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ORIGINAL ARTICLE

Type of proton-pump inhibitor and risk of iron deficiency in kidney transplant recipients – results from the TransplantLines Biobank and Cohort Study

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SUMMARY

Proton-pump inhibitors (PPIs) have been associated with iron deficiency (ID) in kidney transplant recipients (KTRs). Gastric acid plays a pivotal role in the intestinal absorption of non-heme iron, but the pharmacodynamics of PPIs differs in potency of acid suppression. We hypothesized that the risk of ID might be lower in KTRs using a less potent PPI. In a cohort of 724 KTRs from the TransplantLines Biobank and Cohort Study (NCT03272841), PPI use was associated with ID [odds ratio (OR) 2.02; 95% CI 1.36–2.98]. Compared with no PPI use, the point estimate of the odds ratio for risk of ID for pantoprazole (OR 1.55; 95%CI 0.78–3.10) was lower than for esomeprazole and omeprazole (3.58; 95%CI 1.73–7.40 and 1.96; 95%CI 1.31–2.94, respectively). When comparing pantoprazole users with omeprazole users on an equipotent dose (≤20 omeprazole equivalents (OE)/day) omeprazole, but not pantoprazole was associated with ID, although the lack of a significant effect of pantoprazole on the risk of ID could be caused by a lack of power. Furthermore, risk of ID was higher among users of a high PPI dose (≥ 20 OE/day) and OE as continuous variable was also independently associated with ID, indicating that risk of ID is higher while using a more potent PPI. Further investigation seems warranted to confirm whether pantoprazole leads to less ID in KTRs.

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Key words
iron, iron deficiency, kidney transplantation, potency, proton-pump inhibitors

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Introduction

Use of proton-pump inhibitors (PPIs) is common practice after kidney transplantation. PPIs are mainly prescribed to prevent peptic ulcer disease from the high doses of corticosteroids in the post-transplantation period [1]. Although evidence is mounting regarding the potential side effects of PPIs, PPIs are rarely discontinued, even when the treatment indication has been resolved.

Previously, our group identified that PPI use is associated with an increased risk of iron deficiency (ID) in kidney transplant recipients (KTRs) [2]. Importantly, ID has been shown to be independently associated with

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an increased risk of death in this patient setting [3]. Although PPIs are generally considered as a pharmacologic class, the pharmacodynamic profile of PPIs is different in terms of their potency to decrease gastric acid secretion [4,5]. For this reason, the World Health Organization defined daily dosages with equivalent effect based on treatment of gastroesophageal reflux disease [6]. Other surrogate markers widely used to investigate the efficacy of PPIs are the mean 24-hour intragastric pH and the pH4time, which represents the percentage of time over a 24-hour period wherein intragastric pH exceeds 4 [7,8]. In a meta-analysis including 57 clinical studies, the potency of different PPIs was compared using intragastric pH-monitoring data [4]. Compared with omeprazole (equivalent of 1.0), the reported relative potencies were 0.23 for pantoprazole, 0.90 for lansoprazole, 1.60 for esomeprazole, and 1.82 for rabeprazole.

Considering the fact that gastric acid plays a crucial role in the absorption of non-heme iron from the intestinal tract [9], we hypothesized that the risk of ID might be lower in KTRs using a less potent PPI. In this study, we investigated the risk of ID associated with use of different PPIs and explored whether this effect was dose-dependent when comparing PPIs based on their daily dose expressed as omeprazole equivalents (OE).

Patients and methods

Study design and participants

In January 2020, we performed a data extraction of the TransplantLines Biobank and Cohort Study (ClinicalTrials.gov identifier: NCT03272841) regarding KTRs who were at least ≥ 1 year post-transplantation. At that moment, 848 KTRs were included of which 53 KTRs had no data available on iron status parameters because of logistic problems during the biobanking process and therefore not related to health status of the individuals, resulting in a cohort of 795 KTRs [10]. TransplantLines is an ongoing prospective cohort study among all types of solid organ transplant recipients, as described previously [10]. In the TransplantLines study, both new transplant candidates and transplant recipients, are included. Transplant recipients with a functioning graft for at least ≥ 1 year post-transplantation were included at their next outpatient clinic visit. All study procedures, including the laboratory measurement of iron status parameters, were performed during the single study visit at the outpatient clinic of the University Medical Center Groningen (UMCG), The Netherlands, between September 2015 and November 2019. Follow-up of the study participants is performed every 5 years. The study protocol was approved by the local Institutional Review Board (IRB identifier: 2014-077) and adheres to the UMCG Biobank regulation. All procedures performed as part of the study were in adherence with the World Medical Association Declaration of Helsinki and Declaration of Istanbul. Written informed consent was obtained from all participants. For the current cross-sectional analysis, we excluded KTRs using any form of iron supplementation (n = 33), erythropoietin (EPO)-stimulating agents (n = 25) or both (n = 11). In addition, two rabeprazole users were excluded, as this group was too small to allow meaningful subgroup analyses. Hence, this resulted in 724 KTRs eligible for analyses.

Exposure definition

Medication use, including use of PPIs, was extracted from electronic patient records and verified with the patient during the study visit. Both PPI type and daily dose were registered. To assure a temporal relationship between PPI use and ID, the duration of PPI exposure before the study visit was determined and exposure to different types of PPIs before the study visit was derived from electronic patient records. In case patients were exposed to different PPIs before the study visit, only the period of continuous use of the last PPI was registered and included in statistical analyses. In case PPI treatment was initiated before transplantation, only the duration of PPI exposure between transplantation and the study visit was registered, because information about the total duration of PPI exposure before transplantation was unavailable. Omeprazole equivalents were calculated according to the following formula: OE = daily dose x relative potency PPI according to Kirchheiner *et al.* [4,5]. Compared with omeprazole (equivalent of 1.0), the relative potencies were 0.23 for pantoprazole and 1.60 for esomeprazole.

Covariates

All laboratory parameters were measured using standard in-house laboratory techniques. Iron status parameters were measured once routinely as part of the study protocol and not based on clinical indication. ID was defined as transferrin saturation (TSAT) < 20% and ferritin < 300 µg/l as described in literature previously [2,3,11–13]. Iron deficiency anemia was defined as anemia (Hb < 13 g/dl (males) or < 12 g/dl (females)) in combination with ID. Estimated glomerular filtration

rate (eGFR) was calculated using Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) using serum creatinine. Urinary protein excretion ≥ 0.5 g/24 hour was considered proteinuria. Alcohol use and smoking behavior were assessed using questionnaires. To prevent indication bias, we gathered information about prior upper gastrointestinal disease potentially leading to bleeding (i.e. reported history of gastritis, esophagitis, and peptic ulcer disease). In addition, information about previous severe gastrointestinal hemorrhage leading to hospital admission was obtained from electronic patient records.

Statistical analyses

We performed statistical analyses using Statistical Package for Social Sciences, version 23.0 (SPSS statistics, IBM Corp, Armonk, NY). Study characteristics are described as means with standard deviations (SD) for normally distributed data, medians with interquartile ranges (IQRs) for skewed data, or number with percentage for categorical variables. Differences between the combined group of different PPI treatment groups vs. non-users were assessed by student t-tests, Mann-Whitney U tests, or Chi-squared tests as appropriate. Differences between the different PPI treatment groups vs. non-users were assessed by using one-way ANOVA with Gabriel's procedure for multiple comparisons in case of normally distributed variables. For skewed and nominal variables, we performed Mann-Whitney U tests and Chi-squared tests, respectively, with a Bonferroni correction. Logistic regression analyses were used to investigate the association between different PPIs and ID, in which non-users were assigned as reference group. Linear regression analyses were used to investigate the association between different PPIs and iron status parameters (i.e., serum iron, serum ferritin, TSAT, and hemoglobin levels). Both linear and logistic regression models were adjusted for age, sex, body mass index (BMI), history of upper gastrointestinal disease or history of gastrointestinal hemorrhage, eGFR, proteinuria, time since transplantation, smoking behavior, alcohol use (categorized as no use, 1–7 units/week, >7 units/week), high-sensitivity C-reactive protein (hs-CRP), use of calcineurin inhibitors, proliferation inhibitors, prednisolone, platelet inhibitors, beta-blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, and angiotensin-2 receptor blockers. We performed these adjustments based on what is known from literature to be a potential confounder and based on significant differences in covariates at baseline between the different PPI users and non-users. The variables hs-CRP, ferritin, and time since transplantation were natural log transformed to obtain a normal distribution. In addition, we performed a dose–response analysis in which KTRs were categorized based on daily intake of PPIs defined in OE: no PPI, low-dose PPI (≤ 20 OE/day), and high-dose PPI (> 20 OE/day). A trend analysis with adjustment for the same potential confounders was conducted using the median daily OE per subgroup, entered in linear regression analyses as continuous variable. Furthermore, we investigated whether OE as continuous variable was independently associated with ID, to test our hypothesis that KTRs using PPIs with a higher potency and/or a higher dose are at increased risk of ID. Next, a time–response analysis was performed. For this analysis, KTRs who were using a PPI before transplantation were excluded ($n = 193$), because it was uncertain when PPI treatment was initiated in these cases. The duration of PPI exposure was categorized as follows: non-exposed, 0.1–2.0 years, and > 2 years [14,15]. Thereafter, we investigated risk of ID among users of a low PPI dose (≤ 20 OE/day). In this way, risk of ID among pantoprazole users could be compared with risk of ID among omeprazole users on an equivalent standard dose. As sensitivity analysis, we excluded H2-receptor antagonist (H2RA) users and re-assessed the risk of ID associated with the different PPIs. In another sensitivity analysis, we investigated whether adjustment for duration of PPI exposure would alter the association between the different PPIs and ID. For this analysis, time since transplantation was excluded as potential confounder because exposure duration and time since transplantation are related. In addition, we investigated whether a significant interaction effect between treatment and exposure duration was present. To investigate this, both main effects (different PPI treatment groups and duration of exposure) and their cross product terms were entered in a logistic regression analysis. Finally, we investigated whether the association between the different PPIs and ID remained when an alternative definition of ID was used. As no uniform consensus or guideline for the assessment of ID in this population currently exists, we used the definition of ID as proposed by the European Renal Best Practice Group and endorsed by the United Kingdom-based National Institute for Health and Care Excellence guideline (NG8) (TSAT < 20% and ferritin < 100 µg/l) [16,17]. For all analyses, the odds ratios (OR) and *P*-values of the potential confounders can be found in Tables S1–S4 of the Supplemental Material. In total, 1.4% of all demographic data were missing (specified in

footer of Table 1). Multiple imputations ($n = 5$) were performed for missing data, using the MCMC setting with predictive mean matching. Except for the baseline table, all analyses were performed using the imputed dataset. In all analyses, a two-sided P value <0.05 was considered significant, except regarding the analyses between the different treatment groups and non-users where a Bonferroni correction has been applied (two-sided P-value 0.05/3 = 0.0167).

Results

Study characteristics

We included 724 KTRs (age 56 ± 13 years, 61% males), with a mean eGFR of 52.8 ml/min/1.73 m^2 (Table 1). Median [IQR] ferritin level was 93 [43–183] μg/l and mean TSAT was 24 ± 10%. PPIs were used by 507 (70%) of the included 724 KTRs, of whom 403 (80%) used omeprazole, 57 (11%) used pantoprazole, and 47 (9%) used esomeprazole. Of the 507 PPI users, 193 (38%) patients received PPI treatment already before transplantation and continued with PPI treatment after transplantation, 277 patients (55%) received PPI treatment prophylactically and routinely after transplantation, and 37 patients (7%) initiated PPI treatment in the post-transplantation period. All PPI users had a higher BMI, more often a history of gastrointestinal disorders and used more often antiplatelet drugs and beta-blockers, as compared with non-users. When specifically assessing the differences among the types of PPIs, age was clearly increased in omeprazole and pantoprazole users, as compared with non-users. Also, time between transplantation and study visit was shorter in omeprazole users compared with non-users. Point estimates for hemoglobin levels and eGFR among all the different treatment groups seemed lower as compared with non-users, but our study did not pinpoint them as significant differences. However, differences in hemoglobin levels and eGFR were borderline significant when comparing the combined group of different PPI treatment groups versus non-users. Iron status parameters, including serum iron, ferritin, and TSAT, were significantly lower in omeprazole and esomeprazole users, whereas significant results were not shown for pantoprazole users, as compared with non-users.

PPI use and risk of ID

Use of PPIs was associated with ID, independent of adjustment for potential confounders (OR, 2.02; 95% confidence interval (CI), 1.36–3.00, $P < 0.001$, Table 2). When comparing the different PPIs with non-use, both omeprazole and esomeprazole were significantly associated with ID (OR, 1.96; 95% CI, 1.31–2.94, $P = 0.001$ and OR, 3.58; 95% CI, 1.73–7.40, $P = 0.001$, respectively), whereas pantoprazole had a lower, not statistically significant, point estimate with a wide CI for the risk of ID (OR, 1.55; 95% CI, 0.78–3.10, $P = 0.2$) (Table 2).

Dose–response analysis

To account for the different potencies of PPIs on gastric acid suppression, we performed a dose–response analysis using the daily dose of each PPI, expressed as OE, according to Kirchheiner *et al.* [4,5]. KTRs were categorized as follows: no PPI, low-dose PPI (≤ 20 OE/day), and high-dose PPI (> 20 OE/day). There was a significant trend toward a higher risk of ID among users of a high PPI dose (P_{trend} <0.001, Table 3). Similarly, we found that OE as continuous variable was independently associated with ID (OR per 20 units increase, 1.25; 95% CI, 1.07–1.46, $P = 0.005$), independent of adjustment for potential confounders.

Time–response analysis

In a time–response analysis, the risk of ID in patients exposed to PPIs for more than 2 years was not significantly increased compared with patients exposed for 0.1–2.0 years (Table 4).

Risk of ID among low-dose PPI users

To compare PPI users on a low dose (≤ 20 OE/day), we specifically assessed the risk of ID in this group. Low-dose PPI users consisted of all pantoprazole users ($n = 57$, median daily dose (DD) of 40 mg, OE 9.2 mg) and omeprazole users ($n = 290$, median DD of 20 mg, OE 20 mg). When comparing risk of ID within this group, again omeprazole was significantly associated with ID, whereas pantoprazole had a lower, not statistically significant point estimate with a wide CI for risk of ID (OR, 1.82; 95% CI, 1.17–2.82, $P = 0.007$ vs. OR, 1.58; 95% CI, 0.77–3.23, $P = 0.2$, respectively), independent of adjustment for potential confounders.

Association between PPIs and iron status parameters

Next to the definition of ID, we assessed the association between PPI use and the individual iron status

Table 1. Descriptive statistics of 724 kidney transplant recipients from the TransplantLines study.

Characteristics	Total population	Non-PPI users	Esomeprazole	Omeprazole	Pantoprazole	$P^{\#}$
Number of participants, *n* (%)	724 (100)	217 (29.9)	47 (6.5)	403 (55.7)	57(7.9)	n/a
Daily dose, mg/day	20 [0–40]	-	40 [40–40]	20 [20–40]	40 [40–40]	n/a
Omeprazole equivalents	20 [0–20]	-	64 [64–64]	20 [20–40]	9.2 [9.2–9.2]	n/a
Duration of exposure, *n* (%)		-				n/a
0.1–2.0 years	211 (29.1)	-	18 (38.3)	165 (40.9)	28 (49.1)	
> 2.0 years	296 (40.9)	-	29 (61.7)	238 (59.1)	29 (50.9)	
Demographics						
Age, y	56 ± 13	53 ± 14	56 ± 12	57 ± 13**	61 ± 10***	<0.001
Men, *n* (%)	443 (61.2)	127 (58.5)	29 (61.7)	256 (63.5)	31 (54.4)	0.3
BMI, kg/m^2	27.4 ± 4.8	26.6 ± 4.4	28.6 ± 5.3*	27.6 ± 4.8*	28.4 ± 5.2*	0.001
Time since transplantation, y	3.8 [1.0–10.0]	5.2 [1.9–13.4]	6.0 [1.0–11.0]	2.5 [1.0–7.9]***	4.0 [1.0–10.4]	<0.001
History of GI disorders, *n* (%)	72 (9.9)	6 (2.8)	11 (23.4)***	45 (11.2)***	10 (17.5)***	<0.001
Lifestyle parameters						
Smoker, *n* (%)[a]	65 (11.2)	23 (12.8)	6 (15.0)	30 (9.5)	6 (13.0)	0.4
Alcohol consumer, *n* (%)[b]						0.2
None	232 (38.0)	63 (33.7)	17 (43.6)	123 (36.8)	29 (56.9)	
1–7 units/week	254 (41.6)	87 (46.5)	19 (48.7)	133 (39.8)	15 (29.4)	
> 7 units/week	125 (20.5)	37 (19.8)	3 (7.7)	78 (23.4)	7 (13.7)	
Laboratory parameters						
Hb, g/dl	13.7 ± 1.8	13.9 ± 1.8	13.7 ± 1.9	13.5 ± 1.7	13.7 ± 1.8	0.02
MCV, fl	89.3 ± 5.5	90.1 ± 5.3	88.9 ± 5.6	88.7 ± 5.4*	89.9 ± 6.7	0.008
Iron deficiency, *n* (%)	265 (36.6)	53 (24.4)	25 (53.2)***	166 (41.2)***	21 (36.8)	<0.001
Iron deficiency anemia, *n* (%)	87 (12.0)	11 (5.1)	7 (14.9)	62 (15.4)***	7 (12.3)	<0.001
Iron, µmol/l	14.2 ± 5.4	15.2 ± 5.4	12.2 ± 3.6**	13.9 ± 5.5*	14.0 ± 5.8	0.001
Ferritin, µg/l	93 [43–183]	111 [65–190]	86 [32–152]	78 [35–180]***	81 [48–215]	<0.001
TSAT, %	24 ± 10	26 ± 10	21 ± 7**	23 ± 10**	23 ± 10	<0.001
eGFR, ml/min/1.73 m^2	52.8 ± 17.5	55.2 ± 17.4	49.7 ± 16.3	51.9 ± 17.3	52.8 ± 19.6	0.02
Proteinuria (≥ 0.5 g/24h), *n* (%)[c]	101 (14.7)	27 (13.0)	11 (25.6)	59 (15.4)	4 (7.3)	0.4
Hs-CRP, mg/l[d]	1.8 [0.7–4.5]	1.6 [0.6–4.2]	2.6 [1.3–7.0]	1.8 [0.8–4.2]	2.3 [0.8–6.0]	0.1
Medication use						
Platelet inhibitors, *n* (%)	175 (24.2)	23 (10.6)	16 (34.0)***	107 (26.6)***	29 (50.9)***	<0.001
ACE inhibitors, *n* (%)	161 (22.2)	50 (23.0)	16 (34.0)	80 (19.9)	15 (26.3)	0.7
Angiotensin-2 receptor blockers, *n* (%)	61 (8.4)	17 (7.8)	1 (2.1)	36 (8.9)	7 (12.3)	0.7
Beta-blockers, *n* (%)	405 (55.9)	89 (41.0)	34 (72.3)***	244 (60.5)***	38 (66.7)**	<0.001
Calcium channel blockers, *n* (%)	458 (63.3)	153 (70.5)	32 (68.1)	240 (59.6) *	33 (57.9)	0.008

Table 1. Continued.

Characteristics	Total population	Non-PPI users	Esomeprazole	Omeprazole	Pantoprazole	$P^{\#}$
Proliferation inhibitors, *n* (%)	630 (87.0)	189 (87.1)	40 (85.1)	354 (87.8)	47 (82.5)	0.9
Calcineurin inhibitors, *n* (%)	599 (82.7)	166 (76.5)	41 (87.2)	347 (86.1)**	45 (78.9)	0.004
Prednisolone, *n* (%)	708 (97.8)	210 (96.8)	45 (95.7)	398 (98.8)	55 (96.5)	0.3
H2-receptor antagonists, *n* (%)	23 (3.2)	23 (10.6)	0 (0)	0 (0)***	0 (0)*	<0.001

BMI, body mass index; History of GI disorders, history of gastrointestinal disorders; Hb, hemoglobin; MCV, mean corpuscular volume; TSAT, transferrin saturation; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitive C-reactive protein; ACE inhibitors, angiotensin-converting enzyme inhibitors. History of GI disorders included a reported history of gastritis, esophagitis, and peptic ulcer disease or severe gastrointestinal hemorrhage leading to hospital admission. Proliferation inhibitors included azathioprine or mycophenolic acid use. Calcineurin inhibitors included ciclosporin or tacrolimus use.

Data are presented as mean ± SD, median with interquartile ranges (IQR) or number with percentages (%).

$^{\#}P$-value for differences between the combined group of different PPI treatment groups versus non-users; *$P < 0.05$ versus non-users; **$P < 0.01$ versus non-users; ***$P < 0.001$ versus non-users.

[a]Available in 80.2%.

[b]Available in 84.4%.

[c]Available in 95.0%.

[d]Available in 98.8%.

parameters. We identified that PPI use in general was inversely associated with serum iron, ferritin, and TSAT (Table 5). Of the different PPIs, only pantoprazole was not associated with serum iron and TSAT, and less significantly with serum ferritin, as compared with omeprazole and esomeprazole (Table 5).

Sensitivity analyses for risk of ID

In the first sensitivity analysis, in which H2RAs users ($n = 23$) were excluded from statistical analyses, the association between the different PPIs and ID remained (Table S5). In a second sensitivity analysis, we additionally adjusted for the duration of PPI exposure. Again the association between different PPIs and ID remained essentially the same (Table S6). Moreover, no significant interaction between the different PPI treatment groups and exposure duration was present ($P_{interaction} > 0.1$ for all). Next, we investigated whether the association between the different PPIs and ID remained present when an alternative definition of ID was used (TSAT < 20% and ferritin < 100 µg/l). The association between different PPIs and ID remained materially unchanged and independent of adjustment for potential confounders when the alternative definition of ID was used (Table S7).

Discussion

This study explored the effect of different PPIs on the risk of ID in KTRs. The main findings of this study are: (i) omeprazole (10–80 mg) and esomeprazole (20–80 mg) were significantly associated with ID, whereas the point estimate of the odds ratio for risk of ID appears to be lower for pantoprazole (20–80 mg); (ii) based on OE, KTRs on a high PPI dose (≥ 20 OE/day) had the highest risk of ID; (iii) among the PPI users, duration of PPI exposure was not associated with an increased risk of ID; and (iv) when comparing pantoprazole users with omeprazole users on an equivalent maintenance dose (≤ 20 OE/day), again omeprazole was significantly associated with ID, whereas pantoprazole had a lower, not statistically significant point estimate with a wide CI for risk of ID. Furthermore, OE as continuous variable was independently associated with ID, indicating that risk of ID is higher while using PPIs with a higher potency or daily dose.

In a previous study, we found that KTRs using PPIs have an increased risk of ID compared with KTRs who are not using PPIs [2]. This observation was confirmed in the current study, using data from an independent

Table 2. Association between PPI use and iron deficiency in 724 kidney transplant recipients.

	Median DD	Median OE	N events	Iron deficiency OR (95% CI)	*P*
PPI	20 mg	20 OE	265	2.02 (1.36–3.00)	<0.001
No PPI	n/a	n/a	53	reference	n/a
Esomeprazole	40 mg	64 OE	25	3.58 (1.73–7.40)	0.001
Omeprazole	20 mg	20 OE	166	1.96 (1.31–2.94)	0.001
Pantoprazole	40 mg	9.2 OE	21	1.55 (0.78–3.10)	0.2

DD, daily dose; OE, omeprazole equivalent; N events, number of events; OR, odds ratio.

Analyses were adjusted for: age, sex, BMI, history of upper gastrointestinal disease or history of gastrointestinal hemorrhage, eGFR, proteinuria, time since transplantation, smoking, alcohol use, hs-CRP, CNIs, proliferation inhibitors, prednisolone, antiplatelet drugs, beta-blockers, ACE inhibitors, calcium channel blockers and angiotensin-2 receptor blockers.

Table 3. Dose–response analysis.

	Categories of PPI use			
	No PPI OR (95% CI)	Low-dose PPI OR (95% CI)	High-dose PPI OR (95% CI)	P_{trend}
Iron deficiency				
Number of subjects, n (%)	217 (29.9)	348 (47.9)	161 (22.2)	
Crude	1.00 (reference)	2.04 (1.40–2.98)	2.66 (1.72–4.13)	<0.001
Adjusted model	1.00 (reference)	1.80 (1.18–2.72)	2.61 (1.61–4.24)	<0.001

PPI, proton-pump inhibitor; OR, odds ratio.

Analyses were adjusted for: age, sex, BMI, history of upper gastrointestinal disease or history of gastrointestinal hemorrhage, eGFR, proteinuria, time since transplantation, smoking, alcohol use, hs-CRP, CNIs, proliferation inhibitors, prednisolone, antiplatelet drugs, beta-blockers, ACE inhibitors, calcium channel blockers, and angiotensin-2 receptor blockers. Low-dose PPI was defined as ≤ 20 omeprazole equivalents/day, high-dose PPI was defined as > 20 omeprazole equivalents/day.

cohort. The novel observation that pantoprazole (up to 80 mg/day) might be linked with a lower risk of ID may have important implications for clinical practice. Given the high prevalence of ID in this population and the previously reported associated risk of mortality [3], the results of the current study argue against the frequent use of PPIs in the post-transplant setting and justify further investigation in larger cohort studies regarding the risk of ID associated with the different types of PPIs. PPIs are frequently prescribed after kidney transplantation, mainly to prevent peptic ulcer disease that may be induced by concomitant use of corticosteroids and platelet inhibitors, among others. When comparing the effectiveness of pantoprazole and omeprazole for treatment of acute gastric ulcers and duodenal ulcers, pantoprazole does not seem to be inferior to omeprazole [18,19]. Moreover, one might postulate that the pH4time needed for effective gastric protection, may be lower than needed for treatment of acute peptic ulcers. Pantoprazole might therefore be a safe choice as low-dose PPI therapy in KTRs [5]. In the field of cardiology, pantoprazole has long been preferred over other PPIs because of absence of an interaction with clopidogrel through the cytochrome P450 enzyme system, although controversy about this interaction still exists in literature [20,21]. Because platelet inhibitors are often used by KTRs, this might be an additional reason for prescribing pantoprazole.

Our findings are in agreement with results from two large population-based studies, both demonstrating an increased risk of ID in long-term PPI users [14,15]. However, this is the first study suggesting a relatively lower risk of ID in pantoprazole users. Importantly, this relatively lower risk was also present when pantoprazole users (median DD of 40 mg, OE 9.2 mg) were compared with low-dose omeprazole users (median DD of 20 mg, OE 20 mg), which supports our hypothesis that pantoprazole is less potent, although the possibility of lack of power influencing this finding should be acknowledged. Despite comparing equivalent standard dosages (20 mg omeprazole vs. 40 mg pantoprazole), the potency of pantoprazole is indeed lower (9.2 OE vs. 20 OE), thereby possibly interfering less with reduction of ferric (Fe^{3}) to ferrous (Fe^{2+}) iron and subsequently

Table 4. Time–response analysis.

PPI Exposure (years)	Cases (events)	OR (95% CI)	*P*
Reference: non-exposed	217 (53)	-	-
0.1–2.0	102 (39)	1.42 (0.77–2.61)	0.3
> 2.0 years	212 (79)	2.23 (1.37–3.61)	0.001
Non-exposed	217 (53)	-	-
Reference: 0.1–2.0	102 (39)	-	-
>2.0 years	212 (79)	1.57 (0.82–2.99)	0.2

PPI, proton-pump inhibitor; OR, odds ratio.

Analyses were adjusted for: age, sex, BMI, history of upper gastrointestinal disease or history of gastrointestinal hemorrhage, eGFR, proteinuria, time since transplantation, smoking, alcohol use, hs-CRP, CNIs, proliferation inhibitors, prednisolone, antiplatelet drugs, beta-blockers, ACE inhibitors, calcium channel blockers, and angiotensin-2 receptor blockers. Only new PPI users were included in this analysis, KTR who were using a PPI before transplantation were excluded (n = 193).

iron absorption. Another explanation for this finding can be sought in a direct effect on iron metabolism through the iron regulatory hormone hepcidin, which inhibits iron absorption through deactivation of the iron exporter ferroportin [22]. According to an *in vitro* study in HepG2 cells, all PPIs enhanced hepcidin mRNA expression; however, pantoprazole showed the weakest effect [23]. These data indicate that PPIs do not only influence iron absorption by altering the acidic environment of the gastrointestinal tract, but potentially also by modulating hepcidin expression (Fig. 1).

Although switching to a less potent PPI might potentially be able to decrease the iatrogenic component of ID after kidney transplantation, another perhaps even more important factor is reduction of inappropriate PPI use. It is estimated that up to 60% of PPI prescriptions may be unnecessary [24–26]. Because in our study PPIs were used by the majority of KTRs and PPIs were associated with ID, we hope that the current study also stimulates clinicians to re-evaluate treatment indication and to prescribe PPIs only for an evidence-based indication. In the UMCG, it is currently standard care to initiate PPI treatment after transplantation for ulcer prophylaxis. This is, however, not standard policy in transplant centers worldwide, probably because of an absence of scientific data and guideline recommendation that support the routine use of PPIs post-transplant. Added to this, previous studies performed among chronic kidney disease (CKD) populations and transplant populations show that PPI use is associated with a higher risk of hypomagnesaemia, acute kidney injury, incident CKD, CKD progression and even premature mortality [27,28]. Although the evidence for these adverse effects mainly comes from observational studies, it does emphasize the need to avoid unnecessary use of PPIs after transplantation.

Our study has several strengths and limitations. The main strength is the large well-phenotyped cohort of KTRs having data available on iron status parameters, which were measured without medical indication. Another strength is that we tried to minimize indication bias by specifically adjusting for history of upper gastrointestinal disorders which may lead to gastrointestinal bleeding and subsequent treatment with a more potent PPI. Moreover, the use of H2RAs for more than 2 years has also been linked to ID in the study from Lam *et al.* [15]. We therefore performed a sensitivity analysis in which H2RA users were excluded, which did not materially alter the association between type of PPI and risk of ID.

We also acknowledge several limitations of this study. Because of the observational design, the different PPIs studied were not evenly distributed among groups. Use of omeprazole (n = 403) or esomeprazole (n = 47) was associated with ID, whereas use of pantoprazole (n = 57) was not. For the former two, there was enough evidence to show clear positive associations, whereas for the latter, the study could not detect any significant effects, which might pinpoint a power problem rather than a true lack of effect. Possibly, the effect of pantoprazole on ID could have been significant with an increased sample size; however, the effect of pantoprazole would most likely not be as strong as the other two PPI groups (especially esomeprazole). The power issue potentially also applies to the subgroup analysis comparing low-dose omeprazole users versus pantoprazole users. Second, most of our study participants were Caucasians which hampers generalizability to other populations. This is especially important in light of the CYP2C19 genotypes, which are known to influence the pharmacokinetics of PPIs [29]. In Caucasian populations, approximately 36% of the population has a mutation on the CYP2C19 gene (*1/*17 or *17/*17 genotype), which is associated with a faster hepatic metabolism of PPIs. In contrast, 50% of the Asian population has a mutation on one allele (wt/*2, wt/*3 genotype) and 25% of the population has a mutation on both alleles (*2 /*2 or *3 / *3 genotype), which results in the poor metabolizer phenotype [29,30]. The fact that PPIs show a genotype-dependent acid

Table 5. Linear associations between PPI type and serum iron, ferritin, TSAT, and hemoglobin levels in 724 kidney transplant recipients.

	N	Iron, μmol/l β (95% CI)	*P*	Ln Ferritin, μg/l β (95% CI)	*P*	TSAT, % β (95% CI)	*P*	Hemoglobin, g/dl β (95% CI)	*P*
PPI (All)	724	−0.93 (−1.80; −0.06)	0.04	−0.37 (−0.53; −0.21)	<0.001	−2.36 (−3.96; −0.76)	0.004	−0.24 (−0.50; 0.03)	0.08
PPI type									
No PPI	217	Reference	n/a	Reference	n/a	Reference	n/a	Reference	n/a
Esomeprazole	47	−2.42 (−4.08; −0.77)	0.004	−0.42 (−0.73; −0.11)	0.007	−4.50 (−7.55; −1.44)	0.004	0.13 (−0.37; 0.64)	0.6
Omeprazole	403	−0.83 (−1.73; 0.06)	0.07	−0.37 (−0.53; −0.21)	<0.001	−2.20 (−3.84; −0.56)	0.009	−0.30 (−0.57; −0.03)	0.03
Pantoprazole	57	−0.38 (−1.95; 1.19)	0.6	−0.32 (−0.61; −0.03)	0.03	−1.77 (−4.65; 1.11)	0.2	−0.01 (−0.49; 0.47)	0.9

PPI, proton-pump inhibitor; CI, confidence interval; TSAT, transferrin saturation.

Analyses were adjusted for: age, sex, BMI, history of upper gastrointestinal disease or history of gastrointestinal hemorrhage, eGFR, proteinuria, time since transplantation, smoking, alcohol use, hs-CRP, CNIs, proliferation inhibitors, prednisolone, antiplatelet drugs, beta-blockers, ACE inhibitors, calcium channel blockers, and angiotensin-2 receptor blockers.

inhibition makes this a possible confounder for which we could not adjust in this study. Moreover, the extent to which acid inhibition is affected by these different CYP2C19 polymorphisms appears to differ between PPI types [7]. Third, it cannot be entirely excluded that the results of the time–response analysis were affected by the exclusion of KTRs who used PPIs before transplantation. In this analysis, the effect of duration might be underestimated for this non-random subset of KTRs using PPIs before transplantation. Fourth, we excluded KTRs using iron supplementation or EPO-stimulating agents which might have caused sampling bias, because these KTRs are more likely to be sensitive to ID. However, we deliberately excluded these KTRs as iron supplementation could affect the outcome measures of our study. Similarly, we excluded KTRs with no data available on iron status parameters. Although these samples were not available because of logistic problems and most likely not related to the health status of the KTRs, we cannot fully exclude some informative missingness. Another limitation of the current study is that additional erythrocyte and reticulocyte parameters to assess ID were unavailable and information about treatment adherence and the indication of PPI treatment before transplantation were unavailable. Despite adjustment for various confounding factors, residual confounding cannot be excluded and conclusions about causality cannot be drawn because of the cross-sectional nature of this study. Furthermore, we cannot exclude the possibility that our results are subjected to reverse causation, although we tried to minimize the possibility of occurrence of reverse causation by taking into account the duration of PPI exposure for each included KTR in a sensitivity analysis (Table S2). Ideally, to answer a study hypothesis, there should be three clearly separated periods, namely: first, collecting data on covariates (ignoring later changes of covariates); second, recording PPI use or non-use; and third, to subsequently evaluate the outcome ID. Because we were not able to separate these three periods apart from each other, our study needs to be mainly regarded as a pure data description of our cohort. Nevertheless, no randomized clinical trials are currently available to confirm or disprove our findings. This study underscores once more the need for such trials. Finally, it is currently unknown whether the prevention of ID or the correction of ID after transplantation also leads to better clinical outcomes. It has been established that ID correction has positive effects on exercise capacity and cardiovascular endpoints in patients with heart failure and patients with end-stage renal disease [12,31,32]. Whether this also holds true for KTRs has

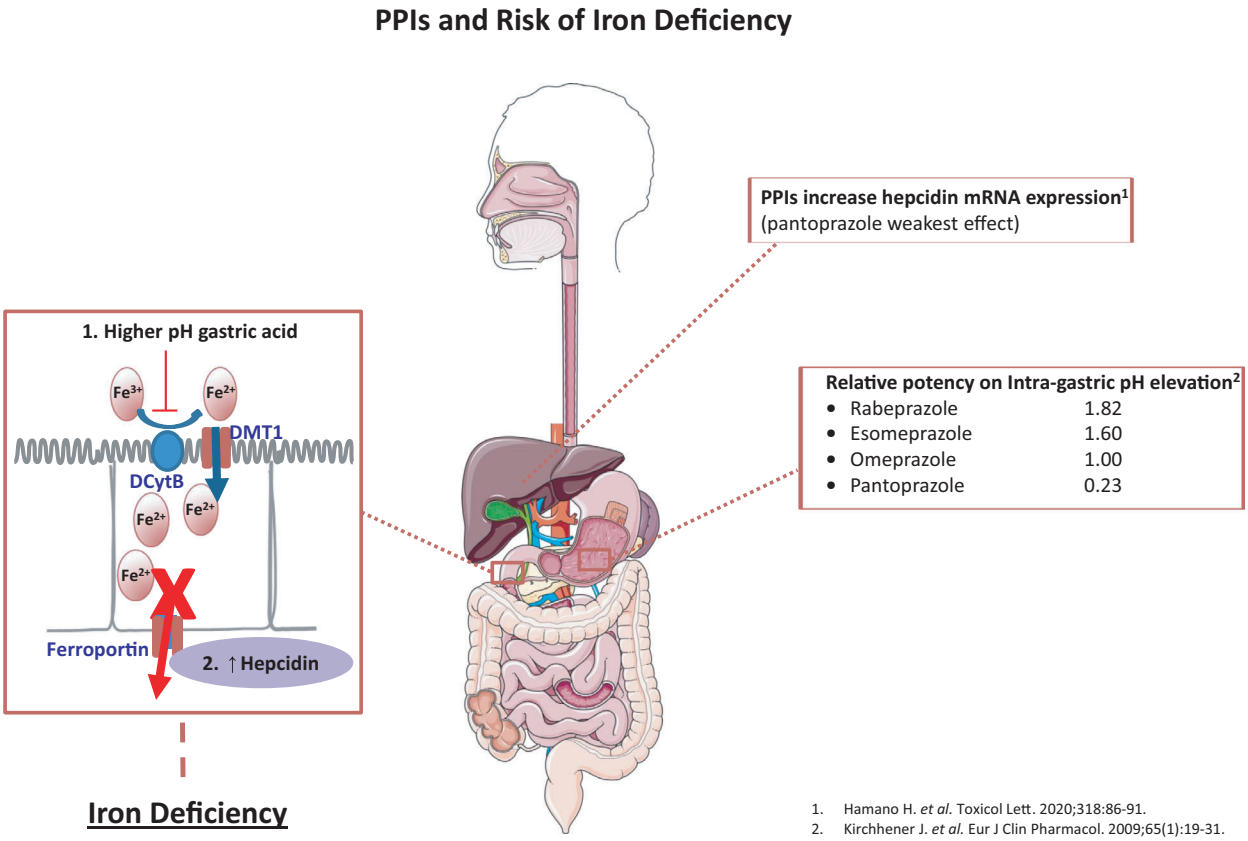

Figure 1 Potential mechanisms explaining the relationship between PPI use and iron deficiency. Potential mechanisms of PPI-induced iron deficiency: 1) PPIs increase the intragastric pH, thereby reducing the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron by the apical membrane ferrireductase duodenal cytochrome B (DCytB), and subsequently, iron absorption through the divalent metal transporter 1 (DMT1), 2) PPIs have been shown to increase hepcidin mRNA expression. The iron regulatory hormone hepcidin inhibits iron absorption through internalization and degradation of the iron exporter ferroportin located among others on the duodenal enterocytes, thereby reducing intestinal iron uptake. DCytB, duodenal cytochrome B; DMT1, divalent metal transporter 1; mRNA, messenger-RNA.

yet to be investigated. A randomized controlled trial investigating the effect of ferric (III) carboxymaltose versus placebo on exercise capacity and quality of life in KTRs is currently ongoing in our center (EFFECT-KTx, ClinicalTrial.gov NCT03769441).

In conclusion, we identified that PPI use is associated with ID in KTRs, but that among the different types of PPIs, pantoprazole, as a less potent PPI, seems to be associated with a relatively lower risk of ID. Future studies will need to delineate in more detail whether switching to a less potent PPI might reduce the iatrogenic component of ID after kidney transplantation.

Authorship

Research idea and study design: RMD, JSJV, AWGN, SJLB, MFE; data acquisition: RMD, JSJV, AWGN, GA, GH, MFE; data analysis/interpretation: RMD, JSJV, MFE; statistical analysis: RMD; manuscript writing: RMD, JSJV, MFE; manuscript review: AWGN, GA, GH, SPB, DJT, HB, SJLB, MHB; supervision or mentorship: SPB, DJT, HB, SJLB, MHB, MFE. Each author contributed important intellectual content during manuscript drafting or revision, accepts personal accountability for the authors own contributions, and agrees to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Conflict of interest

Dr. Touw has received grants (to employer) from ZonMw, Astellas and Chiesi Pharmaceuticals for

research outside this project. Dr. de Borst is the principal investigator of a clinical trial supported by the Dutch Kidney Foundation (grant number: 17OKG18) and Vifor Fresenius Medical Care Renal Pharma. He received consultancy fees (to employer) from Amgen, Astra Zeneca, Bayer, Kyowa Kirin, Sanofi Genzyme, and Vifor Fresenius Medical Care Renal Pharma. Dr. Eisenga received speaking fees from Vifor Pharma. None of these entities had any role in the design, collection, analysis, and interpretation of data for the current study, nor in writing the report or the decision to submit the report for publication. Parts of this study were presented in abstract form at the 57th ERA-EDTA Congress 2020.

Data availability statement

All data underlying the results presented in this study can be made available by the data manager of the TransplantLines study, by mailing to datarequest.transplantlines@umcg.nl.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Association between PPI use and iron deficiency in 724 kidney transplant recipients.

Table S2. Dose-response analysis.

Table S3. Time-response analysis.

Table S4. Linear associations between PPI type and serum iron, ferritin, TSAT and hemoglobin levels in 724 kidney transplant recipients.

Table S5. Association between PPI use and iron deficiency in 701 kidney transplant recipients, H2RA users excluded (n = 23).

Table S6. Association between PPI use and iron deficiency in 724 kidney transplant recipients with additional adjustment for duration of PPI exposure.

Table S7. Association between PPI use and iron deficiency in 724 kidney transplant recipients using alternative definition of ID (TSAT < 20% and ferritin < 100 μg/L).

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