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ORIGINAL RESEARCH ARTICLE

Long-Term Proton Pump Inhibitor Use is Associated with Vascular Calcification in Chronic Kidney Disease: A Cross-Sectional Study Using Propensity Score Analysis

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Abstract
Background Proton pump inhibitors (PPIs) are a class of drugs that is extensively used for common gastrointestinal disorders and often prescribed long-term for years. Long-term PPI treatment is associated with an increased risk of fractures in the general population. Several studies have suggested a relationship between vascular calcification, which is a predictor of cardiovascular morbidity and mortality, impaired bone metabolism and fractures. In dialysis patients, vascular calcifications are widespread and are connected to bone health.
Objective The aim of this study was to assess the association between the use of PPIs and vascular calcifications involving the aorta and iliac arteries in haemodialysis patients.
Methods Between November 2008 and November 2009, 387 patients receiving long-term dialysis treatment ($\geq$1 year) were enrolled in a multicentre (18 Dialysis Units), cross-sectional study. Overall, 76.2 % of patients were receiving long-term PPI treatment. The main outcome measure was calcification of the aorta and iliac arteries in relation to PPI use. Standardized radiographs were sent to the coordinating centre for centralized evaluation in duplicate by two physicians who were blind to PPI status.
Results Arterial calcifications were significantly more common in the PPI group ($p < 0.01$). Also, the rates of aortic and iliac calcifications considered separately were higher (+12.2 %, $p = 0.0254$; and +13.6 %, $p = 0.0211$, respectively). After correction for the propensity score, the odds ratios [ORs] (95 % CI) related to PPI use were aorta 1.89 (1.01–3.54), $p = 0.048$; iliac arteries 2.27 (1.31–3.92), $p = 0.003$; aorta and iliac arteries 2.59 (1.48–4.53), $p = 0.008$. The ORs (95 % CI) related to the association of warfarin + PPI were aorta 2.19 (0.95–5.00), $p = 0.06$; iliac arteries 2.90 (1.07–7.86), $p = 0.036$; aorta and iliac arteries 2.69 (1.03–6.96), $p = 0.042$.
Conclusion In haemodialysis patients, long-term treatment with PPIs, especially in the presence of warfarin treatment, is associated with vascular calcifications.

For the PPI-VC (Proton Pump Inhibitors-Vascular Calcification) Study Group.

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1 Introduction

Proton pump inhibitors (PPIs) are a class of drugs that is extensively used for common gastrointestinal disorders where the inhibition of gastric acid secretion is desirable, such as gastroduodenal ulcer, dyspepsia and gastroesophageal reflux disease. They are often prescribed long-term for years, as they are considered to be well tolerated drugs with a very good safety profile [1]. Long-term PPI treatment has been found to be associated with an increased risk of hip fracture after 1 year of treatment [2]; the strength of the association increases over time and after 7 years or more of treatment the association extends to all osteoporosis-related fractures [2, 3].

Several studies have suggested a relationship between vascular calcification, impaired bone metabolism, fractures and increased mortality [4, 5]. Indeed, aortic calcifications are a strong predictor for low bone density and fragility fractures [6]. In dialysis patients, vascular calcifications are also widespread and connected to bone health [7].

We performed a cross-sectional study in haemodialysis patients to establish whether long-term ongoing treatment with PPIs is associated with vascular calcifications by calculating odds ratios (ORs) corrected according to the propensity score analysis [8], which allows studying the causal effect of treatment in non-randomized observational studies of efficacy accounting for the 'confounding by indication'.

2 Materials and Methods

2.1 Study Design

This was a cross-sectional investigation involving all patients who underwent haemodialysis at 18 Italian Dialysis Centres in 2008–2009. The centres were coordinated by Consiglio Nazionale delle Ricerche (CNR—National Research Institute of Italy) at the subsidiary in Padua, which was the sponsor of the study. The sponsor monitored the conduct of the trial, collated and analysed the data, and drafted the manuscript. The investigators at all the centres reviewed and approved the manuscript.

All the local Ethics Committees were duly notified about the conduct of the study and all patients gave their consent to the use of their medical records for the study.

2.2 Study Population and Assessments

The cohort of 387 patients was enrolled between November 2008 and November 2009.

We included adult patients of both sexes who had been receiving haemodialysis for more than 1 year, provided that they gave their informed consent in writing to the use of their medical records for the study.

We excluded patients who had a life expectancy of less than 6 months, who had any evidence of cancer (with the exception of basalioma) or who had any condition that, according to the investigator, could interfere with the outcome of the study.

Routine laboratory tests [serum calcium (Ca), phosphorus (P), parathyroid hormone (PTH), high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, triglycerides, albumin, 25-hydroxyvitamin D (25-OH-vitamin D)] were performed at each of the dialysis centres; serum PTH data were standardized according to Souberbielle [9]. Dose of delivered dialysis was measured as a single pool dialyser clearance of urea (K) multiplied by the duration of the dialysis treatment (T, in minutes) divided by the volume of distribution of urea in the body (V, in mL) [KT/V]. Vascular calcification assessments were centralized at CNR in Padua, using Witteman's method [10]. The main outcome measure was calcification of the aorta and iliac arteries in relation to PPI use.

Local radiologists were asked to collect lateral radiographs of the abdominal aorta taken in the standing position using standard radiographic equipment; at least a 4 cm tract anterior to the lumbar spines was to be visible and the film distance was 100 cm. The estimated dose of radiation was about 15 mGy. The radiographs were sent to CNR for blinded evaluation in duplicate by two physicians who were blind to the PPI status of the patient. Vascular calcifications were quantified by measuring the length of calcific deposits along the anterior and posterior wall of the aorta (mild 0.1–5 cm, moderate 5.1–10 cm and severe >10 cm). They also evaluated the presence or absence of calcifications of the iliac arteries in the same radiograph (mild 0.1–3 cm, moderate 3.1–5 cm and severe >5 cm). Any differences were resolved by consensus.

Long-term concomitant use of warfarin, a drug that is known to induce calcifications by inhibiting the vitamin K cycle, was noted and its association with vascular calcifications was assessed.

2.3 Statistical Analysis

The data are shown as mean ± standard deviation for quantitative variables or median for not normal or strongly asymmetric variables, and frequency percentages for all discrete variables.

For discrete variables, the differential distribution between patients receiving long-term treatment with a PPI and those who were not was analysed by Chi-squared test or Fisher's exact method. The comparisons between means were evaluated using generalized linear models; the Levene's test was performed to test the homoscedasticity of

Table 1 Main characteristics of patients by PPI status

Characteristics	Patients receiving long-term PPI treatment ($n = 295$)	Patients not receiving long-term PPI treatment ($n = 92$)	p-value
Sex, female [n (%)]	121 (41.0)	24 (26.1)	**0.001**
Age, years [mean ± SD]	64.21 ± 13.63	63.97 ± 15.44	0.884
Weight, kg [mean ± SD]	69.75 ± 14.71	71.58 ± 14.17	0.293
Height, cm [mean ± SD]	166.52 ± 9.16	169.12 ± 9.39	0.018
BMI, kg/cm^2 [mean ± SD]	25.07 ± 4.49	24.98 ± 4.17	0.87
Current or former smoker [n (%)]	115 (39.0)	37 (40.7)	0.775
Current or former alcohol drinker [n (%)]	63 (22.4)	19 (23.8)	0.802
Medical history			
Dialysis duration, months, median	51.00	47.50	0.400
Type of dialysis [n (%)]			
Bicarbonate dialysis	143 (48.5)	46 (50.0)	0.884
HF	24 (8.1)	8 (8.7)	
HDF	81 (27.5)	21 (22.8)	
AFB	39 (13.2)	15 (16.3)	
Other types of dialysis	8 (2.7)	2 (2.2)	
Previous kidney transplant [n (%)]	37 (12.5)	17 (18.5)	0.151
Hypertension [n (%)]	235 (79.7)	69 (75.0)	0.341
Angina [n (%)]	57 (19.3)	7 (7.6)	**0.008**
Myocardial infarction [n (%)]	62 (21.0)	11 (12.0)	0.052
Atrial fibrillation [n (%)]	45 (15.3)	6 (6.5)	**0.031**
Heart failure [n (%)]	31 (10.5)	8 (8.7)	0.614
Diabetes mellitus [n (%)]	64 (21.7)	21 (22.8)	0.819
Peripheral vascular disease [n (%)]	189 (64.1)	64 (69.6)	0.396
No asymptomatic	75 (25.4)	23 (25.0)	
Intermittent claudication	25 (8.5)	3 (3.3)	
Amputation	6 (2.0)	2 (2.2)	
Cerebrovascular accident [n (%)]			
No	262 (75.7)	84 (91.3)	0.701
Stroke	17 (5.8)	3 (3.3)	
Other type	16 (5.4)	5 (5.4)	
Vertebral fractures [n (%)]	163 (55.3)	51 (55.4)	0.976
Routine biochemical profile			
Ca, mg/dL [mean ± SD]	9.15 ± 0.85	9.06 ± 0.66	0.385
P, mg/dL [mean ± SD]	4.77 ± 1.27	4.75 ± 1.27	0.880
Alkaline phosphatase, U/L [median]	84.00	76.50	**0.042**
PTH, pg/mL [median]	248.00	212.50	0.187
Albumin, g/dL [mean ± SD]	3.82 ± 0.51	3.80 ± 0.43	0.730
CRP, mg/L [median]	1.90	1.00	**0.030**
KT/V [mean ± SD]	1.25 ± 0.27	1.27 ± 0.25	0.545
Aluminium, μg/L [median]	12.00	13.00	0.786
Total cholesterol, mg/dL [mean ± SD]	168.37 ± 39.62	169.35 ± 46.53	0.843
Triglycerides, mg/dL [median]	152.00	132.50	0.128
HDL cholesterol, mg/dL [mean ± SD]	42.10 ± 12.64	43.01 ± 12.93	0.548
LDL cholesterol, mg/dL [mean ± SD]	93.04 ± 35.03	97.14 ± 43.22	0.376
25(OH)D, ng/mL [median]	28.80	31.85	0.272
BGP total, μg/L [median]	187.00	163.00	0.996
BGP undercarboxylated, μg/L [median]	11.10	10.05	0.163
MGP total, nmol/L [median]	18.90	17.80	0.923
MGP undercarboxylated, nmol/L [median]	557.00	609.90	0.923

Statistically significant p-values are shown in bold

25(OH)D 25-hydroxy-vitamin D, *AFB* acetate-free biofiltration, *BGP* bone GLA protein, *BMI* body mass index, *Ca* serum calcium, *CRP* C-reactive protein, *GLA* gamma-carboxyglutamic, *HDF* haemodiafiltration, *HDL* high-density lipoprotein, *HF* haemofilatration, *KT/V* dialyser clearance of urea (K) multiplied by the duration of the dialysis treatment (T, in minutes) divided by the volume of distribution of urea in the body, *LDL* low-density lipoprotein, *MGP* matrix GLA protein, *P* phosphorus, *PPI* proton pump inhibitor, *PTH* parathyroid hormone

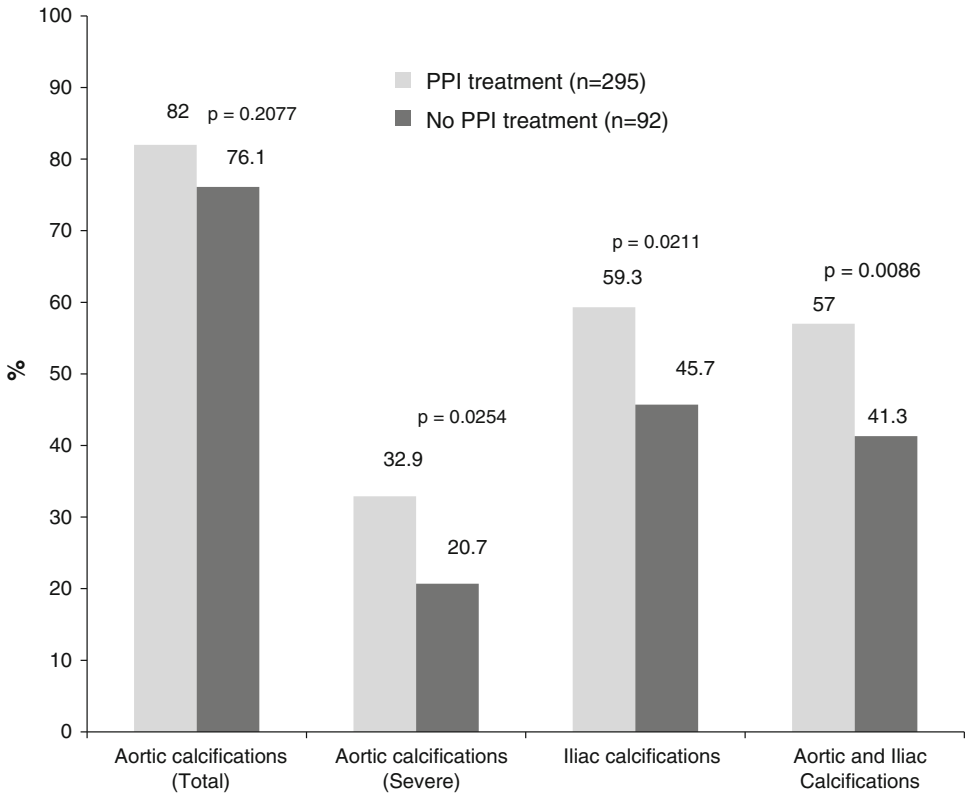

Fig. 1 Proportions of patients with aortic and iliac calcifications by PPI status. *PPI* proton pump inhibitor

variances, and when its assumption was violated, the Welch's ANOVA was used. The comparison between medians was performed considering the Wilcoxon sum rank test.

Given the observational nature of our study, the relationship between PPI treatment, alone or in combination with warfarin, and the likelihood of iliac and aortic calcifications, was investigated by the analysis of the propensity score [8, 11], a statistical technique that takes into account the 'confounding by indication' in observational studies of efficacy and which is considered superior to standard covariance analysis in this type of study [12]. In order to calculate the propensity score, we constructed a preliminary multiple logistic regression model having PPI treatment (0 = no; 1 = yes) as the dependent variable and all variables that differ (with $p \leq 0.10$) between treated and untreated patients (namely sex, age, height, type of dialysis, hypertension, angina, myocardial infarction, atrial fibrillation, peripheral vascular disease, cerebrovascular accident, vertebral fractures, Ca, alkaline phosphatase, C-reactive protein [CRP], total cholesterol, triglycerides, bone gamma-carboxyglutamic [GLA] protein total and matrix GLA protein [MGP] total) as independent variables. By using the routine command in SPSS (IBM SPSS Statistics, Armonk, NY, USA), in the setting of a multiple logistic regression analysis, for each patient we calculated the estimated probability of being treated with a PPI, conditional to the series of risk factors listed above. Finally, we used this score as a covariate to adjust for the 'confounding by indication' in logistic regression analyses modelling the relationship between PPI treatment and vascular calcifications. The same analysis was carried out for the combination of PPI/warfarin. All statistical analyses were performed using SAS statistical package (version 9.2, SAS Software, Cary, NC, USA) at the CNR in Padua.

3 Results

3.1 Participants

A total of 387 patients were enrolled: 295 (76.2 %) were receiving long-term PPI treatment and the remaining 92 were the non-PPI group. Patients were also receiving long-term treatment with many other drugs; the drugs that were given to ≥10 % of patients were, in order of frequency, heparin (98.7 %), oral calcitriol (45.7 %), sevelamer hydrochloride (42.1 %), β-blockers (37.2 %), calcium carbonate (34.1 %), statins (32.6 %), aluminum-based binders (24.8 %), paricalcitol (19.9 %), cinacalcet (19.4 %), insulin (15.0 %), lanthanum (14.5 %), warfarin (11.9 %) and thyroid hormone (10.3 %). The main characteristics of patients in terms of demography, medical history, type of dialysis and routine biochemical profile are

shown by PPI status in Table 1. An imbalance was found in sex, with a higher percentage of women among subjects receiving PPI treatment (41 vs 26.1 %, $p = 0.001$). The proportion of patients suffering from angina and atrial fibrillation were significantly higher in the group receiving long-term PPI treatment than in those who were not (19.3 vs 7.6 %, $p = 0.008$, and 15.3 vs 6.5 %, $p = 0.031$, respectively). However, after data adjustment for propensity, these associations did not attain the statistical significance (p = NS); a history of acute myocardial infarction tended to be more common among patients receiving long-term PPI treatment (21 vs 12 %, $p = 0.052$). No other parameters differed according to long-term PPI treatment, except alkaline phosphatase and CRP (higher median in the PPI group 84.0 vs 76.5 U/L, $p = 0.042$, and 1.9 mg/L vs 1.0, $p = 0.030$, respectively).

3.2 Extent of Vascular Calcification

A total of 312 patients (80.6 %) had aortic calcifications: mild 81 (20.9 %), moderate 115 (29.7 %), severe 116 (30 %). In addition, 217 (56.1 %) had iliac artery calcifications; 206 (53.2 %) had calcifications in both vascular districts. The proportion of patients who had both aortic and iliac calcifications was significantly higher in the PPI group (Fig. 1): 168 (57.0 %) vs 38 (41.3 %) [$p = 0.009$]. Significant differences were found also when considering the proportion of patients with aortic (+12.2 %, $p = 0.025$) and iliac (+13.6 %, $p = 0.021$) calcifications, regardless of the presence of calcifications in other sites.

Amongst patients with aortic calcifications, the proportion of patients receiving long-term treatment with warfarin increased with the severity of the calcifications (mild 6.2 %, moderate 10.4 %, severe 23.3 %); very few patients with no aortic calcifications were receiving long-term treatment with warfarin (2.7 %) [$p < 0.0001$]. The same was true for iliac calcifications (mild 8.3 %, moderate 16.5 %, severe 25.9 %, no calcifications 5.3 %). Amongst patients with moderate and severe iliac calcifications, the proportion of patients receiving long-term PPI treatment was higher (83.5 and 85.2 %, respectively) than amongst those without iliac calcifications or with only mild iliac calcifications (70.6 and 69.8 %, respectively) [$p = 0.017$]. There was a trend amongst patients with aortic calcifications to receive long-term PPI treatment more frequently (83.6 % amongst patients with severe aortic calcifications vs 70.7 % amongst patients with no aortic calcifications) [$p = 0.068$].

Logistic regression models identified male sex, PPI use and warfarin use as predictors of vascular calcifications. Either on crude analysis (Fig. 2) or after correction for the propensity score, the ORs (95 % CI) related to PPI use were statistically significant: aorta 1.89 (1.01–3.54), $p = 0.048$; iliac arteries 2.27 (1.31–3.92), p = 0.003; aorta and iliac arteries 2.59 (1.48–4.53), $p = 0.008$ (Fig. 2). The combined use of PPI and warfarin treatment was strongly related to both aortic and iliac calcifications ($p < 0.0001$) [Table 2]. Accordingly, in a logistic regression model including the propensity score, the combined use of warfarin + PPI resulted in a significant correlation of iliac and/or aortic calcifications [OR 2.69 (1.03–6.96), $p = 0.042$]. A separate analysis of aorta and iliac arteries showed that the combination of these drugs was significantly related to the iliac calcifications [OR 2.90 (1.07–7.86), $p = 0.036$] and barely failed to correlate with aortic calcifications [OR 2.19 (0.95–5.00), $p = 0.06$].

4 Discussion

This cross-sectional study shows that there is an association between long-term PPI treatment and vascular calcifications in haemodialysis patients.

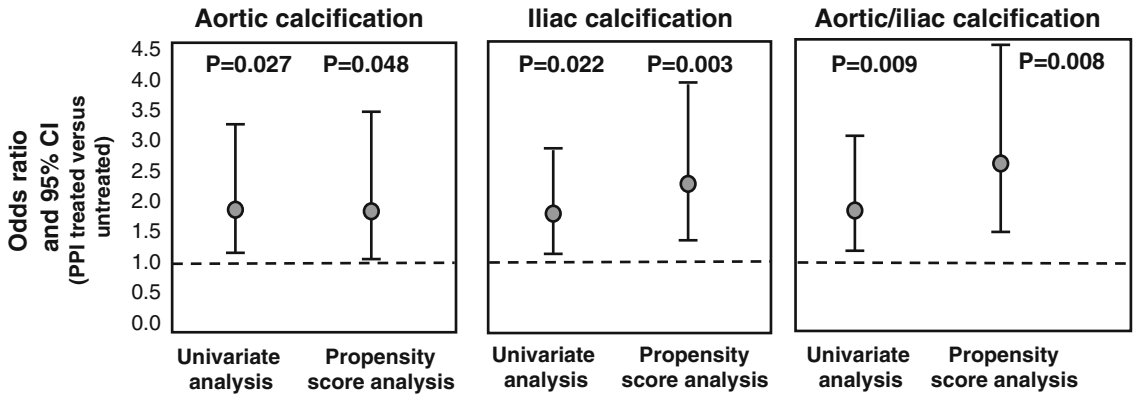

Fig. 2 Odds ratios of vascular calcification of PPI treated versus untreated subjects. Data are adjusted by traditional covariance analysis and by the propensity score approach. *PPI* proton pump inhibitor

Table 2 Presence and severity of vascular calcifications by warfarin and PPI use

Calcification	Patients not treated with either warfarin or PPIs ($n = 86$)	Patients not treated with warfarin but treated with PPIs ($n = 255$)	Patients treated with warfarin and PPIs ($n = 40$)	p-value
Aortic calcifications [n (%)]				**0.0001**
None	21 (24.4)	52 (20.4)	1 (2.5)	
Mild	25 (29.1)	51 (20.0)	5 (12.5)	
Moderate	24 (27.9)	79 (31.0)	10 (25.0)	
Severe	16 (18.6)	73 (28.6)	24 (60.0)	
Iliac calcifications [n (%)]				**<0.0001**
None	47 (54.7)	114 (44.7)	6 (15.0)	
Mild	15 (17.4)	29 (11.4)	4 (10.0)	
Moderate	17 (19.8)	79 (31.0)	17 (42.5)	
Severe	7 (8.1)	33 (12.9)	13 (32.5)	
Aortic and iliac calcifications [n (%)]				**0.0001**
None	18 (20.9)	46 (18.0)	0 (0.0)	
Aortic or iliac	32 (37.2)	74 (29.0)	7 (17.5)	
Aortic and iliac	36 (41.9)	135 (52.9)	33 (82.5)	

Statistically significant p-values are highlighted in bold

PPI proton pump inhibitor

To our knowledge, this is the first time that this association has been reported. The association is strong, as it was found for all the parameters selected (both aortic and iliac calcifications considered together and separately). Moreover, logistic regression showed that the association is independent of the other factors that influence calcification. In particular, we could not identify an association with calcium and phosphorus levels, whose average levels were within the accepted range by the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [13]. Logistic regression, however, did find an association between vascular calcification and other cardiovascular risk factors, namely age, male sex and abnormalities in the lipid profile, as was to be expected [14]. It also identified an association between vascular calcifications and the use of warfarin, which has already been reported [15, 16].

The study was not designed to assess the association of PPI and cardiovascular disease, so no claims can be made. Nevertheless, as the study showed that PPIs increase vascular calcification, which has proved to be an independent risk factor for cardiovascular events and mortality in haemodialysis patients [17, 18], we speculate that long-term treatment with PPIs might be associated with a higher incidence of cardiovascular outcomes and believe that the issue is worthy of further investigation.

Another important finding was the interaction between PPI and warfarin use, which increased the risk of vascular calcification nearly threefold. To our knowledge, this is the first time that this effect has been reported, whereas the mechanism whereby warfarin may increase the risk of vascular calcification is well known: it is a vitamin K antagonist, which interferes with the function of vitamin K-dependent proteins, including osteocalcin, involved in the regulation of bone mineralization, and MGP, which inhibits vascular calcification [19].

The phenomenon would be easier to detect in a population at risk of vascular disease, such as haemodialysis patients, but may occur in the general population as well, as vascular calcification has proved to be a cardiovascular risk factor in subjects devoid of renal and cardiovascular disease [20, 21]. In addition, a recent Danish retrospective, nationwide study showed that in aspirin-treated patients with first time myocardial infarction, treatment with PPIs was associated with an increased risk of adverse cardiovascular events, namely recurrent myocardial infarction, stroke or cardiovascular death [22].

Recent meta-analyses have shown that PPIs increase the risk of hip, spine and any site fractures [23, 24]. However, the possibility that the findings may be due to unidentified confounding factors has been raised, as a mechanism of action has not been established [25]. We could not confirm an increased prevalence of fractures in PPI-treated haemodialysis patients.

The mechanisms underlying the association of PPI use with vascular calcifications are unknown. A possible, although speculative, explanation for the phenomenon is the reduction in magnesium serum levels induced by long-term PPI treatment. Over the last few years, several cases of severe hypomagnesemia have been reported during long-term treatment with PPIs. Magnesium is absorbed

from the gut both by a passive paracellular pathway and by an active transcellular route involving channel proteins TRPM6/7, which intervene when low quantities of magnesium are ingested. It is believed that PPIs interfere with the active transport of magnesium and that their effects become clinically significant in the carriers of heterozygotic mutations of TRPM6/7 [26–28]. Magnesium, which is deposited in large quantities in bone, is essential for bone health and severe osteoporosis has been reported in patients receiving long-term PPI treatment [29]. Moreover, low magnesium serum levels are associated with the presence of peripheral vascular calcifications [30, 31]. It has been shown experimentally that magnesium actively prevents arterial calcification and osteogenic differentiation of vascular smooth muscle cells by increasing the expression of anti-calcification proteins, including osteopontin, bone morphogenetic protein-7 (BMP-7) and MGP [32]. This hypothesis could not be explored in our patient population as data on serum magnesium levels are not available. In addition, serum levels may not correctly identify patients with a reduced magnesium pool.

Our findings regarding new potential adverse effects of PPIs are hypothesis generating as they derive from a non-prospective study. A limitation of the present study, and of observational studies in general, is the lack of a priori randomization of treatment assignment. Anyway, the strength of observational studies is the ability to estimate treatment effect in *real-world* conditions; regression considering propensity scores is a well recognized technique to compare groups (treated vs not treated), adjusting for the most important pitfall in observational studies of efficacy, that is the 'confounding by indication' [12]. Another limitation of the study is the small size (92 patients) of the control group not treated with PPI. However, widespread use of PPIs in dialysis patients is a reality, which can be due to the high prevalence of gastrointestinal disorders associated with the uraemic state [33]. Although these are limitations of our study that should be taken into consideration, the possibility of increased vascular calcifications should be further evaluated with a prospective study, involving the large number of patients treated with PPIs in the general population.

5 Conclusion

In haemodialysis patients, we have shown that long-term treatment with PPIs, especially in the presence of warfarin treatment, is associated with vascular calcification, which is a risk factor for cardiovascular events and cardiovascular mortality in haemodialysis patients, and for cardiovascular events in elderly subjects without renal impairment. Additional studies are warranted to explore the association among long-term PPI treatment, arterial calcifications and cardiovascular events.

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Author contributions Maria Fusaro drafted the protocol, co-ordinated the trial (logistics, compliance with good clinical practice), interpreted the results and drafted the manuscript; Marianna Noale and Giovanni Tripepi drafted the statistical section of the protocol and performed the statistical analysis; Angela D'Angelo, Lorenzo Calò and Angelo Pica contributed to the clinical sections of the protocol; Davide Miozzo contributed to the co-ordination of the trial; Sandro Giannini and Maurizio Gallieni reviewed the protocol and interpreted the results.

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Conflict of interest The authors have no conflicts of interest to declare that are directly relevant to the content of this study.

References

1. Thomson AB, Sauve MD, Kassam N, Kamitakahara H. Safety of the long-term use of proton pump inhibitors. World J Gastroenterol. 2010;16(19):2323–30.

2. Yang YX, Lewis JD, Epstein S, Metz DC. Long-term proton pump inhibitor therapy and risk of hip fracture. JAMA. 2006; 296(24):2947–53.
3. Targownik LE, Lix M, Metge CJ, Prior HJ, Leung S, Leslie WD. Use of proton pump inhibitors and risk of osteoporosis-related fractures. CMAJ. 2008;179(4):319–26.
4. Cannata-Andia JB, Roman-Garcia P, Hruska K. The connections between vascular calcification and bone health. Nephrol Dial Transplant. 2011;26(11):3429–36.
5. Russo D, Corrao S, Battaglia Y, et al. Progression of coronary artery calcification and cardiac events in patients with chronic renal disease not receiving dialysis. Kidney Int. 2011;80(1): 112–8.
6. Schulz E, Arfai K, Liu X, Sayre J, Gilsanz V. Aortic calcification and the risk of osteoporosis and fractures. J Clin Endocrinol Metab. 2004;89(9):4246–53.
7. Moe SM, O'Neill KD, Reslerova M, Fineberg N, Persohn S, Meyer CA. Natural history of vascular calcification in dialysis and transplant patients. Nephrol Dial Transplant. 2004;19(9): 2387–93.
8. Rosenbaum PR, Rubin DB. Assessing sensitivity to an unobserved binary covariate in an observational study with binary outcome. J R Stat Soc. 1983;45(2):212–8.
9. Souberbielle JCP, Roth H, Fouque DP. Parathyroid hormone measurement in CKD. Kidney Int. 2010;77(2):93–100.
10. Witteman JC, Grobbee DE, Valkenburg HA, et al. J-shaped relation between change in diastolic blood pressure and progression of aortic atherosclerosis. Lancet. 1994;343(8896):504–7.
11. Adamina M, Guller U, Weber WP, Oertli D. Propensity scores and the surgeon. Br J Surg. 2006;93(4):389–94.
12. D'Agostino RB Jr. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med. 1998;17(19):2265–81.
13. KDIGO clinical practice guideline for the diagnosis. Evaluation, prevention and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. Kidney Int Suppl. 2009;76(Suppl. 113):S1–130.
14. Nakagami H, Osako MK, Morishita R. New concept of vascular calcification and metabolism. Curr Vasc Pharmacol. 2011;9(1): 124–7.
15. Danziger J. Vitamin K-dependent proteins, warfarin and vascular calcification. Clin J Am Soc Nephrol. 2008;3(5):1504–10.
16. Beazley KE, Deasey S, Lima F, Nurminskaya MV. Transglutaminase 2-mediated activation of beta-catenin signalling has a critical role in warfarin-induced vascular calcification. Arterioscler Thromb Vasc Biol. 2012;32(1):123–30.
17. Inoue T, Ogawa T, Ishida H, Ando Y, Nitta K. Aortic arch calcification evaluated on chest X-ray is a strong independent predictor of cardiovascular events in chronic hemodialysis patients. Heart Vessels. 2011;27(2):135–42.
18. Noordzij M, Cranenburg EM, Engelsman LF, et al. Progression of aortic calcification is associated with disorders or mineral metabolism and mortality in chronic dialysis patients. Nephrol Dial Transplant. 2011;26(5):1662–9.
19. Fusaro M, Crepaldi G, Maggi S, et al. Bleeding, vertebral fractures and vascular calcifications in patients treated with warfarin: hope for lower risks with alternative therapies. Curr Vasc Pharmacol. 2011;9(6):763–9.
20. Schousboe JT, Taylor BC, Kiel DP, Ensrud KE, Wilson KE, McCloskey EV. Abdominal aortic calcification detected on lateral spine images from a bone densitometer predicts incident myocardial infarction or stroke in older women. J Bone Miner Res. 2008;23(3):409–16.
21. Bolland MJ, Wang TK, van Pelt NC, et al. Abdominal aortic calcification on vertebral morphometry images predicts incident myocardial infarction. J Bone Miner Res. 2010;25(3):505–12.
22. Charlot M, Grove EL, Hansen PR, et al. Proton pump inhibitor use and risk of adverse cardiovascular events in aspirin treated patients with first time myocardial infarction: nationwide propensity score matched study. BMJ. 2011;342:d2690.
23. Yu EW, Bauer SR, Bain PA, Bauer DC. Proton pump inhibitors and risk of fractures: a meta-analysis of 11 international studies. Am J Med. 2011;124(6):519–26.
24. Ngamruengphong S, Leontiadis GI, Radni S, Dentino A, Nugent K. Proton pump inhibitors and risk of fracture: a systematic review and meta-analysis of observational studies. Am J Gastroenterol. 2011;106(7):1209–18.
25. Targownik LE, Leslie WD. The relationship among proton pump inhibitors, bone disease and fracture. Expert Opin Drug Saf. 2011;10(6):901–12.
26. Kuipers MT, Thang HD, Arntzenius AB. Hypomagnesaemia due to use of proton pump inhibitors: a review. Neth J Med. 2009;67(5):169–72.
27. Hoorn EJ, van der Hoek J, de Man RA, Kuipers EJ, Bolwerk C, Zietse R. A case series of proton pump inhibitor–induced hypomagnesemia. Am J Kidney Dis. 2010;56(1):112–6.
28. Regolisti G, Cabassi A, Parenti E, Maggiore U, Fiaccadori E. Severe hypomagnesemia during long-term treatment with a proton pump inhibitor. Am J Kidney Dis. 2010;56(1):168–1274.
29. Kanazawa I, Yamamoto M, Yamaguchi T, Yamauchi M, Yano S, Sugimoto T. A case of magnesium deficiency associated with insufficient parathyroid hormone action and severe osteoporosis. Endocr J. 2007;54(6):935–40.
30. Ishimura E, Lokuno S, Kitatani K, Tsuchida T, Yamakawa T, Shioi A, Inaba M, Nishizawa Y. Significant association between the presence of peripheral vascular calcification and lower serum magnesium in hemodialysis patients. Clin Nephrol. 2007;68(4): 222–7.
31. Meema HE, Oreopooulos DG, Rapoport A. Serum magnesium level and arterial calcification in end-stage renal disease. Kidney Int. 1987;32(3):388–94.
32. Montezano AC, Zimmerman D, Yusuf H, et al. Vascular smooth muscle cell differentiation to an osteogenic phenotype involves TRPM7 modulation by magnesium. Hypertension. 2010;56(3): 453–62.
33. Strid H, Fjell A, Simrén M, Björnsson ES. Impact of dialysis on gastroesophageal reflux, dyspepsia, and proton pump inhibitor treatment in patients with chronic renal failure. Eur J Gastroenterol Hepatol. 2009;21(2):137–42.