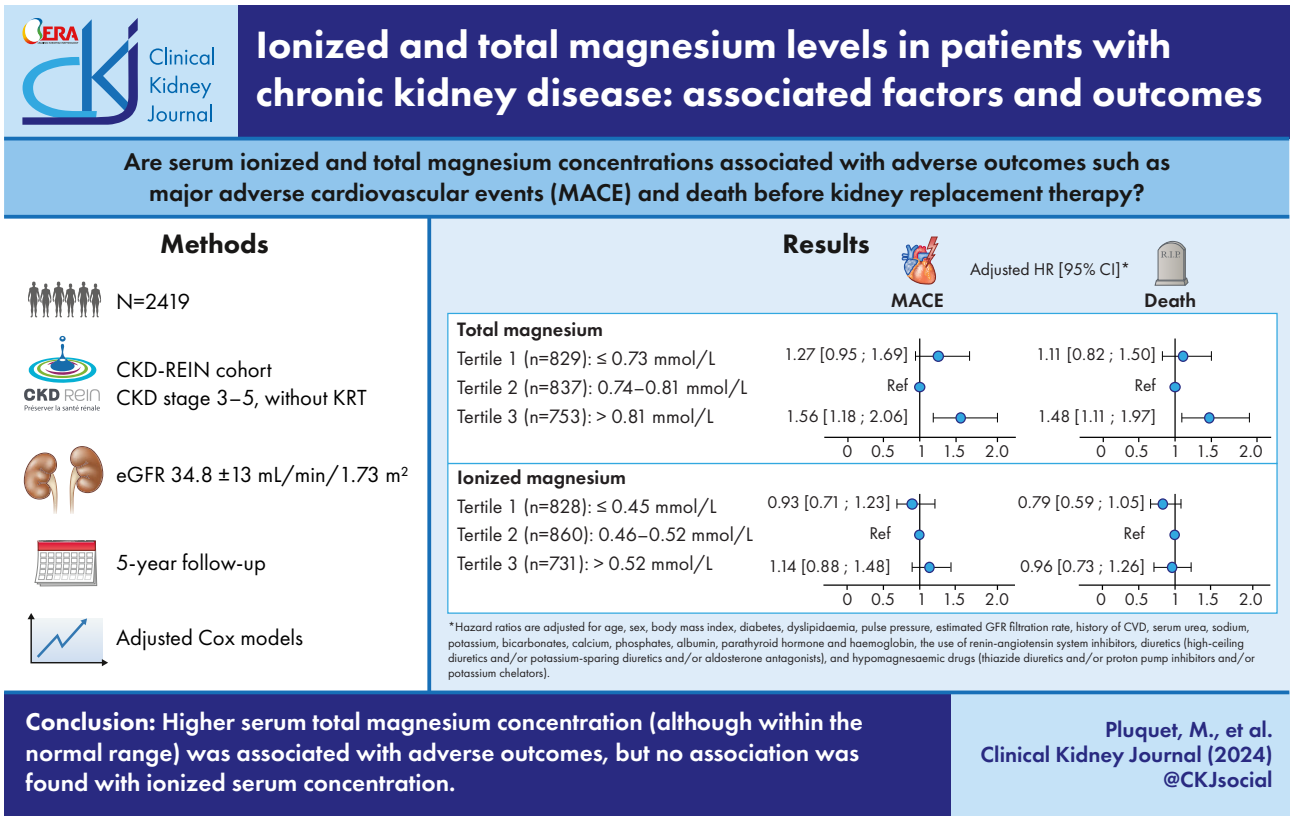
Conclusions. Our analysis of a large cohort of patients with moderate-to-advanced CKD demonstrated that individuals with higher serum tMg concentrations, although still within the normal range, had a greater likelihood of MACE and mortality. However, serum iMg levels were not associated with these outcomes.

GRAPHICAL ABSTRACT



Keywords: cardiovascular disease, chronic kidney disease, magnesium, mortality

KEY LEARNING POINTS

What was known:

- In patients with chronic kidney disease (CKD), previous studies have shown an association between high or low serum total magnesium (tMg) levels and all-cause mortality, with conflicting findings regarding cardiovascular (CV) events.
- However, no study has investigated the association between ionized Mg (iMg) and CV events or mortality in patients with CKD.

This study adds:

- In 3033 patients with moderate-to-advanced CKD, individuals with elevated serum tMg concentrations, although still within the normal range, had a greater likelihood of major adverse cardiovascular events and mortality, even after adjustment for confounding factors.
- We failed to show an association between serum iMg on one hand and the occurrence of CV events or mortality on the other.

Potential impact:

- tMg is routinely assayed in hospital laboratories and might be a useful variable to monitor in CKD patients.
- The safety of magnesium supplementation requires further evaluation.

INTRODUCTION

Chronic kidney disease (CKD) is considered a major public health problem worldwide [1]. All patients with CKD should be considered to have a high risk of cardiovascular disease (CVD), although the underlying pathophysiological mechanisms are not fully understood [2, 3]. Indeed, conventional cardiovascular (CV) risk factors do not appear to fully explain the elevated risk of CVD.

Magnesium is the fourth most abundant cation in the body. Although only about 1% of the total magnesium (tMg) is located outside the cells, this is nevertheless a major fraction because it is involved in a multitude of enzyme reactions and essential physiological processes [4–6], such as the inhibition of vascular smooth muscle cell calcification [7], the regulation of inflammation and immune responses [8]. Magnesium homeostasis depends mainly on absorption through the intestine and excretion by the kidney [9]. In late-stage CKD, the growing impairment in renal excretion typically leads to the accumulation of magnesium in the blood [10]. However, hypomagnesaemia is also common in patients with CKD, due to variations in dietary magnesium intake and the use of drugs that can induce hypomagnesaemia (such as thiazide diuretics and proton pump inhibitors) [9, 11].

In serum, around 20%–30% of the tMg in circulation is bound to proteins and is believed to be biologically inert. Ionized magnesium (iMg) comprises roughly 60%–70% of tMg in circulation and is regarded as the active form of magnesium. It is plausible that iMg could serve as a more physiologically significant marker compared with tMg. The total serum magnesium concentration is most frequently used in routine clinical practice [12]. In fact, the fraction of iMg is not usually measured and is difficult to calculate [13, 14]. Recent studies of various non-CKD cohorts have evidenced a moderate correlation between tMg and iMg [15, 16]. Given the variations and/or impairments in serum binding proteins observed in patients with CKD, one could expect to find the same moderate correlation in this population. However, data on levels of iMg in patients with CKD are lacking.

A large body of data from studies of the general population shows that both hypermagnesaemia and hypomagnesaemia are associated with greater mortality and worse CVD outcomes [17–20]. Along the same lines, the results of a registry-based study of a cohort of more than 140 000 haemodialysis patients highlighted a U-shaped relationship between tMg levels and both CVD and non-CVD mortality rates [21]. All-cause mortality is the most frequently studied outcome in CKD populations [22–29]. The association between magnesium levels and CV outcomes has not been frequently evaluated, and the few published data are conflicting [22, 23, 25, 28]. Furthermore, most studies of the link between magnesium and patient outcomes have focused on tMg concentrations and not iMg, and some studies have reported that tMg and iMg are not related in the same way to the predicted clinical outcomes [15, 16].

Thus, the objectives of the present study were to (i) describe the serum iMg concentration in patients at various CKD stages, (ii) measure the correlation between iMg and tMg concentrations, (iii) identify associated factors of the iMg concentration and (iv) determine whether serum total and/or iMg concentrations are associated with adverse outcomes (such as fatal or non-fatal major CV events and mortality) before kidney replacement therapy (KRT, defined as the initiation of chronic dialysis, or kidney transplantation) in patients with CKD, after adjustment for the estimated glomerular filtration rate (eGFR) and other risk factors.

MATERIALS AND METHODS

The results of this cohort study are reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline [30].

Study design and participants

Chronic Kidney Disease–Renal Epidemiology and Information Network (CKD-REIN) is a French prospective cohort of adult patients (>18 years of age) with a confirmed diagnosis of CKD, with eGFR <60 mL/min/1.73 m^2 (stage 3–5), who were not on maintenance dialysis nor had undergone kidney transplantation by study entry. Details of the study protocol have already been published [31]. Patients were recruited from 40 nationally representative nephrology outpatient facilities in France with respect to geography and facility legal status, public or private. A total of 3033 patients were included during a routine nephrology outpatient appointment between 2013 and 2016, and were followed up by clinical research associates (CRAs) for 5 years or until 31 December 2020. The study protocol was approved by the institutional review board at the French National Institute of Health and Medical Research (INSERM; reference: IRB00003888) and was registered at ClinicalTrials.gov (NCT03381950).

For the purposes of the present analysis, we excluded patients who did not have a serum collection at baseline (n = 442), patients for whom the tMg assay could not be performed (n = 3) and patients for whom serum samples were collected >90 days after inclusion in the study (n = 169). Hence, a total of 2419 patients were included in the present analysis (Supplementary data, Fig. S1).

Study data

CRAs collected data from patient interviews and medical records at baseline and then annually. The patients' characteristics were recorded, and the patients were screened for a history of hypertension, diabetes and CVD. Height and weight were measured to calculate body mass index (BMI). A specific electronic case report form (linked to the international Anatomical Therapeutic and Chemical thesaurus) was used by the CRAs to record the drugs prescribed to patients in the 3 months prior to the enrolment visit. Standard blood and urine tests (those recommended by the French health authorities for the routine management of CKD) were carried out for all patients in their usual medical laboratory. The GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation [32]. The urinary albumin-to-creatinine ratio (ACR) was determined by either measurement or estimation using an equation based on proteinuria measurements [33]. We therefore classified patients according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guideline stages as follows: A1 (normal or minimal increase), ACR <3 mg/mmol or albumin excretion rate (AER) <30 mg/24 h; A2 (moderate increase), ACR 3–30 mg/mmol or AER 30–300 mg/24 h; A3 (severe increase), ACR = 30 mg/mmol or AER = 300 mg/24 h [34].

tMg and iMg measurements

Serum samples were collected at the time of patient's enrolment, stored at 4°C, and aliquoted within 6 h without further processing. All samples were stored at –80°C at the Biobanque de Picardie (Amiens-Picardie University Hospital, Amiens, France; BRIF number: BB-0033-00017). We used direct potentiometry

with an ion-selective electrode (Stat Profile® PRIME™ ES Comp, Nova Biomedical, Waltham, MA, USA) to assay iMg levels. In healthy adults, the serum iMg concentration ranges from 0.43 to 0.54 mmol/L [35]. We used a photometric technique (a modified xylidyl blue reaction) to assay tMg assays in the same serum samples (Atellica® CH, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA; reference range 0.66–1.07 mmol/L). For three patients whose serum iMg concentration was below the assay's limit of quantification (0.10 mmol/L), we set the value of 0.10 mmol/L. Hypomagnesaemia was defined as a tMg concentration below 0.66 mmol/L, and hypermagnesaemia as a magnesium concentration equal or above 1.07 mmol/L.

Study outcomes

Hospitalizations occurring during study follow-up were identified from medical reports, hospital records and/or patient interview; deaths were ascertained from death certificates, hospital records, reports by family members and by linkage with the national vital status registry. A physician reviewed and coded all events using the International Classification of Diseases, Tenth Revision. Then, based on these codes, this physician identified and assessed CV events according to the Cardiovascular and Stroke Endpoint Definitions for Clinical Trials [36]. One senior cardiologist further adjudicated all CV deaths.

The primary endpoint was the occurrence of the first fatal or non-fatal major CV event (MACE), defined as any CV death, myocardial infarction, stroke and hospitalization for heart failure. The secondary endpoint was the occurrence of death (i) whatever the cause and (ii) from CV cause.

Only events that occurred before KRT were analysed. KRT events were defined as initiation of maintenance dialysis or pre-emptive kidney transplantation, identified from medical records, patient interviews, and/or by linkage with the national REIN registry. KRT events and non-CV deaths before KRT were considered competing events for CV events and CV mortality. Patients were censored at the date of the competing event, the end of their 5-year follow-up, or at study loss to follow-up.

Statistical analysis

Baseline characteristics were described first for the study population as a whole ($n = 2419$). We then compared subgroups of patients by the serum tMg tertiles at baseline [Tertile 1 (T1), ≤0.73 mmol/L; T2, 0.74–0.81 mmol/L; T3, >0.81 mmol/L]. Continuous variables were reported as the mean [standard deviation (SD)] or the median (first–third quartiles), depending on the distribution. Categorical variables were reported as the frequency (percentage). Depending on the distribution, we used a chi-squared test or Fisher's exact test to compare values of categorical variables, and an analysis of variance or the Kruskal–Wallis test to compare values of continuous variables.

Multivariable linear regressions (based on sociodemographic variables, clinical variables and prescription drugs) were used to estimate independent associations with the serum iMg concentration and the serum tMg concentration (expressed as a non-standardized coefficient [95% confidence interval (CI)], and as a standardized coefficient). Our choice of the variables included in multivariable models was based on a literature review and a P-value <.2 in a univariable analysis. Hypomagnesaemic drugs were those negatively associated with magnesium concentration in multivariable models.

We used cause-specific Cox models to test the crude and adjusted associations between the risk of a first CV event on one hand and the serum ionized, tMg concentration at baseline on the other. Cox models were adjusted for a set of confounding factors selected from a directed acyclic graph (Supplementary data, Fig. S2) [37]. This approach enables the selection of an optimal set of adjustment factors [i.e. closing non-causal pathways between exposure (magnesaemia) and outcome (CV event)]. The selected covariates were: age, sex, history of CVD, diabetes, dyslipidaemia, BMI, pulse pressure, eGFR, levels of serum urea, sodium, potassium, bicarbonate, calcium, phosphate, serum albumin, parathyroid hormone (PTH) and haemoglobin, and prescriptions of renin–angiotensin system inhibitors, hypomagnesaemic drugs [thiazide diuretics, proton pump inhibitors (PPIs) and/or potassium chelators] and other diuretics (defined as all diuretics other than thiazide diuretics, such as high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists). We also tested the association between the baseline serum magnesium concentration (ionized and total) and all-cause mortality, as well as CV mortality. The adjustment factors were the same as those used to assess the risk of MACE (Supplementary data, Fig. S3). We tested the proportional hazards hypothesis by analysing the Schoenfeld residuals for each model. Using restricted cubic splines in the adjusted Cox models [38], we investigated the functional relationship between the serum magnesium concentration (ionized and total) and the CV risk, all-cause and CV mortality.

After assuming that data were missing at random, we managed missing covariate data with multivariate imputation by chained equations with predictive mean matching in the mice package in R [39, 40]. A total of 25 datasets were created, and the number of iterations was set to 20. All covariates present in the final Cox models were included in the imputation model. Fitted Cox models were generated for each dataset, and pooled regression coefficients were obtained using Rubin's rules.

All tests were two-tailed, and the threshold for statistical significance was set to $P < .05$. All statistical analyses were performed with R software (version 4.1.3) [40].

RESULTS

Characteristics of the patients at baseline

Of the 3033 patients enrolled in the CKD-REIN cohort, 2419 were analysed (Supplementary data, Fig. S1). The median age was 68 (60–76) years, 66% of the patients were men and the mean (SD) eGFR was 34.8 (13.4) mL/min/1.73 m^2; 52% of the patients had a history of CVD and 41% had diabetes (Table 1). For the study population as a whole, the mean (SD) serum tMg and iMg concentrations were, respectively, 0.77 (0.10) mmol/L and 0.48 (0.09) mmol/L. Compared with patients in T1 and T2, patients in T3 were older, were more likely to have a history of CVD history, had higher serum phosphate, PTH and urea concentrations, and had a lower baseline eGFR. However, patients in T1 had more prescribed daily medications, including higher use of PPI, thiazide diuretics or potassium chelators, and had more frequently diabetes than those of T2 or T3. A small but significant negative correlation was found for serum magnesium (ionized and total) and the eGFR ($r = –0.19$, $P < .001$ for both total and ionized). The more advanced CKD stages were associated with higher serum ionized and tMg concentrations (Table 2).

Associations between iMg and tMg

The serum ionized and tMg concentrations were strongly correlated ($r = 0.89$, $P < .001$). The vast majority of patients had a

Table 1: Baseline characteristics of the study population.

		tMg (mmol/L)				
	Total (N = 2419)	T1 ≤0.73 (N = 829)	T2 0.74–0.81 (N = 837)	T3 >0.81 (N = 753)	P-value	Imputed data (N = 2419) (%)
tMg (mmol/L)	0.77 (0.10)	0.66 (0.07)	0.78 (0.02)	0.88 (0.06)	<.001	0
iMg (mmol/L)	0.48 (0.09)	0.40 (0.08)	0.49 (0.04)	0.57 (0.07)	<.001	0
Age (years)	68 (60; 76)	67 (58; 75)	68 (60; 75)	70 (63; 77)	<.001	0
Men (%)	66	68	68	63	.049	0
Level of studies (%)					.23	1
<9 years	14	16	14	13		
9–11 years	49	48	48	52		
≥12 years	36	36	38	35		
Alcohol abuse	1	2	1	1	.16	0.7
Smoking status (%)					.12	0.6
Non-smoker	40	40	38	44		
Current	12	13	14	10		
Past	47	48	48	46		
Hypertension (%)	96	97	96	96	.46	0.2
Diabetes (%)	41	51	39	33	<.001	0.2
Dyslipidaemia (%)	66	68	68	63	.35	0.4
History of CVD (%)	52	50	51	56	.04	0.5
Gastrointestinal bleeding (%)	4	4	3	4	.65	4.3
BMI (kg/m^2)	28.8 (5.8)	29.6 (6.0)	28.5 (5.6)	28.1 (5.7)	<.001	1.9
Systolic blood pressure (mmHg)	142 (21)	143 (21)	143 (20)	141 (21)	.13	2.1
Pulse pressure (mmHg)	64 (19)	64 (19)	64 (18)	64 (19)	.88	2.3
eGFR (mL/min/1.73m^2)	34.8 (13)	36.9 (14)	36.1 (14)	31.0 (12)	<.001	1.4
Albumin- or protein-to-creatinine ratio classes (%)					.02	8.5
Normal or minimal increase (A1)	28	25	30	30		
Moderate increase (A2)	32	31	31	34		
Severe increase (A3)	40	44	39	37		
CRP (mg/L)	2.5 (1.1; 5.9)	2.7 (1.2; 6.1)	2.4 (1.0; 5.4)	2.6 (1.3; 6.5)	.12	0.8
Sodium (mmol/L)	140 (2.8)	140 (2.8)	140 (2.7)	140 (2.8)	.90	0.5
Potassium (mmol/L)	4.5 (0.5)	4.5 (0.5)	4.6 (0.5)	4.5 (0.5)	.10	0.4
Bicarbonates (mmol/L)	24.9 (3.4)	24.5 (3.4)	25.0 (3.4)	25.2 (3.4)	<.001	8.9
Calcium (mmol/L)	2.35 (0.13)	2.34 (0.15)	2.36 (0.12)	2.35 (0.12)	<.001	2.5
Phosphates (mmol/L)	1.15 (0.23)	1.14 (0.24)	1.14 (0.21)	1.18 (0.24)	<.001	3.8
Serum albumin (g/L)	40.6 (4.1)	39.8 (4.3)	40.8 (4.0)	41.1 (3.8)	<.001	0.1
Urea (mmol/L)	13.3 (10.2; 18.1)	12.8 (10.1; 16.9)	12.9 (9.80; 17.2)	14.8 (11.1; 20.5)	<.001	6.7
Total cholesterol (mmol/L)	4.83 (1.29)	4.74 (1.29)	4.91 (1.30)	4.84 (1.27)	.04	9.3
HDL-cholesterol (mmol/L)	1.32 (0.47)	1.25 (0.44)	1.32 (0.48)	1.38 (0.47)	<.001	10.0
LDL-cholesterol (mmol/L)	2.69 (1.09)	2.58 (1.08)	2.76 (1.10)	2.73 (1.08)	.005	11.8
Triglycerides (mmol/L)	1.53 (1.10; 2.25)	1.72 (1.19; 2.52)	1.51 (1.08; 2.22)	1.45 (1.04; 1.98)	<.001	9.6
Haemoglobin (g/dL)	13.0 (1.7)	12.9 (1.7)	13.3 (1.7)	12.9 (1.6)	<.001	0.8
PTH (pg/mL)	79 (49; 129)	73 (46; 122)	73 (46; 118)	95 (58; 150)	<.001	12.9
25-OH vitamin D (ng/mL)	28 (19; 37)	27 (18; 36)	28 (21; 37)	28 (20; 37)	.24	10.6
Number of prescriptions per patient	8 (5; 10)	8 (6; 11)	7 (5; 10)	8 (5; 10)	<.001	0.3
PPIs (%)	32	39	30	28	<.001	0.3
Diuretics (%)	54	53	51	59	.01	0.3
Thiazide diuretics	21	27	21	16	<.001	0.3
High-ceiling diuretics	35	27	33	46	<.001	0.3
Potassium-sparing diuretics	5	5	4	5	.49	0.3
Beta-blockers (%)	42	43	40	43	.38	0.3
Calcium channel blockers (%)	47	45	48	48	.43	0.3
Renin–angiotensin system inhibitors (%)	76	80	78	70	<.001	0.3
Corticosteroids (%)	7	7	6	8	.42	0.3
Potassium chelator (%)	13	15	12	12	.049	0.3
Phosphate binders (%)	4	3	3	5	.06	0.3
Magnesium supplementation (%)	0.4	0.4	0.5	0.4	.93	0.3
Medications for constipation (%)	4	3	4	6	.02	0.3
Lithium (%)	0.2	0.02	0.2	0.3	.34	0.3

Data are presented as the mean (SD) or the median (first–third quartiles), or frequency (%).
CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2: Serum iMg and tMg concentrations, by CKD stage.

		CKD stage			
	Total (*N* = 2394)	Stage 2–3A (*N* = 531)	Stage 3B (*N* = 884)	Stage 4–5 (*N* = 979)	*P*-value
iMg (mmol/L)	0.48 (0.09)	0.46 (0.08)	0.47 (0.09)	0.50 (0.10)	<.001
tMg (mmol/L)	0.77 (0.10)	0.74 (0.09)	0.76 (0.10)	0.79 (0.11)	<.001

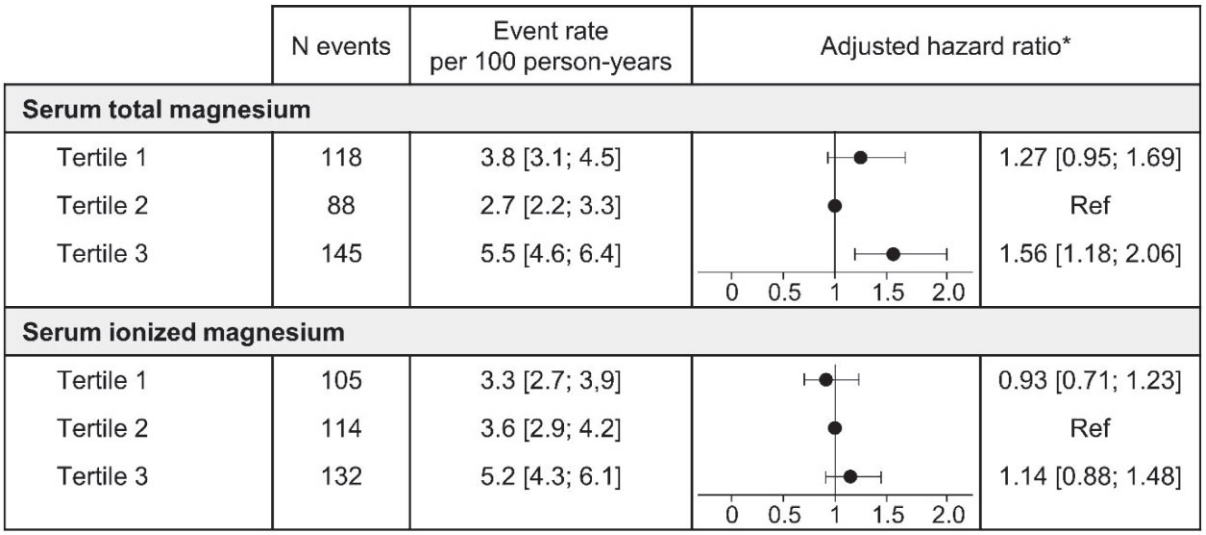


Figure 1: Number of events, incidence rates and adjusted HRs for MACEs, as a function of the baseline serum tMg and iMg concentrations. Serum tMg: T1 (≤0.73 mmol/L), T2 (0.74–0.81 mmol/L), T3 (>0.81 mmol/L). Serum iMg: T1 (≤0.45 mmol/L), T2 (0.46–0.52 mmol/L), T3 (>0.52 mmol/L). HRs are adjusted for age, sex, BMI, diabetes, dyslipidaemia, pulse pressure, eGFR, history of CVD, levels of serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, PTH and haemoglobin, the use of renin–angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists) and hypomagnesaemic drugs (thiazide diuretics, PPIs and/or potassium chelators).

normal tMg concentration (88%); 11.5% showed hypomagnesaemia (<0.66 mmol/L) and only 0.5% showed hypermagnesaemia (≥1.07 mmol/L). In T1 for iMg, one-third of patients showed hypomagnesaemia and two-thirds were in the normal range of tMg levels (Supplementary data, Table S1). In T2, over 99% of the patients were in the normal range. In T3, only 2% of patients showed hypermagnesaemia, and 98% were in the normal range.

Factors associated with magnesium levels

In linear regression models, both ionized and tMg levels were independently positively associated with age, serum levels of phosphate, albumin and urea, and prescriptions for high-ceiling diuretics and laxatives; and negatively associated with diabetes, BMI, eGFR, serum levels of triglycerides, and prescriptions for PPIs and potassium chelators. Additionally, the serum iMg level was negatively associated with systolic blood pressure, serum levels of calcium and prescriptions for thiazide diuretics (Supplementary data, Table S2), while the serum tMg level was positively associated with the serum calcium and bicarbonate, and negatively associated with sodium and 25-OH vitamin D levels (Supplementary data, Table S3). Diabetes, prescription for high-ceiling diuretics, serum urea levels, prescriptions for PPIs and potassium chelators were the variables that most strongly predicted iMg levels (Supplementary data, Table S2). For tMg levels, the best predictors were prescriptions for high-ceiling diuretics, diabetes, urea and serum albumin levels, and kidney function (Supplementary data, Table S3).

Major CV events

Over a median (interquartile range) follow-up period of 4.9 (3.3; 5.1) years, 351 patients experienced a first MACE. The crude incidence rate (95% CI) was 3.9 (3.5; 4.3) per 100 person-years. The incidence rate was higher in patients in T3 and (to a lesser extent) in T1 than in patients in T2 (Fig. 1, Supplementary data, Table S4). The adjusted hazard ratio (HR) (95% CI) for MACEs associated with the baseline serum tMg level was 1.27 (0.95; 1.69) for patients in T1 and 1.56 (1.18; 2.06) for patients in T3, relative to patients in T2 (Fig. 1, Supplementary data, Table S5). A restricted cubic spline analysis showed that the relationship between the serum tMg level and the MACE risk was non-linear, with a greater risk at high concentrations (Fig. 2A).

The adjusted HR (95% CI) for MACEs associated with the baseline serum iMg level was 0.93 (0.71; 1.23) for patients in T1 and 1.14 (0.88; 1.48) for patients in T3, relative to patients in T2 (Fig. 1, Supplementary data, Table S6). Restricted cubic spline analysis confirmed this non-significant trend (Fig. 2B).

Mortality

In our study, 314 patients died before KRT resulting in a crude incidence rate (95% CI) of 3.3 (3.0; 3.7) per 100 person-years.

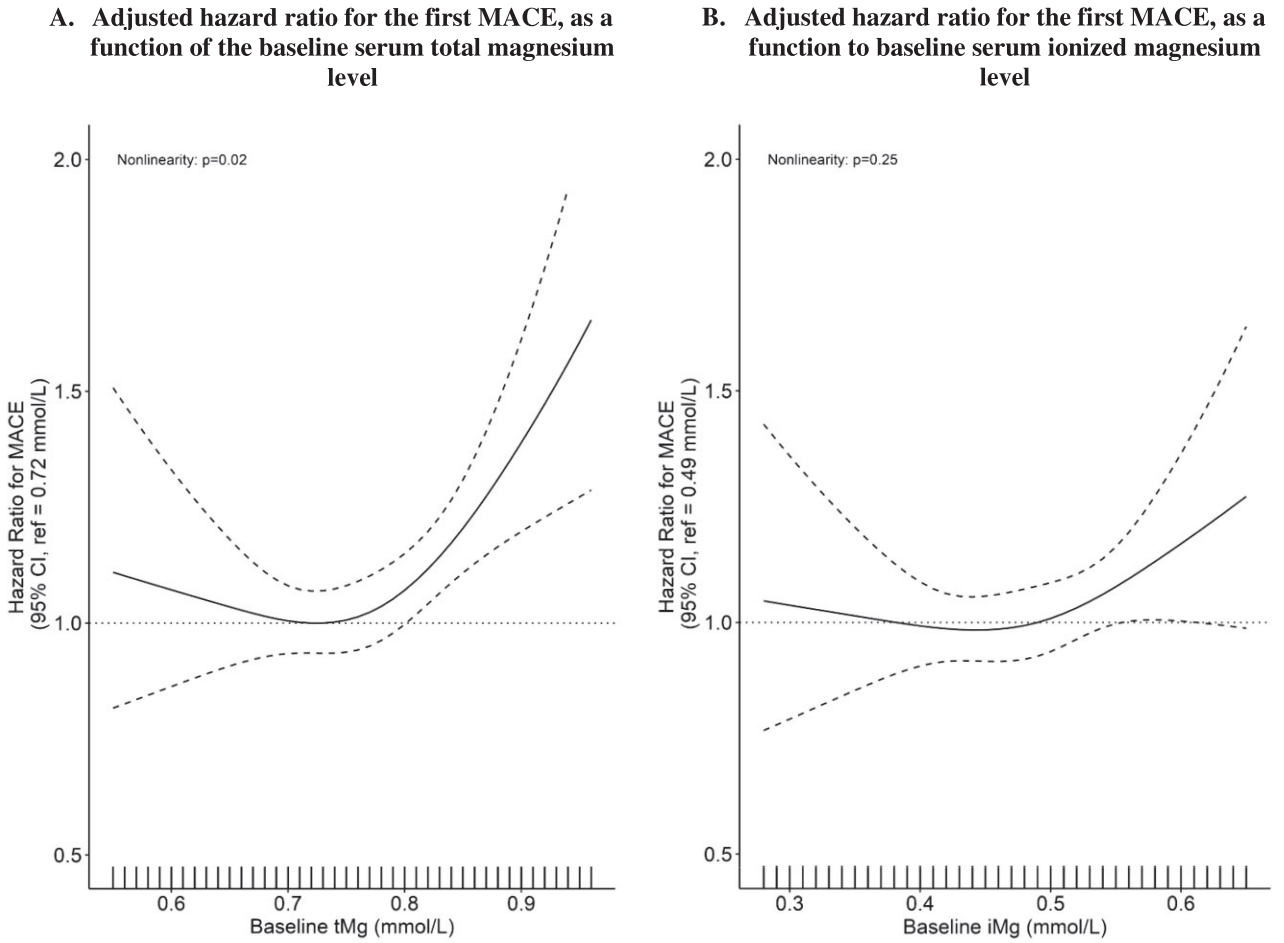


Figure 2: Adjusted HRs for the occurrence of the first MACE, as a function of the baseline serum tMg and iMg concentrations, using restricted cubic spline term. (**A**) Adjusted HR for the first MACE, as a function of the baseline serum tMg level. (**B**) Adjusted HR for first MACE, as a function of baseline serum iMg level. HRs are adjusted for age, sex, BMI, diabetes, dyslipidaemia, pulse pressure, eGFR, history of CVD, levels of serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, PTH and haemoglobin, the use of renin–angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists) and hypomagnesaemic drugs (thiazide diuretics, PPIs and/or potassium chelators). Dotted lines correspond to the 95% CI. The lines on the *x*-axis correspond to patient values. The 5% most extreme values of serum magnesium concentrations have been removed in this graph.

The incidence rate was higher in patients in T3 and (to a lesser extent) T1 than in patients in T2 for both ionized and tMg (Fig. 3, Supplementary data, Table S4). After multiple adjustments, the risk of death before KRT according to serum tMg concentration was 1.11 (0.82; 1.50) for patients in T1 and 1.48 (1.11; 1.97) for patients in T3, compared with patients in T2 (Fig. 3, Supplementary data, Table S7). The adjusted risk of death before KRT according to serum iMg concentration did not differ significantly from one tertile to another (Fig. 3, Supplementary data, Table S8). The same trends have been found when the analysis was restricted to CV mortality, the risk being increased in patients in T3 of serum tMg [HR (95% CI) 1.98 (1.27; 3.10)] and non-significantly in patients in T1 [HR (95% CI) 1.38 (0.85; 2.25)], relative to patients in T2 (Fig. 4, Supplementary data, Table S9). The risk of CV death was not found to be significantly associated with serum iMg levels in our study (Fig. 4, Supplementary data, Table S10). Restricted cubic spline analyses confirmed these trends (Supplementary data, Figs S4 and S5).

DISCUSSION

Our analysis of 2419 non-dialysed CKD patients from a large prospective cohort showed that both ionized and tMg levels increased with the CKD stage, were strongly correlated, and were associated with similar factors. Higher serum tMg levels, although still within the normal range, were significantly associated with an elevated risk of MACEs, all-cause mortality and CV mortality. However, the serum iMg level was not associated with these outcomes.

In our study, the serum magnesium concentrations (iMg and tMg) tended to rise with the CKD stage; indeed, magnesium excretion is known to decrease as renal function declines [10]. The correlation between iMg and tMg concentrations was very strong ($r = 0.89$; $P < .001$). This finding contrasts with published data on intensive care unit patients, where the correlation was weak and up to 85% of the patients with a low tMg level had a normal iMg level [15]. However, our finding is in line with the results of a small, single-centre study of chronic haemodialysis patients ($n = 42$), in which the ionized and tMg levels were

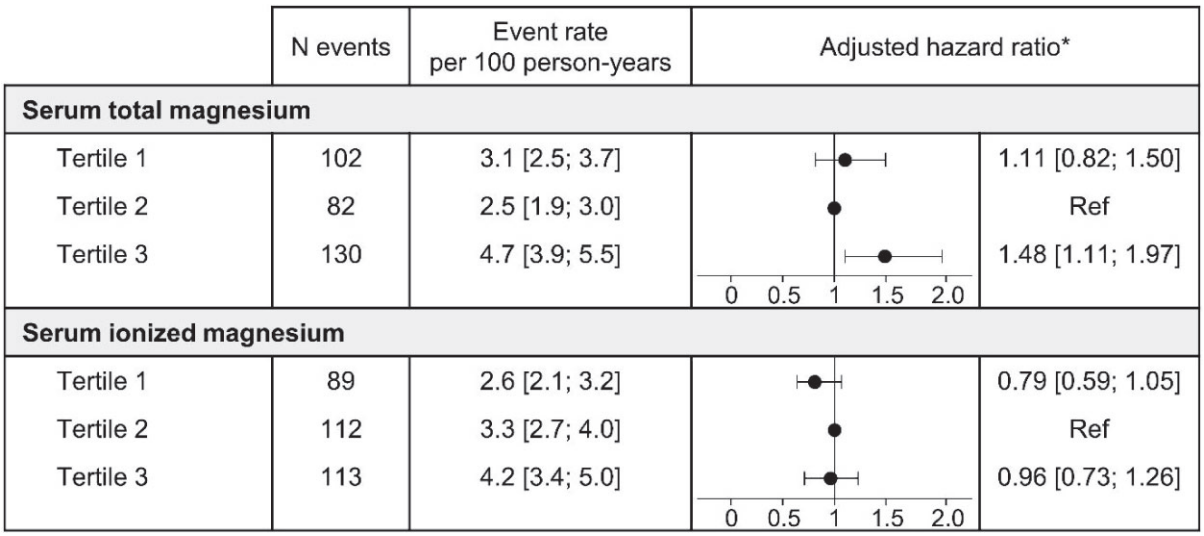


Figure 3: Number of events, incidence rates and adjusted HRs for overall mortality, as a function of the baseline serum tMg and iMg concentrations. Serum tMg: T1 (≤0.73 mmol/L), T2 (0.74–0.81 mmol/L), T3 (>0.81 mmol/L). Serum iMg: T1 (≤0.45 mmol/L), T2 (0.46–0.52 mmol/L), Tertile 3 (>0.52 mmol/L). HRs are adjusted for age, sex, BMI, diabetes, dyslipidaemia, pulse pressure, eGFR, history of CVD, levels of serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, PTH and haemoglobin, the use of renin–angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists) and hypomagnesaemic drugs (thiazide diuretics, PPIs and/or potassium chelators).

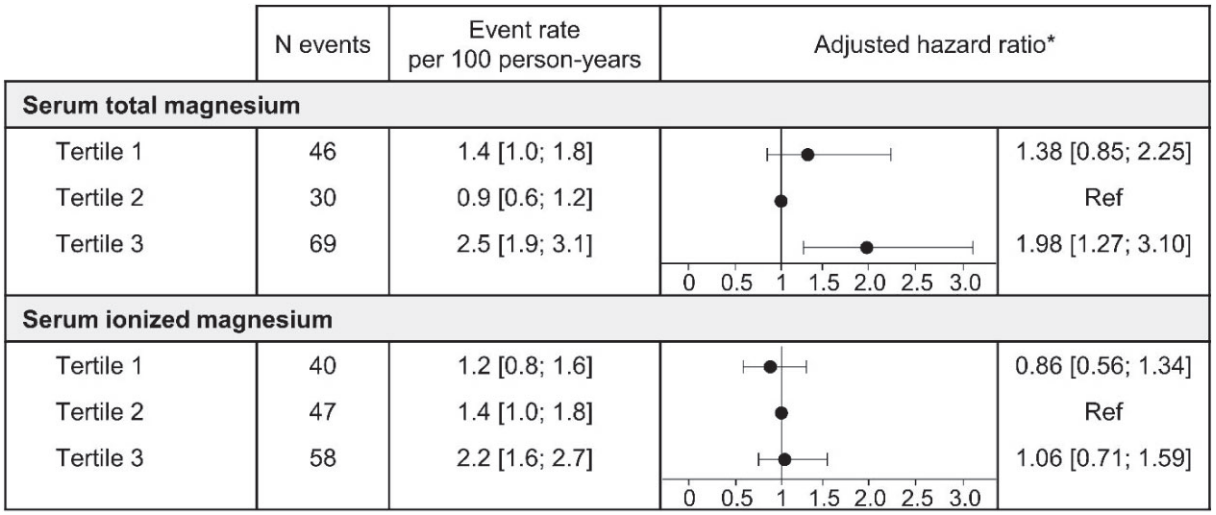


Figure 4: Number of events, incidence rates and adjusted HRs for CV mortality, as a function of the baseline serum tMg and iMg concentrations. Serum tMg: T1 (≤0.73 mmol/L), T2 (0.74–0.81 mmol/L), T3 (>0.81 mmol/L). Serum iMg: T1 (≤0.45 mmol/L), T2 (0.46–0.52 mmol/L), T3 (>0.52 mmol/L). HRs are adjusted for age, sex, BMI, diabetes, dyslipidaemia, pulse pressure, eGFR, history of CVD, levels of serum urea, sodium, potassium, bicarbonates, calcium, phosphates, albumin, PTH and haemoglobin, the use of renin–angiotensin system inhibitors, diuretics (high-ceiling diuretics, potassium-sparing diuretics and/or aldosterone antagonists) and hypomagnesaemic drugs (thiazide diuretics, PPIs and/or potassium chelators).

strongly correlated ($r = 0.88$; $P < .001$) [41]. It is noteworthy that the degree of inter-individual variability was lower for iMg than for tMg.

We found that serum magnesium concentrations were not particularly elevated, as might have been the case in patients with a decreased kidney function [10]. This could be due, at least in part, to the use of hypomagnesaemic drugs [9]. We found that the use of PPIs, thiazide diuretics and potassium chelators was associated with a decrease of serum magnesium levels, either ionized for thiazide diuretics, or both total and ionized for PPIs and potassium chelators. Indeed, hypomagnesaemia is a known adverse effect of these classes of medications, and is mentioned in the summaries of product characteristics. Furthermore, around 40% of patients suffered from diabetes, a factor known to be associated with a decrease in magnesaemia [42]. These factors could partially explain why patients in our cohort have magnesium concentrations close to normal. Overall, we found that ionized and tMg were associated with the same factors: age, eGFR, diabetes, BMI, serum calcium, phosphate, albumin, urea and triglycerides, and prescriptions of PPIs, high-ceiling diuretics, potassium chelators and laxatives. These predictors of magnesium concentration have already been described in

the literature for tMg levels [22, 24, 25, 43, 44]. So, we extended these findings for iMg levels.

With regard to the reference values for tMg (0.66–1.07 mmol/L), 11.5% of patients showed hypomagnesaemia and 0.5% showed hypermagnesaemia. Similar values were found in a study of more than 10 000 patients (at CKD stages 3–4) in the Cleveland Clinic registry: 12.4% of the patients had a magnesium concentration <1.7 mg/dL (0.70 mmol/L) and 1.9% had a concentration >2.6 mg/dL (1.07 mmol/L) [24].

Our present study highlighted an elevated risk of MACE and mortality in T3 of the serum tMg concentration. Of note, the elevation of tMg is moderate (>0.81 mmol/L). A similar, non-significant trend was found for the serum iMg level and the risk of MACE. In a study of more than 65 000 hospitalized patients, a U-shaped association between the tMg level and mortality was found [19]. A registry-based study of a large cohort of haemodialysis patients also found that high levels of tMg were associated with both CVD and non-CVD mortality [21]. Of note, the levels of tMg in this dialysis cohort were higher than in our non-dialysed CKD population. In studies of non-dialysed CKD patients, the tMg level (but not the iMg level) has sometimes (but not always) been linked to mortality or CV disease. Some studies (such as the present one) have found an association between a high tMg level on one hand and CV events [23] and all-cause mortality [22–24] on the other, whereas other studies failed to show an association with CV outcomes [22, 25]. Furthermore, low tMg levels have been associated with mortality in CKD populations [22, 24, 25, 27]. In the present study, we failed to find an association between low tMg levels and studied outcomes.

Moreover, we failed to show an association between serum iMg on one hand and the occurrence of CV events or mortality on the other. This lack of association with iMg (the biologically active fraction) might suggest that serum tMg is not causally related to MACE and mortality, and that confounding factors affecting both tMg and the occurrence of these events caused the significant association in our study. The ionized fraction of magnesium might not be affected by these unknown confounding factors, which would explain why the association with MACE and mortality is not significant in our study. In contrast to tMg, iMg is not routinely assayed in hospital laboratories and is used in a research setting only. Furthermore, the measurement of iMg is not straightforward because the result can be influenced by other ions (especially calcium), temperature, pH, dilution of the sample and/or the lack of normative reference values [45].

It has been suggested that magnesium supplementation can reduce the CV burden in general and the vascular calcification burden in CKD patients in particular. A randomized, controlled trial in patients with stage 3–4 CKD showed that the median in the coronary artery calcification (CAC) score at 2 years was significantly smaller in participants taking magnesium oxide than in controls [46]. In another recent, randomized, controlled trial, supplementation with magnesium hydroxide for 12 months did not slow the progression of CAC (relative to a placebo) in 148 patients with stage 3b–4 CKD [47]. It is noteworthy that although no MACEs were reported in the placebo group, six (three sudden cardiac deaths, two strokes and one incident heart failure) were reported in the magnesium group. Hence, the safety of magnesium supplementation requires further evaluation, in addition to determination of whether lowering tMg concentrations will impact outcomes in the setting of kidney disease.

The present study had several strengths. First, it is (to the best of our knowledge) the first to have investigated the association between both serum tMg and iMg concentrations and the occurrence of CV events and mortality in CKD patients not undergoing KRT. Secondly, our analysis of a large number of patients from a nationally representative, multicentre, prospective cohort enabled comprehensive adjustments for confounding factors. Thirdly, the serum iMg and tMg concentrations were measured centrally, which probably reduced the variability between measurements. Lastly, all CV events were assessed carefully, according to standardized definitions.

Our study also had some limitations. First, 99% of the body's magnesium is located in the intracellular space (mainly in the bones), and so only 1% is present in extracellular fluids. Hence, the serum magnesium concentration might not adequately reflect the body's magnesium status; it might be more relevant to measure the intracellular magnesium concentration in specific tissues or cell types. However, this evaluation is particularly complex and, in contrast to a tMg assay, is not carried out on a routine basis. Secondly, the magnesium concentrations were measured once (at baseline), and so we were not able to explore putative associations between time-varying concentrations and outcomes. Lastly, the lack of data on the participants' diet prevented us from adjusting our analyses for the magnesium intake through diet.

In conclusion, we highlighted a strong correlation between total and iMg levels. Our analysis of a large cohort of patients with moderate-to-advanced CKD demonstrated that (i) elevated serum tMg concentrations (albeit still within the normal range) were associated with a greater likelihood of MACE and mortality but (ii) elevated serum iMg levels were not associated with these outcomes. Moreover, the safety of magnesium supplementation requires further evaluation.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work—even one in which the author was not directly involved—are appropriately investigated and resolved, including with documentation in the literature if appropriate.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

M.P., S.M.L., S.L., S.K. and M.M. have nothing to declare. Z.A.M. reports having received grants for CKD-REIN and other research projects from Amgen, Baxter, Fresenius Medical Care, GlaxoSmithKline, Merrck Sharp & Dohme-Chibret, Sanofi-Genzyme, Lilly, Otsuka, AstraZeneca, Vifor and the French government, as well as fees and grants to charities from AstraZeneca, Boehringer Ingelheim and GlaxoSmithKline. N.A.P. declare financial support from pharmaceutical companies integrating the public–private partnership of the CKD-REIN cohort: Fresenius Medical Care, GlaxoSmithKline (GSK), Vifor France and Boeringher Ingelheim; all grants are made to Paris Saclay University.

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