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Original Research Article

Oral vitamin B12 supplementation in pernicious anemia: a prospective cohort study

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A B S T R A C T

Background: The absorption of vitamin B12 is hindered in pernicious anemia (PA) owing to intrinsic factor deficiency. Traditionally, intramuscular vitamin B12 injections were the standard treatment, bypassing the impaired absorption. Although there is potential for oral vitamin B12 supplementation through passive enteral absorption, it is not commonly prescribed in PA owing to limited studies assessing its efficacy.

Objectives: We aimed to assess the efficacy of oral vitamin B12 supplementation in PA.

Methods: We enrolled participants diagnosed with incident vitamin B12 deficiency related to PA. The diagnosis of PA was based on the presence of classical immune gastritis and of anti-intrinsic factor and/or antiparietal cell antibodies. To evaluate the vitamin B12 status, we measured total plasma vitamin B12, plasma homocysteine, and plasma methylmalonic acid (pMMA) concentration and urinary methylmalonic acid–to–creatinine ratio. Participants were treated with oral cyanocobalamin at a dosage of 1000 µg/d throughout the study duration. Clinical and biological vitamin B12 deficiency related features were prospectively and systematically assessed over the 1-y study duration.

Results: We included 26 patients with vitamin B12 deficiency revealing PA. Following 1 mo of oral vitamin B12 supplementation, 88.5% of patients were no longer deficient in vitamin B12, with significant improvement of plasma vitamin B12 [407 (297–485) compared with 148 (116–213) pmol/L; $P < 0.0001$], plasma homocysteine [13.5 (10.9–29.8) compared with 18.6 (13.7–46.8) µmol/L; $P < 0.0001$], and pMMA [0.24 (0.16–0.38) compared with 0.56 (0.28–1.09) pmol/L; $P < 0.0001$] concentrations than those at baseline. The enhancement of these biological parameters persisted throughout the 12-month follow-up, with no patients showing vitamin B12 deficiency by the end of the follow-up period. The median time to reverse initial vitamin B12 deficiency abnormalities ranged from 1 mo for hemolysis to 4 mo for mucosal symptoms.

Conclusions: Oral supplementation with 1000 µg/d of cyanocobalamin has been shown to improve vitamin B12 deficiency in PA.

Keywords: pernicious anemia, vitamin B12 deficiency, atrophic gastritis, vitamin B12, anemia

Introduction

Vitamin B12 (cobalamin) deficiency is a common condition, affecting ~5% of the general population and 10%–20% of individuals aged 65 y and older [1–5]. Vitamin B12 deficiency is accountable for various manifestations, including hematologic, mucosal, and neurologic impairments [6,7]. Pernicious anemia (PA) stands out as one of its main causes [8–10]. PA is an autoimmune atrophic gastritis characterized by the destruction of gastric parietal cells and the absence of intrinsic factor, the intestinal vitamin B12 carrier protein essential for its active enteral absorption through the cubilin–amnionless receptor [10,11]. Historically, the treatment of PA-related vitamin B12

Abbreviations: IM, intramuscular; PA, pernicious anemia; PA-APCA, pernicious anemia with antiparietal cell antibodies (without intrinsic factor); PA-IFA, pernicious anemia with anti-intrinsic factor antibodies (with or without APCA); pMMA, plasma methylmalonic acid; uMMA/C, urinary methylmalonic acid/urinary creatinine; tB12, total plasma vitamin B12.

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deficiency consisted in intramuscular (IM) vitamin B12 administration to bypass the compromised enteral absorption. In the 1950s and 1960s, it was demonstrated that ~1% of ingested vitamin B12 can be passively absorbed without the presence of intrinsic factor [12–15]. Considering that the daily recommended intake of vitamin B12 is estimated to be 2.4 μg, it is noteworthy that this requirement can be easily fulfilled with the currently available supplements [16,17]. This discovery opened up the possibility of oral supplementation for individuals with PA [14,15,18]. Nevertheless, the adoption of oral supplementation for patients with PA remains relatively uncommon, likely owing to the limited supporting evidence [18,19]. Although the effectiveness of vitamin B12 oral supplementation has been extensively demonstrated and documented, it is essential to acknowledge that, in these studies, PA was excluded [20–23], inadequately defined [24], or represented only a small portion of the studied population [25–27]. The most extensive prospective study dedicated to evaluating oral supplementation in PA was limited to 10 patients, with a single assessment conducted after a 3-mo period of supplementation [28]. Hence, although the theoretical effectiveness of oral vitamin B12 supplementation is recognized, experts openly acknowledge the dearth of long-term data concerning such supplementation in PA. These gaps in comprehensive data may account for the skepticism prevailing within the medical community and likely play a role in the relatively limited adoption of oral supplementation in the context of PA [29,30].

In this study, our objective was to evaluate the effect of oral vitamin B12 supplementation on both the biological and clinical manifestations of vitamin B12 deficiency in a prospective cohort of individuals with PA over a 12-mo follow-up.

Methods

Ethics

This study was approved by the Bioethics Committee of the Angers University Hospital (no. 2018/63) and by the French data protection authority (CNIL, no. 2018-021). It was conducted in compliance with the tenets of the Declaration of Helsinki and according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations for cohort studies. Written consent was obtained from enrolled patients.

Study population

We prospectively enrolled both inpatients and outpatients aged 18 y and older, at Angers University Hospital between November 2018 and October 2021, presenting with vitamin B12 deficiency secondary to PA. We excluded individuals who had received oral vitamin B12 supplementation for ≥7 d before the inclusion visit, had been administered ≥7000 μg of cyanocobalamin (or an equivalent dose) orally before the inclusion visit, or had ever received IM vitamin B12 supplementation. We also excluded individuals with conditions known to impair enteral vitamin B12 absorption, such as gastrectomy, radiation-induced gastritis, ileal resection, Crohn disease, and Imerslund–Grasbeck disease.

Definition of vitamin B12 deficiency

Vitamin B12 deficiency was defined as follows: *1*) total plasma vitamin B12 (tB12) ≤ 148 pmol/L (200 ng/L) or *2*) the combination of tB12 between 148 and 258 pmol/L (201 and 350 ng/L) and elevated plasma homocysteine (≥13 μmol/L for females, ≥15 μmol/L for males, and ≥20 μmol/L for patients older than 65 y), plasma methylmalonic acid (pMMA, ≥0.35 μmol/L) [31], or urinary methylmalonic acid–to–creatinine ratio (uMMA/C, ≥1.45 μmol/mmol) [10,32–36]. In case of folate deficiency [serum folate < 9.1 nmol/L (4 μg/L)] [37], plasma homocysteine was not considered to define vitamin B12 deficiency [38,39]. In case of severe renal failure with MDRD (modification of diet in renal disease) clearance of <30 mL/min/1.73 m^2, plasma homocysteine and pMMA were not considered to define vitamin B12 deficiency [32]. This definition of vitamin B12 deficiency was used both at baseline for patient inclusions and during follow-up to assess the effectiveness of oral vitamin B12 supplementation.

Diagnosis of PA

The diagnosis of PA was defined by the presence of vitamin B12 deficiency along with either *1*) the presence of anti-intrinsic factor antibodies (IFAs) or *2*) the presence of antiparietal cell antibodies (APCA) along with typical (atrophic gastritis affecting the fundus with sparing of the antrum) or suggestive gastric histology (non-atrophic fundus gastritis with sparing of the antrum, atrophy of both antrum and fundus, large fundus metaplasia with sparing of the antrum) [8,40]. IFA are highly specific (98%–100%) for PA [41], unlike APCA (50%–90%) [42,43]. Hence, in cases with presence of APCA, the confirmation of PA required the presence of documented autoimmune gastritis [44].

Participants were subsequently divided into 2 groups: PA-IFA group with presence of IFA, with or without APCA, and PA-APCA group with presence of APCA only.

Vitamin B12 supplementation

Participants with PA received a daily oral cyanocobalamin supplementation of 1000 μg throughout the entire study period [17,28,45]. In case of high tB12 levels, defined as a tB12 measurement >922 pmol/L (1250 ng/L) or 2 consecutive measurements >738 pmol/L (1000 ng/L), the dose was reduced to 1000 μg/wk.

Follow-up, data collection, and definitions of biological and clinical abnormalities

Participants were monitored for 12 mo with standardized clinical and biological assessments conducted at baseline (M0), as well as at 1, 3, 6, 9 and 12 mo after the initiation oral vitamin B12 supplementation (M1, M3, M6, M9, and M12 visits). Clinical and biological features associated with vitamin B12 deficiency were evaluated at each visit: clinical signs of anemia (fatigue, dyspnea, and palor), signs of polyneuropathy, pyramidal signs, proprioceptive disorders, Hunter glossitis, lingual dysesthesia, oral ulcerations, anemia, other cytopenias, and macrocytosis. Total plasma vitamin B12, plasma homocysteine and pMMA were measured at each visit, while uMMA/C was assessed at M0, M6, and M12. *Helicobacter pylori* gastritis was systematically investigated by a 13C-urea breath test (HELIKIT) and histologic analysis of gastric biopsies. Treatment compliance was evaluated using the Girerd questionnaire, comprising 6 items and scored on a scale from 0 to 6 (a score of 0/6 indicated very good compliance and a score ≥3/6 indicated poor compliance) [46–48]. Iron deficiency was defined by either ferritin of ≤30 μg/L or the combination of ferritin of ≤100 μg/L and transferrin saturation coefficient <20% in patients with C-reactive protein ≥10 mg/L [49,50]. Anemia was defined by a hemoglobin concentration of <120 g/L in females and <130 g/L in males, and macrocytosis by a mean corpuscular volume of >98 fL. Severe undernutrition was defined as having BMI (in kg/m^2) of <17 for individuals aged between 18 and 70 y; having BMI of <18 for those aged

70 y and older; cases with a weight loss of ≥10% in 1 mo or ≥15% over 6 mo; or cases with an albumin concentration of <30 g/L [51,52]. A polyneuropathy was defined as the presence of ≥2 of the 3 following signs, whether or not associated with motor impairment: distal paresthesia, distal hypoesthesia, and reduced osteotendinous reflexes. Pyramidal signs encompassed Hoffman sign, Babinski sign, and intense osteotendinous reflexes with an enlargement of the reflex area.

Biological assays

tB12 was quantified by chemiluminescent competitive immunoassay with the ADVIA Centaur system (Siemens Healthcare Diagnostics). Plasma homocysteine was quantified by HPLC-MS/MS (Agilent 1200 Infinity Series; Agilent Technologies; Triple Quad 4500; SCIEX). pMMA was quantified using HPLC-MS/MS (Agilent 1200 Infinity Series/Triple Quad 5500). uMMA was quantified by gas chromatography-tandem mass spectrometry (QP 2010S; Shimadzu).

IFA were tested using immunodot (Biermer atrophic gastritis dot; Alphadia). APCA were assessed using both immunodot (Biermer atrophic gastritis dot; Alphadia) and indirect immunofluorescence on rat stomach tissue (Biosystems).

Statistical analysis

Quantitative variables were presented as medians and quartiles and compared between 2 groups using Student *t* test or Mann–Whitney test according to the normality of the distribution, assessed by the d'Agostino–Pearson test. To assess changes in quantitative variables within a same group from baseline to follow-up, we used the paired *t* test or the Wilcoxon matched signed-rank test, depending on the normality of the distribution. Categorical variables were presented as absolute values and percentages and compared using the χ^2 or Fisher exact test. We presented the evolution of clinical and biological features over time using Kaplan–Meier curves. Patients were censored in case of loss of follow-up. The curves were compared using the log-rank test. To depict the evolution of tB12 over time following the introduction of oral B12 supplementation, we compared linear and logarithmic models. Akaike Information Criteria were used to select the model that best fits the evolution of tB12. Missing data were addressed using the Missing Completely at Random and pairwise deletion method. The type I error was set at 5%. The analyses were performed using the Graphpad Prism v6.01 software.

Results

Description of the population

We included 26 participants with vitamin B12 deficiency that uncovered PA (Supplemental Figure 1). Their main characteristics at baseline are detailed in Table 1. The study population, with a median age of 63 (49–74) y, comprised 17 of 26 (66.4%) females. IFA were identified in 10 of the 26 (38.5%) patients (isolated IFA in 2 patients and IFA in conjunction with APCA in 8 patients), while APCA were identified in 16 of the 26 (61.5%) patients without concomitant IFA presence. No statistical difference was observed between the groups in terms of tB12 [146 (120–176) compared with 152 (111–225) pmol/L; $P = 0.73$]; homocysteine [27.7 (18.1–44.3) compared with 14.4 (11.3–50.8) μmol/L; $P = 0.28$]; pMMA [0.76 (0.28–1.04) compared with 0.48 (0.32–1.60) μmol/L; $P = 0.83$]; and uMMA/C [0.39 (0.29–1.23) compared with 0.41 (0.33–2.92); $P = 0.81$] values at baseline for PA-IFA and PA-APCA groups, respectively.

TABLE 1
Description of the study population at baseline.

	Whole PA population ($n = 26$)	PA-IFA ($n = 10$)	PA-APCA ($n = 16$)
General characteristics			
Age (y)	63 (49–74)	67 (49–84)	63 (49–67)
Sex (females)	17 (65.4)	7 (70.0)	10 (62.5)
Concurrent causes of vitamin B12 deficiency			
Chronic PPI use	1 (3.8)	0 (0)	1 (6.3)
Biguanide (Metformin)	3 (11.5)	0 (0)	3 (18.8)
Severe undernutrition	1 (3.8)	1 (10.0)	0 (0)
Vegetarian or vegetarian diet	1 (3.8)	0 (0)	1 (6.3)
Helicobacter pylori gastritis	1 (3.8)	0 (0)	1 (6.3)
Clinical features at baseline			
Signs of anemia (fatigue, pallor, dyspnea)	9 (34.6)	3 (30.0)	6 (37.5)
Polyneuropathy	8 (30.8)	2 (20.0)	6 (37.5)
Pyramidal signs	7 (26.9)	1 (10.0)	6 (37.5)
Proprioceptive disorder	2 (7.7)	0 (0)	2 (12.5)
Hunter glossitis	5 (19.2)	2 (20.0)	3 (18.8)
Tongue dysesthesia	3 (11.5)	2 (20.0)	1 (6.3)
Recurrent oral ulcerations	3 (11.5)	2 (20.0)	1 (6.3)
Biological parameters at baseline			
Total plasma vitamin B12 (pmol/L)	148 (116–213)	146 (120–176)	152 (111–225)
Plasma homocysteine (μmol/L)	18.6 (13.7–46.8)	27.7 (18.1–44.3)	14.4 (11.3–50.8)
pMMA (μmol/L)	0.56 (0.28–1.09)	0.76 (0.28–1.04)	0.48 (0.32–1.60)
uMMA/C (μmol/mmol of creatinine)	0.40 (0.31–2.80)	0.39 (0.29–1.23)	0.41 (0.33–2.92)
Hemoglobin (g/dL)	12.1 (10.8–12.7)	11.0 (10.5–13.7)	12.3 (11.7–12.7)
MCV (fL)	93.8 (88.2–99.6)	93.8 (87.7–99.6)	93.3 (89.4–98.4)
Platelets (g/L)	248 (199–307)	251 (197–344)	248 (203–305)
Neutrophils (g/L)	4.5 (3.3–5.7)	4.2 (3.1–5.8)	4.5 (4.1–5.7)
Lymphocytes (g/L)	1.4 (1.2–1.9)	1.5 (1.1–1.7)	1.4 (1.2–2.2)
Haptoglobin (g/L)	1.1 (0.4–1.7)	1.6 (0.4–1.9)	1.0 (0.5–1.5)
Serum ferritin (μg/L)	43.5 (18.2–130.5)	148.0 (31.5–211.7)	28.5 (15.0–74.2)
Plasma folates (nmol/L)	19.8 (14.1–29.1)	17.0 (12.7–25.2)	23.9 (16.4–36.1)
MDRD creatinine clearance (mL/min/1.73 m^2)	102 (78–123)	78 (65–108)	115 (100–132)

Abbreviations: MCV, mean corpuscular volume; MDRD, Modification of Diet in Renal Disease; PA, pernicious anemia; PA-APCA, pernicious anemia with antiparietal cell antibodies (without IFA); PA-IFA, pernicious anemia with anti-intrinsic factor antibodies (with or without APCA); pMMA, plasma methylmalonic acid; PPI, proton pump inhibitors; uMMA/C, urinary methylmalonic acid/urinary creatinine.

Three patients with IFA did not undergo gastroscopy owing to their extreme age or frailty. Among the 23 of the 26 (88.5%) patients who underwent gastric biopsies, the histologic analysis revealed fundal atrophy and inflammatory infiltrates with antral sparing in 21 (91.3%). In 1 patient with isolated APCA, the biopsy specimen showed fundal metaplasia and inflammatory infiltrates without atrophy, while 1 patient with isolated IFA displayed intense fundal inflammatory infiltrate with antral sparing.

At the time of PA diagnosis and before vitamin B12 supplementation, 12 of the 26 (46.2%) participants presented with neurologic involvement (polyneuropathy, pyramidal signs, or proprioceptive impairment); 8 of the 26 (30.8%) with mucosal involvement (glossitis, tongue dysesthesias, or oral ulcerations); and 15 of the 26 (57.7%) with anemia.

The median Girerd score, assessing compliance with vitamin B12 supplementation, was 0 (0–0), indicating a very high level of adherence within the study population. We noted a temporary interruptions or reductions in vitamin B12 supplementation in 6 of the 26 (23.1%) patients, primarily attributable to changes in prescriptions on renewal by the general practitioner or during hospitalization. Additionally, the cyanocobalamin dosage was lowered to 1000 µg/wk in 3 patients owing to high tB12 levels, as stipulated in the protocol. None of these 3 patients with experienced signs of intoxication. During the 1-year follow-up, 3 patients died from causes unrelated to PA with no more vitamin B12 deficiency at previous assessment, and 1 patient was lost to follow-up at the last visit.

Correction of vitamin B12 deficiency through oral cyanocobalamin supplementation

tB12, plasma homocysteine, and pMMA significantly improved after 1 mo of oral vitamin B12 supplementation (Figure 1 and Supplemental Table 1). uMMA/C was measured solely after 6 mo of oral supplementation and also showed improvement (Figure 1 and Supplemental Table 1). The improvements were sustained and persisted throughout the 1-y follow-up (Figure 1 and Supplemental Table 1).

Vitamin B12 deficiency was corrected within 1 mo of oral vitamin B12 supplementation in 23 of the 26 (88.5%) patients, and all participants had their deficiency corrected by the end of the follow-up. No difference was observed between the PA-IFA and PA-APCA groups (Figure 2A). Interestingly, after just 1 mo of supplementation, 9 of the 10 (90.0%) patients in the PA-IFA group and 14 of the 16 (87.5%) patients in the PA-APCA group no longer had vitamin B12 deficiency.

The logarithmic model was the most suitable model for depicting the evolution of tB12 over time during oral supplementation in both PA-IFA and PA-APCA groups (probability ratios based on Akaike

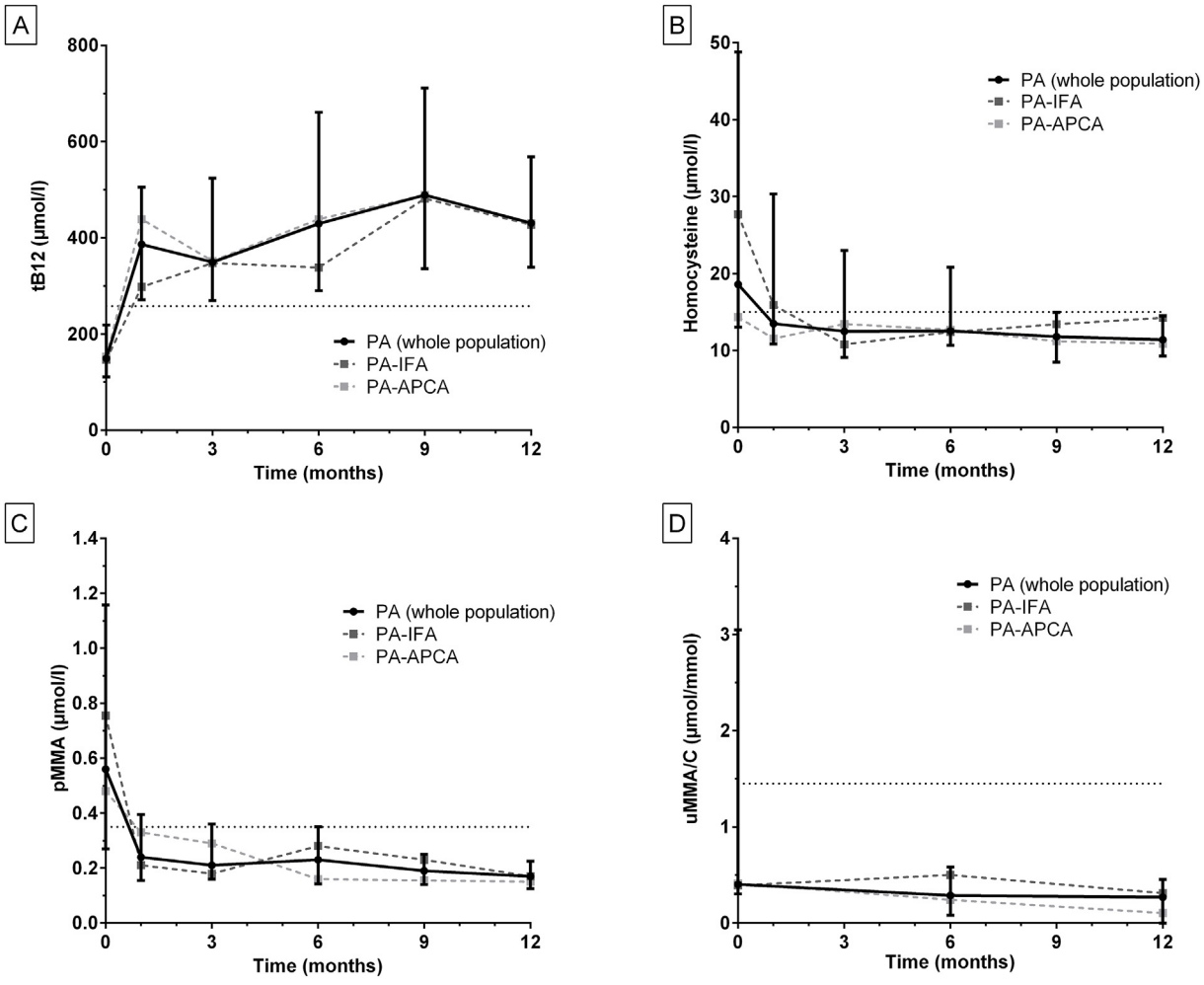

FIGURE 1. Changes in biological parameters associated with vitamin B12 deficiency in pernicious anemia following the initiation of oral vitamin B12 supplementation (cyanocobalamin 1000 µg/d). Dashed lines represent the threshold between the normal values and values associated with vitamin B12 deficiency (258 pmol/L for vitamin B12, 15 µmol/L for homocysteine, 0.35 µmol/L for MMAp, and 1.45 µmol/mmol for uMMA/C). Data are presented as means and SD. PA, pernicious anemia; PA-APCA, pernicious anemia with antiparietal cell antibodies (without IFA); PA-IFA, pernicious anemia with anti-intrinsic factor antibodies (with or without APCA); tB12, total plasma vitamin B12.

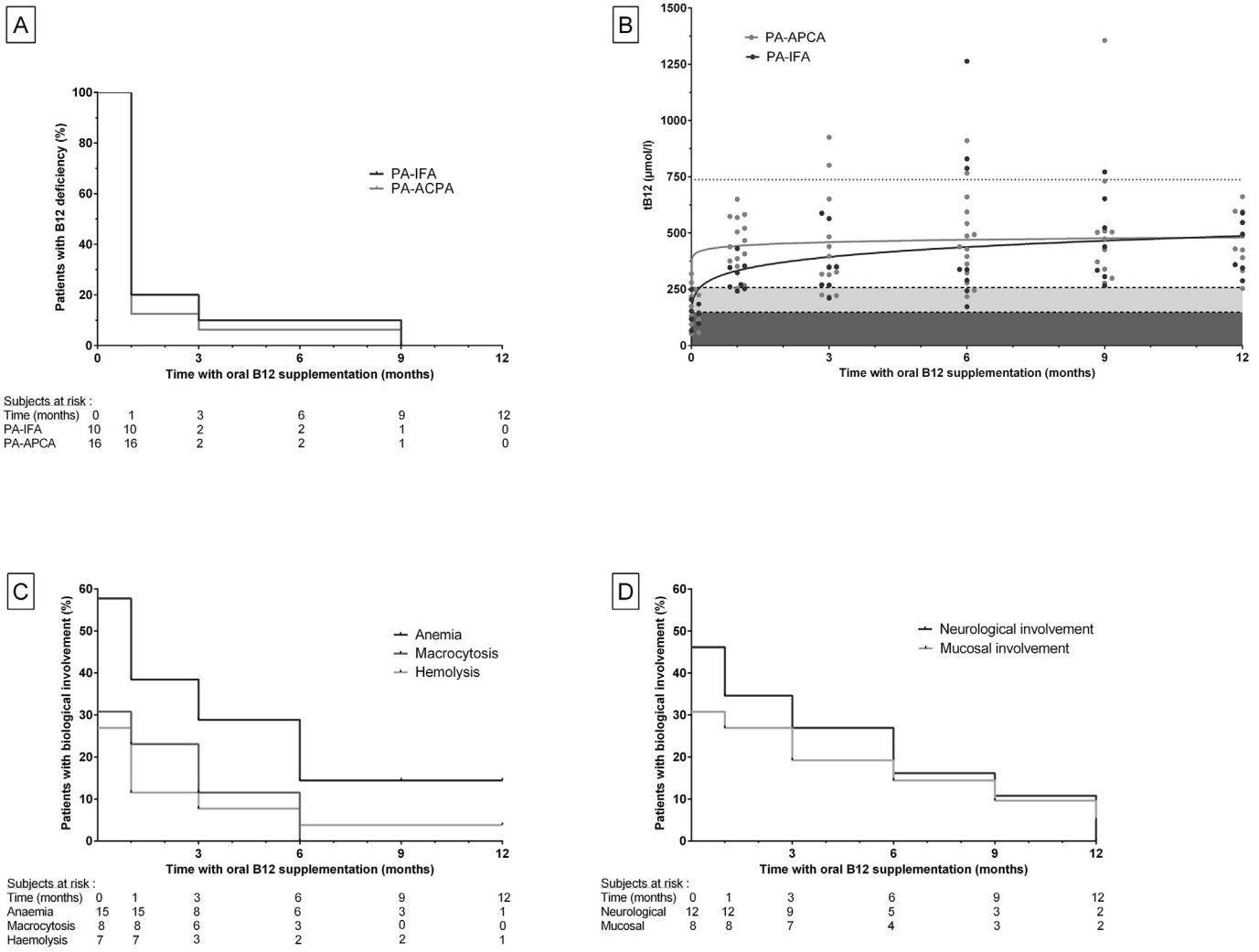

FIGURE 2. Correction of biological and clinical features following the initiation of oral vitamin B12 supplementation (cyanocobalamin, 1000 µg/d) in PA according to immunological markers: (A) correction of vitamin B12 deficiency; (B) changes in tB12; (C) improvement of blood parameters; (D) correction of clinical involvements. In panel (B), the light gray zone (tB12 between 148 and 258 pmol/L) corresponds to tB12 values that may reflect vitamin B12 deficiency in case of concomitant elevation of homocysteine, pMMA, or uMMA/C, and the dark gray zone (tB12 <148 pmol/L) corresponds to tB12 values defining a vitamin B12 deficiency without needing to know the values of the other parameters. Data are presented as means and SD. APCA, antiparietal cell antibodies; IFA, anti-intrinsic factor antibodies; PA, pernicious anemia; PA-APCA, pernicious anemia with antiparietal cell antibodies (without IFA); PA-IFA, pernicious anemia with anti-intrinsic factor antibodies (with or without APCA).

Information Criteria in favor of logarithmic model compared with that of linear model: 1.73 for PA-IFA curve and 29.72 for PA-ACPA curve) (Figure 2B).

Correction of clinical and biological features related to vitamin B12 deficiency

Figure 2C, D present the resolution of the key biological and clinical involvement associated with vitamin B12 deficiency. The median duration to reverse the initial abnormalities related to vitamin B12 deficiency was as follows: 1 (1–4) mo for hemolysis, 3 (2–6) mo for macrocytosis, 3 (1–6) mo for anemia, 3 (2–7) mo for neurologic manifestations (polyneuropathy, signs of pyramidal irritation, and proprioceptive disorders), and 4 (3–10) mo for mucosal involvement (glossitis, lingual dysesthesias, and oral ulcerations).

Of the 3 patients with persistent anemia after 12 mo of oral vitamin B12 supplementation, 1 patient was diagnosed with myelodysplastic syndrome, another participant had bone marrow aplasia, and the third patient experienced severe iron deficiency resistant to oral iron supplementation. Consequently, this latter participant benefited from intravenous iron supplementation at the end of the follow-up. The patient with persistent hemolysis at the conclusion of the study had normal tB12, plasma homocysteine, and pMMA concentrations since the first month of oral vitamin B12 supplementation. Furthermore, a negative-result Coombs test was observed, and investigations are underway with the hypothesis of corpuscular hemolytic anemia.

Discussion

The experimental demonstration of passive enteral absorption of vitamin B12, independent of intrinsic factor, theoretically opened the way to oral vitamin B12 supplementation in PA [12–15]. However, only a few studies have evaluated the efficacy of the oral route in PA, and these studies involved a very limited number of participants with a brief follow-up period [25–28]. This likely contributes to the hesitancy

of clinicians to recommend oral supplementation in PA [18,19,30]. In this study, we conducted a prospective demonstration of the clinical and biological improvement under oral vitamin B12 supplementation over a 12-mo follow-up in 26 patients with PA. To our knowledge, this study represents the longest and most extensive standardized prospective follow-up assessing the effects of oral vitamin B12 supplementation in individuals with PA.

In our study, patients with PA exhibited similar characteristics to those previously observed in other cohorts, including age, sex ratio, and frequency of mucosal and neurologic involvements, with the exception of hematologic involvement [53,54]. However, the hematologic features observed in our patients with PA (anemia in 57.7% and macrocytosis in 30.8% of the patients with PA before supplementation) aligned more closely with the cohorts of vitamin B12–deficient patients (anemia in 30%–37% and macrocytosis in 45%–54% of the patients) [25,55] rather than with the cohorts focusing solely on PA (anemia in 83%–89% and macrocytosis in 88%–95% of the patients) [53,54]. Indeed, as we included participants based on vitamin B12 deficiency, even in the absence of hematologic features, early diagnosis of PA was occasionally established before the onset of anemia. Additionally, we noted that the frequency of anemia was higher than that of macrocytosis among our patients with PA, in contrast to cohorts of vitamin B12–deficient patients with vitamin B12 deficiency. This difference may be attributed to concurrent iron deficiency in our cohort (14 of the 26 patients at baseline).

Our study revealed that within just 1 mo of oral supplementation, 88.5% of patients with PA were no longer deficient in vitamin B12. This finding underscores that vitamin B12 deficiency in PA can be quickly rectified through oral supplementation [28,45]. A daily supplementation of 1000 μg cyanocobalamin proved effective in maintaining tB12 concentrations within the normal range for 23 of 26 patients. However, in 3 patients, a dose reduction was necessary although dosages as high as 2000 μg/d of cyanocobalamin have been suggested [25], we confirmed that 1000 μg/d is sufficient for correcting the initial deficiency and maintaining a stable vitamin B12 concentration for the majority of patients thereafter [30]. We demonstrated that this dosage not only enabled the attainment of normal tB12, homocysteine, pMMA, and uMMA/c concentrations but also sustained them within the normal range over an extended follow-up.

Improvements of hematologic and neurologic damages with oral vitamin B12 supplementation has been reported [24,25,28]. However, before our study, the effectiveness on mucosal damage had not been documented. More importantly, this study provides precise insights into the timeframes for the correction of these involvements following the initiation of oral vitamin B12 treatment: 1 mo for hemolysis, 3 mo for macrocytosis and anemia, 3 mo for neurologic manifestations, and 4 mo for mucosal involvements. Consequently, if patients do not exhibit improvement beyond these timeframes, further investigations into alternative causes should be actively pursued.

The response to supplementation was similar between participants with IFA or APCA, both of whom exhibited high oral vitamin B12 requirements, thereby validating previous retrospective findings [44]. These outcomes reinforce the uniqueness of PA, wherein different immunologic profiles coexist [56]. However, we noted that the tB12 concentrations 1 mo after supplementation were higher in participants without IFA (PA-APCA group). This difference could be attributed to the tendency of PA-IFA patients to present with a more severe vitamin B12 deficiency at diagnosis, also characterized by higher baseline levels of plasma homocysteine and pMMA, which may need a slightly longer time to correct intracellular vitamin B12 deficiency. Otherwise, we could hypothesize that patients without IFA may retain residual intrinsic factor that enables minimal active absorption of vitamin B12.

Few studies compared oral and IM routes for supplementing patients with vitamin B12 deficiency. However, none of them was specifically dedicated to PA. These studies indicated a similar efficacy of both administration routes in correcting vitamin B12 deficiency stemming from various causes. Nevertheless PA cases in these studies were either a minority—7 of the 33 patients in the study by Kuzminski et al. [25], with 5 being orally supplemented; poorly defined—11 of the 60 patients had APCA in the study by Bolaman et al. [24], without demonstrating atrophic gastritis; or not described in detail [23]. Although this study did not directly compare oral and IM supplementations, we believe that the oral route should be preferred for several reasons: *1*) this study has demonstrated its remarkable short-term and long-term efficacy in addressing markers of vitamin B12 deficiency and reversing its clinical and biological consequences; *2*) the use of antiaggregant or anticoagulant medications, which are frequently prescribed to elderly individuals where PA is prevalent [9], theoretically contraindicates IM injections; *3*) oral route aligns with the preference of the majority of patients [27]; *4*) the IM route does not eliminate the risk of noncompliance (discontinuation of treatment resulting from factors such as the discomfort associated with injections or the lack of perceived benefits) [57], whereas we described excellent compliance with oral supplementation; and *5*) oral supplementation offers medicoeconomic advantages [24,58,59]. From a pathophysiologic perspective, Kuzminski et al. [25] observed an increase in pMMA concentrations in the days preceding the next scheduled injection in patients receiving monthly high-dosage IM supplementation. This phenomenon that may correspond to a dose-end effect that could result in a functional cellular deficiency [25]. In contrast, daily oral intake closely mirrors the natural absorption process associated with food consumption, offering a more physiologic approach. Considering these reasons and in light of the demonstrated efficacy of the oral route in previous preliminary studies and in this prospective study, we believe that oral route surpasses IM supplementation and that IM supplementation should be reserved for limited and specific cases.

Our study has several limitations. First, it was an open-label study without a comparison group treated with the IM route. However, it is worth noting that the main outcomes, which are grounded in biological data, help mitigate the inherent biases associated with the open-label nature of this study. Regarding the absence of IM-treated group, it is important to acknowledge that the efficacy of the IM route has already been well-established. However, it is essentially to consider the constraints of IM route. Thus, the prevailing question pertains more to the hypothetical inferiority of oral route than to the IM route. Given that this study demonstrated the rapid efficacy of the oral route and its excellent tolerance and compliance, we believe it provides ample evidence in support of its use.

Second, in our cohort, none of the participants exhibited with combined spinal cord sclerosis. Therefore, we could not draw conclusions about the efficacy of the oral route in this specific presentation. However, it is noteworthy that some patients did present with other severe forms of vitamin B12 deficiency, such as pancytopenia and intramedullar abortion, and they responded successfully to oral supplementation.

Third, we acknowledge that the place of APCA in PA may be subject of debate among some authors, and the diagnosis of PA could be a point of discussion for participants with only APCA. However, we want to emphasize that all those participants (PA-APCA group) had the classical gastric histologic pattern associated with PA, and ultimately, their responses to oral vitamin B12 supplementation were similar to those with IFA.

In conclusion, in this prospective study, which stands as the largest cohort evaluating the efficacy of the oral route in supplementing patients with PA, we have demonstrated that oral route with cyanocobalamin at a daily dose of 1000 μg was highly effective. This supplementation swiftly corrected vitamin B12 deficiency, normalized all parameters assessing cellular vitamin B12 deficiency (homocysteine, pMMA, and uMMA/C), and improved hematologic, neurologic, and mucosal involvements. Importantly, the correction was rapid and endured throughout the 1-y follow-up. This study strongly advocates for oral vitamin B12 supplementation at a daily dosage of 1000 μg, particularly given the numerous advantages offered by oral route compared with those by IM injections.

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Author contributions

The authors' responsibilities were as follows – VL, GU: designed research; VL, EV, GR, M-CC, ED, SH, CL, CA, JMCdlB, PR, GU: conducted research; EV, M-CC, OB, JMCdlB, PR: provided essential reagents; VL, GU: analyzed data and performed statistical analysis; VL, GU: wrote article; GU, had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflict of interest.

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Data availability

Data described in the manuscript will be made available on reasonable request to the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.05.019.

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