Long-term use of proton pump inhibitors and vitamin B12 status in elderly individuals

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SUMMARY

Background
Some studies have shown that short-term use of proton pump inhibitors decreases the absorption of vitamin B12, but the results of studies into long-term proton pump inhibitor use and vitamin B12 deficiency are inconsistent.

Aim
To investigate whether long-term proton pump inhibitor use is associated with an abnormal vitamin B12 status in elderly individuals.

Methods
One hundred and twenty-five long-term (>3 years) proton pump inhibitor users aged 65 years and above were recruited from general practices. Their 125 partners (who did not use proton pump inhibitors) served as the reference group. Vitamin B12 status was determined by serum levels of vitamin B12 and homocysteine, and mean corpuscular volume.

Results
No differences in mean vitamin B12 levels were observed between the long-term proton pump inhibitor users and their partners [345 (s.d. 126) pM vs. 339 (s.d. 133) pM, P = 0.73], even after adjustment for age, gender, Helicobacter pylori status and C-reactive protein levels (P = 0.87). Four proton pump inhibitor users and three partners had vitamin B12 levels <150 pM (3% vs. 2%, P = 1.00). No differences between the groups were observed in homocysteine levels and mean corpuscular volume.

Conclusions
No association between long-term proton pump inhibitor use and vitamin B12 status was observed. Regular testing for low vitamin B12 levels in elderly patients on long-term treatment with proton pump inhibitors is therefore not recommended.

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INTRODUCTION

Many elderly patients use proton pump inhibitors (PPIs) for various indications, such as gastro-oesophageal reflux disease, peptic ulcers and chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs). PPIs reduce the formation of gastric acid in the stomach by inhibiting H⁺K⁺-ATPase in parietal cells. As gastric acid is essential for the release of vitamin B12 from food, PPI use may lead to vitamin B12 deficiency.

In many reviews on the aetiology and diagnosis of vitamin B12 deficiency, it is stated that PPI use is a cause of vitamin B12 deficiency. Screening for vitamin B12 deficiency in patients on long-term treatment with PPIs has even been recommended. Short-term studies have shown that PPI use indeed decreases the absorption of protein-bound vitamin B12. However, the results of studies into long-term PPI use and vitamin B12 deficiency are inconsistent. Some studies have reported a decline in serum vitamin B12 during long-term PPI use, but it is not clear whether this decline is clinically significant.

The aim of this study was to investigate whether long-term use of PPIs is associated with a clinically significant vitamin B12 deficiency in the elderly. For this purpose, the vitamin B12 status was compared between long-term users of PPIs aged 65 years and above and their partners as a reference group.

PARTICIPANTS AND METHODS

Participants

Long-term users of PPIs aged 65 years and over who were living in the community were selected in general practices associated with the Leiden Primary Care Research Network, the Netherlands. Patients were identified using the Electronic Patient Records (EPRs). Long-term use of PPIs was defined as prescription of >270 Defined Daily Doses (DDD) of PPIs (ATC codes A02BC01 through A02BC05) per year for 3 years or more (>810 DDD in 3 years).

As serum vitamin B12 levels may be influenced by dietary habits and nutritional status, partners who shared the same dwelling (or other housemates) served as the reference group. Couples were eligible for study participation if according to the EPR: (i) the partner had not used PPIs in the preceding 3 years and (ii) the PPI user and partner had not used parenteral vitamin B12 supplements, folic acid supplements, H₂-blockers and antacids for the preceding 3 years. Couples were excluded by the general practitioners if the PPI user or partner suffered from chronic alcohol abuse, had severe cognitive impairment, had severe psychological problems or was terminally ill.

Methods

General practitioners invited eligible couples by mail to participate in the study. The invitation letter included a patient information form, an informed consent form and a short questionnaire on demographic characteristics, dietary intake and the use of oral vitamin supplements and additional stomach medication. Those couples who returned the consent form and questionnaire were contacted by telephone to schedule a visit to the laboratory for blood sampling. Blood sampling and analyses were performed by the ‘SCAL Medical Diagnostic Centre’ in Leiden, the Netherlands. This is a certified medical laboratory, which is frequently used by general practitioners in and around Leiden. As the laboratory measurements were carried out in the day-to-day phlebotomy setting of the SCAL laboratory, it was not possible to perform all measurements in one batch. Therefore, the PPI user and partner were asked to visit the laboratory together to reduce differences in test conditions within each couple. In cases of physical impairment, the participants were visited at home by a phlebotomist from the SCAL laboratory. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre.

Measurements

Questionnaires. To assess whether eating habits and daily menus were equal within each household, the participants (patients and partners) completed a short questionnaire on dietary intake. All participants were asked to indicate how many days in the past week they had consumed seven different foods or food groups containing vitamin B12: (i) cooked meat, (ii) cold cuts of meat, (iii) chicken, (iv) cheese, (v) eggs, (vi) milk and (vii) yogurt. Possible answers for each question were: none (0 points), 4 days or less (1 point) and more than 4 days (2 points). The dietary vitamin...
B12 intake was estimated by adding together the points for these seven questions.

Participants were also asked about their height, weight, smoking habits, alcohol use, level of education, use of over-the-counter oral (multi)vitamin supplements and stomach medication.

The general practitioners were interviewed to obtain information on the starting date of PPI use and the reason for PPI use.

**Laboratory measurements.** The vitamin B12 status was determined by serum levels of vitamin B12, serum levels of homocysteine and mean corpuscular volume (MCV). These three indicators for vitamin B12 status were used, because serum levels of vitamin B12 alone may not accurately reflect true vitamin B12 status.\(^7,24,25\)

Concentrations of vitamin B12, homocysteine, albumin, C-reactive protein (CRP), MCV and *Helicobacter pylori* status were measured in every participant. CRP levels were measured as a general marker of health status. All assays were performed using commercially available kits and methods. MCV was determined in anticoagulated whole blood (EDTA-coated tubes) samples using Bayer Advia 120 [Bayer, Leverkusen, Germany; interassay coefficients of variation (CV): \(~1\%\)]. Concentrations of other variables were performed in serum samples from clotted blood. Vitamin B12 concentrations were measured with the Abbott method using the Axsym apparatus (Abbott, Abbott Park, IL, USA; interassay CVs: \(~10\%)\). Homocysteine concentrations were determined with Beckman LX20 (Beckman, Brea, CA, USA; interassay CVs: \(~13\%)\). Concentrations of albumin and CRP were measured using Roche Integra 800 (Roche, Basel, Switzerland; interassay CV: \(~2\%)\) and interassay CV: \(~2\%)\) respectively). For *Helicobacter* serology the QuickVue method was used (Quidel, San Diego, CA, USA; sensitivity 90% and specificity 78%).\(^26\)

**Sample size**

Previous research has shown that the mean vitamin B12 level between 65 and 80 years of age is \(~295 \text{ pM}\) with a standard deviation \(~160 \text{ pM}\).\(^27\) Therefore, to detect a clinically relevant difference of \(~50 \text{ pM}\) between the long-term PPI group and the partner group, 110 patients and 110 partners were needed, assuming that the correlation between the vitamin B12 levels of the PPI user and partner was 0.5 (power 90%, significance level 0.05). The selection and inclusion of participants continued until at least 110 couples had participated in the study.

**Data analysis**

As the long-term PPI users were matched to their partners, the data were analysed as paired data. Differences in continuous variables between the long-term users of PPIs and partners were analysed with paired \(t\)-tests. Differences in age and CRP levels were analysed with the Wilcoxon signed ranks test, as these variables were not normally distributed. Differences in categorical variables were analysed with McNemar’s test. Differences in continuous variables within the PPI group were analysed with independent \(t\)-tests. To adjust for possible confounding variables, i.e. age, gender, *H. pylori* status and CRP levels (as a measure of the presence of disease), we used linear mixed models. \(P < 0.05\) was considered statistically significant.

The statistical analyses were performed with SPSS version 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

**RESULTS**

Two hundred and six couples from 39 general practices in and around Leiden, the Netherlands, were invited to participate in the study. One hundred and twenty-five couples completed the questionnaires and agreed to blood sampling (response 61%). All couples \((n = 124)\) were married couples or couples who lived together as husband and wife, except one couple which consisted of a mother (PPI user) and a son.

For 116 PPI users we obtained information from the general practitioners on the starting date of PPI use and the reason for PPI use. The median duration of PPI use was 6 years (interquartile range: 5–10). Most PPI users (66%) received PPIs for gastro-oesophageal reflux disease. Five per cent of the PPI users received PPIs for the treatment of peptic ulcers, 5% for chronic use of NSAIDs and 25% for other reasons.

Table 1 shows the demographic characteristics, dietary intake of vitamin B12 and use of over-the-counter oral vitamin B12/folic acid supplements and stomach medication of the long-term PPI users \((n = 125)\) compared to their partners \((n = 125)\). We observed no differences between the study groups in

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gender, age, level of education, smoking behaviour, dietary vitamin B12 intake, \textit{H. pylori} status, CRP levels, use of over-the-counter oral vitamin B12/folic acid supplements and use of stomach medication. PPI users had higher body mass indices and lower albumin levels compared with their partners (paired \textit{t}-test \(P < 0.05\)).

The vitamin B12 status of the long-term PPI users and their partners is presented in Table 2. The prevalence of vitamin B12 deficiency (serum level <150 pM) was similar in both groups: four (3\%) PPI users and three (2\%) partners (McNemar’s test \(P = 1.00\)). No differences in mean serum levels of vitamin B12 were observed between the long-term PPI users [mean 345 (s.d. 126) pM] and their partners [mean 339 (s.d. 133) pM, paired \textit{t}-test \(P = 0.73\)]. Adjustment for gender, age, \textit{H. pylori} status and CRP levels did not change the results [linear mixed model: adjusted mean difference = 2 pM (95\% CI: −27 to 32), \(P = 0.87\)]. Moreover, exclusion of participants that used over-the-counter oral vitamin B12/folic acid supplements or other stomach medication did not change the

<p>| Table 1. Sociodemographic and clinical characteristics of long-term users of proton pump inhibitors (PPIs) aged 65 years and above and their partners |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>PPI users ((n = 125))</th>
<th>Partners ((n = 125))</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>69 (55%)</td>
<td>56 (45%)</td>
</tr>
<tr>
<td>Age ([\text{years}; \text{median (IQR)}])</td>
<td>72.9 (68.2–76.4)</td>
<td>73.2 (68.5–76.2)</td>
</tr>
<tr>
<td>Low level of education ((\leq \text{primary school}))</td>
<td>40 (33%)</td>
<td>38 (32%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (7%)</td>
<td>10 (8%)</td>
</tr>
<tr>
<td>Body mass index ([\text{kg/m}^2])</td>
<td>27.1 (4.6)</td>
<td>25.7 (3.0)</td>
</tr>
<tr>
<td>Dietary vitamin B12 intake score*</td>
<td>9 (2)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>Over-the-counter oral vitamin B12/folic acid supplements†</td>
<td>26 (21%)</td>
<td>23 (19%)</td>
</tr>
<tr>
<td>Over-the-counter stomach medication</td>
<td>12 (10%)</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Positive \textit{Helicobacter pylori} status‡</td>
<td>40 (32%)</td>
<td>50 (41%)</td>
</tr>
<tr>
<td>Albumin ([\text{g/L}])</td>
<td>43.7 (2.8)</td>
<td>44.4 (2.5)</td>
</tr>
<tr>
<td>C-reactive protein ([\text{mg/L}; \text{median (IQR)}])</td>
<td>1.0 (1.0–2.6)</td>
<td>1.0 (1.0–2.6)</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean (s.d.) unless stated otherwise; differences were analysed with paired \textit{t}-tests or Wilcoxon signed ranks tests. Categorical data are presented as number (%); differences were analysed with McNemar’s tests.

* Based on weekly use of meat, chicken, milk, yoghurt, cheese and eggs (range: 0–14). † Multivitamins. No stand-alone vitamin B12 or folic acid supplements were used. ‡ Serology.

<p>| Table 2. Differences in vitamin B12 status between long-term users of proton pump inhibitors (PPIs) aged 65 years and above and their partners |
|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>PPI users ((n = 125))</th>
<th>Partners ((n = 125))</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12 ([\text{pM}])</td>
<td>345 (126)</td>
<td>339 (133)</td>
</tr>
<tr>
<td>Low vitamin B12 levels (&lt;150 \text{ pM})</td>
<td>4 (3%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Homocysteine ([\mu\text{M}])</td>
<td>12.6 (4.4)</td>
<td>13.1 (4.2)</td>
</tr>
<tr>
<td>Elevated homocysteine levels (&gt;13.5 \mu\text{M})</td>
<td>43 (35%)</td>
<td>48 (40%)</td>
</tr>
<tr>
<td>MCV ([\text{fL}])</td>
<td>91 (5)</td>
<td>91 (4)</td>
</tr>
<tr>
<td>Elevated MCV (&gt;100 \text{ fL})</td>
<td>3 (2%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean (s.d.); differences were analysed with paired \textit{t}-tests. Categorical data are presented as number (%); differences were analysed with McNemar’s tests. MCV, mean corpuscular volume.
results [PPI users 329 (s.d. 119) pm (n = 75) vs. partners 323 (s.d. 127) pm (n = 75), P = 0.75]. Within the PPI group, vitamin B12 levels of participants that had been using PPIs for 6 years or more (median duration of PPI use) did not differ from vitamin B12 levels of participants that had been using PPIs for 3–5 years [mean 349 (s.d. 127) pm (n = 59) vs. mean 342 (s.d. 114) pm (n = 42), independent t-test P = 0.77]. In addition, no differences in vitamin B12 levels were found between participants who had been using PPIs for 6 years or more and their partners [349 (s.d. 127) pm (n = 59) vs. 340 (s.d. 145) pm (n = 59), paired t-test P = 0.69].

We did not observe any differences in homocysteine levels [mean 12.6 (s.d. 4.4) μM vs. mean 13.1 (s.d. 4.2) μM, paired t-test P = 0.38] and MCV [mean 91 (s.d. 5) fl vs. mean 91 (s.d. 4) fl, paired t-test P = 0.69] between long-term users of PPIs and their partners (Table 2). Similar results were obtained after adjustment for possible confounders: homocysteine [adjusted mean difference = −0.4 μM (95% CI: −1 to 0.5), P = 0.42] and MCV [adjusted mean difference = 0.1 fl (95% CI: −0.9 to 1), P = 0.88].

DISCUSSION

In this cross-sectional study, the vitamin B12 status of long-term PPI users aged 65 years and over was compared with that of their partners who did not use PPIs. Long-term PPI use was neither associated with vitamin B12 deficiency, nor with other parameters that are clinically associated with vitamin B12 deficiency, such as elevated homocysteine levels and elevated MCV. These results suggest that there is no need to screen elderly long-term PPI users for vitamin B12 deficiency, not even after 6 years of therapy.

Gastric acid has been shown to be required for the release of vitamin B12 from food.5, 6 As a result, PPI use is often mentioned as a cause of vitamin B12 deficiency.7–11 Still, evidence for a relation between PPI use and clinical vitamin B12 deficiency is scarce. Some short-term studies show that PPIs have a direct effect on protein-bound vitamin B12 absorption.15–17 However, the relation between long-term PPI use and vitamin B12 deficiency is unclear. Two case reports indicated that vitamin B12 levels decreased under long-term PPI therapy.18, 19 Moreover, serum levels of vitamin B12 significantly declined in 21 achlorhydric patients with Zollinger–Ellison syndrome on long-term treatment with omeprazole (mean follow-up period 6.3 years).21 In contrast, no significant differences in mean levels of vitamin B12 were found before and during long-term omeprazole therapy (36–81 months) in 25 patients with gastro-oesophageal reflux disease.16 Furthermore, in 34 patients with various peptic diseases, vitamin B12 levels remained constant during 3 years of treatment with omeprazole. After 3 years of follow-up, however, vitamin B12 levels did show a significant decline, but in all patients vitamin B12 levels remained within the normal ranges.14, 20 These varying outcomes could possibly be explained by the small sample sizes, different patient populations, as well as differences in follow-up duration. In our large sample of elderly patients in general practice, we did not see any association between long-term PPI use and vitamin B12 status. Thus, PPIs reduce the absorption of vitamin B12, but long-term PPI use does not seem to lead to a clinically significant deficiency of vitamin B12, not even after 6 years of PPI therapy when the body stores of vitamin B12 are likely to be low due to prolonged inhibition of vitamin B12 absorption.20 Possibly, residual gastric acidity or the consumption of sufficient amounts of unbound (freely absorbed) vitamin B12 keeps the vitamin B12 status of long-term PPI users within the normal ranges.16, 20

Our study has multiple strengths. We studied a large sample of elderly patients on long-term PPI use in general practice. Inclusion was restricted to subjects using PPIs for at least 3 years. As the vitamin B12 status may be influenced by nutritional habits, we used the partners of the long-term PPI users as a reference group, rather than an independent sample of elderly individuals. We hereby circumvented daily vitamin B12 intake as a possible confounder, because the partners of the PPI users shared the same place of residence and their dietary habits were likely close to those of the PPI users. Our results were therefore not likely to be influenced by differences in dietary habits and may be generalized to all elderly PPI users. Moreover, in addition to measuring vitamin B12 levels directly, we considered other parameters that are clinically associated with vitamin B12 deficiency, such as homocysteine levels and MCV.

A possible limitation of our study is the cross-sectional design, as opposed to prospectively measuring vitamin B12 levels in elderly subjects from the start of PPI treatment. However, we do not think that this impedes a causal interpretation of the results. PPI use could not have induced a large decline in vitamin B12 levels, as the vitamin B12 levels of those participants...
that had been using PPIs for 6 years or more were similar to the vitamin B12 levels of their partners. This lack of effect on vitamin B12 levels, even with prolonged PPI use, supports our main outcome.

Another limitation of our study may be that the results were influenced by a 'healthy-user' effect. As PPI users who had received parenteral vitamin B12 supplements were excluded from the study, PPI users who had developed vitamin B12 deficiency caused by PPI use may have been excluded. However, the 'healthy-user' effect in our study might be minimal, as the guideline on stomach complaints of the Dutch College of General Practitioners does not include a statement on vitamin B12 deficiency as a complication of (long-term) PPI use and does not advise Dutch general practitioners to monitor vitamin B12 levels in patients on long-term PPI use.29

In conclusion, in this cross-sectional study, no association was observed between long-term PPI use and vitamin B12 status in the elderly. Based on this finding, regular testing for low vitamin B12 levels in elderly individuals on long-term PPI therapy is not recommended.

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REFERENCES


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