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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033687
Article Type:	Research
Date Submitted by the Author:	16-Aug-2019
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Keywords:	Vitamin B 12 Deficiency, Administration Oral, Equivalence Trial,, Injections Intramuscular, primary health care, Patient preference

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

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Abstract

Objectives: To compare the effectiveness of oral versus intramuscular VB12 in patients aged ≥65 years with VB12 deficiency.

Design: Pragmatic, randomized, noninferiority, multicenter trial in patients ≥65 years in 22 primary healthcare centres in Madrid (Spain). **Participants**: 283 adults with VB12 deficiency were randomly assigned to oral (n=140) or intramuscular (n=143) treatment arms. **Interventions:** The intramuscular arm received 1mg VB12 on alternate days in weeks 1–2, 1mg/week in weeks 3–8, and 1mg/month in weeks 9–52. The oral arm received 1mg/day in weeks 1–8 and 1 mg/week in weeks 9–52.

Main outcomes: Serum VB12 concentration normalization at 8, 26, and 52 weeks. Non-inferiority would be declared if the difference between arms is 10% or less. Secondary outcomes included symptoms, adverse events, adherence to treatment, quality of life, patient preferences and satisfaction.

Results: At week 8, the differences in the percentage of patients who normalized serum VB12 levels between the oral and intramuscular arm were -0.7% (95% CI: -3.2 to 1.8) analyzed per protocol (PPT) and 4.8% (95% CI: -1.3 to 10.9) by intention-to-treat (ITT). At week 52, these differences were -6.3% (95% CI: -11.9 to -0.1) and -6.8% (95% CI: -16.6 to 2.9), respectively. Factors affecting the success rate at week 52 were age, OR=0.95 (95% CI: 0.91 to 0.99), and having reached VB12 levels ≥281pg/mL at week 8, OR= 8.1 (95% CI: 2.4 to 27.3). Under a Bayesian framework, noninferiority probabilities (Δ>-10%) at week 52 were 0.036 (PPT) and 0.060 (ITT). Quality of life and adverse effects were comparable across groups. 83.4% of patients preferred the oral route.

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Conclusions: Oral administration was not less effective than intramuscular administration at 8 weeks. Although differences were found between administration routes at week 52, the probability that the differences were below the noninferiority threshold was very low.

Trial registration: ClinicalTrials.gov (NCT 01476007) and EUDRACT (2010-024129-20).

Funding: Ministerio de Sanidad y Consumo Español. Instituto de Salud Carlos III (ISCIII). European Regional Development Fund.

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Strengths and limitations of this study

- The present trial is the largest ever designed to compare the effectiveness of oral versus intramuscular VB12 in patients aged ≥65 years with VB12 deficiency. It offers the longest follow-up period and it is the first to evaluate, in addition to VB12 levels, clinical signs and symptoms, health-related quality of life, and patient preferences.
- The study design did not allow for masking the patients to the received treatment. However, these limitations were compensated for by the objective measurement of the main outcome variable.
- The loss of patients was low at 8 and 26 weeks and higher at 52 weeks. This effect has been observed in long follow-up pragmatic clinical trials.

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INTRODUCTION

Vitamin B12 (VB12) is an essential nutrient for the synthesis of cellular DNA. Daily needs in adults range from 1 to 2 μg/day. The Western diet is estimated to contain 7–30 μg/day of cobalamin, of which 1–5 μg is absorbed and stored (estimated reserves of 2–5 mg), and therefore, symptoms resulting from a VB12 deficit would not appear until 3–5 years after establishing a low-ingestion or poor-absorption regimen.[1] VB12 deficiency can lead to hematological and neuropsychiatric disorders,[2] as well as cardiovascular risk factors.[3] The prevalence of VB12 deficiency in the elderly is highly variable across studies, which report values of 1.5% to 15%.[4-7]

In primary care, the most commonly observed causes of VB12 deficiency are related to abnormalities in digestion or absorption [8] or the consequences of surgical resection.[9] A deficiency stemming solely from dietary habits is rare and usually affects strict vegans.[10] In the elderly, different alterations in the processes involved in VB12 absorption increase the prevalence of this deficit, which can appear in the absence of specific symptoms, thereby hindering its diagnosis. [11]

The traditional treatment for VB12 deficiency consists of intramuscular (IM) injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum*.[9,12,13] The vitamin may, however, be administered orally. Several studies have shown serum VB12 concentrations to normalize after taking large oral doses.[14,15] Studies taking into consideration the patients´ preferences have found differences in favor of the oral route.[16,17] Furthermore, oral treatment could avoid injection nuisances, reduce unnecessary travel for the patients or nurses, and minimize treatment costs.[18]

Some authors have questioned the use of oral administration while others favor it, although no firm conclusions can be drawn due to the methodological limitations of the evidence the authors provide.[9, 19,20,21] The 2018 Cochrane Review[4] includes three randomized clinical trials comparing

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the effectiveness of oral and IM administration. There are differences among the trials in terms of treatment regimens and follow-up duration, ranging from 3 to 4 months, and average age of the patients, as well as the frequency and VB12 daily dose for both routes. In terms of outcomes, adverse events, and cost, the overall quality of the evidence was low due to the small number of studies and limited sample sizes.[22-24] In their conclusions, the authors state the need for trials with improved methods for random allocation and masking, larger sample sizes, and information on other relevant outcome variables that are preferably conducted in the primary care setting.

The aim of this study was to compare the effectiveness of oral- and IM-administered VB12 in the normalization of serum VB12 concentrations at 8, 26, and 52 weeks in patients aged ≥65 years with VB12 deficiency treated at primary healthcare centres (PHC). Secondary outcomes included the safety (adverse events), quality of life, and adherence to treatment. Additional aims were to describe patient preferences and satisfaction with treatment and to explore the immediate response (8 weeks) as a normalization predictor of one-year outcomes to propose clinical recommendations.

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METHODS

Study Design and Participants

A pragmatic, randomized, multicenter, noninferiority clinical trial with a duration of 12 months was conducted in a PHC. On ethical grounds, a placebo-controlled trial was not appropriate.[25] Methodological issues of this trial have been published elsewhere (Supplement 1).[26]

Competitive recruitment was performed in 22 PHC in Madrid (Spain) from July 2014 to November 2016. Eligible patients were 65 years of age or older, had been attending a PHC for consultation on any medical matter, and had a serum VB12 concentration of <211 pg/mL. The last criterion was modified before the start of recruitment due to the requirements of the laboratory; the threshold selected in the protocol was <179 pg/mL. Written informed consent was obtained from all participants.

Randomization and Masking

Physicians centrally randomized patients using an electronic online platform by simple randomization at a 1:1 ratio. Neither the patients nor the health professionals were blinded.

Intervention

The pharmaceutical formulations used in the study are commercially available in Spain (Optovite® vials). The treatment regimen was : a) IM route: 1 mg of cyanocobalamine on alternate days during weeks 1–2, 1 mg/week during weeks 3–8 and 1 mg/month from weeks 9–52; b) oral route: 1 mg/day of cyanocobalamin for 8 weeks and 1 mg/week from weeks 9–52. The period between 1-8 weeks was considered as the charging period.

Outcomes

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The main outcome was the normalization of serum VB12 concentrations (≥211 pg/mL) at 8, 26, and 52 weeks. The secondary outcomes were the serum VB12 concentrations (pg/mL), adverse events, adherence to treatment (good adherence was considered greater than 80%), quality of life (EQ-5D-5L) and patient preferences and satisfaction were assessed. Anamnesis, demographic and lifestyle information, clinical variables, analytical variables, and concomitant treatment were recorded.[26]

Procedures

After signing the consent form, those who agreed to participate had serum VB12 concentrations determined. If the VB12 value was <211 pg/mL, a hemogram, biochemical analysis, and anti-intrinsic factor antibody levels were assessed.[26] The patients also received a medication diary to be filled out daily. Baseline data were collected by the family physician and/or a nurse. IM treatments were administered by nurses in the health centres. The follow-up visits were conducted during weeks 8, 26 and 52.[26]

Statistical Analysis

Sample size. Assuming that 70% of patients reach a serum VB12 concentration of ≥ 211 pg/mL in both groups, for a threshold of noninferiority of 10%, statistical power of 60% with significance set at $p<0.05$ and a 5% loss to follow-up, the final sample size was word 320 (160 in each arm).

As recommended for noninferiority studies, both PPT and ITT analyses were performed, with the null hypothesis being a lack of differences between treatments at the three monitoring points. Comparing both arms, we calculated the difference between the percentage of patients in each treatment arm whose serum VB12 concentrations became normalized at 8, 26, and 52 weeks, with their 95% CI. If the confidence intervals do not fall outside the noninferiority limit

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(10%), it can be concluded that the oral treatment is not inferior to the intramuscular treatment.[27,28] In ITT analyses, missing values for the main outcome variable were added using the 'last observation carried forward' (LOCF) method.[29]

To explore factors affecting the normalization of serum VB12 concentration at 52 weeks, serum VB12 levels were studied at 8 weeks. A receiver operating characteristic (ROC) curve was built to determine the likelihood ratios of each cutpoint after the charging period to "predict" the normalization of levels at the end of the study.[3] After this, a generalized linear model (GLM) was built (function logit) [30,31]. The normalization of serum VB12 levels at 52 weeks was the dependent variable, and the treatment group was the independent variable. Variables considered significant by the researchers from a clinical perspective were included in the model. To test the noninferiority hypothesis, adding the information contained in these data to previous knowledge, additional statistical analyses were performed using a Bayesian approach. Secondary outcome variables were analyzed using the appropriate statistical tests, and their means or proportions were used to estimate differences between groups. All analyses were performed using STATA 14 and EPIDAT 4.2 software.

Patient involvement

Patients were not involved in the development of plans for recruitment, design, outcome measures, or implementation of the study conduct. No patients were asked to advise on the interpretation or writing of the results. Patients have explained the experience of participating in the study on the occasion of International Clinical Trial´s day in *Radio Nacional de España (RNE)*. We will pursue patient and public involvement in the development of an appropriate method for further dissemination.

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RESULTS

Characteristics of the Study Participants

A total of 2342 patients were offered participation and 2152 provided informed consent. A total of 307 patients showed a VB12 deficit (14.3%), 283 of whom were allocated to receive VB12 treatment via the IM route (n=143) or orally (n=140). The follow-up period (52 weeks) was completed by 229 patients (80.9%) (Figure 1).

The average age was 75.18 (6.34), and 58.3% of the patients were women. Table 1 describes the baseline characteristics of the patients included in the trial. No significant differences were found between groups at baseline for demographic and medical characteristics or for the study endpoints.

Table 1. Baseline Characteristics at Baseline by Group

Variable	No. (%)		
	Oral route (n=140)	IM route (n=143)	Total (n=283)
Sociodemographic data			
Women	87 (62.1)	78 (54.5)	165 (58.3)
Age (years), mean (SD)	74.2 (5.8)	76.2 (6.7)	75.2 (6.3)
Educational level			
Illiteracy-Incomplete-Primary education	110 (79.1)	116 (84.7)	226 (81.9)
Secondary – Higher education	29 (20.9)	21 (15.3)	50 (18.1)
Social occupational class [a]			
Class I - IV	31 (27.7)	33 (27.3)	64 (27.5)
Class V - VI	81 (72.3)	88 (72.7)	169 (72.5)
Living alone	32 (21.4)	30 (22.2)	62 (21.9)
Clinical data			
Tobacco habit			
Ex-smoker	27 (19.7)	25 (18.4)	52 (19.0)
Smoker	9 (6.6)	10 (7.4)	19 (7.0)
Nonsmoker	101 (73.7)	101 (74.3)	202 (74.0)
Vegetarian	2 (1.4)	0 (0)	2 (0.7)
Having undergone gastrectomy	1 (0.7)	2 (1.4)	3 (1.1)
Symptoms			

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Paresthesia	33 (23.6)	45 (31.5)	78 (27.6)
Asthenia	43 (30.7)	54 (37.8)	97 (34.3)
Loss of appetite	12 (8.6)	30 (21.0)	42 (14.8)
Sadness	37 (26.4)	53 (37.1)	90 (31.8)
Showing ≥1 symptoms	70 (50.0)	83 (58.0)	153 (54.1)
Signs			
Glossitis	2 (1.4)	9 (6.3)	11 (3.9)
Position sensitivity	2 (1.4)	1 (0.7)	3 (1.1)
Vibration sensitivity	15 (10.7)	13 (9.1)	28 (9.9)
Showing ≥1 altered signs	16 (11.4)	21 (14.7)	37 (13.1)
Hemogram-Clinical Biochemistry			
Vitamin B12 (pg/mL), mean (SD)	173.1 (27.3)	166.4 (32.6)	169.7 (6.3)
Anemia	16 (11.4)	27 (18.9)	43 (15.2)
Hematocrit (%), mean (SD)	42.4 (4.0)	41.9 (4.2)	442.1 (4.1)
MCV (fL), mean (SD)	92.1 (6.7)	94.3 (7.4)	93.2 (7.1)
Anti-intrinsic factor antibody	15 (11.0)	15 (10.5)	30 (10.8)
Medication			
Proton-pump inhibitors	57 (40.7)	64 (44.8)	121 (42.8)
Metformin	69 (49.3)	56 (39.2)	125 (44.2)
Scales			
MMSE [b], mean (SD)	30.8 (4.6)	30.2 (4.8)	30.5 (4.7)
EQ-5D-5L-Utilities, mean (SD)	0.9 (0.1)	0.8 (0.2)	0.8 (0.2)

[a]Neoweberian occupational social class (CSO-SEE12). Gac Sanit. 2013;27(3):263–272.
[b]Mini Mental State Examination. Maximum score= 35 points. Normal score= 30–35. Borderline score= 24–29 points. Scores < 24 points in patients aged >65 years and scores < 29 points in patients aged <65 years suggest cognitive impairment.

Primary Outcomes

At week 8, the difference in the success rate between the oral and IM routes was -0.7% (95%CI: -3.2% to 1.8%) and 4.8% (95%CI: -1.3% to 10.9%) with the PPT and ITT analyses, respectively. At week 26, these differences were -12.9% (95%CI: -17.9% to -6.1%) and -3.2% (95%CI: -11.8% to 5.4%), respectively. At week 52, these differences were -6.3% (95%CI: -11.9% to -0.07%) and -6.8% (95%CI: -16.6% to 2.9%), respectively (Figure 2).

In the PPT analysis under a Bayesian approach, the probabilities of differences in the treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively. In the ITT analysis, these values were 0.000, 0.015,

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and 0.060 at weeks 8, 26, and 52, respectively (Supplement 2). The result of the likelihood ratio for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 to predict normalization at the end of the study is shown in Supplement 3. The level at the 5th percentile of the distribution was selected as the most useful value as it showed the best classification ability. When patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they had reached levels over 281 pg/mL (12~1/negative likelihood ratio).

In the ITT analysis, the factors affecting the success rate at week 52 were age, for each year of increase in age the success rate decreased 5% and having attained VB12 levels of ≥281 pg/mL at week 8, which yielded a success rate 8.1 times higher (Table 2).

Table 2. Factors Associated with VB12 Concentration ≥ 211 pg/ml at Week 52

Variable	Odds ratio	Robust std. error	P>z	95% CI
IM vs. oral route	1.10	0.370	0.776	(0.57 to 2.13)
Age	0.95	0.022	0.025	(0.91 to 0.99)
VB12 concentration >281 pg/ml at week 8	8.10	5.014	0.001	(2.41 to 27.25)
Constant	0.78	0.622	0.755	(0.16 to 3.72)

GLM, N=265. Variance function: V(u) = u*(1-u/1) [Binomial]. Link function: g(u) = ln(u/(1-u)) [Logit]. AIC= 0.89967. BIC = -1225.89.

The mean levels of VB12 for each follow-up visit were above the normalization threshold in both groups, although these values were much greater in the IM group (Supplement 4). In 51 patients (36 IM and 5 oral), the levels of VB12 in week 8 were above the normal range limit of the laboratory (≥911 pg/mL), so the treatment regimen was changed from the initial planned pattern.

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Secondary Outcomes

In terms of quality of life and the presence of signs related to VB12 deficiency, no significant differences were found between treatment arms at any of the follow-up visits (Table 3).

Table 3. Secondary Outcomes (Quality of Life and Exploratory Findings) at Weeks 8, 26 and 52

Visit	Oral route		IM route		Means difference
	N	Mean (SD)	N	Mean (SD)	(95% CI)
Quality of life (EQ-5D-5L Index)					
Baseline	139	0.855 (0.139)	137	0.817 (0.197)	0.066 (-0.002 to 0.078)
Week 8	134	0.853 (0.158)	134	0.822 (0.204)	0.031 (-0.013 to 0.075)
Week 26	128	0.853 (0.153)	128	0.826 (0.191)	0.027 (-0.016 to 0.070)
Week 52	112	0.824 (0.179)	112	0.823 (0.194)	0.001 (-0.047 to 0.049)
At least one altered sign (glossitis and/or altered vibration sensitivity and/or altered position sensitivity)					
Visit	**N**	**n (%)**	**N**	**n (%)**	**Proportions difference (95% CI)**
Baseline	140	16 (11.4%)	143	21 (14.7)	-3.3 (-11.1% to 4.6)
Week 8	135	15 (11.1%)	130	13 (10.0)	1.1 (-6.3% to 8.5)
Week 26	131	14 (10.7%)	122	12 (9.8)	0.9 (-6.6% to 8.3)
Week 52	122	14 (12.5%)	117	9 (7.7)	3.8 (-3.7% to 11.2)

Eleven adverse events were reported and none of them were severe; five (3.57%) occurred with patients in the oral arm and six (4.20%) with patients in the IM arm, yielding a difference of -0.63% (95%CI: -5.12% to 3.87%). Three patients withdrew from the study: one patient in the oral group due to urticaria, and two in the IM group due to reddening and pruritic facial erythema and generalized itching (mainly in the cheeks with scarce urticariform lesions). In three other cases, treatment for the adverse events was prescribed (constipation and erythema), and in five cases, it was not necessary to take further measures.

At week 8, adherence to treatment was evaluated in 265 patients, of whom 95.5% were adherent (97.8% oral and 93.8% IM); the difference between the groups was 3.9% (95%CI: -0.1

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to 8.7). At week 52, adherence was evaluated in 229 patients, of whom 220 (96.1%) were adherent (98.2% oral and 94.0% IM); the difference was 4.2% (95%CI: -0.7 to 9.1).

Overall, 89.5% of the patients reported being satisfied or very satisfied with the treatment via the oral route (91.3%) and the IM route (87.6%). These differences were not statistically significant.

83.4% of patients preferred the oral route (97.6% among the patients receiving VB12 orally vs. 68.6% of the patients in the IM group); the difference was 29.0% (95%CI: 20.3 to 37.7).

DISCUSSION

Main findings of the study

Supplementing VB12 in patients with VB12 deficiency, whether orally or intramuscularly, achieves the normalization of VB12 levels in most cases. The oral route was not inferior to the IM route during the charging period. Formally, the pre-established conditions for determining the noninferiority of oral administration were not met for the complete follow-up period, but these results merit a deeper analysis.

Differences between the administration routes were found at 26 and 52 weeks. The IM maintenance treatment of 1 mg/month was effective in maintaining VB12 levels, while oral administration of 1 mg/week had a probability of being inferior (by more than 10%) to the IM route by 20% in the most unfavorable scenario (PPT). However, given that no strategy was superior in the charging period, and in view of the model results showing that when VB12 levels reached ≥ 281 pg/mL during the charging period, the success rate at 12 months was 8 times higher, the probability of differences between groups to surpass Δ was very low, independent of

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the administration route. The most plausible explanation for the observed difference between routes might be that in patients below this threshold, the maintenance oral dose should be higher than the dose used in the present study. Some authors have recommended that an oral dose of 2 mg/week be administered as a maintenance dose.[32]

The incidence of adverse events was very low and similar for oral and intramuscular administration, and nonserious adverse events were found. These findings were similar to other studies.[4] Patients' preferences can be a decisive factor for determining the administration route. In this trial, similar to previous studies,[16] there was a clear preference for the oral route, especially among the patients assigned to this group.

The effect of VB12 supplements on quality of life remains unclear,[33,34] but the presents results show that the treatment route does not improve patients´ perception of their health-related quality of life or related symptoms.

We did not find significant differences in adherence. However, in usual practice, adherence with the oral route could be more compromised than with the IM route, and this factor should be taken into consideration to personalize prescription.

Comparison with other studies

The comparison with other studies is difficult, due to the treatment different doses used, but especially because of the follow-up length had been inferior to 4 months and the number of patients included were small.

As far as we know, the present trial is the largest clinical trial with the longest follow-up period, and it is the first to evaluate, in addition to VB12 levels, clinical signs and symptoms, health-related quality of life, and patient preferences. The 3 clinical trials[22-24] described in the 2018 Cochrane Systematic Review[4] had a duration between 3 and 4 months and included a total

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of 153 patients. In the Saraswathy trial, patients in the oral route at 3 months normalised levels 20/30 (66.7%) vs 27/30 (90%) of the patients in the IM route.[24] In Kuzminski's patients in the oral route at 4 months normalised levels 18/18 (100%) vs 10/14 (71.4%) of the patients in the IM route.[22] These differences were statistically non-significant in both studies.

Strengths and limitations

Our study was pragmatic[35] in both the inclusion and diagnostic methods criteria. The majority of the patients with deficits included in this study did not present any symptomatology or very low level symptoms, with no anemia, which is the common profile of most patients who present with VB12 deficits in primary care. The study design did not allow for masking the patients to the received treatment. However, these limitations were compensated for by the objective measurement of the main outcome variable.

As occurs in all pragmatic clinical trials, patient recruitment was complicated, and the sample size only reached 88.4% of the calculated necessary size, which predicts a loss of power of the study. Hence, the analysis was complemented using Bayesian methods that allow for studying *a posteriori* the likelihood of a difference between two outcomes to exceed a certain limit.[36] Under this approach, the *a posteriori* probability for differences to exceed the proposed Δ=-10% was not significant during the charging period, and the probabilities were low but not negligible in the PPT analysis and low in the ITT analysis over the complete follow-up period.

The loss of patients was low at 8 and 26 weeks and higher at 52 weeks. This effect has been observed in long follow-up pragmatic clinical trials. Missing data were greater in the IM arm, during the interval between randomization and initiation of treatment (6% IM vs 1% oral), over 8 weeks (9% IM vs 4% oral) and over 26 weeks (15% IM vs 6%). These differences could represent a lower acceptability of the IM route by patients, since the missing data were mostly

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due to patient abandonment. At 52 weeks, the number of losses in both arms was similar (20% oral and 18% IM), and in the case of oral treatment, several of those losses corresponded to withdrawals for not achieving particular levels of VB12.

Implications of the study findings

On the basis of our results and the available evidence, we propose the administration of VB12 doses of 1 mg/day by the oral route during the charging period. Subsequently, the recommended dose would vary as a function of the VB12 levels reached in the charging period. For VB12 concentrations between the normal levels of 211 pg/mL (in our laboratory) and 281 pg/mL (the 5th percentile of the distribution in this trial), a dose of 2 mg/week is suggested. When the levels reached in the charging period are between 281 and 380 pg/mL (the 20th percentile of the distribution), it could be appropriate to perform an analysis between 8 and 26 weeks to confirm that normal levels are maintained. All the patients that reach levels of 380 pg/mL by week 8 could be maintained at the initial doses (1 mg/week) without subsequent analyses during the year of follow-up.

If choosing the IM route, the proposed dose for the IM route during the first weeks may be excessive for patients with VB12 deficiency. The scheduled IM dose should be reconsidered in the first two weeks based on VB12 levels, and in light of this outcome, the scheduled dose could be limited to 1 mg/week. Nevertheless, these recommendations must be assessed in further research.

Oral administration of VB12 in patients older than 65 years is shown as a strategy that is probably as effective as the intramuscular route, without adverse effects and preferred by patients. We also must highlight the potential benefit of using the oral route in terms of safety for patients with coagulant problems, for whom IM-administered medication is relatively

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contraindicated. A small number of patients may require additional follow-up after the 8 weeks if certain levels of VB12 in blood are not reached.

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- Healthcare Centres (managers)*: study coordination development in each healthcare centre with principal investigator supervision.
- Clinical Investigators: collected the data for the study, which included recruiting patients, consenting, blood testing, applying an intervention, collecting data, and arranging and performing follow-up for patients.
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Acknowledgments

To Margarita Blázquez Herranz, Instituto de Salud Carlos III, for making the impossible possible.

To Juan Carlos Abánades Herranz for trusting us and his contribution to facilitate our work.

To Antonio Jesús Alemany López and Marta Sánchez-Celaya, Managing Director of Primary Healthcare for accepting to sponsor this research after the dissolution of CAIBER.

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To Fundación para la Investigación e Innovación Biomédica de Atención Primaria (FIIBAP) for the grant awarded for the publishing of the research.

To Fernando Cava Valencia and Raquel Guillén Santos from the Clinical Laboratory BRSALUD.

To Elena Tolosa Oropesa from the Pharmacy Department. Gerencia Asistencial de Atención Primaria de Madrid.

To our colleagues from the Research Unit, including the assistants Marcial Caboblanco Muñoz, Juan Carlos Gil and Isabel Morales Ropero for their unconditional support; researchers Juan A. López Rodríguez, Jaime Barrio Cortes, Cristina Lozano Hernandez and Medical Residents Daniel Toledo Bartolomé, Irene Wijers and Palmira Jurado Macías for their technical advice to finalize this paper.

To Javier Zamora Romero, head of the Unit of Biostatistics, Hospital Ramón y Cajal of Madrid , for the revision of the manuscript.

To all the professionals from the participating healthcare centers.

To all patients who contributed to this research.

Competing Interests

The authors declare that they have no competing interests.

Funding

The trial was financed by Ministerio de Sanidad y Consumo Español through their call for independent clinical research, Orden Ministerial SAS/2377, 2010 (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122); CAIBER -Spanish Clinical Research Network, Instituto de Salud

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Carlos III (ISCIII) (CAI08/010044) and Gerencia Asistencial de Atención Primaria de Madrid. This study is also supported by the Spanish Clinical Research Network (SCReN), funded by ISCIII-Subdirección General de Evaluación y Fomento de la Investigación, project number PT13/0002/0007, within the National Research Program I+D+I 2013-2016 and co-funded with European Union ERDF funds (European Regional Development Fund).

The funders of the study had no role in the study design, data collection, onsite monitoring, data analysis, data interpretation, or writing of the manuscript.

Ethics approval

Madrid Region Clinical Research Ethics Committee on February 8th, 2011.

Data sharing statement

Individual de-identified participant data will be shared at kindly request. This data will include every variable used in the showed analysis and they will available for five years under request to the correspondence author.

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Figure legends

Figure 1. Trial profile

Figure 2. Differences in proportions of patients recovering levels of VB12 (≥211 pg/ml) between oral and intramuscular routes

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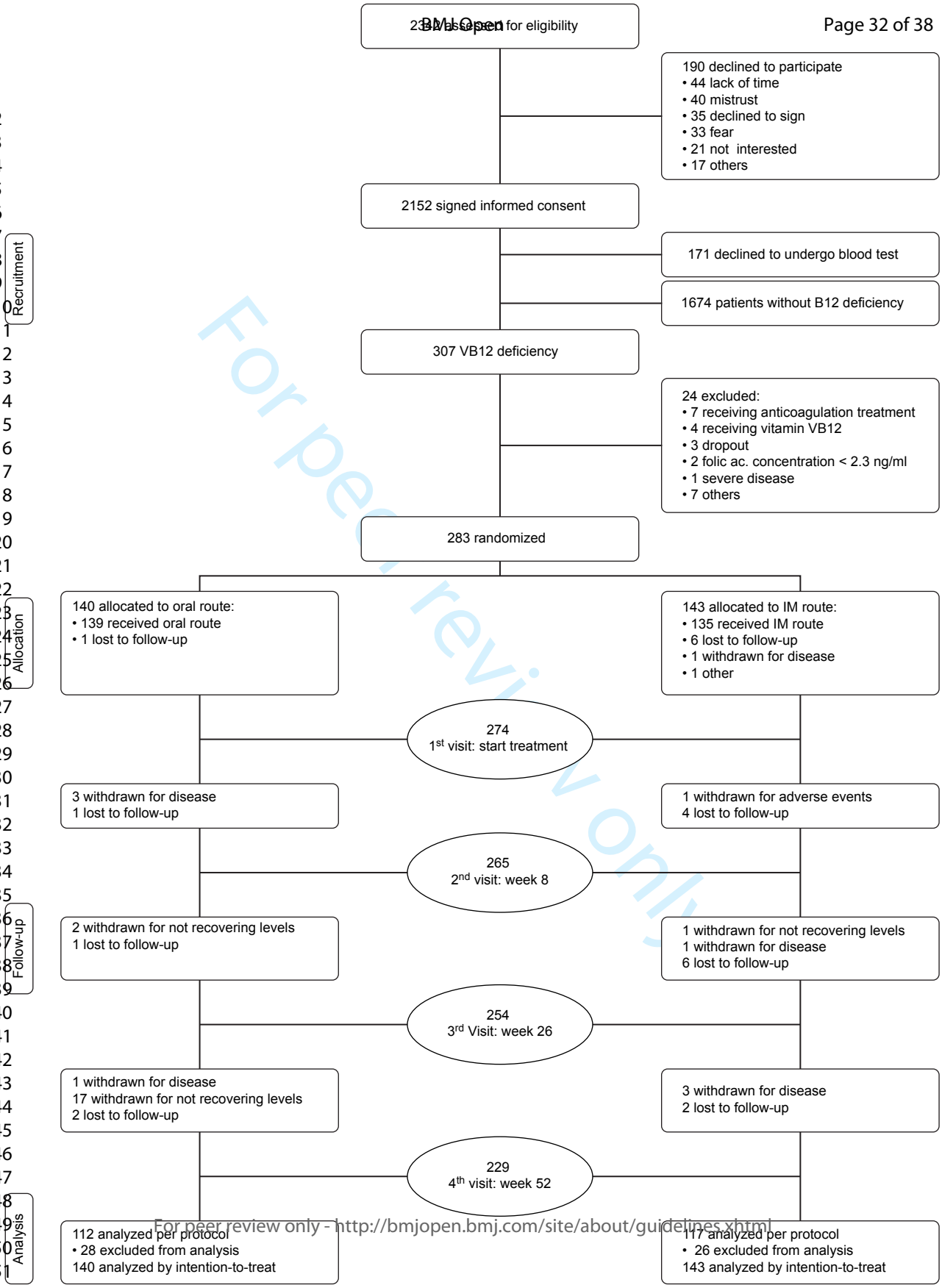

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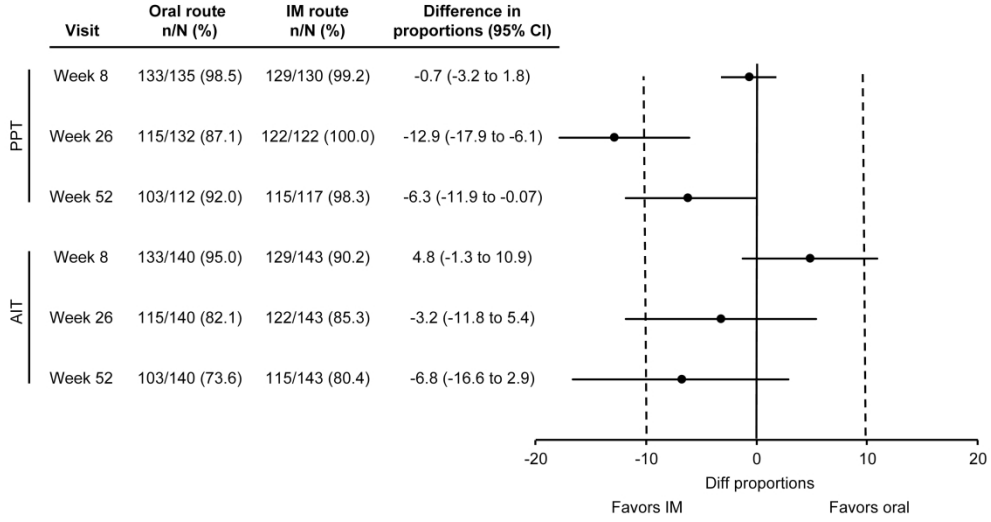

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Supplement 2: Bayesian Analysis

Bayesian analysis is a highly appropriate analysis strategy when working with small sample sizes. Previous knowledge about the studied item can be taken advantage of by means of the assessment of the plausibility of a given hypothesis after incorporating the new observed data.[1]

The noninferiority hypothesis, formally $\Delta < -10\%$, was tested, taking into account the observed results but also taking into account the results of the trials by Kuzminski et al.[2] and Saraswathy et al.[3]

P1 denotes the percentage of patients who responded to VB12 oral administration, and P0 represents the percentage of those responding to VB12 intramuscular administration. Bayesian analysis allows for calculating the probability of P1 being equal to or smaller than P0 by a specified magnitude, the noninferiority limit ($\Delta < -10\%$). For each of the parameters P1 and P0, both measured at 8, 26 and 52 weeks, we selected *a priori* distributions from the family of beta distributions with parameters **a** and **b**, which are related to the proportions of those responding in each trial arm. The gamma distribution represents the *a priori* hypothesis of the distribution of differences. According to the results of both trials by Kuzminski et al.[2] and Saraswathy et al.,[3] included in the review by Wang et al.,[4] 79.1% and 84.1% of patients normalized their VB12 levels in the oral and IM treatment groups, respectively.[4] The respective CIs associated with these prior data were calculated, and parameters were chosen (**a** and **b** in the beta distribution) such that the maximum density intervals of these distributions approximately coincided with the CI previously obtained (see Figure 1). Beta distributions for the success rate in each arm of the trial were obtained using binomial data. A total of 10000 simulations were made from these *a posteriori* distributions, and the corresponding differences, P1-P0, were calculated yielding an *a posteriori* distribution of differences. This distribution was used to derive simulation-based estimates of the probability of relevant magnitudes concerning Δ: P1-P0>0.10 at weeks 8, 26, and 52. Both PPT and ITT analyses were performed. EPIDAT 4.2 software was used for all computations.

Table 1 shows the *a posteriori* probability of differences in treatment effectiveness between oral and IM routes at different weeks (8, 26 and 52). The probabilities of the differences in treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively (per protocol analysis). In the intention-to-treat (ITT) analysis, these values were 0.000, 0.015, and 0.060 at weeks 8, 26, and 52, respectively.

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Figure 1. A priori distributions of the differences between oral and intramuscular treatment

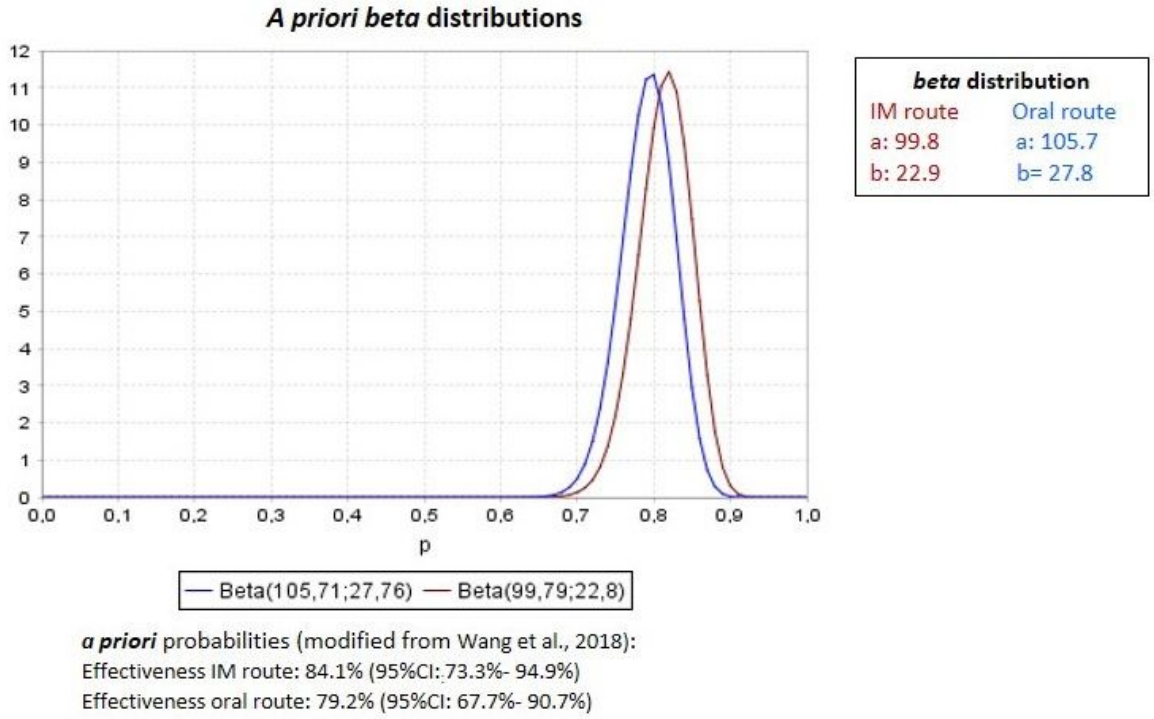

Table 1. *A posteriori* probability of differences in treatment effectiveness between oral and IM routes at 8, 26, and 52 weeks.

A posteriori **probability (Δ < -10%)**	**Week 8**	**Week 26**	**Week 52**
Per-protocol analysis	0.001	0.201	0.036
Intention-to-treat analysis	0.000	0.015	0.060

Δ: threshold of non-inferiority

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Supplement 3: Receiver Operating Characteristic (ROC) Curve

To explore factors affecting the normalization of serum VB12 concentration (yes/no) at 52 weeks, serum VB12 levels were studied at 8 weeks (at the end of the "charging period"). An ROC curve was built to determine the likelihood ratios for each cutpoint after the charging period to "predict" the normalization of levels (serum VB12 levels ≥211 pg/mL) at the end of the study.[1]

Table 1 shows the results of the likelihood ratios for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 ("charging period") to predict normalized VB12 serum levels at the end of the study. In Figure 1, the ROC curve is plotted. The level at the 5th percentile of the distribution was selected as the most useful value as it showed best classification ability and because when patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they did reach levels over 281 pg at week 8 (12~1/negative likelihood ratio).

References

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Table 1. Exploring the value of several cutpoints of OB12 serum levels at week 8 to "predict" normalization of values of Vit B12 at the end of the study

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-	Percentil
≥ 281	0.977	0.273	94.30%	1.3435	0.0841	5
≥ 328	0.963	0.546	94.30%	2.1193	0.0673	10
≥ 353	0.931	0.636	91.70%	2.5608	0.1081	15
≥ 389	0.895	0.818	89.10%	4.9197	0.129	20
≥ 421	0.839	0.818	83.80%	4.617	0.1962	25

LR+: Positive Likelihood ratio. LR-: Negative Likelihood ratio

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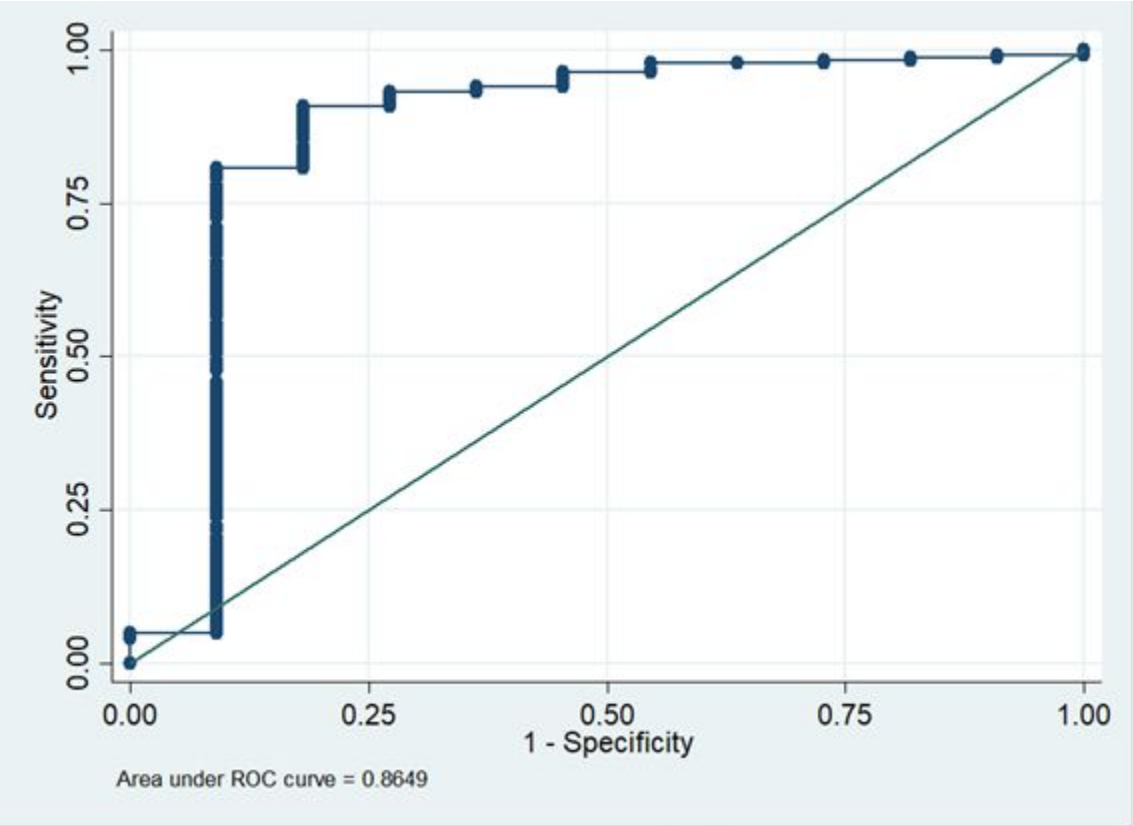
Figure 1. ROC curve



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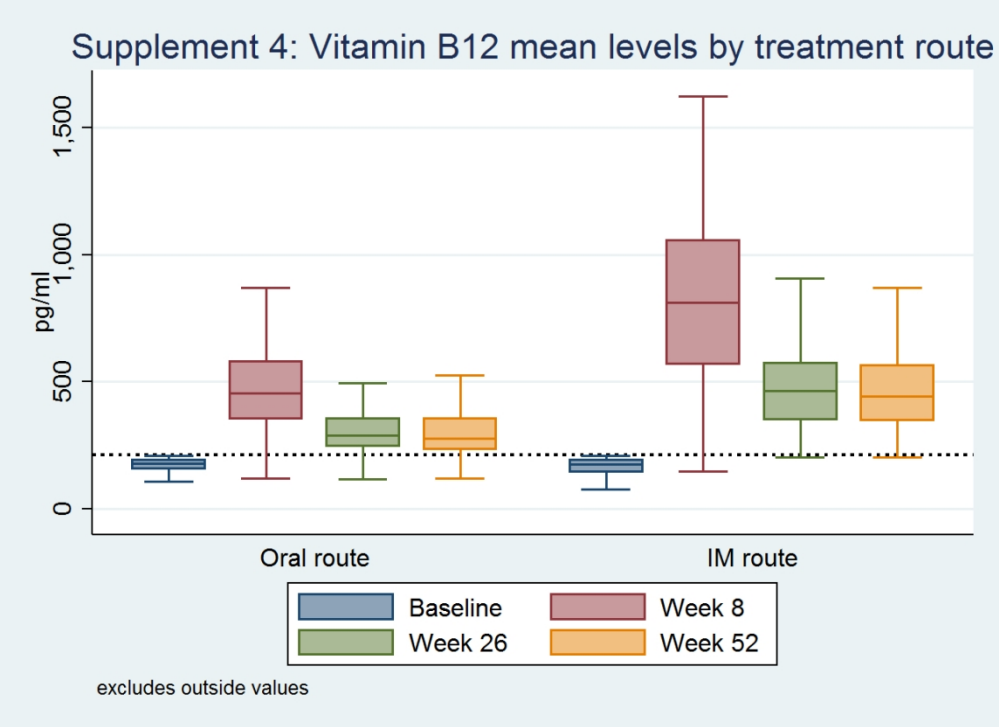

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CONSORT Statement 2006 - Checklist for Non-inferiority and Equivalence Trials

Items to include when reporting a non-inferiority or equivalence randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned"), *specifying that the trial is a non-inferiority or equivalence trial.*	Page 1 and 2
INTRODUCTION Background	2	Scientific background and explanation of rationale, *including the rationale for using a non-inferiority or equivalence design.*	Page 5 and 6
METHODS Participants	3	Eligibility criteria for participants *(detailing whether participants in the non-inferiority or equivalence trial are similar to those in any trial(s) that established efficacy of the reference treatment)* and the settings and locations where the data were collected.	Page 7 Supplement 1
Interventions	4	Precise details of the interventions intended for each group *detailing whether the reference treatment in the non-inferiority or equivalence trial is identical (or very similar) to that in any trial(s) that established efficacy,* and how and when they were actually administered.	Page 7
Objectives	5	Specific objectives and hypotheses, *including the hypothesis concerning non-inferiority or equivalence.*	Page 6
Outcomes	6	Clearly defined primary and secondary outcome measures *detailing whether the outcomes in the non-inferiority or equivalence trial are identical (or very similar) to those in any trial(s) that established efficacy of the reference treatment* and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	Page 8
Sample size	7	How sample size was determined *detailing whether it was calculated using a non-inferiority or equivalence criterion and specifying the margin of equivalence with the rationale for its choice.* When applicable, explanation of any interim analyses and stopping rules (*and whether related to a non-inferiority or equivalence hypothesis*).	Page 8
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	Page 7 Supplement 1
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	Page 7 Supplement 1

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Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	Page 7 and 8
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	Not blinded
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s), *specifying whether a one or two-sided confidence interval approach was used.* Methods for additional analyses, such as subgroup analyses and adjusted analyses.	Page 8 and 9 Supplement 2 Supplement 3
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	Page 7 and 8 Figure 1
Baseline data	15	Baseline demographic and clinical characteristics of each group.	Page 9 and 10 Table 1
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was *"intention-to-treat" and/or alternative analyses were conducted.* State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	Figure 1 and Figure 2
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval). *For the outcome(s) for which non-inferiority or equivalence is hypothesized, a figure showing confidence intervals and margins of equivalence may be useful.*	Page 11 to 13 Table 2 Table 3 Figure 2 Supplement 2
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	Page 12
Adverse events	19	All important adverse events or side effects in each intervention group.	Page 13
DISCUSSION Interpretation	20	Interpretation of the results, taking into account the *non-inferiority or equivalence hypothesis and any other* study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	Page 14 to16
Generalizability	21	Generalizability (external validity) of the trial findings.	Page 14 to 16
Overall evidence	22	General interpretation of the results in the context of current evidence.	Page 16 and 17

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033687.R1
Article Type:	Research
Date Submitted by the Author:	18-Nov-2019
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Primary Subject Heading:	General practice / Family practice
Secondary Subject Heading:	Geriatric medicine, Evidence based practice, Health services research
Keywords:	Vitamin B 12 Deficiency, Administration Oral, Equivalence Trial,, Injections Intramuscular, primary health care, Patient preference

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

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Abstract

Objectives: To compare the effectiveness of oral versus intramuscular vitamin B12 (VB12) in patients aged ≥65 years with VB12 deficiency.

Design: Pragmatic, randomized, noninferiority, multicenter trial in patients ≥65 years in 22 primary healthcare centres in Madrid (Spain). **Participants**: 283 adults with VB12 deficiency were randomly assigned to oral (n=140) or intramuscular (n=143) treatment arm. **Interventions:** The intramuscular arm received 1mg VB12 on alternate days in weeks 1–2, 1mg/week in weeks 3–8, and 1mg/month in weeks 9–52. The oral arm received 1mg/day in weeks 1–8 and 1 mg/week in weeks 9–52.

Main outcomes: Serum VB12 concentration normalization (≥ 211 pg/mL) at 8, 26, and 52 weeks. Noninferiority would be declared if the difference between arms is 10% or less. Secondary outcomes included symptoms, adverse events, adherence to treatment, quality of life, patient preferences and satisfaction.

Results: At week 8, the percentage of patients in each arm who achieved normal B12 levels was well above 90%; the differences in this percentage between the oral and intramuscular arm were -0.7% (95% CI: -3.2 to 1.8) by per-protocol (PPT) analysis and 4.8% (95% CI: -1.3 to 10.9) by intention-to-treat (ITT) analysis. At week 52, the percentage of patients who achieved normal B12 levels was 73.6% in the oral arm and 80.4% in the intramuscular (IM) arm; these differences were -6.3% (95% CI: -11.9 to -0.1) and -6.8% (95% CI: -16.6 to 2.9), respectively. Factors affecting the success rate at week 52 were age, OR=0.95 (95% CI: 0.91 to 0.99), and having reached VB12 levels ≥281 pg/mL at week 8, OR= 8.1 (95% CI: 2.4 to 27.3). Under a Bayesian framework, noninferiority probabilities (Δ>-10%) at week 52 were 0.036 (PPT) and 0.060 (ITT). Quality of life and adverse effects were comparable across groups. 83.4% of patients preferred the oral route.

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Conclusions: Oral administration was no less effective than intramuscular administration at 8 weeks. Although differences were found between administration routes at week 52, the probability that the differences were below the noninferiority threshold was very low.

Trial registration: ClinicalTrials.gov (NCT 01476007) and EUDRACT (2010-024129-20).

Funding: Ministerio de Sanidad y Consumo Español. Instituto de Salud Carlos III (ISCIII). European Regional Development Fund.

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Strengths and limitations of this study

- This is the largest and longest follow-up randomized clinical trial in patients aged ≥65 years with VB12 deficiency.
- In addition to VB12 levels, this study incorporates patient-reported outcomes such as symptoms, quality of life, and patient preferences.
- The study design did not allow patient blinding; however, the main outcome measurement was objective.
- The rates of loss to follow-up were low at week 8 and week 26 and higher at week 52, consistent with pragmatically designed clinical trials.

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INTRODUCTION

Vitamin B12 (VB12) is an essential nutrient for the synthesis of cellular DNA. It is generally accepted that daily needs in adults range from 1 to 2 μg/day, [1] but other standards recently recommend 3-4 μg per day.[2] The Western diet is estimated to contain 7–30 μg/day of cobalamin, of which 1–5 μg is absorbed and stored (estimated reserves of 2–5 mg); therefore, symptoms resulting from a VB12 deficit would not appear until 3–5 years after establishing a low-ingestion or poor-absorption regimen.[1] VB12 deficiency can lead to hematological and neuropsychiatric disorders,[3] as well as cardiovascular risk factors.[4] The prevalence of VB12 deficiency in the elderly is highly variable across studies, which report values of 1.5% to 15%.[5–8]

In primary care, the most commonly observed causes of VB12 deficiency are related to abnormalities in digestion (atrophic gastritis, achlorhydria) or absorption (autoimmune pernicious anaemia, chronic pancreatitis, Crohn's disease, the effect of medications that alter the mucosa of the ileum such as metformin, antacids -proton-pump inhibitors and H2-receptor antagonists-, antibiotics, and colchicine)[9] or the consequences of surgical resection.[10] A deficiency stemming solely from dietary habits is rare and usually affects strict vegans.[11] In the elderly, different alterations in the processes involved in VB12 absorption increase the prevalence of this deficit, which can appear in the absence of specific symptoms, thereby hindering its diagnosis. [12]

The traditional treatment for VB12 deficiency consists of intramuscular (IM) injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum*.[10,13,14]. The vitamin may, however, be administered orally. Several studies have shown serum VB12 concentrations to normalize after taking large oral doses.[15,16] Studies taking into consideration the patients´ preferences have found differences in

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favour of the oral route.[17,18] Furthermore, oral treatment could avoid injection nuisances, reduce unnecessary travel for the patients or nurses, and minimize treatment costs.[19]

Some authors have questioned the use of oral administration while others favour it, although no firm conclusions can be drawn due to the methodological limitations of the evidence the authors provide.[10,20–22] The 2018 Cochrane Review[5] includes three randomized clinical trials comparing the effectiveness of oral and IM administration. There are differences among the trials in terms of treatment regimens and follow-up duration, ranging from 3 to 4 months, and average age of the patients, as well as the frequency and VB12 daily dose for both routes. In terms of outcomes, adverse events, and cost, the overall quality of the evidence was low due to the small number of studies and limited sample sizes.[23–25] In their conclusions, the authors state the need for trials with improved methods for random allocation and masking, larger sample sizes, and information on other relevant outcome variables that are preferably conducted in the primary care setting.

The aim of this study was to compare the effectiveness of oral- and IM-administered VB12 in the normalization of serum VB12 concentrations at 8, 26, and 52 weeks in patients aged ≥65 years with VB12 deficiency treated at primary healthcare centres (PHC). Secondary outcomes included safety (adverse events), quality of life, and adherence to treatment. Additional aims were to describe patient preferences and satisfaction with treatment and to explore the immediate response (8 weeks) as a normalization predictor of one-year outcomes to propose clinical recommendations.

METHODS

Study design and participants

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A pragmatic, randomized, multicenter, noninferiority clinical trial with a duration of 12 months was conducted in a PHC. On ethical grounds, a placebo-controlled trial was not appropriate.[26] Methodological issues of this trial have been published elsewhere (Supplement 1).[27]

Competitive recruitment was performed in 22 PHC in Madrid (Spain) from July 2014 to November 2016. Eligible patients were 65 years of age or older and had been attending a PHC for consultation on any medical matter. Patients were assessed for eligibility and invited to participate consecutively by their general practitioners. Written informed consent was obtained from all participants. A blood test was performed, and in patients with a serum concentration of VB12 of <211 pg/mL, the remaining inclusion and exclusion criteria were evaluated. The cut-off value selected in the protocol was <179 pg/mL; this value was modified by the laboratory following the recommendations of the provider. This change took place prior to the beginning of the recruitment. Patient recruitment was always performed using the same methodology and cut-off point. The procedures for measurement of the biomarkers were ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY, USA).

Randomization and masking

Patients were allocated by simple randomization at a 1:1 ratio to oral or intramuscular administration of vitamin B12. The randomization system was incorporated into the electronic data collection system to assure allocation concealment. Because of the nature of the intervention, patients and general practitioners were aware of their treatment allocation. Analysis was performed by the trial statistician, who was blinded to allocation.

Intervention

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The pharmaceutical formulations used in the study are commercially available in Spain (Optovite® vials). Its pharmaceutical presentation is in silk-screen-printed clear glass ampoules that are presented in PVC blister support. The treatment regimen was : a) IM route: 1 mg of cyanocobalamin on alternate days during weeks 1–2, 1 mg/week during weeks 3–8 and 1 mg/month during weeks 9–52; b) oral route: 1 mg/day of cyanocobalamin for 8 weeks and 1 mg/week during weeks 9–52. The period between 1-8 weeks was considered the charging period. In the oral route, the medication was provided to the patient at the health centre, along with instructions for self-administration at home. The information sheet explained to the patient the procedure for oral administration, i.e., how to open the ampoule and dilute its contents in a glass, then drink it.

In the IM route, the medication was administered by the nurse at the health centre.

Outcomes

The main outcome was the normalization of serum VB12 concentrations (≥211 pg/mL) at 8, 26, and 52 weeks. The secondary outcomes were the serum VB12 concentrations (pg/mL), adverse events, adherence to treatment (number of vials for the oral arm and the number of injections for the IM arm during each visit; good adherence was considered greater than 80%), quality of life (EQ-5D-3L) [28] and patient preferences and satisfaction were assessed. Anamnesis, demographic and lifestyle information, clinical variables, analytical variables, and concomitant treatment were recorded.[27]

Procedures

After signing the consent form, those who agreed to participate had serum VB12 concentrations determined. If the VB12 value was <211 pg/mL, a hemogram, biochemical analysis, and anti-

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intrinsic factor antibody levels were assessed.[27] The patients also received a medication diary to be filled out daily. Baseline data were collected by the family physician and/or a nurse. IM treatments were administered by nurses in the health centres. The follow-up visits were conducted during weeks 8, 26 and 52.[27]

Statistical analysis

Sample size. Assuming that 70% of patients reach a serum VB12 concentration of ≥ 211 pg/mL in both groups, for a threshold of noninferiority of 10%, statistical power of 60% with significance set at $p<0.05$ and a 5% loss to follow-up, the final sample size was word 320 (160 in each arm).

As recommended for noninferiority studies, both PPT and ITT analyses were performed, with the null hypothesis being that there were differences between treatments at the three monitoring points. Comparing both arms, we calculated the difference between the percentage of patients in each treatment arm whose serum VB12 concentrations became normalized at 8, 26, and 52 weeks, with their 95% CI. If the confidence intervals do not fall outside the noninferiority limit (10%), it can be concluded that the oral treatment is not inferior to the intramuscular treatment.[29,30] In ITT analyses, missing values for the main outcome variable were added using the 'last observation carried forward' (LOCF) method.[31]

To explore factors affecting the normalization of serum VB12 concentration at 52 weeks, serum VB12 levels were studied at 8 weeks. A receiver operating characteristic (ROC) curve was built to determine the likelihood ratios of each cutpoint after the charging period to “predict” the normalization of levels at the end of the study. After this, a generalized linear model (GLM) was built (function logit). [32,33] The normalization of serum VB12 levels at 52 weeks was the dependent variable, and the treatment group was the independent variable. Variables considered

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significant by the researchers from a clinical perspective were included in the model. To test the noninferiority hypothesis, adding the information contained in these data to previous knowledge, additional statistical analyses were performed using a Bayesian approach. Secondary outcome variables were analyzed using the appropriate statistical tests, and their means or proportions were used to estimate differences between groups. All analyses were performed using STATA 14 and EPIDAT 4.2 software.

Patient involvement

Patients were not involved in the development of plans for recruitment, design, outcome measures, or implementation of the study conduct. No patients were asked to advise on the interpretation or writing of the results. Patients explained the experience of participating in the study on the occasion of International Clinical Trial´s day in *Radio Nacional de España (RNE)*. We will pursue patient and public involvement in the development of an appropriate method for further dissemination.

RESULTS

Characteristics of the study participants

A total of 2342 patients were offered participation, and 2152 provided informed consent. A total of 307 patients showed a VB12 deficit (14.3%), 283 of whom were allocated to receive VB12 treatment via the IM route (n=143) or orally (n=140). The follow-up period (52 weeks) was completed by 229 patients (80.9%) (Figure 1).

The average age was 75.18 (6.34), and 58.3% of the patients were women. Table 1 describes the baseline characteristics of the patients included in the trial. No relevant differences

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were found between groups at baseline for demographic and medical characteristics or for the study endpoints.

Table 1. Baseline characteristics at baseline by group

Variable	No. (%)		
	Oral route (n=140)	**IM route (n=143)**	**Total (n=283)**
Sociodemographic data			
Women	87 (62.1)	78 (54.5)	165 (58.3)
Age (years), mean (SD)	74.2 (5.8)	76.2 (6.7)	75.2 (6.3)
Educational level			
Illiteracy	4 (2.9)	7 (5.1)	11 (4.0)
Incomplete education	48 (34.5)	46 (33.6)	94 (34.1)
Primary education	58 (41.7)	63 (46.0)	121 (43.8)
Secondary education	16 (11.5)	10 (7.3)	26 (9.4)
Tertiary education	4 (2.9)	4 (2.9)	8 (2.9)
Higher education	9 (6.5)	7 (5.1)	16 (5.8)
Social occupational class [a]			
Class I - IV	31 (27.7)	33 (27.3)	64 (27.5)
Class V - VI	81 (72.3)	88 (72.7)	169 (72.5)
Living alone	32 (21.4)	30 (22.2)	62 (21.9)
Clinical data			
Tobacco habit			
Ex-smoker	27 (19.7)	25 (18.4)	52 (19.0)
Smoker	9 (6.6)	10 (7.4)	19 (7.0)
Nonsmoker	101 (73.7)	101 (74.3)	202 (74.0)
Vegetarian	2 (1.4)	0 (0)	2 (0.7)
Having undergone gastrectomy	1 (0.7)	2 (1.4)	3 (1.1)
Symptoms			
Paresthesia	33 (23.6)	45 (31.5)	78 (27.6)
Asthenia	43 (30.7)	54 (37.8)	97 (34.3)
Loss of appetite	12 (8.6)	30 (21.0)	42 (14.8)
Sadness	37 (26.4)	53 (37.1)	90 (31.8)
Showing ≥1 symptom	70 (50.0)	83 (58.0)	153 (54.1)
Signs			
Glossitis	2 (1.4)	9 (6.3)	11 (3.9)
Position sensitivity	2 (1.4)	1 (0.7)	3 (1.1)
Vibration sensitivity	15 (10.7)	13 (9.1)	28 (9.9)

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Showing ≥1 altered sign	16 (11.4)	21 (14.7)	37 (13.1)
Hemogram-Clinical Biochemistry			
Vitamin B12 (pg/mL), mean (SD)	173.1 (27.3)	166.4 (32.6)	169.7 (6.3)
Anemia [b]	16 (11.4)	27 (18.9)	43 (15.2)
Hematocrit (%), mean (SD)	42.4 (4.0)	41.9 (4.2)	442.1 (4.1)
MCV (fL), mean (SD)	92.1 (6.7)	94.3 (7.4)	93.2 (7.1)
Anti-intrinsic factor antibody	15 (11.0)	15 (10.5)	30 (10.8)
Medication			
Proton-pump inhibitors (PPI)	57 (40.7)	64 (44.8)	121 (42.8)
Metformin PPI and metformin	69 (49.3) 33 (23.6)	56 (39.2) 30 (21.0)	125 (44.2) 63 (22.3)
Scales			
MMSE [c], mean (SD)	30.8 (4.6)	30.2 (4.8)	30.5 (4.7)
EQ-5D-Utilities, mean (SD)	0,817 (0,169)	0,855 (0,139)	0,836 (0,171)

[a]Neoweberian occupational social class (CSO-SEE12). Gac Sanit. 2013;27(3):263–272.
[b]Anaemia was defined by the World Health Organization criteria (haemoglobin <12 g/dL in women and <13 g/dL in men). https://www.who.int/vmnis/indicators/haemoglobin
[c]Mini Mental State Examination. Maximum score= 35 points. Normal score= 30–35. Borderline score= 24–29 points. Scores < 24 points in patients aged >65 years and scores < 29 points in patients aged <65 years suggest cognitive impairment.

Primary outcomes

At week 8, the difference in the success rate between the oral and IM routes was -0.7% (95%CI: -3.2% to 1.8%) and 4.8% (95%CI: -1.3% to 10.9%) with the PPT and ITT analyses, respectively. At week 26, these differences were -12.9% (95%CI: -17.9% to -6.1%) and -3.2% (95%CI: -11.8% to 5.4%), respectively. At week 52, these differences were -6.3% (95%CI: -11.9% to -0.07%) and -6.8% (95%CI: -16.6% to 2.9%), respectively (Figure 2).

In the PPT analysis under a Bayesian approach, the probabilities of differences in the treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively. In the ITT analysis, these values were 0.000, 0.015, and 0.060 at weeks 8, 26, and 52, respectively (Supplement 2). The result of the likelihood ratio for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 to predict normalization at the end of the study is shown in Supplement 3. The level at the 5th

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percentile of the distribution was selected as the most useful value because it showed the best classification ability. When patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they had reached levels over 281 pg/mL (12~1/negative likelihood ratio).

In the ITT analysis, the factors affecting the success rate at week 52 were age, for each year of increase in age, the success rate decreased by 5%, and having attained VB12 levels of ≥281 pg/mL at week 8, which yielded a success rate 8.1 times higher (Table 2).

Table 2. Factors associated with VB12 concentrations ≥ 211 pg/ml at week 52

Variable	Odds ratio	Robust std. error	P>z	95% CI
IM vs. oral route	1.10	0.370	0.776	(0.57 to 2.13)
Age	0.95	0.022	0.025	(0.91 to 0.99)
VB12 concentration >281 pg/ml at week 8	8.10	5.014	0.001	(2.41 to 27.25)
Constant	0.78	0.622	0.755	(0.16 to 3.72)

GLM, N=265. Variance function: V(u) = u*(1-u/1) [Binomial]. Link function: g(u) = ln(u/(1-u)) [Logit]. AIC= 0.89967. BIC = -1225.89.

The mean levels of VB12 for each follow-up visit were above the normalization threshold in both groups, although these values were much greater in the IM group (Supplement 4). In 51 patients (36 IM and 5 oral), the levels of VB12 in week 8 were above the normal range limit of the laboratory (≥911 pg/mL), so the treatment regimen was changed from the initial planned pattern.

Secondary outcomes

In terms of quality of life and the presence of signs related to VB12 deficiency, no significant differences were found between treatment arms at any of the follow-up visits (Table 3).

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Table 3. Secondary outcomes (Quality of life and exploratory findings) at weeks 8, 26 and 52

Visit	Oral route		IM route		Mean difference
	N	Mean (SD)	N	Mean (SD)	(95% CI)
Quality of life (EQ-5D-5L Index)					
Baseline	139	0.855 (0.139)	137	0.817 (0.197)	0.066 (-0.002 to 0.078)
Week 8	134	0.853 (0.158)	134	0.822 (0.204)	0.031 (-0.013 to 0.075)
Week 26	128	0.853 (0.153)	128	0.826 (0.191)	0.027 (-0.016 to 0.070)
Week 52	112	0.824 (0.179)	112	0.823 (0.194)	0.001 (-0.047 to 0.049)
At least one altered sign (glossitis and/or altered vibration sensitivity and/or altered position sensitivity)					
Visit	**N**	**n (%)**	**N**	**n (%)**	**Proportion difference (95% CI)**
Baseline	140	16 (11.4%)	143	21 (14.7)	-3.3 (-11.1% to 4.6)
Week 8	135	15 (11.1%)	130	13 (10.0)	1.1 (-6.3% to 8.5)
Week 26	131	14 (10.7%)	122	12 (9.8)	0.9 (-6.6% to 8.3)
Week 52	122	14 (12.5%)	117	9 (7.7)	3.8 (-3.7% to 11.2)

Eleven adverse events were reported and none of them were severe; five (3.57%) occurred with patients in the oral arm and six (4.20%) with patients in the IM arm, yielding a difference of -0.63% (95%CI: -5.12% to 3.87%). Three patients withdrew from the study: one patient in the oral group due to urticaria, and two in the IM group due to reddening and pruritic facial erythema and generalized itching (mainly in the cheeks with scarce urticariform lesions). In three other cases, treatment for the adverse events was prescribed (constipation and erythema), and in five cases, it was not necessary to take further measures (Table 4).

Table 4. Description of adverse events by patient and route of administration

Route	Adverse event	Action
IM route	Constipation	Administration of specific treatment
	Generalized itching and hives on the cheeks	Withdrawal
	Dyspepsia	Treatment not required
	Constipation	Administration of specific treatment
	Redness and pruritic facial erythema	Withdrawal
	Erythema on forearms	Administration of specific treatment
Oral route	Urticaria on the neck and arms	Treatment not required
	Occasional postprandial dyspepsia	Treatment not required
	Occasional postprandial dyspepsia	Treatment not required
	Urticaria	Withdrawal
	Increased irritability and nervousness	Treatment not required

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At week 8, adherence to treatment was evaluated in 265 patients, of whom 95.5% were adherent (97.8% oral and 93.8% IM); the difference between the groups was 3.9% (95%CI: -0.1 to 8.7). At week 52, adherence was evaluated in 229 patients, of whom 220 (96.1%) were adherent (98.2% oral and 94.0% IM); the difference was 4.2% (95%CI: -0.7 to 9.1).

Overall, 89.5% of the patients reported being satisfied or very satisfied with the treatment via the oral route (91.3%) and the IM route (87.6%). The difference was 3.7% (95% CI: -4.0% to 11.3%).

A total of 83.4% of patients preferred the oral route (97.6% among the patients receiving VB12 orally vs. 68.6% of the patients in the IM group); the difference was 29.0% (95%CI: 20.3 to 37.7).

DISCUSSION

Main findings of the study

Supplementing VB12 in patients with VB12 deficiency, whether orally or intramuscularly, achieves the normalization of VB12 levels in most cases. The oral route was not inferior to the IM route during the charging period. Formally, the pre-established conditions for determining the noninferiority of oral administration were not met for the complete follow-up period, but these results merit a deeper analysis.

Differences between the administration routes were found at 26 and 52 weeks. The IM maintenance treatment of 1 mg/month was effective in maintaining VB12 levels, while oral administration of 1 mg/week had a probability of being inferior (by more than 10%) to the IM route by 20% in the most unfavourable scenario (PPT). However, given that no strategy was superior in the charging period, and in view of the model results showing that when VB12 levels

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reached ≥ 281 pg/mL during the charging period, the success rate at 12 months was 8 times higher, the probability that the differences between groups would exceed Δ was very low, independent of the administration route. The most plausible explanation for the observed difference between routes might be that in patients below this threshold, the maintenance oral dose should be higher than the dose used in the present study. Some authors have recommended that an oral dose of 2 mg/week be administered as a maintenance dose.[34]

The incidence of adverse events was very low and similar for oral and intramuscular administration, and nonserious adverse events were found. These findings were similar to other studies.[5] Patients' preferences can be a decisive factor for determining the administration route. In this trial, similar to previous studies,[17] there was a clear preference for the oral route, especially among the patients assigned to this group.

The effect of VB12 supplements on quality of life remains unclear,[35,36] but the present results show that the treatment route does not improve patients´ perception of their health-related quality of life or related symptoms.

We did not find significant differences in adherence. Adherence to the treatment via the IM route was lower than expected. Although drug administration was assured once the patient attended the consultation, the patient could choose not to attend appointments for various reasons. However, in usual practice, adherence with the oral route could be more compromised than with the IM route, and this factor should be taken into consideration to personalize prescription.

Comparison with other studies

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The comparison with other studies is difficult, due to the treatment different doses used, but especially because of the follow-up length had been inferior to 4 months and the number of patients included was small.

As far as we know, the present trial is the largest clinical trial with the longest follow-up period, and it is the first to evaluate, in addition to VB12 levels, clinical signs and symptoms, health-related quality of life, and patient preferences. The 3 clinical trials[23–25] described in the 2018 Cochrane Systematic Review[5] had a duration between 3 and 4 months and included a total of 153 patients. In the Saraswathy trial, patients in the oral route at 3 months normalised levels 20/30 (66.7%) vs 27/30 (90%) of the patients in the IM route.[25] In Kuzminski's patients in the oral route at 4 months normalised levels 18/18 (100%) vs 10/14 (71.4%) of the patients in the IM route.[23] These differences were statistically non-significant in both studies.

Two studies have recently been published add evidence in favour of oral and sublingual administration of VB12.[37,38] The follow-up of Molerio's study reached 24 months versus 12 months in our study. However, Molerio J performed a prospective uncontrolled study that included 26 patients submitted to total gastrectomy. All patients received oral VB12 supplementation (1 mg/day), and all of them maintained normalization V12 at 6, 12, 18, and 24 months. There was a progressive increase in serum V12 levels within the first 12 months, which remained stable thereafter.[37] The long-term effectiveness of the oral route in absorption-deficient people such as gastrectomized patients would support the results of our study.

Bensky et al. compared the efficacy of sublingual vs. intramuscular administration of vitamin B12 in a retrospective observational study from the computerized pharmacy records of Maccabi Health Service (MHS). Among 4281 patients treated with VB12 supplements (830 (19.3%) with IM and 3451 (80.7%) with sublingual tablets, the IM group achieved a significant

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increase in VB12 levels compared with the sublingual group, OR 1.85, CI 95% 1.5-2.3. [38] Although this study has a large sample size, the important methodological limitations on its effectiveness (retrospective design; reliance on clinical records; absence of epidemiological information such as patient age and sex or the aetiology of the deficit) should be considered in the interpretation of their results.

Strengths and limitations

Our study was pragmatic[39] in both the inclusion and diagnostic methods criteria. The majority of the patients with deficits included in this study presented no symptomatology or very low-level symptoms, with no anemia, which is the common profile of most patients who present with VB12 deficits in primary care. The study design did not allow for masking the patients to the received treatment. However, these limitations were compensated for by the objective measurement of the main outcome variable.

As occurs in all pragmatic clinical trials, patient recruitment was complicated, and the sample size reached only 88.4% of the calculated necessary size, which implies that the power of the study was limited. Hence, the analysis was complemented using Bayesian methods that allow for studying *a posteriori* the likelihood of a difference between two outcomes to exceed a certain limit.[40] Under this approach, the *a posteriori* probability for differences to exceed the proposed Δ=-10% was not significant during the charging period, and the probabilities were low but not negligible in the PPT analysis and low in the ITT analysis over the complete follow-up period.

Loss to follow-up was low at 8 and 26 weeks and higher at 52 weeks. This effect has been observed in pragmatic clinical trials with long follow-up periods. Missing data were greater in the IM arm, during the interval between randomization and initiation of treatment (6% IM vs 1% oral), over 8 weeks (9% IM vs 4% oral) and over 26 weeks (15% IM vs 6%). These

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differences could represent a lower acceptability of the IM route by patients, since the missing data were mostly due to patient dropout. At 52 weeks, the numbers of losses in the two arms were similar (20% oral and 18% IM), and in the case of oral treatment, several of those losses were withdrawals occasioned by not achieving particular levels of VB12.

Implications of the study findings

On the basis of our results and the available evidence, we propose the oral administration of VB12 at 1 mg/day during the charging period. Subsequently, the recommended dose would vary as a function of the VB12 levels reached during the charging period. For VB12 concentrations between the normal levels of 211 pg/mL (in our laboratory) and 281 pg/mL (the 5th percentile of the distribution in this trial), a dose of 2 mg/week is suggested. When the levels reached in the charging period are between 281 and 380 pg/mL (the 20th percentile of the distribution), it may be appropriate to perform an analysis between 8 and 26 weeks to confirm that normal levels are maintained. All patients who reach a level of 380 pg/mL by week 8 could be maintained at the initial dosage (1 mg/week) without subsequent analyses during the year of follow-up.

If the IM route is chosen, the proposed dose for this route during the first few weeks may be excessive for patients with VB12 deficiency. The scheduled IM dose should be reconsidered in the first two weeks based on VB12 levels, and the scheduled dose could be limited to 1 mg/week if warranted by the outcome. Nevertheless, these recommendations must be assessed in further research.

Oral administration of VB12 in patients older than 65 years is probably as effective as intramuscular administration, and it also lacks adverse effects and is preferred by patients. We must also highlight the potential benefit of the oral route in terms of safety for patients with

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coagulation problems, for whom IM-administered medication is often contraindicated. A small number of patients may require additional follow-up after 8 weeks if a certain concentration of VB12 in blood is not reached.

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Authors' Contributions

- Trial Management Committee: TSC; EEM; IDC; JMF; RRF; SGE.
- Healthcare Centres (managers)*: JEMS, TGG,MAV, IGG, PGE,CVMC,MNA, FGBG,RBM,CDL,NCR,AHD, RFG,JHH,BPG study coordination development in each healthcare centre with principal investigator supervision.
- Technical Support Group**: participated in different phases of the design and development of the research. MLSP; CMR; BMB; MAJ; coordinated the pharmaceutical aspects.
- Clinical Investigators: collected the data for the study, which included recruiting patients, obtaining consent, performing blood tests, applying interventions, collecting data, and arranging and performing follow-up for patients.
- Statistical analysis: TSC; JMF; IDC; EEM with the collaboration of the Research Unit (JGM and MMM).
- Writing Committee: TSC; EEM; IDC; JMF, SGE, and RRF wrote the manuscript. All authors in the OB12 Group read and approved the final manuscript.

Acknowledgements

To Margarita Blázquez Herranz, Instituto de Salud Carlos III, for making the impossible possible.

To Juan Carlos Abánades Herranz for trusting us and his contribution to facilitate our work.

To Antonio Jesús Alemany López and Marta Sánchez-Celaya, Managing Director of Primary Healthcare for accepting to sponsor this research after the dissolution of CAIBER.

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To the Fundación para la Investigación e Innovación Biomédica de Atención Primaria (FIIBAP) for the grant awarded for the publishing of the research.

To Fernando Cava Valencia and Raquel Guillén Santos from the Clinical Laboratory BRSALUD.

To Elena Tolosa Oropesa from the Pharmacy Department, Gerencia Asistencial de Atención Primaria de Madrid.

To our colleagues from the Research Unit, including the assistants Marcial Caboblanco Muñoz, Juan Carlos Gil and Isabel Morales Ropero for their unconditional support; project manager Pilar Pamplona-Gardeta, researchers Juan A. López Rodríguez, Jaime Barrio Cortes, Cristina Lozano Hernandez and Medical Residents Daniel Toledo Bartolomé, Irene Wijers and Palmira Jurado Macías for their technical advice in finalizing this paper.

To Javier Zamora Romero, head of the Unit of Biostatistics, Hospital Ramón y Cajal of Madrid, for revising the manuscript.

To all the professionals from the participating healthcare centers.

To all patients who contributed to this research.

Competing Interests

The authors declare that they have no competing interests.

Funding

The trial was financed by Ministerio de Sanidad y Consumo Español through their call for independent clinical research, Orden Ministerial SAS/2377, 2010 (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122); CAIBER – Spanish Clinical Research Network, Instituto de Salud

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Carlos III (ISCIII) (CAI08/010044); and Gerencia Asistencial de Atención Primaria de Madrid. This study is also supported by the Spanish Clinical Research Network (SCReN), funded by ISCIII-Subdirección General de Evaluación y Fomento de la Investigación, project number PT13/0002/0007, within the National Research Program I+D+I 2013-2016 and co-funded with European Union ERDF funds (European Regional Development Fund).

The funders of the study had no role in the study design, data collection, onsite monitoring, data analysis, data interpretation, or writing of the manuscript.

Ethics approval

Madrid Region Clinical Research Ethics Committee on February 8th, 2011.

Data sharing statement

Individual de-identified participant data will be shared upon reasonable request. These data will include every variable used in the analysis shown in this report, and they will be available for five years upon request to corresponding author.

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OB12 Group
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Figure legends

Figure 1. Trial profile

Figure 2. Difference between the oral and intramuscular routes in the proportion of patients whose VB12 levels returned to normal (≥211 pg/ml)

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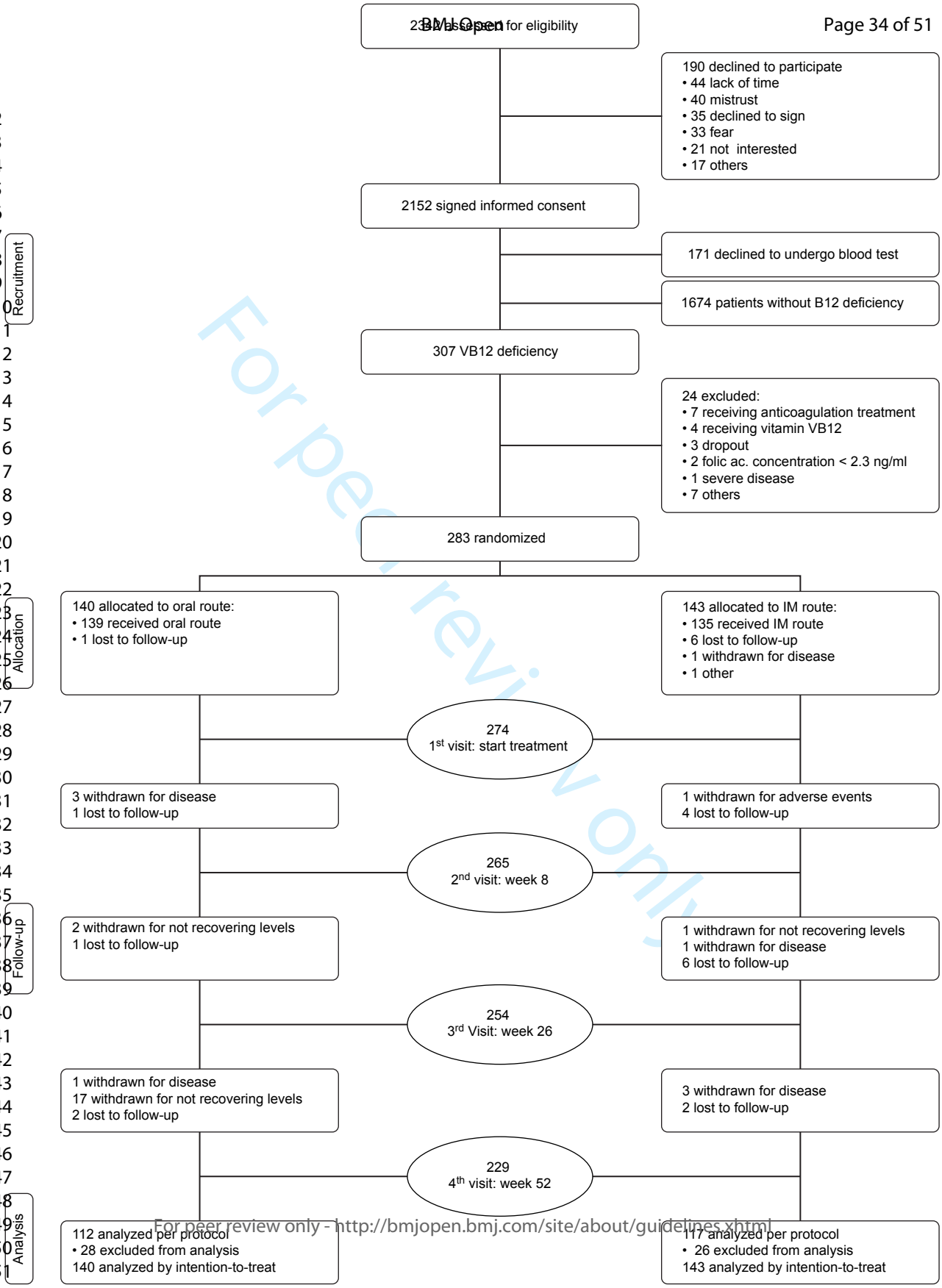

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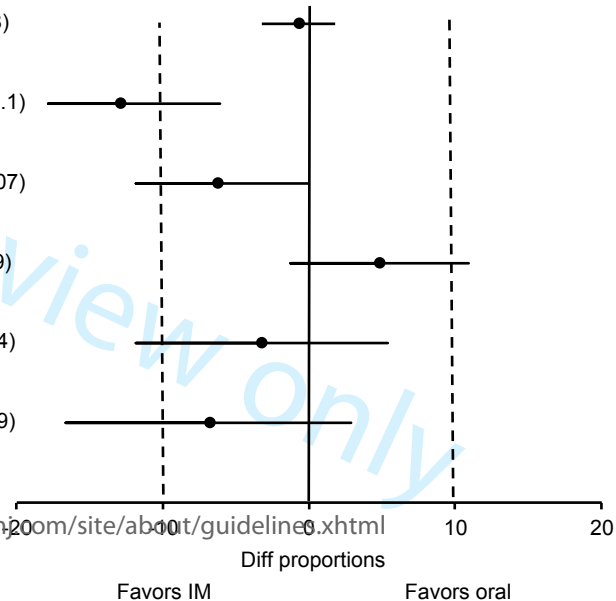
	Visit	Oral route n/N (%)	IM route n/N (%)	Difference in proportions (95% CI)
BPT	Week 8	133/135 (98.5)	129/130 (99.2)	-0.7 (-3.2 to 1.8)
	Week 26	115/132 (87.1)	122/122 (100.0)	-12.9 (-17.9 to -6.1)
	Week 52	103/112 (92.0)	115/117 (98.3)	-6.3 (-11.9 to -0.07)
AHT	Week 8	133/140 (95.0)	129/143 (90.2)	4.8 (-1.3 to 10.9)
	Week 26	115/140 (82.1)	122/143 (85.3)	-3.2 (-11.8 to 5.4)
	Week 52	103/140 (73.6)	115/143 (80.4)	-6.8 (-16.6 to 2.9)



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Sanz-Cuesta *et al. BMC Public Health* 2012, **12**:394
http://www.biomedcentral.com/1471-2458/12/394

BMC Public Health

STUDY PROTOCOL

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Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12)

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Abstract

Background: The oral administration of vitamin B12 offers a potentially simpler and cheaper alternative to parenteral administration, but its effectiveness has not been definitively demonstrated. The following protocol was designed to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the treatment of patients ≥65 years of age with vitamin B12 deficiency.

Methods/design: The proposed study involves a controlled, randomised, multicentre, parallel, non-inferiority clinical trial lasting one year, involving 23 primary healthcare centres in the Madrid region (Spain), and patients ≥65 years of age. The minimum number of patients required for the study was calculated as 320 (160 in each arm). Bearing in mind an estimated 8-10% prevalence of vitamin B12 deficiency among the population of this age group, an initial sample of 3556 patients will need to be recruited.

Eligible patients will be randomly assigned to one of the two treatment arms. In the intramuscular treatment arm, vitamin B12 will be administered as follows: 1 mg on alternate days in weeks 1 and 2, 1 mg/week in weeks 3–8,and 1 mg/month in weeks 9–52. In the oral arm, the vitamin will be administered as: 1 mg/day in weeks 1–8 and 1 mg/week in weeks 9–52. The main outcome variable to be monitored in both treatment arms is the normalisation of the serum vitamin B12 concentration at weeks 8, 26 and 52; the secondary outcome variables include the serum concentration of vitamin B12 (in pg/ml), adherence to treatment, quality of life (EuroQoL-5D questionnaire), patient

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3satisfaction and patient preferences. All statistical tests will be performed with intention to treat and per protocol. Logistic regression with random effects will be used to adjust for prognostic factors. Confounding factors or factors that might alter the effect recorded will be taken into account in analyses.

Discussion: The results of this study should help establish, taking quality of life into account, whether the oral administration of vitamin B12 is an effective alternative to its intramuscular administration. If this administration route is effective, it should provide a cheaper means of treating vitamin B12 deficiency while inducing fewer adverse effects. Having such an alternative would also allow patient preferences to be taken into consideration at the time of prescribing treatment.

Trial registration: This trial has been registered with ClinicalTrials.gov, number NCT 01476007, and under EUDRACT number 2010-024129-20.

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Background

Vitamin B12 (cyanocobalamin), along with other derivatives of folic acid, is a nutrient essential for the synthesis of DNA. Its deficiency is manifested through changes in the number and morphology of erythrocytes, leucocytes and platelets, and by neurological alterations owed to the progressive demineralisation of the nervous system (a consequence of defective myelin synthesis). Vitamin B12 is found mostly in food of animal origin. It is separated from ingested food through the action of the gastric acid, and in the duodenum the vast majority binds to intrinsic factor (IF). The vitamin B12/IF complex formed, which is very resistant to digestion, is then absorbed by endocytosis in the terminal ileum. Only 1-2% of vitamin B12 absorption occurs independent of IF [1]. Daily vitamin B12 requirements vary between 1 and 2 μg/day in adults [2]. A balanced diet, however, provides somewhere between 7 and 30 μg/day. Some of this excess can be stored (some 2–5 mg), meaning that deficiency symptoms may not occur until 3–5 years after the diet fails to provide sufficient vitamin B12 or its absorption becomes inadequate [3].

In the primary healthcare setting, the most commonly seen causes of vitamin B12 deficiency are related to abnormalities of digestion (atrophic gastritis, achlorhydria or the consequences of gastrectomy) or absorption (autoimmune pernicious anaemia, chronic pancreatitis, Crohn's disease, the effect of medications that alter the mucosa of the ileum, or the consequences of surgical resection), and, to a lesser extent, a lack of exogenous supply. The exact prevalence of vitamin B12 deficiency in industrialised countries is unknown; indeed, different studies using different definitions have reported it as between 5% and 60% [4]. Results have even differed widely between similar studies using an identical definition of deficiency, and after stratifying by age [5]. In Spain, the prevalence of vitamin B12 deficiency may reach 18% according to a meta-analysis of the studies undertaken up to 1999 [6]. However, population-based studies performed in Catalonia and the Canary Islands [7,8], both of which used a serum vitamin B12 cut-off of 200 pg/ml, returned values of 1.9% and 3.4% respectively. What does appear to be constant in all studies reviewed for the present work is that the prevalence of deficiency is greater among people aged 65–76 years. For example, the above Catalonian and Canary Island studies returned values of 3.8% and 8.5% for these age groups. Among elderly patients belonging to the Framingham cohort, Lidenbaun [9] observed a prevalence of over 5.3%. Other authors [10,11], however, report figures of 30-40% in elderly people with degenerative neuropsychiatric disorders and those receiving institutionalised care.

In the elderly, the symptoms of vitamin B12 deficiency caused by deficient diets and/or digestive and/or absorption problems can be nonspecific, making a diagnosis of deficiency more difficult. For example, up to 40% of elderly people show no haematological alterations. Further, neurological symptoms may appear before those of anaemia; indeed, only about 60% of elderly people with vitamin B12 deficiency are anaemic [12].

In primary healthcare in Spain, vitamin B12 deficiency is diagnosed via the determination of the serum concentration of the vitamin. Some studies [13-17] have described the limitations of trying to diagnose vitamin B12 deficiency exclusively via the measurement of this concentration, and report blood methylmalonic acid (MMA) and homocysteine concentrations to be more sensitive markers capable of detecting subclinical deficiency.

The traditional treatment of vitamin B12 deficiency is the intramuscular injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum* [4,18,19]. The vitamin may, however, be offered orally. In some circles this route has been regarded as an effective alternative to parenteral administration since the 1950s, during which time several studies showed serum vitamin B12 concentration to normalise after taking large oral doses. These results prompted the spread of oral administration in Sweden and Canada [3]. In the former country, 13% of the population over 70 years of age

now receives treatment for vitamin B12 deficiency, with two of every three patients treated via the oral route [20]. However, in the rest of the world, the parenteral route remains the most used. Indeed, controversy still surrounds the advantages and effectiveness of the oral route. Some authors question its use [21] while others favour it, although the methodological limitations of the evidence they provide means no firm conclusions can be drawn. In reviews of the literature published between 1999 and 2007, Daly-Youcef [4] and Andrés E [19] concluded that orally administered vitamin B12 provided effective treatment for adult and elderly patients with deficiencies, although they highlighted that further studies were needed to determine its effectiveness in patients with severe neurological symptoms. Federicia [22], who reviewed the treatment criteria followed in different studies, concluded oral administration to be effective, but recommended further work to confirm this. Shatsky[23], who examined evidence derived from the use of oral and intramuscular administration, indicated that high dose oral administration appeared to be safe, effective and cost-effective, although long term clinical trials were required to confirm this. In a prospective study performed in Spain involving commercially available multi-vitamin supplements, Rabuñal et al. [24] reported the effectiveness and tolerance of oral vitamin B12 to be excellent, but also indicated that the dosage to be used was yet to fully established. In 2005, a Cochrane review [3] was published that examined two randomised clinical trials - those reported by Kuzminski [2] and Bolaman [25] - that studied the effectiveness of oral vs. intramuscular administration of vitamin B12 for the treatment of its deficiency. The Kuzminski trial involved 33 patients (18 in the oral arm and 15 in the intramuscular arm), while the Bolaman trial involved 60 (26 in the oral arm and 15 in the intramuscular arm). The Cochrane concluded that orally administered vitamin B12 appeared to be as effective as the intramuscular route with respect to the short-term haematological and neurological responses observed in patients with deficiencies, but highlighted methodological limitations in both trials. A large clinical trial was called for in the primary healthcare setting, where a high percentage of patients with vitamin B12 deficiency is seen. The Cochrane review also underscored the need to include a measurement of the quality of life as an outcome, and patient preference at the time of prescribing treatment. Among other variables, three studies [24,26,27] have recorded patient views on the administration route, and record a high level of acceptance of the oral route, the advantages of which include avoiding the displacement of patients to receive injections, avoiding the discomfort of injection, and a reduction in treatment costs [28,29].

A further question still to be answered is that of the optimum dose when using the oral route [3].

In summary, despite many studies indicating the oral administration of vitamin B12 to be easy, effective and less costly than intramuscular administration, their designs, and in some cases their methodological limitations, mean that debate still surrounds the effectiveness of the oral route. This may help explain why it is little used by health professionals [30].

Although some authors [31,32] recommend the use of moderately high doses (which have obtained the best results), studies are still being performed to investigate this. In a randomised clinical trial involving five treatment arms with doses of between 2.5 μg/day and 1000 μg/day, Eussen [33] concluded that a dose of at least 600 μg/day was required to obtain adequate results. However, in guidelines published in 2012, the British Columbia Medical Association (Canadian Ministry of Health) recommended a dose of 1000 μg/day for pernicious anemia or food-bound cobalamin malabsorption [34].

The proposed study examines the questions that, according to the Cochrane review mentioned above [3], are still to be answered, via a clinical trial (of ample duration and with a large number of patients) in the primary healthcare setting. As recommended, one of the outcomes examined is quality of life. The results obtained should provide high quality scientific evidence of use when taking treatment decisions in the primary healthcare centres, while allowing patient preference of administration route to be taken into consideration. The results may reveal oral treatment with vitamin B12 to be, as Lederle [35] put it, “medicine’s best kept secret”.

Aim

The aim of the proposed protocol is to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the normalisation of serum vitamin B12 concentrations at 8, 26 and 52 weeks of treatment, in patients aged ≥65 years with vitamin B12 deficiency treated at primary healthcare centres in the Madrid region, Spain. The secondary outcomes to be measured include the safety of both administration routes, quality of life (measured using the EuroQoL-5D questionnaire) and adherence to treatment. Patient preferences and satisfaction with treatment will also be recorded, along with patient sociodemographic profiles, lifestyle habits, and the clinical manifestation of each patient’s deficiency.

Methods/design

Study type

This study takes the form a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the

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primary healthcare setting, with a duration of one year. For ethical reasons, a placebo controlled trial would not be appropriate [36].

The study involves 23 primary healthcare centres in the Madrid region of Spain. The research team is composed of a clinical assistance group of 169 general practitioners and nurses, and a technical group of 22 health professionals including doctors of different specialities, nurses and pharmacists. For the undertaking of fieldwork, these 191 team members are divided into smaller groups (with similar numbers of clinical and technical personnel), each in charge of one of five subprojects. Each subproject is led by a member of the technical personnel. Together, these five leaders form the coordination group for the trial as a whole.

The trial protocol was approved by the Madrid Region Clinical Research Ethics Committee (*Comité Ético de Investigación Clínica Regional de la Comunidad de Madrid*) on February 8th 2011, and has been registered with ClinicalTrials.gov number NCT 01476007, and under EUDRACT number 2010-024129-20 [Oral Versus Intramuscular Cobalamin to treat Cobalamin Deficiency: Noninferiority randomised controlled trial, pragmatic and multi-center in the primary healthcare setting (OB12 project)].

Patients

1. Inclusion criteria: all participants must:
   - be ≥65 years of age
   - be attending a primary healthcare centre for consultation on some medical matter
   - provide their informed consent to be included
   - have a serum B12 concentration of <179 pg/ml.
2. Exclusion criteria: patients meeting any of the following conditions will be excluded:
   - having been treated (under medical prescription) in the last five years for vitamin B12 deficiency
   - serious neurological or psychiatric symptoms, including psychotic problems
   - dementia preventing the giving of informed consent to take part
   - atrophy of the optic nerve
   - serum folic acid concentration of <2.3 ng/ml
   - stage 4 kidney disease 4 (estimated glomerular filtration rate [GFR] 15–29 ml/min)
   - having received/suffering malabsorption-related:
     - surgery or diseases affecting the jejunum-ileum
     - inflammatory-intestinal disease, e.g., Crohn's disease, ulcerative colitis
     - celiac disease
   - chronic pancreatitis
   - myelodisplasia or malignant blood disease
   - haemophilia or other coagulation problems contraindicating parenteral administration
   - severe systemic disease
   - having been involved in any other trial involving the administration of any experimental treatment in the 28 days prior to the start of the present study
   - being treated for HIV, HVB or HVC infection
   - hypersensitivity to vitamin B12, or any of the vitamin preparation's excipients
   - receiving anticoagulation treatment
   - being away from home and with no intention of residing for the following year in the health district where consultation was made
   - failing to meet any inclusion criterion
   - limitations regarding oral treatment

Randomisation

Participants will be enrolled consecutively by their general practitioners when attending a primary healthcare centre in the study area (Figure 1). All patients without reason to be excluded will be invited to participate. Those patients that accept this invitation will provide written, informed consent to be included. A blood sample will then be taken and part of this used to determine the serum vitamin B12 concentration (pg/ml). In those returning a value of <179 pg/ml (defined as vitamin B12 deficiency by the reference analytical laboratory analysing the samples collected), the remaining fraction of the sample will be analysed to provide a haemogram (reticulocyte, erythrocyte, leucocyte and platelets counts), the values of biochemical variables (glucose, creatinine, GOT, GPT, GGT and ferritin), the folic acid concentration, and an anti-IF antibody count. Those who meet all inclusion criteria, and no exclusion criteria, will then be randomly assigned to one arm of the treatment, i.e., oral or intramuscular administration of vitamin B12. This will be performed by means of a simple randomisation process performed by the electronic data collection system. This guarantees that neither researcher nor patient has any choice with respect to the group to which the latter is assigned.

Sample size

The sample size required was determined bearing in mind the results of Kuzminski et al. [2]. In the latter study the parenteral administration of vitamin B12 was associated with an increase in serum concentrations of the vitamin of >200 pg/ml at 4 months in over 70% of patients. For the present trial, the level of non-inferiority of the oral treatment is set at a difference (delta) in response compared to the parenteral treatment of ≤10%. This threshold was set given its importance from a clinical rather than a statistical viewpoint, and since it falls within the range normally accepted for this type of study [37].

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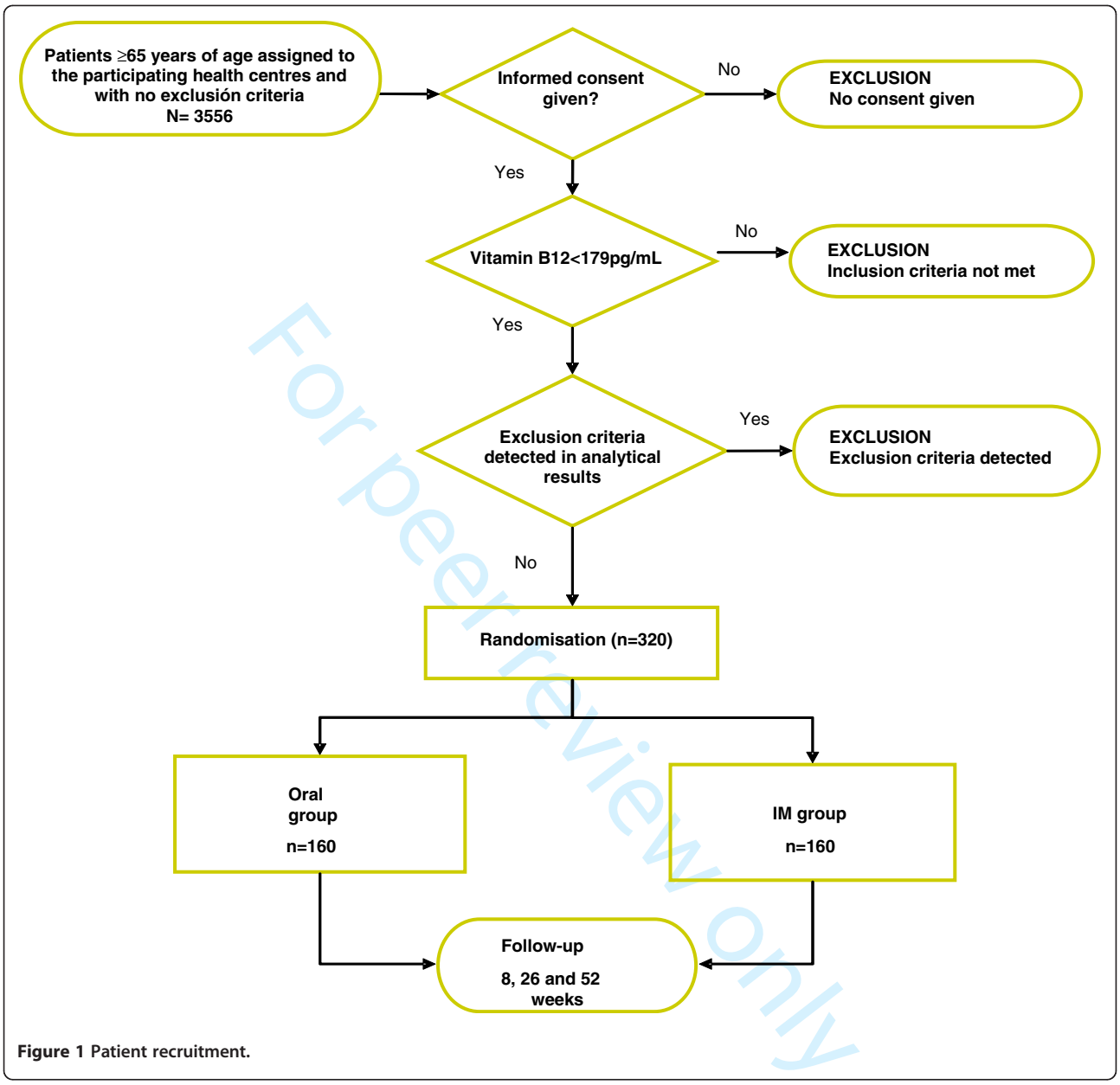

Figure 1 Patient recruitment.

Assuming that the percentage of patients showing an increase in serum vitamin B12 concentration to above 179 pg/ml in both groups is 70%, means the study requires at least 304 patients (152 in each arm) for a threshold of non-inferiority of 10% and a statistical power of 60% with significance set at $p < 0.05$. Given the type of patients to be studied, i.e., patients who have come to the health centres for consultation, plus the fact that their own family doctors are members of the research team, a loss to follow-up of under 5% is expected. The minimum starting sample size for each arm was therefore deemed to be n = 160. With an expected prevalence of vitamin B12 deficiency of 8-10% (a figure of 9% was used in calculations), the original number of patients to be enrolled so that 320 with a vitamin B12 deficiency can be guaranteed is 3556.

Blinding

In studies with the present design it is impossible to blind the patient to the treatment received. However, this limitation is compensated for by the objective measurement of the main outcome variable (the serum vitamin B12 concentration) and the randomisation of the patients to the treatment groups. Further, the persons charged with the statistical analysis of the data will be blind to the identity of the patients in each treatment arm.

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The intervention

The pharmaceutical formulations to be used in the study are commercially available in Spain. The treatments will involve:

- Intramuscular route: 1 mg of vitamin B12 on alternate days during weeks 1 and 2; 1 mg/week over weeks 3–8 (i.e., for 6 weeks); and 1 mg/month from weeks 9–52
- Oral route: 1 mg/day of vitamin B12 for 8 weeks; 1 mg/week from weeks 9–52

Patients in both arms will undergo analytical monitoring in weeks 8, 26 and 52. They will receive appointments for the appropriate dates. The response to treatment will be recorded alongside adherence to treatment and the appearance of any adverse effects.

Work plan

Before work begins, the project will be presented to all the research team members in a special meeting. Training sessions lasting 2–3 h will also be held at each participating health centre. These will involve a review of the inclusion and exclusion criteria, provide instructions regarding the intervention, and examine the ethical requirements to be met for the trial to be held.

The procedures to be followed and information to be recorded at each of a patient's visits to a participating health centre is as follows:

- Selection Visit
  - Signing of informed consent
  - Assessment of inclusion/exclusion criteria
  - Recording of demographic data (age and sex)
  - Analysis: serum vitamin B12. If concentration is <179 pg/ml the following analyses are to be requested: haemogram, biochemical analysis (glucose, creatinine, GOT/GPT/GGT), ferritin, folic acid, anti-IF antibody level. If serum vitamin B12 concentration is >179 pg/ml: patient preference questionnaire
  - Randomisation of patients to treatment group
- Visit 1 (start of treatment)
  - Anamnesis: record whether the patient lives alone or with others, lifestyle habits, use of alcohol, whether a vegan diet is followed, whether the patient has undergone gastrectomy
  - Symptoms: record paresthesia, asthenia, loss or reduction of appetite, sadness or change in state of mind, concomitant pharmacological treatment
  - Physical examination: for Hunter's glositis, positional and vibrational sensitivity
  - Questionnaires: Lobo cognitive mini-exam, EuroQoL-5D
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
- Visit 2 (week 8)
  - Anamnesis: record lifestyle habits and use of alcohol
  - Symptoms: if pathological at the first visit, record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
  - Physical examination: if pathological at the first visit examine for Hunter's glositis, positional and vibrational sensitivity
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Questionnaires: EuroQoL-5D
  - Assessment of adverse effects
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
  - Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given
- Visit 3 (week 26)
  - Anamnesis: record lifestyle habits and use of alcohol
  - Symptoms: if pathological at the first visit, record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
  - Physical examination: if pathological at the first visit examine for Hunter's glositis, positional and vibrational sensitivity
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Questionnaire: EuroQoL-5D
  - Assessment of adverse effects
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
  - Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given
- Visit 4 (week 52)
  - Anamnesis: record lifestyle habits and use of alcohol

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- Symptoms: record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
- Physical examination: for Hunter's glositis, positional and vibrational sensitivity
- Record concomitant treatment
- Questionnaires: EuroQoL-5D, satisfaction and preferences
- Assessment of haemogram and serum vitamin B12 concentration
- Assessment of adverse effects
- Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given

Variables

Outcome variables

The main outcome to be measured is the normalisation of the serum vitamin B12 concentration (>179 pg/ml) at 8, 26 and 52 weeks. The secondary outcomes will be the serum vitamin B12 concentration (pg/ml), adverse events (description, moment of onset and resolution, intensity, cause, steps taken), adherence to treatment (measured at each patient visit via the number of vials used for patients in the oral arm, and the number of injections given in the intramuscular arm), quality of life (measured using the EuroQoL-5D questionnaire), and patient satisfaction and preferences.

Anamnesis, demographic and lifestyle information

Including age, sex, whether the patient lives alone or with others, whether a vegan diet is followed, and the use of alcohol (g/week).

Clinical variables

Symptoms such as paresthesia, asthenia, loss or reduction of appetite, sadness or change in state of mind (anamnesis), Hunter's glositis, positional and vibrational sensitivity (all via physical examination), and cognitive decline (Lobo test).

Analytical variables

Haemogram (complete blood cell and platelet count) and biochemical analysis (folic acid, glucose, creatinine, GOT, GPT, GGT, ferritin, anti-IF antibodies). Blood analyses will be performed in plasma or serum as required and under standard conditions.

Concomitant treatment

Recording of the taking of protein pump inhibitors, H2 receptor antagonists, antacids, potassium, metformin, colchicine, neomycin, p-aminosalicylic acid, parenteral chloramphenicol, Fe, vitamin C and other vitamin supplements.

Losses and withdrawals

Patients will be removed from the trial if any of the following conditions are met:

- Serum vitamin B12 concentration still <179 pg/ml after 8 weeks of treatment. Treatment will be deemed to have failed in these patients, and they will be further studied and treated outside the trial according to normal clinical practice.
- Serious adverse events.
- Voluntary withdrawal or violation of the protocol.

At least two attempts will be made to contact by telephone those patients who do not come for their scheduled visits. All patients will be informed that they can abandon the study at any time without this affecting their future medical treatment in any way.

Analysis

Descriptive analysis of the patients

The trial will involve a descriptive statistical analysis of the baseline characteristics of patients in both treatment arms. Quantitative variables will be described in terms of their measure of central tendency, mean or median (for those showing asymmetric distributions), and the corresponding dispersion, standard deviation or interquartile range. Qualitative variables will be described in terms of proportions and their corresponding confidence intervals.

Baseline comparisons

The Student t test or Mann–Whitney U test (when the normal hypothesis is rejected) will be used to determine whether the two treatment arms are comparable based on their quantitative baseline characteristics and known prognostic factors. Comparisons on qualitative variables will be undertaken using the Pearson Chi-squared test or Fisher's Exact test as required. If cases of inequality are detected, the confounding factors will be defined and appropriate adjustments made.

Analysis of effectiveness of treatment (main outcome) at the three monitoring points

Intention-to-treat and per-protocol analyses will both be performed, as is recommended for non-inferiority studies [38].

The effectiveness of treatment will be analysed by examining the therapeutic success achieved in each arm at 8, 26 and 52 weeks, determining the 95% confidence interval for the percentage of patients in each treatment arm whose serum vitamin B12 concentrations become normalised. If the confidence intervals do not fall outside the non-inferiority limit (10%), it can be concluded that

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the oral treatment is not inferior to the intramuscular treatment. The within-patient percentage change in serum vitamin B12 concentration at each monitoring point will be determined, and the confidence intervals for the difference in the mean values for each arm calculated.

If the distribution of confounding factors differs in the two arms, explicative regression analysis will be performed in which the dependent variable will be the normalisation of the serum vitamin B12 concentration, and the independent variable will be the treatment group.

Repeated measures ANOVA will be used to examine the change in serum vitamin B12 concentration in each group at each monitoring point.

Safety analysis

The incidence of adverse events in the two arms will be compared using the Pearson Chi-squared test or Fisher's Exact test as required.

Quality of life analysis

The perception of quality of life by the patients of each arm will be assessed by comparing the EuroQol 5D scores (determined using a visual analogue scale) and the transformation of these scores into utility-based quality of life values.

Analysis of adherence to treatment

Adherence to treatment will be examined via the counting of oral doses taken in the oral arm, and the number of injections given in the intramuscular arm. An operative indicator variable will then be defined to describe the degree of adherence.

Ethics

The trial has been approved by the Madrid Region Clinical Research Ethics Committee (February 8th 2011). It will be performed by qualified medical and scientific staff. The rights and welfare of the patients will be respected at all times. All patients will be adequately informed, both verbally and in writing, of the nature of the trial, its aim, and its risks and possible benefits. Given that the study is a non-inferiority trial, all patients will be informed that the oral treatment is expected to be as effective as the standard intramuscular treatment. Signed, dated consent to be included will be required from each patient.

Spanish law regarding the use of human subjects in clinical trials will be adhered to. The trial will respect all basic ethical principles of autonomy, justice, goodness of intent and absence of malintent according to the standards of good clinical practice enshrined in the Declaration of Helsinki (Seoul, 2008) and the Oviedo Agreement (*Convenio de Oviedo*) (1997).

Discussion

From a clinical point of view, the results obtained will help establish whether the oral administration of vitamin B12 is as effective as intramuscular treatment in the normalisation of serum vitamin B12 concentrations in patients ≥65 years of age with a deficiency. Knowledge in this respect is important since oral administration should provide these patients with greater autonomy, improve patient satisfaction with treatment, and reduce treatment costs. Patients receiving anti-coagulation treatment, for whom intramuscular treatment may be contraindicated, should also benefit. The possibility of taking an oral preparation would also allow patient preferences to be taken into account when deciding on what treatment to prescribe; indeed, patient preference is a factor of prime importance in clinical decision-taking. The possibility of providing treatment options in normal clinical practice rests on two conditions being met: 1) that quality scientific information supports the effectiveness of the therapeutic options on offer, and 2) that heterogeneous groups of patients have recorded their satisfaction with these options. The present trial provides for information in this respect to be gathered [39] and therefore treatment preferences to be taken into account at the time of prescription.

The trial is also designed to provide information on the effect of the normalisation of serum vitamin B12 concentrations by both treatments on patient-perceived quality of life. Physicians commonly assume that taking oral supplements will be associated with a feeling of greater well-being, although this has never been proven [40]. The present trial should also throw light on this.

The trial suffers from the practical limitation of having to enrol a large number of patients to meet its sample size requirements. However, a high degree of motivation is expected of the research team since its clinical assistance members are those involved in the enrolment process. Further, the fact that the patients to be enrolled will be seeking medical help (although not necessarily for vitamin B12 deficiency) suggests few will be lost to follow-up. A further possible limitation is the low statistical power used in the calculation of the sample size. The 60% power contemplated requires a sample size of 304 patients (152 in each arm) – higher powers would increase the sample size required and the enrolment of such numbers cannot be guaranteed. However, given the results reported in previous studies (2,25,31-33) that used moderate/high doses of vitamin B12, it should be possible to demonstrate the non-inferiority of the oral treatment with this power level. If the 95% confidence interval were to cross the non-inferiority threshold, i.e., showing the results to be inconclusive, the intramuscular treatment would remain the treatment of choice. To determine the degree of adherence to treatment (and thus avoid outcome dilution effects) [41], the

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number of doses taken orally and received by injection will be recorded. The characteristics of all the original 320 patients will be recorded to provide insight into the type of patient left in the study after any withdrawals, as recommended by the CONSORT group [41,42]. Basic information (age, sex, etc.) on potentially eligible patients who decline to take part will also be recorded. This type of information is of use when assessing the possible extrapolation of the trial results to more general populations.

The decision not to take serum methylnalonic acid and homocysteine concentrations into account as diagnostic markers and outcome variables was made bearing in mind that these are not normally determined, either at diagnosis or during follow-up, in patients with a vitamin B12 deficiency.

Finally, given the pragmatic nature of the proposed trial, the decision was taken to include consecutive patients seeking medical help at the participating centres, thus ensuring the enrolment of subjects similar to those that would be seen in normal clinical practice.

Abbreviations

Fe: Ferrum; g: Gram; GFR: Glomerular filtration rate; GGT: Gamma-glutamyl transpeptidase; GOT: Glutamic oxaloacetic transaminase; GP: General practitioner; GPT: Glutamic-pyruvic transaminase; HIV: Human immunodeficiency virus; HVB: Hepatitis B virus; HVC: Hepatitis C virus; IF: Intrinsic factor; µg: Microgram; MMA: Methylmalonic acid; mg: Milligrams; ng: Nanograms; pg: Picograms.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PGE y RRF conceived of the study and participated in its design. TSC; RRF; SGE; ldCG; JMF; EEM; participated in the design and coordination of the study. FRS; MGS; RGG; MAMS; COL; MLSP; CMR; BMB; AVP; FGBG; JEMS; RRB; GAC; LMCB; EPC; MRB; MTRM; SSD; SMI; RRG; IBL; MVN; JSD; TGG; MDC; AAB participated in different phases of the design. TSC; RRF; SGE; ldCG; JMF; EEM directed the writing of the manuscript. All authors OB12 Group read and approved the final manuscript.

Funding

This study was funded by the *Ministerio de Sanidad, Política Social e Igualdad*, Spain (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122) and by CAIBER - Spanish Clinical Research Network. The authors thank the following persons for their contributions to this work: Dolores Otero-Criado, Carlos Carvajales Fernández, Rosa Zurdo-Baz and Raisa González-Pérez.

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Received: 4 May 2012 Accepted: 31 May 2012
Published: 31 May 2012

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doi:10.1186/1471-2458-12-394
Cite this article as: Sanz-Cuesta *et al.*: Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). *BMC Public Health* 2012 **12**:394.

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Supplement 2: Bayesian Analysis

Bayesian analysis is a highly appropriate analysis strategy when working with small sample sizes. Previous knowledge about the studied item can be taken advantage of by means of the assessment of the plausibility of a given hypothesis after incorporating the new observed data.[1]

The noninferiority hypothesis, formally $\Delta < -10\%$, was tested, taking into account the observed results but also taking into account the results of the trials by Kuzminski et al.[2] and Saraswathy et al.[3]

P1 denotes the percentage of patients who responded to VB12 oral administration, and P0 represents the percentage of those responding to VB12 intramuscular administration. Bayesian analysis allows for calculating the probability of P1 being equal to or smaller than P0 by a specified magnitude, the noninferiority limit ($\Delta < -10\%$). For each of the parameters P1 and P0, both measured at 8, 26 and 52 weeks, we selected *a priori* distributions from the family of beta distributions with parameters **a** and **b**, which are related to the proportions of those responding in each trial arm. The gamma distribution represents the *a priori* hypothesis of the distribution of differences. According to the results of both trials by Kuzminski et al.[2] and Saraswathy et al.,[3] included in the review by Wang et al.,[4] 79.1% and 84.1% of patients normalized their VB12 levels in the oral and IM treatment groups, respectively.[4] The respective CIs associated with these prior data were calculated, and parameters were chosen (**a** and **b** in the beta distribution) such that the maximum density intervals of these distributions approximately coincided with the CI previously obtained (see Figure 1). Beta distributions for the success rate in each arm of the trial were obtained using binomial data. A total of 10000 simulations were made from these *a posteriori* distributions, and the corresponding differences, P1-P0, were calculated yielding an *a posteriori* distribution of differences. This distribution was used to derive simulation-based estimates of the probability of relevant magnitudes concerning Δ: P1-P0>0.10 at weeks 8, 26, and 52. Both PPT and ITT analyses were performed. EPIDAT 4.2 software was used for all computations.

Table 1 shows the *a posteriori* probability of differences in treatment effectiveness between oral and IM routes at different weeks (8, 26 and 52). The probabilities of the differences in treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively (per protocol analysis). In the intention-to-treat (ITT) analysis, these values were 0.000, 0.015, and 0.060 at weeks 8, 26, and 52, respectively.

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Figure 1. A priori distributions of the differences between oral and intramuscular treatment

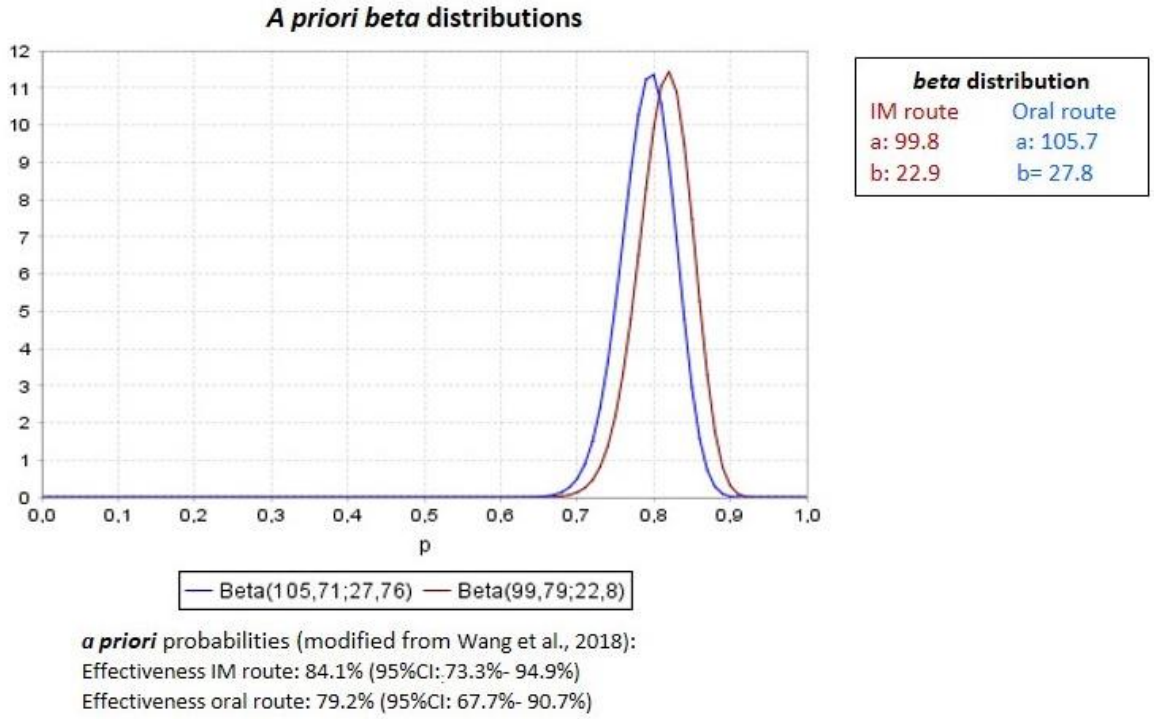

Table 1. *A posteriori* probability of differences in treatment effectiveness between oral and IM routes at 8, 26, and 52 weeks.

A posteriori **probability (Δ < -10%)**	**Week 8**	**Week 26**	**Week 52**
Per-protocol analysis	0.001	0.201	0.036
Intention-to-treat analysis	0.000	0.015	0.060

Δ: threshold of non-inferiority

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Supplement 3: Receiver Operating Characteristic (ROC) Curve

To explore factors affecting the normalization of serum VB12 concentration (yes/no) at 52 weeks, serum VB12 levels were studied at 8 weeks (at the end of the "charging period"). An ROC curve was built to determine the likelihood ratios for each cutpoint after the charging period to "predict" the normalization of levels (serum VB12 levels ≥211 pg/mL) at the end of the study.[1]

Table 1 shows the results of the likelihood ratios for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 ("charging period") to predict normalized VB12 serum levels at the end of the study. In Figure 1, the ROC curve is plotted. The level at the 5th percentile of the distribution was selected as the most useful value as it showed best classification ability and because when patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they did reach levels over 281 pg at week 8 (12~1/negative likelihood ratio).

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Table 1. Exploring the value of several cutpoints of OB12 serum levels at week 8 to "predict" normalization of values of Vit B12 at the end of the study

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-	Percentil
≥ 281	0.977	0.273	94.30%	1.3435	0.0841	5
≥ 328	0.963	0.546	94.30%	2.1193	0.0673	10
≥ 353	0.931	0.636	91.70%	2.5608	0.1081	15
≥ 389	0.895	0.818	89.10%	4.9197	0.129	20
≥ 421	0.839	0.818	83.80%	4.617	0.1962	25

LR+: Positive Likelihood ratio. LR-: Negative Likelihood ratio

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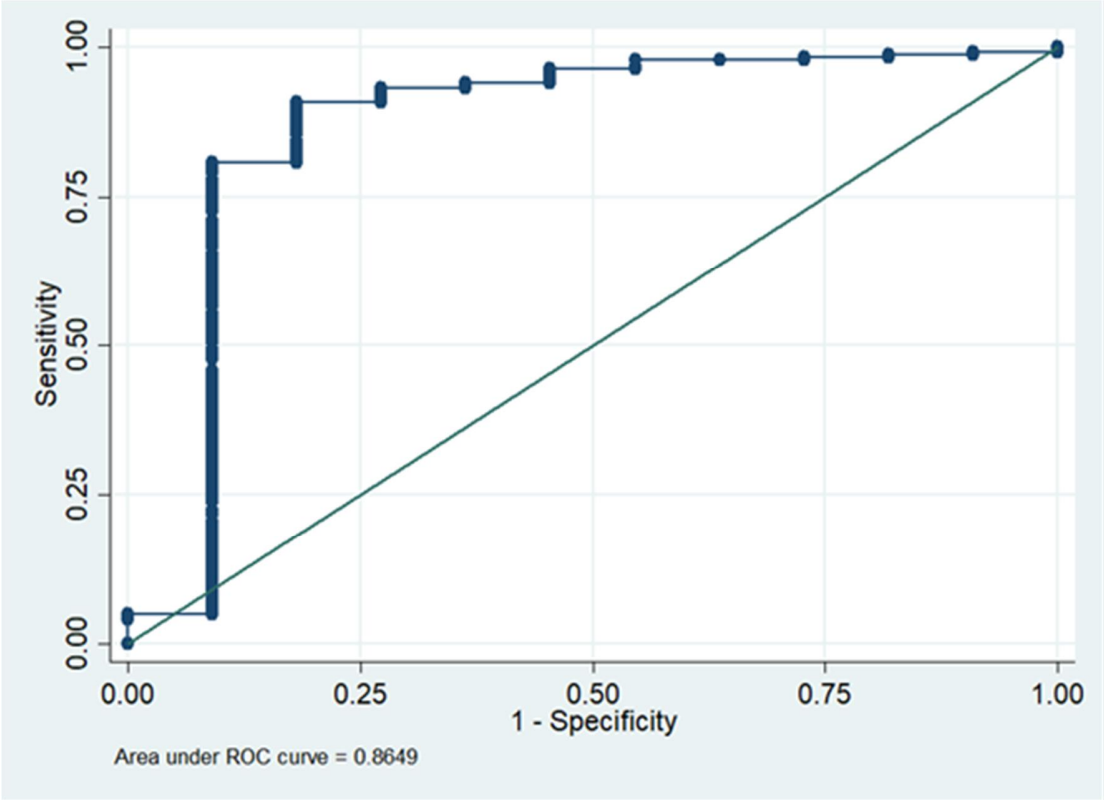
Figure 1. ROC curve



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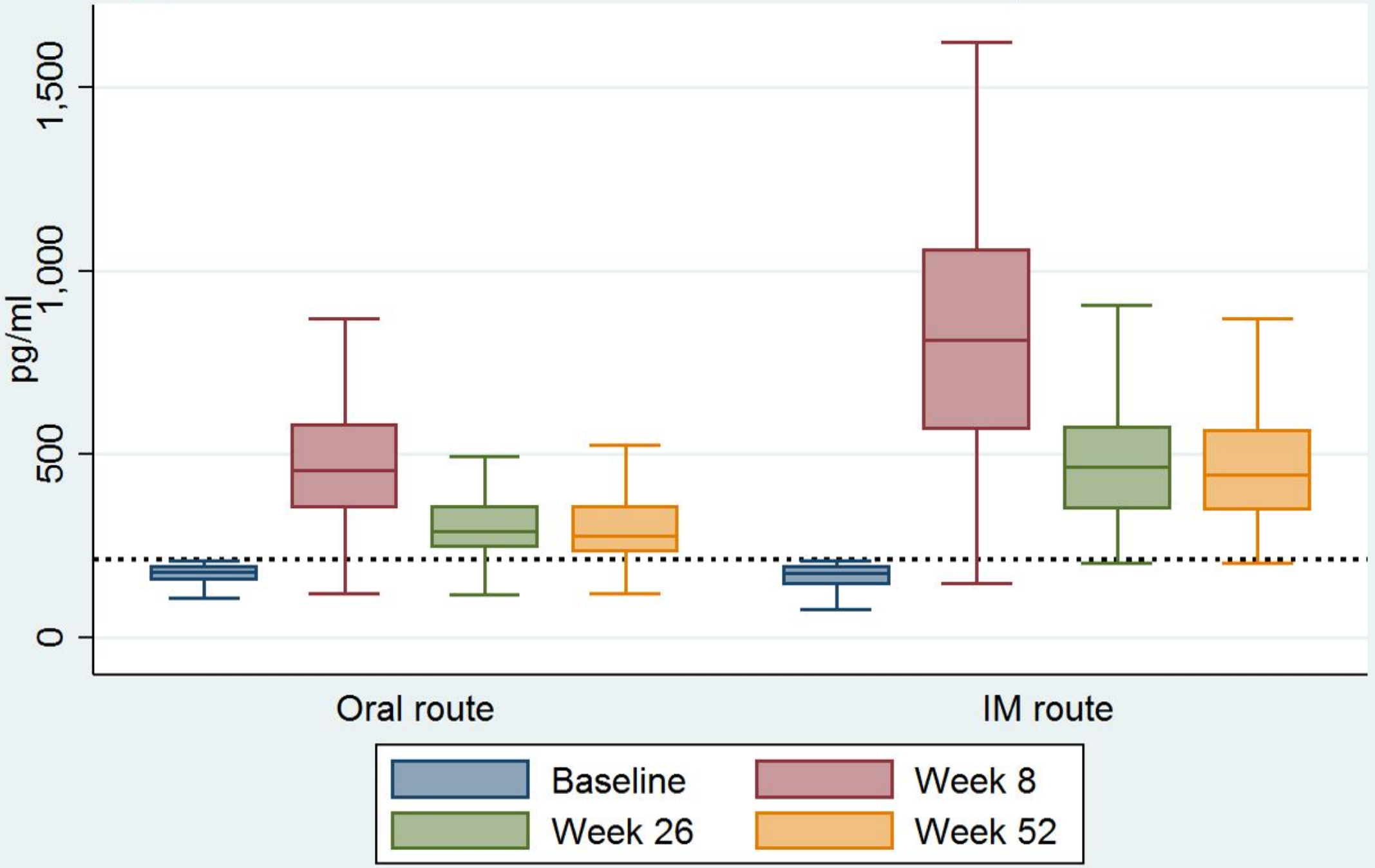
Supplement 4: Vitamin B12 mean levels by treatment route



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CONSORT Statement 2006 - Checklist for Non-inferiority and Equivalence Trials

Items to include when reporting a non-inferiority or equivalence randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned"), *specifying that the trial is a non-inferiority or equivalence trial.*	Page 1 and 2
INTRODUCTION Background	2	Scientific background and explanation of rationale, *including the rationale for using a non-inferiority or equivalence design.*	Page 5 and 6
METHODS Participants	3	Eligibility criteria for participants *(detailing whether participants in the non-inferiority or equivalence trial are similar to those in any trial(s) that established efficacy of the reference treatment)* and the settings and locations where the data were collected.	Page 7 Supplement 1
Interventions	4	Precise details of the interventions intended for each group *detailing whether the reference treatment in the non-inferiority or equivalence trial is identical (or very similar) to that in any trial(s) that established efficacy,* and how and when they were actually administered.	Page 7
Objectives	5	Specific objectives and hypotheses, *including the hypothesis concerning non-inferiority or equivalence.*	Page 6
Outcomes	6	Clearly defined primary and secondary outcome measures *detailing whether the outcomes in the non-inferiority or equivalence trial are identical (or very similar) to those in any trial(s) that established efficacy of the reference treatment* and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	Page 8
Sample size	7	How sample size was determined *detailing whether it was calculated using a non-inferiority or equivalence criterion and specifying the margin of equivalence with the rationale for its choice*. When applicable, explanation of any interim analyses and stopping rules (*and whether related to a non-inferiority or equivalence hypothesis*).	Page 8
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	Page 7 Supplement 1
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	Page 7 Supplement 1

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Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	Page 7 and 8
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	Not blinded
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s), *specifying whether a one or two-sided confidence interval approach was used.* Methods for additional analyses, such as subgroup analyses and adjusted analyses.	Page 8 and 9 Supplement 2 Supplement 3
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	Page 7 and 8 Figure 1
Baseline data	15	Baseline demographic and clinical characteristics of each group.	Page 9 and 10 Table 1
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was *"intention-to-treat" and/or alternative analyses were conducted*. State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	Figure 1 and Figure 2
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval). *For the outcome(s) for which non-inferiority or equivalence is hypothesized, a figure showing confidence intervals and margins of equivalence may be useful.*	Page 11 to 13 Table 2 Table 3 Figure 2 Supplement 2
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	Page 12
Adverse events	19	All important adverse events or side effects in each intervention group.	Page 13
DISCUSSION Interpretation	20	Interpretation of the results, taking into account the *non-inferiority or equivalence hypothesis and any other* study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	Page 14 to16
Generalizability	21	Generalizability (external validity) of the trial findings.	Page 14 to 16
Overall evidence	22	General interpretation of the results in the context of current evidence.	Page 16 and 17

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033687.R2
Article Type:	Research
Date Submitted by the Author:	28-Jan-2020
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Primary Subject Heading:	General practice / Family practice
Secondary Subject Heading:	Geriatric medicine, Evidence based practice, Health services research
Keywords:	Vitamin B 12 Deficiency, Administration Oral, Equivalence Trial,, Injections Intramuscular, primary health care, Patient preference

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

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Abstract

Objectives: To compare the effectiveness of oral versus intramuscular vitamin B12 (VB12) in patients aged ≥65 years with VB12 deficiency.

Design: Pragmatic, randomized, noninferiority, multicenter trial in patients ≥65 years in 22 primary healthcare centres in Madrid (Spain). **Participants**: 283 adults with VB12 deficiency were randomly assigned to oral (n=140) or intramuscular (n=143) treatment arm. **Interventions:** The intramuscular arm received 1mg VB12 on alternate days in weeks 1–2, 1mg/week in weeks 3–8, and 1mg/month in weeks 9–52. The oral arm received 1mg/day in weeks 1–8 and 1 mg/week in weeks 9–52.

Main outcomes: Serum VB12 concentration normalization (≥ 211 pg/mL) at 8, 26, and 52 weeks. Noninferiority would be declared if the difference between arms is 10% or less. Secondary outcomes included symptoms, adverse events, adherence to treatment, quality of life, patient preferences and satisfaction.

Results: At week 8, the percentage of patients in each arm who achieved normal B12 levels was well above 90%; the differences in this percentage between the oral and intramuscular arm were -0.7% (95% CI: -3.2 to 1.8) by per-protocol (PPT) analysis and 4.8% (95% CI: -1.3 to 10.9) by intention-to-treat (ITT) analysis. At week 52, the percentage of patients who achieved normal B12 levels was 73.6% in the oral arm and 80.4% in the intramuscular (IM) arm; these differences were -6.3% (95% CI: -11.9 to -0.1) and -6.8% (95% CI: -16.6 to 2.9), respectively. Factors affecting the success rate at week 52 were age, OR=0.95 (95% CI: 0.91 to 0.99), and having reached VB12 levels ≥281 pg/mL at week 8, OR= 8.1 (95% CI: 2.4 to 27.3). Under a Bayesian framework, noninferiority probabilities (Δ>-10%) at week 52 were 0.036 (PPT) and 0.060 (ITT). Quality of life and adverse effects were comparable across groups. 83.4% of patients preferred the oral route.

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Conclusions: Oral administration was no less effective than intramuscular administration at 8 weeks. Although differences were found between administration routes at week 52, the probability that the differences were below the noninferiority threshold was very low.

Trial registration: ClinicalTrials.gov (NCT 01476007) and EUDRACT (2010-024129-20).

Funding: Ministerio de Sanidad y Consumo Español. Instituto de Salud Carlos III (ISCIII). European Regional Development Fund.

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Strengths and limitations of this study

- This is the largest and longest follow-up randomized clinical trial in patients aged ≥65 years with VB12 deficiency.
- In addition to VB12 levels, this study incorporates patient-reported outcomes such as symptoms, quality of life, and patient preferences.
- The study design did not allow patient blinding; however, the main outcome measurement was objective.
- The rates of loss to follow-up were low at week 8 and week 26 and higher at week 52, consistent with pragmatically designed clinical trials.

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INTRODUCTION

Vitamin B12 (VB12) is an essential nutrient for the synthesis of cellular DNA. It is generally accepted that daily needs in adults range from 1 to 2 μg/day, [1] but other standards recently recommend 3-4 μg per day.[2] The Western diet is estimated to contain 7–30 μg/day of cobalamin, of which 1–5 μg is absorbed and stored (estimated reserves of 2–5 mg); therefore, symptoms resulting from a VB12 deficit would not appear until 3–5 years after establishing a low-ingestion or poor-absorption regimen.[1] VB12 deficiency can lead to hematological and neuropsychiatric disorders,[3] as well as cardiovascular risk factors.[4] The prevalence of VB12 deficiency in the elderly is highly variable across studies, which report values of 1.5% to 15%.[5–8]

In primary care, the most commonly observed causes of VB12 deficiency are related to abnormalities in digestion (atrophic gastritis, achlorhydria) or absorption (autoimmune pernicious anaemia, chronic pancreatitis, Crohn's disease, the effect of medications that alter the mucosa of the ileum such as metformin, antacids -proton-pump inhibitors and H2-receptor antagonists-, antibiotics, and colchicine)[9] or the consequences of surgical resection.[10] A deficiency stemming solely from dietary habits is rare and usually affects strict vegans.[11] In the elderly, different alterations in the processes involved in VB12 absorption increase the prevalence of this deficit, which can appear in the absence of specific symptoms, thereby hindering its diagnosis. [12]

The traditional treatment for VB12 deficiency consists of intramuscular (IM) injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum*.[10,13,14]. The vitamin may, however, be administered orally. Several studies have shown serum VB12 concentrations to normalize after taking large oral doses.[15,16] Studies taking into consideration the patients´ preferences have found differences in

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favour of the oral route.[17,18] Furthermore, oral treatment could avoid injection nuisances, reduce unnecessary travel for the patients or nurses, and minimize treatment costs.[19]

Some authors have questioned the use of oral administration while others favour it, although no firm conclusions can be drawn due to the methodological limitations of the evidence the authors provide.[10,20–22] The 2018 Cochrane Review[5] includes three randomized clinical trials comparing the effectiveness of oral and IM administration. There are differences among the trials in terms of treatment regimens and follow-up duration, ranging from 3 to 4 months, and average age of the patients, as well as the frequency and VB12 daily dose for both routes. In terms of outcomes, adverse events, and cost, the overall quality of the evidence was low due to the small number of studies and limited sample sizes.[23–25] In their conclusions, the authors state the need for trials with improved methods for random allocation and masking, larger sample sizes, and information on other relevant outcome variables that are preferably conducted in the primary care setting.

The aim of this study was to compare the effectiveness of oral- and IM-administered VB12 in the normalization of serum VB12 concentrations at 8, 26, and 52 weeks in patients aged ≥65 years with VB12 deficiency treated at primary healthcare centres (PHC). Secondary outcomes included safety (adverse events), quality of life, and adherence to treatment. Additional aims were to describe patient preferences and satisfaction with treatment and to explore the immediate response (8 weeks) as a normalization predictor of one-year outcomes to propose clinical recommendations.

METHODS

Study design and participants

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A pragmatic, randomized, multicenter, noninferiority clinical trial with a duration of 12 months was conducted in a PHC. On ethical grounds, a placebo-controlled trial was not appropriate.[26] Methodological issues of this trial have been published elsewhere (Supplement 1).[27]

Competitive recruitment was performed in 22 PHC in Madrid (Spain) from July 2014 to November 2016. Eligible patients were 65 years of age or older and had been attending a PHC for consultation on any medical matter. Patients were assessed for eligibility and invited to participate consecutively by their general practitioners. Written informed consent was obtained from all participants. A blood test was performed, and in patients with a serum concentration of VB12 of <211 pg/mL, the remaining inclusion and exclusion criteria were evaluated. The cut-off value selected in the trial register/ trial protocol was <179 pg/mL; this value was modified by the laboratory following the recommendations of the provider. This change took place prior to the beginning of the recruitment. Patient recruitment was always performed using the same methodology and cut-off point. The procedures for measurement of the biomarkers were ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY, USA).

Randomization and masking

Patients were allocated by simple randomization at a 1:1 ratio to oral or intramuscular administration of vitamin B12. The randomization system was incorporated into the electronic data collection system to assure allocation concealment. Because of the nature of the intervention, patients and general practitioners were aware of their treatment allocation. Analysis was performed by the trial statistician, who was blinded to allocation.

Intervention

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The pharmaceutical formulations used in the study are commercially available in Spain (Optovite® vials). Its pharmaceutical presentation is in silk-screen-printed clear glass ampoules that are presented in PVC blister support. The treatment regimen was : a) IM route: 1 mg of cyanocobalamin on alternate days during weeks 1–2, 1 mg/week during weeks 3–8 and 1 mg/month during weeks 9–52; b) oral route: 1 mg/day of cyanocobalamin for 8 weeks and 1 mg/week during weeks 9–52. The period between 1-8 weeks was considered the charging period. In the oral route, the medication was provided to the patient at the health centre, along with instructions for self-administration at home. The information sheet explained to the patient the procedure for oral administration, i.e., how to open the ampoule and dilute its contents in a glass, then drink it.

In the IM route, the medication was administered by the nurse at the health centre.

Outcomes

The main outcome was the normalization of serum VB12 concentrations (≥211 pg/mL) at 8, 26, and 52 weeks. The secondary outcomes were the serum VB12 concentrations (pg/mL), adverse events, adherence to treatment (number of vials for the oral arm and the number of injections for the IM arm during each visit; good adherence was considered greater than 80%), quality of life (EQ-5D-3L) [28] and patient preferences and satisfaction were assessed. Anamnesis, demographic and lifestyle information, clinical variables, analytical variables, and concomitant treatment were recorded.[27]

Procedures

After signing the consent form, those who agreed to participate had serum VB12 concentrations determined. If the VB12 value was <211 pg/mL, a hemogram, biochemical analysis, and anti-

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intrinsic factor antibody levels were assessed.[27] The patients also received a medication diary to be filled out daily. Baseline data were collected by the family physician and/or a nurse. IM treatments were administered by nurses in the health centres. The follow-up visits were conducted during weeks 8, 26 and 52.[27]

Statistical analysis

Sample size. Assuming that 70% of patients reach a serum VB12 concentration of ≥ 211 pg/mL in both groups, for a threshold of noninferiority of 10%, statistical power of 60% with significance set at $p<0.05$ and a 5% loss to follow-up, the final sample size was word 320 (160 in each arm).

As recommended for noninferiority studies, both PPT and ITT analyses were performed, with the null hypothesis being that there were differences between treatments at the three monitoring points. Comparing both arms, we calculated the difference between the percentage of patients in each treatment arm whose serum VB12 concentrations became normalized at 8, 26, and 52 weeks, with their 95% CI. If the confidence intervals do not fall outside the noninferiority limit (10%), it can be concluded that the oral treatment is not inferior to the intramuscular treatment.[29,30] In ITT analyses, missing values for the main outcome variable were added using the 'last observation carried forward' (LOCF) method.[31]

To explore factors affecting the normalization of serum VB12 concentration at 52 weeks, serum VB12 levels were studied at 8 weeks. A receiver operating characteristic (ROC) curve was built to determine the likelihood ratios of each cutpoint after the charging period to “predict” the normalization of levels at the end of the study. After this, a generalized linear model (GLM) was built (function logit). [32,33] The normalization of serum VB12 levels at 52 weeks was the dependent variable, and the treatment group was the independent variable. Variables considered

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significant by the researchers from a clinical perspective were included in the model. To test the noninferiority hypothesis, adding the information contained in these data to previous knowledge, additional statistical analyses were performed using a Bayesian approach. Secondary outcome variables were analyzed using the appropriate statistical tests, and their means or proportions were used to estimate differences between groups. All analyses were performed using STATA 14 and EPIDAT 4.2 software.

Patient involvement

Patients were not involved in the development of plans for recruitment, design, outcome measures, or implementation of the study conduct. No patients were asked to advise on the interpretation or writing of the results. Patients explained the experience of participating in the study on the occasion of International Clinical Trial´s day in *Radio Nacional de España (RNE)*. We will pursue patient and public involvement in the development of an appropriate method for further dissemination.

RESULTS

Characteristics of the study participants

A total of 2342 patients were offered participation, and 2152 provided informed consent. A total of 307 patients showed a VB12 deficit (14.3%), 283 of whom were allocated to receive VB12 treatment via the IM route (n=143) or orally (n=140). The follow-up period (52 weeks) was completed by 229 patients (80.9%) (Figure 1).

The average age was 75.2 (6.34), and 58.3% of the patients were women. Table 1 describes the baseline characteristics of the patients included in the trial. No relevant differences

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were found between groups at baseline for demographic and medical characteristics or for the study endpoints.

Table 1. Baseline characteristics at baseline by group

Variable	No. (%)		
	Oral route (n=140)	**IM route (n=143)**	**Total (n=283)**
Sociodemographic data			
Women	87 (62.1)	78 (54.5)	165 (58.3)
Age (years), mean (SD)	74.2 (5.8)	76.2 (6.7)	75.2 (6.3)
Educational level			
Illiteracy	4 (2.9)	7 (5.1)	11 (4.0)
Incomplete education	48 (34.5)	46 (33.6)	94 (34.1)
Primary education	58 (41.7)	63 (46.0)	121 (43.8)
Secondary education	16 (11.5)	10 (7.3)	26 (9.4)
Tertiary education	4 (2.9)	4 (2.9)	8 (2.9)
Higher education	9 (6.5)	7 (5.1)	16 (5.8)
Social occupational class [a]			
Class I - IV	31 (27.7)	33 (27.3)	64 (27.5)
Class V - VI	81 (72.3)	88 (72.7)	169 (72.5)
Living alone	32 (21.4)	30 (22.2)	62 (21.9)
Clinical data			
Tobacco habit			
Ex-smoker	27 (19.7)	25 (18.4)	52 (19.0)
Smoker	9 (6.6)	10 (7.4)	19 (7.0)
Nonsmoker	101 (73.7)	101 (74.3)	202 (74.0)
Vegetarian	2 (1.4)	0 (0)	2 (0.7)
Having undergone gastrectomy	1 (0.7)	2 (1.4)	3 (1.1)
Symptoms			
Paresthesia	33 (23.6)	45 (31.5)	78 (27.6)
Asthenia	43 (30.7)	54 (37.8)	97 (34.3)
Loss of appetite	12 (8.6)	30 (21.0)	42 (14.8)
Sadness	37 (26.4)	53 (37.1)	90 (31.8)
Showing ≥1 symptom	70 (50.0)	83 (58.0)	153 (54.1)
Signs			
Glossitis	2 (1.4)	9 (6.3)	11 (3.9)
Position sensitivity	2 (1.4)	1 (0.7)	3 (1.1)
Vibration sensitivity	15 (10.7)	13 (9.1)	28 (9.9)

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Showing ≥1 altered sign	16 (11.4)	21 (14.7)	37 (13.1)
Hemogram-Clinical Biochemistry			
Vitamin B12 (pg/mL), mean (SD)	173.1 (27.3)	166.4 (32.6)	169.7 (6.3)
Anemia [b]	16 (11.4)	27 (18.9)	43 (15.2)
Hematocrit (%), mean (SD)	42.4 (4.0)	41.9 (4.2)	442.1 (4.1)
MCV (fL), mean (SD)	92.1 (6.7)	94.3 (7.4)	93.2 (7.1)
Anti-intrinsic factor antibody	15 (11.0)	15 (10.5)	30 (10.8)
Medication			
Proton-pump inhibitors (PPI)	57 (40.7)	64 (44.8)	121 (42.8)
Metformin PPI and metformin	69 (49.3) 33 (23.6)	56 (39.2) 30 (21.0)	125 (44.2) 63 (22.3)
Scales			
MMSE [c], mean (SD)	30.8 (4.6)	30.2 (4.8)	30.5 (4.7)
EQ-5D-Utilities, mean (SD)	0,817 (0,169)	0,855 (0,139)	0,836 (0,171)

[a]Neoweberian occupational social class (CSO-SEE12). Gac Sanit. 2013;27(3):263–272.
[b]Anaemia was defined by the World Health Organization criteria (haemoglobin <12 g/dL in women and <13 g/dL in men). https://www.who.int/vmnis/indicators/haemoglobin
[c]Mini Mental State Examination. Maximum score= 35 points. Normal score= 30–35. Borderline score= 24–29 points. Scores < 24 points in patients aged >65 years and scores < 29 points in patients aged <65 years suggest cognitive impairment.

Primary outcomes

At week 8, the difference in the success rate between the oral and IM routes was -0.7% (95%CI: -3.2% to 1.8%) and 4.8% (95%CI: -1.3% to 10.9%) with the PPT and ITT analyses, respectively. At week 26, these differences were -12.9% (95%CI: -17.9% to -6.1%) and -3.2% (95%CI: -11.8% to 5.4%), respectively. At week 52, these differences were -6.3% (95%CI: -11.9% to -0.07%) and -6.8% (95%CI: -16.6% to 2.9%), respectively (Figure 2).

In the PPT analysis under a Bayesian approach, the probabilities of differences in the treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively. In the ITT analysis, these values were 0.000, 0.015, and 0.060 at weeks 8, 26, and 52, respectively (Supplement 2). The result of the likelihood ratio for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 to predict normalization at the end of the study is shown in Supplement 3. The level at the 5th

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percentile of the distribution was selected as the most useful value because it showed the best classification ability. When patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they had reached levels over 281 pg/mL (12~1/negative likelihood ratio).

In the ITT analysis, the factors affecting the success rate at week 52 were age, for each year of increase in age, the success rate decreased by 5%, and having attained VB12 levels of ≥281 pg/mL at week 8, which yielded a success rate 8.1 times higher (Table 2).

Table 2. Factors associated with VB12 concentrations ≥ 211 pg/ml at week 52

Variable	Odds ratio	Robust std. error	P>z	95% CI
IM vs. oral route	1.10	0.370	0.776	(0.57 to 2.13)
Age	0.95	0.022	0.025	(0.91 to 0.99)
VB12 concentration >281 pg/ml at week 8	8.10	5.014	0.001	(2.41 to 27.25)
Constant	0.78	0.622	0.755	(0.16 to 3.72)

GLM, N=265. Variance function: V(u) = u*(1-u/1) [Binomial]. Link function: g(u) = ln(u/(1-u)) [Logit]. AIC= 0.89967. BIC = -1225.89.

The mean levels of VB12 for each follow-up visit were above the normalization threshold in both groups, although these values were much greater in the IM group (Supplement 4). In 51 patients (36 IM and 5 oral), the levels of VB12 in week 8 were above the normal range limit of the laboratory (≥911 pg/mL), so the treatment regimen was changed from the initial planned pattern.

Secondary outcomes

In terms of quality of life and the presence of signs related to VB12 deficiency, no significant differences were found between treatment arms at any of the follow-up visits (Table 3).

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Table 3. Secondary outcomes (Quality of life and exploratory findings) at weeks 8, 26 and 52

Visit	Oral route		IM route		Mean difference
	N	Mean (SD)	N	Mean (SD)	(95% CI)
Quality of life (EQ-5D-5L Index)					
Baseline	139	0.855 (0.139)	137	0.817 (0.197)	0.066 (-0.002 to 0.078)
Week 8	134	0.853 (0.158)	134	0.822 (0.204)	0.031 (-0.013 to 0.075)
Week 26	128	0.853 (0.153)	128	0.826 (0.191)	0.027 (-0.016 to 0.070)
Week 52	112	0.824 (0.179)	112	0.823 (0.194)	0.001 (-0.047 to 0.049)
At least one altered sign (glossitis and/or altered vibration sensitivity and/or altered position sensitivity)					
Visit	**N**	**n (%)**	**N**	**n (%)**	**Proportion difference (95% CI)**
Baseline	140	16 (11.4%)	143	21 (14.7)	-3.3 (-11.1% to 4.6)
Week 8	135	15 (11.1%)	130	13 (10.0)	1.1 (-6.3% to 8.5)
Week 26	131	14 (10.7%)	122	12 (9.8)	0.9 (-6.6% to 8.3)
Week 52	122	14 (12.5%)	117	9 (7.7)	3.8 (-3.7% to 11.2)

Eleven adverse events were reported and none of them were severe; five (3.57%) occurred with patients in the oral arm and six (4.20%) with patients in the IM arm, yielding a difference of -0.63% (95%CI: -5.12% to 3.87%). Three patients withdrew from the study: one patient in the oral group due to urticaria, and two in the IM group due to reddening and pruritic facial erythema and generalized itching (mainly in the cheeks with scarce urticariform lesions). In three other cases, treatment for the adverse events was prescribed (constipation and erythema), and in five cases, it was not necessary to take further measures (Table 4).

Table 4. Description of adverse events by patient and route of administration

Route	Adverse event	Action
IM route	Constipation	Administration of specific treatment
	Generalized itching and hives on the cheeks	Withdrawal
	Dyspepsia	Treatment not required
	Constipation	Administration of specific treatment
	Redness and pruritic facial erythema	Withdrawal
	Erythema on forearms	Administration of specific treatment
Oral route	Urticaria on the neck and arms	Treatment not required
	Occasional postprandial dyspepsia	Treatment not required
	Occasional postprandial dyspepsia	Treatment not required
	Urticaria	Withdrawal
	Increased irritability and nervousness	Treatment not required

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At week 8, adherence to treatment was evaluated in 265 patients, of whom 95.5% were adherent (97.8% oral and 93.8% IM); the difference between the groups was 3.9% (95%CI: -0.1 to 8.7). At week 52, adherence was evaluated in 229 patients, of whom 220 (96.1%) were adherent (98.2% oral and 94.0% IM); the difference was 4.2% (95%CI: -0.7 to 9.1).

Overall, 89.5% of the patients reported being satisfied or very satisfied with the treatment via the oral route (91.3%) and the IM route (87.6%). The difference was 3.7% (95% CI: -4.0% to 11.3%).

A total of 83.4% of patients preferred the oral route (97.6% among the patients receiving VB12 orally vs. 68.6% of the patients in the IM group); the difference was 29.0% (95%CI: 20.3 to 37.7).

DISCUSSION

Main findings of the study

Supplementing VB12 in patients with VB12 deficiency, whether orally or intramuscularly, achieves the normalization of VB12 levels in most cases. The oral route was not inferior to the IM route during the charging period. Formally, the pre-established conditions for determining the noninferiority of oral administration were not met for the complete follow-up period, but these results merit a deeper analysis.

Differences between the administration routes were found at 26 and 52 weeks. The IM maintenance treatment of 1 mg/month was effective in maintaining VB12 levels, while oral administration of 1 mg/week had a probability of being inferior (by more than 10%) to the IM route by 20% in the most unfavourable scenario (PPT). However, given that no strategy was superior in the charging period, and in view of the model results showing that when VB12 levels

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reached ≥ 281 pg/mL during the charging period, the success rate at 12 months was 8 times higher, the probability that the differences between groups would exceed Δ was very low, independent of the administration route. The most plausible explanation for the observed difference between routes might be that in patients below this threshold, the maintenance oral dose should be higher than the dose used in the present study. Some authors have recommended that an oral dose of 2 mg/week be administered as a maintenance dose.[34]

The incidence of adverse events was very low and similar for oral and intramuscular administration, and nonserious adverse events were found. These findings were similar to other studies.[5] Patients' preferences can be a decisive factor for determining the administration route. In this trial, similar to previous studies,[17] there was a clear preference for the oral route, especially among the patients assigned to this group.

The effect of VB12 supplements on quality of life remains unclear,[35,36] but the present results show that the treatment route does not improve patients´ perception of their health-related quality of life or related symptoms.

We did not find significant differences in adherence. Adherence to the treatment via the IM route was lower than expected. Although drug administration was assured once the patient attended the consultation, the patient could choose not to attend appointments for various reasons. However, in usual practice, adherence with the oral route could be more compromised than with the IM route, and this factor should be taken into consideration to personalize prescription.

Comparison with other studies

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The comparison with other studies is difficult, due to the treatment different doses used, but especially because of the follow-up length had been inferior to 4 months and the number of patients included was small.

As far as we know, the present trial is the largest clinical trial with the longest follow-up period, and it is the first to evaluate, in addition to VB12 levels, clinical signs and symptoms, health-related quality of life, and patient preferences. The 3 clinical trials[23–25] described in the 2018 Cochrane Systematic Review[5] had a duration between 3 and 4 months and included a total of 153 patients. In the Saraswathy trial, patients in the oral route at 3 months normalised levels 20/30 (66.7%) vs 27/30 (90%) of the patients in the IM route.[25] In Kuzminski's patients in the oral route at 4 months normalised levels 18/18 (100%) vs 10/14 (71.4%) of the patients in the IM route.[23] These differences were statistically non-significant in both studies.

Two studies have recently been published and add evidence in favour of oral and sublingual administration of VB12.[37,38] The follow-up of Moleiro's study reached 24 months versus 12 months in our study. However, Moleiro et al performed a prospective uncontrolled study that included 26 patients submitted to total gastrectomy. All patients received oral VB12 supplementation (1 mg/day), and all of them maintained normalization V12 at 6, 12, 18, and 24 months. There was a progressive increase in serum V12 levels within the first 12 months, which remained stable thereafter.[37] The long-term effectiveness of the oral route in absorption-deficient people such as gastrectomized patients would support the results of our study.

Bensky et al. compared the efficacy of sublingual vs. intramuscular administration of vitamin B12 in a retrospective observational study from the computerized pharmacy records of Maccabi Health Service (MHS). Among 4281 patients treated with VB12 supplements (830 (19.3%) with IM and 3451 (80.7%) with sublingual tablets, the IM group achieved a significant

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increase in VB12 levels compared with the sublingual group, OR 1.85, CI 95% 1.5-2.3. [38] Although this study has a large sample size, the important methodological limitations on its effectiveness (retrospective design; reliance on clinical records; absence of epidemiological information such as patient age and sex or the aetiology of the deficit) should be considered in the interpretation of their results.

Strengths and limitations

Our study was pragmatic[39] in both the inclusion and diagnostic methods criteria. The majority of the patients with deficits included in this study presented no symptomatology or very low-level symptoms, with no anemia, which is the common profile of most patients who present with VB12 deficits in primary care. The study design did not allow for masking the patients to the received treatment. However, these limitations were compensated for by the objective measurement of the main outcome variable.

As occurs in all pragmatic clinical trials, patient recruitment was complicated, and the sample size reached only 88.4% of the calculated necessary size, which implies that the power of the study was limited. Hence, the analysis was complemented using Bayesian methods that allow for studying *a posteriori* the likelihood of a difference between two outcomes to exceed a certain limit.[40] Under this approach, the *a posteriori* probability for differences to exceed the proposed Δ=-10% was not significant during the charging period, and the probabilities were low but not negligible in the PPT analysis and low in the ITT analysis over the complete follow-up period.

Loss to follow-up was low at 8 and 26 weeks and higher at 52 weeks. This effect has been observed in pragmatic clinical trials with long follow-up periods. Missing data were greater in the IM arm, during the interval between randomization and initiation of treatment (6% IM vs 1% oral), over 8 weeks (9% IM vs 4% oral) and over 26 weeks (15% IM vs 6%). These

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differences could represent a lower acceptability of the IM route by patients, since the missing data were mostly due to patient dropout. At 52 weeks, the numbers of losses in the two arms were similar (20% oral and 18% IM), and in the case of oral treatment, several of those losses were withdrawals occasioned by not achieving particular levels of VB12.

Implications of the study findings

On the basis of our results and the available evidence, we propose the oral administration of VB12 at 1 mg/day during the charging period. Subsequently, the recommended dose would vary as a function of the VB12 levels reached during the charging period. For VB12 concentrations between the normal levels of 211 pg/mL (in our laboratory) and 281 pg/mL (the 5th percentile of the distribution in this trial), a dose of 2 mg/week is suggested. When the levels reached in the charging period are between 281 and 380 pg/mL (the 20th percentile of the distribution), it may be appropriate to perform an analysis between 8 and 26 weeks to confirm that normal levels are maintained. All patients who reach a level of 380 pg/mL by week 8 could be maintained at the initial dosage (1 mg/week) without subsequent analyses during the year of follow-up.

If the IM route is chosen, the proposed dose for this route during the first few weeks may be excessive for patients with VB12 deficiency. The scheduled IM dose should be reconsidered in the first two weeks based on VB12 levels, and the scheduled dose could be limited to 1 mg/week if warranted by the outcome. Nevertheless, these recommendations must be assessed in further research.

Oral administration of VB12 in patients older than 65 years is probably as effective as intramuscular administration, and it also lacks adverse effects and is preferred by patients. We must also highlight the potential benefit of the oral route in terms of safety for patients with

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coagulation problems, for whom IM-administered medication is often contraindicated. A small number of patients may require additional follow-up after 8 weeks if a certain concentration of VB12 in blood is not reached.

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- Technical Support Group**: participated in different phases of the design and development of the research. MLSP; CMR; BMB; MAJ; coordinated the pharmaceutical aspects.
- Clinical Investigators: collected the data for the study, which included recruiting patients, obtaining consent, performing blood tests, applying interventions, collecting data, and arranging and performing follow-up for patients.
- Statistical analysis: TSC; JMF; IDC; EEM with the collaboration of the Research Unit (JGM and MMM).
- Writing Committee: TSC; EEM; IDC; JMF, SGE, and RRF wrote the manuscript. All authors in the OB12 Group read and approved the final manuscript.

Acknowledgements

To Margarita Blázquez Herranz, Instituto de Salud Carlos III, for making the impossible possible.

To Juan Carlos Abánades Herranz for trusting us and his contribution to facilitate our work.

To Antonio Jesús Alemany López and Marta Sánchez-Celaya, Managing Director of Primary Healthcare for accepting to sponsor this research after the dissolution of CAIBER.

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To the Fundación para la Investigación e Innovación Biomédica de Atención Primaria (FIIBAP) for the grant awarded for the publishing of the research.

To Fernando Cava Valencia and Raquel Guillén Santos from the Clinical Laboratory BRSALUD.

To Elena Tolosa Oropesa from the Pharmacy Department, Gerencia Asistencial de Atención Primaria de Madrid.

To our colleagues from the Research Unit, including the assistants Marcial Caboblanco Muñoz, Juan Carlos Gil and Isabel Morales Ropero for their unconditional support; project manager Pilar Pamplona-Gardeta, researchers Juan A. López Rodríguez, Jaime Barrio Cortes, Cristina Lozano Hernandez and Medical Residents Daniel Toledo Bartolomé, Irene Wijers and Palmira Jurado Macías for their technical advice in finalizing this paper.

To Javier Zamora Romero, head of the Unit of Biostatistics, Hospital Ramón y Cajal of Madrid, for revising the manuscript.

To all the professionals from the participating healthcare centers.

To all patients who contributed to this research.

Competing Interests

The authors declare that they have no competing interests.

Funding

The trial was financed by Ministerio de Sanidad y Consumo Español through their call for independent clinical research, Orden Ministerial SAS/2377, 2010 (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122); CAIBER – Spanish Clinical Research Network, Instituto de Salud

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Carlos III (ISCIII) (CAI08/010044); and Gerencia Asistencial de Atención Primaria de Madrid. This study is also supported by the Spanish Clinical Research Network (SCReN), funded by ISCIII-Subdirección General de Evaluación y Fomento de la Investigación, project number PT13/0002/0007, within the National Research Program I+D+I 2013-2016 and co-funded with European Union ERDF funds (European Regional Development Fund).

The funders of the study had no role in the study design, data collection, onsite monitoring, data analysis, data interpretation, or writing of the manuscript.

Ethics approval

Madrid Region Clinical Research Ethics Committee on February 8th, 2011.

Data sharing statement

Individual de-identified participant data will be shared upon reasonable request. These data will include every variable used in the analysis shown in this report, and they will be available for five years upon request to corresponding author.

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Figure legends

Figure 1. Trial profile

Figure 2. Difference between the oral and intramuscular routes in the proportion of patients whose VB12 levels returned to normal (≥211 pg/ml)

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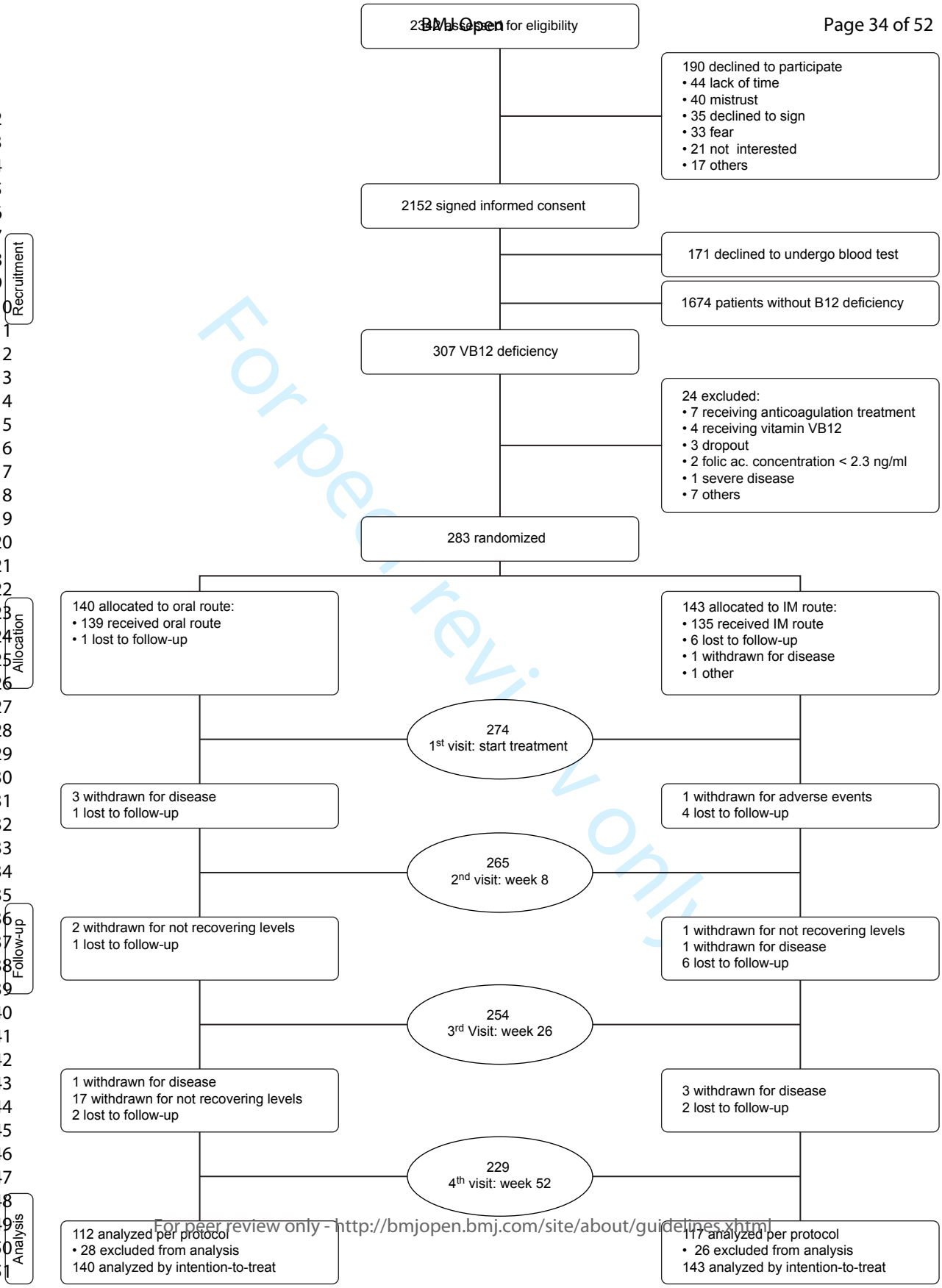

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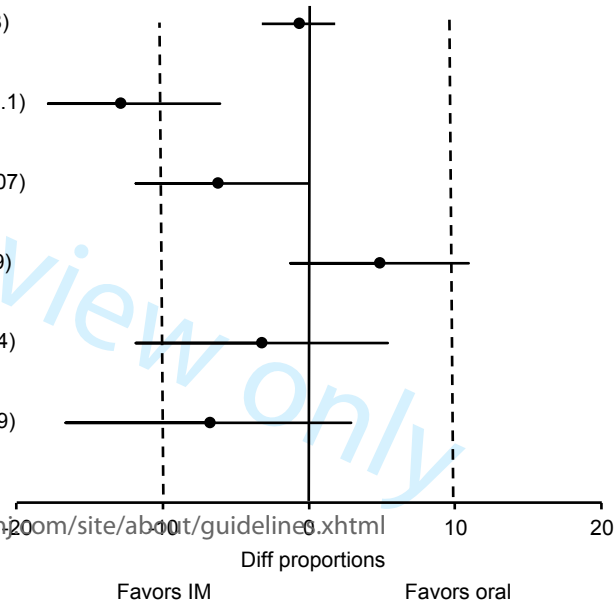
	Visit	Oral route n/N (%)	IM route n/N (%)	Difference in proportions (95% CI)
BPT	Week 8	133/135 (98.5)	129/130 (99.2)	-0.7 (-3.2 to 1.8)
	Week 26	115/132 (87.1)	122/122 (100.0)	-12.9 (-17.9 to -6.1)
	Week 52	103/112 (92.0)	115/117 (98.3)	-6.3 (-11.9 to -0.07)
AHT	Week 8	133/140 (95.0)	129/143 (90.2)	4.8 (-1.3 to 10.9)
	Week 26	115/140 (82.1)	122/143 (85.3)	-3.2 (-11.8 to 5.4)
	Week 52	103/140 (73.6)	115/143 (80.4)	-6.8 (-16.6 to 2.9)



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BPT			
Week 8	133/135 (98.5)	129/130 (99.2)	-0.7 (-3.2 to 1.8)
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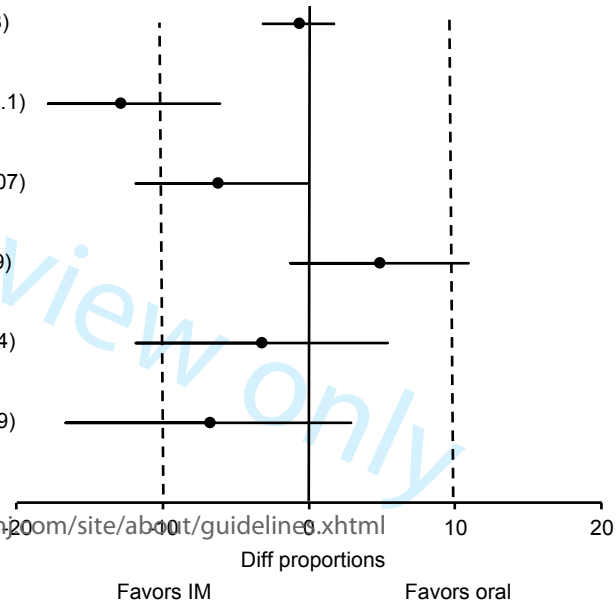
Week 52	103/112 (92.0)	115/117 (98.3)	-6.3 (-11.9 to -0.07)
HT			
Week 8	133/140 (95.0)	129/143 (90.2)	4.8 (-1.3 to 10.9)
Week 26	115/140 (82.1)	122/143 (85.3)	-3.2 (-11.8 to 5.4)
Week 52	103/140 (73.6)	115/143 (80.4)	-6.8 (-16.6 to 2.9)



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BMC Public Health

STUDY PROTOCOL Open Access

Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12)

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Abstract

Background: The oral administration of vitamin B12 offers a potentially simpler and cheaper alternative to parenteral administration, but its effectiveness has not been definitively demonstrated. The following protocol was designed to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the treatment of patients ≥65 years of age with vitamin B12 deficiency.

Methods/design: The proposed study involves a controlled, randomised, multicentre, parallel, non-inferiority clinical trial lasting one year, involving 23 primary healthcare centres in the Madrid region (Spain), and patients ≥65 years of age. The minimum number of patients required for the study was calculated as 320 (160 in each arm). Bearing in mind an estimated 8-10% prevalence of vitamin B12 deficiency among the population of this age group, an initial sample of 3556 patients will need to be recruited.

Eligible patients will be randomly assigned to one of the two treatment arms. In the intramuscular treatment arm, vitamin B12 will be administered as follows: 1 mg on alternate days in weeks 1 and 2, 1 mg/week in weeks 3–8,and 1 mg/month in weeks 9–52. In the oral arm, the vitamin will be administered as: 1 mg/day in weeks 1–8 and 1 mg/week in weeks 9–52. The main outcome variable to be monitored in both treatment arms is the normalisation of the serum vitamin B12 concentration at weeks 8, 26 and 52; the secondary outcome variables include the serum concentration of vitamin B12 (in pg/ml), adherence to treatment, quality of life (EuroQoL-5D questionnaire), patient

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3satisfaction and patient preferences. All statistical tests will be performed with intention to treat and per protocol. Logistic regression with random effects will be used to adjust for prognostic factors. Confounding factors or factors that might alter the effect recorded will be taken into account in analyses.

Discussion: The results of this study should help establish, taking quality of life into account, whether the oral administration of vitamin B12 is an effective alternative to its intramuscular administration. If this administration route is effective, it should provide a cheaper means of treating vitamin B12 deficiency while inducing fewer adverse effects. Having such an alternative would also allow patient preferences to be taken into consideration at the time of prescribing treatment.

Trial registration: This trial has been registered with ClinicalTrials.gov, number NCT 01476007, and under EUDRACT number 2010-024129-20.

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Background

Vitamin B12 (cyanocobalamin), along with other derivatives of folic acid, is a nutrient essential for the synthesis of DNA. Its deficiency is manifested through changes in the number and morphology of erythrocytes, leucocytes and platelets, and by neurological alterations owed to the progressive demineralisation of the nervous system (a consequence of defective myelin synthesis). Vitamin B12 is found mostly in food of animal origin. It is separated from ingested food through the action of the gastric acid, and in the duodenum the vast majority binds to intrinsic factor (IF). The vitamin B12/IF complex formed, which is very resistant to digestion, is then absorbed by endocytosis in the terminal ileum. Only 1-2% of vitamin B12 absorption occurs independent of IF [1]. Daily vitamin B12 requirements vary between 1 and 2 μg/day in adults [2]. A balanced diet, however, provides somewhere between 7 and 30 μg/day. Some of this excess can be stored (some 2–5 mg), meaning that deficiency symptoms may not occur until 3–5 years after the diet fails to provide sufficient vitamin B12 or its absorption becomes inadequate [3].

In the primary healthcare setting, the most commonly seen causes of vitamin B12 deficiency are related to abnormalities of digestion (atrophic gastritis, achlorhydria or the consequences of gastrectomy) or absorption (autoimmune pernicious anaemia, chronic pancreatitis, Crohn's disease, the effect of medications that alter the mucosa of the ileum, or the consequences of surgical resection), and, to a lesser extent, a lack of exogenous supply. The exact prevalence of vitamin B12 deficiency in industrialised countries is unknown; indeed, different studies using different definitions have reported it as between 5% and 60% [4]. Results have even differed widely between similar studies using an identical definition of deficiency, and after stratifying by age [5]. In Spain, the prevalence of vitamin B12 deficiency may reach 18% according to a meta-analysis of the studies undertaken up to 1999 [6]. However, population-based studies performed in Catalonia and the Canary Islands [7,8], both of which used a serum vitamin B12 cut-off of 200 pg/ml, returned values of 1.9% and 3.4% respectively. What does appear to be constant in all studies reviewed for the present work is that the prevalence of deficiency is greater among people aged 65–76 years. For example, the above Catalonian and Canary Island studies returned values of 3.8% and 8.5% for these age groups. Among elderly patients belonging to the Framingham cohort, Lidenbaun [9] observed a prevalence of over 5.3%. Other authors [10,11], however, report figures of 30-40% in elderly people with degenerative neuropsychiatric disorders and those receiving institutionalised care.

In the elderly, the symptoms of vitamin B12 deficiency caused by deficient diets and/or digestive and/or absorption problems can be nonspecific, making a diagnosis of deficiency more difficult. For example, up to 40% of elderly people show no haematological alterations. Further, neurological symptoms may appear before those of anaemia; indeed, only about 60% of elderly people with vitamin B12 deficiency are anaemic [12].

In primary healthcare in Spain, vitamin B12 deficiency is diagnosed via the determination of the serum concentration of the vitamin. Some studies [13-17] have described the limitations of trying to diagnose vitamin B12 deficiency exclusively via the measurement of this concentration, and report blood methylmalonic acid (MMA) and homocysteine concentrations to be more sensitive markers capable of detecting subclinical deficiency.

The traditional treatment of vitamin B12 deficiency is the intramuscular injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum* [4,18,19]. The vitamin may, however, be offered orally. In some circles this route has been regarded as an effective alternative to parenteral administration since the 1950s, during which time several studies showed serum vitamin B12 concentration to normalise after taking large oral doses. These results prompted the spread of oral administration in Sweden and Canada [3]. In the former country, 13% of the population over 70 years of age

now receives treatment for vitamin B12 deficiency, with two of every three patients treated via the oral route [20]. However, in the rest of the world, the parenteral route remains the most used. Indeed, controversy still surrounds the advantages and effectiveness of the oral route. Some authors question its use [21] while others favour it, although the methodological limitations of the evidence they provide means no firm conclusions can be drawn. In reviews of the literature published between 1999 and 2007, Daly-Youcef [4] and Andrés E [19] concluded that orally administered vitamin B12 provided effective treatment for adult and elderly patients with deficiencies, although they highlighted that further studies were needed to determine its effectiveness in patients with severe neurological symptoms. Federicia [22], who reviewed the treatment criteria followed in different studies, concluded oral administration to be effective, but recommended further work to confirm this. Shatsky[23], who examined evidence derived from the use of oral and intramuscular administration, indicated that high dose oral administration appeared to be safe, effective and cost-effective, although long term clinical trials were required to confirm this. In a prospective study performed in Spain involving commercially available multi-vitamin supplements, Rabuñal et al. [24] reported the effectiveness and tolerance of oral vitamin B12 to be excellent, but also indicated that the dosage to be used was yet to fully established. In 2005, a Cochrane review [3] was published that examined two randomised clinical trials - those reported by Kuzminski [2] and Bolaman [25] - that studied the effectiveness of oral vs. intramuscular administration of vitamin B12 for the treatment of its deficiency. The Kuzminski trial involved 33 patients (18 in the oral arm and 15 in the intramuscular arm), while the Bolaman trial involved 60 (26 in the oral arm and 15 in the intramuscular arm). The Cochrane concluded that orally administered vitamin B12 appeared to be as effective as the intramuscular route with respect to the short-term haematological and neurological responses observed in patients with deficiencies, but highlighted methodological limitations in both trials. A large clinical trial was called for in the primary healthcare setting, where a high percentage of patients with vitamin B12 deficiency is seen. The Cochrane review also underscored the need to include a measurement of the quality of life as an outcome, and patient preference at the time of prescribing treatment. Among other variables, three studies [24,26,27] have recorded patient views on the administration route, and record a high level of acceptance of the oral route, the advantages of which include avoiding the displacement of patients to receive injections, avoiding the discomfort of injection, and a reduction in treatment costs [28,29].

A further question still to be answered is that of the optimum dose when using the oral route [3].

In summary, despite many studies indicating the oral administration of vitamin B12 to be easy, effective and less costly than intramuscular administration, their designs, and in some cases their methodological limitations, mean that debate still surrounds the effectiveness of the oral route. This may help explain why it is little used by health professionals [30].

Although some authors [31,32] recommend the use of moderately high doses (which have obtained the best results), studies are still being performed to investigate this. In a randomised clinical trial involving five treatment arms with doses of between 2.5 μg/day and 1000 μg/day, Eussen [33] concluded that a dose of at least 600 μg/day was required to obtain adequate results. However, in guidelines published in 2012, the British Columbia Medical Association (Canadian Ministry of Health) recommended a dose of 1000 μg/day for pernicious anemia or food-bound cobalamin malabsorption [34].

The proposed study examines the questions that, according to the Cochrane review mentioned above [3], are still to be answered, via a clinical trial (of ample duration and with a large number of patients) in the primary healthcare setting. As recommended, one of the outcomes examined is quality of life. The results obtained should provide high quality scientific evidence of use when taking treatment decisions in the primary healthcare centres, while allowing patient preference of administration route to be taken into consideration. The results may reveal oral treatment with vitamin B12 to be, as Lederle [35] put it, “medicine’s best kept secret”.

Aim

The aim of the proposed protocol is to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the normalisation of serum vitamin B12 concentrations at 8, 26 and 52 weeks of treatment, in patients aged ≥65 years with vitamin B12 deficiency treated at primary healthcare centres in the Madrid region, Spain. The secondary outcomes to be measured include the safety of both administration routes, quality of life (measured using the EuroQoL-5D questionnaire) and adherence to treatment. Patient preferences and satisfaction with treatment will also be recorded, along with patient sociodemographic profiles, lifestyle habits, and the clinical manifestation of each patient’s deficiency.

Methods/design

Study type

This study takes the form a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the

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primary healthcare setting, with a duration of one year. For ethical reasons, a placebo controlled trial would not be appropriate [36].

The study involves 23 primary healthcare centres in the Madrid region of Spain. The research team is composed of a clinical assistance group of 169 general practitioners and nurses, and a technical group of 22 health professionals including doctors of different specialities, nurses and pharmacists. For the undertaking of fieldwork, these 191 team members are divided into smaller groups (with similar numbers of clinical and technical personnel), each in charge of one of five subprojects. Each subproject is led by a member of the technical personnel. Together, these five leaders form the coordination group for the trial as a whole.

The trial protocol was approved by the Madrid Region Clinical Research Ethics Committee (*Comité Ético de Investigación Clínica Regional de la Comunidad de Madrid*) on February 8th 2011, and has been registered with ClinicalTrials.gov number NCT 01476007, and under EUDRACT number 2010-024129-20 [Oral Versus Intramuscular Cobalamin to treat Cobalamin Deficiency: Noninferiority randomised controlled trial, pragmatic and multi-center in the primary healthcare setting (OB12 project)].

Patients

1. Inclusion criteria: all participants must:
   - be ≥65 years of age
   - be attending a primary healthcare centre for consultation on some medical matter
   - provide their informed consent to be included
   - have a serum B12 concentration of <179 pg/ml.
2. Exclusion criteria: patients meeting any of the following conditions will be excluded:
   - having been treated (under medical prescription) in the last five years for vitamin B12 deficiency
   - serious neurological or psychiatric symptoms, including psychotic problems
   - dementia preventing the giving of informed consent to take part
   - atrophy of the optic nerve
   - serum folic acid concentration of <2.3 ng/ml
   - stage 4 kidney disease 4 (estimated glomerular filtration rate [GFR] 15–29 ml/min)
   - having received/suffering malabsorption-related:
     - surgery or diseases affecting the jejunum-ileum
     - inflammatory-intestinal disease, e.g., Crohn's disease, ulcerative colitis
     - celiac disease
   - chronic pancreatitis
   - myelodisplasia or malignant blood disease
   - haemophilia or other coagulation problems contraindicating parenteral administration
   - severe systemic disease
   - having been involved in any other trial involving the administration of any experimental treatment in the 28 days prior to the start of the present study
   - being treated for HIV, HVB or HVC infection
   - hypersensitivity to vitamin B12, or any of the vitamin preparation's excipients
   - receiving anticoagulation treatment
   - being away from home and with no intention of residing for the following year in the health district where consultation was made
   - failing to meet any inclusion criterion
   - limitations regarding oral treatment

Randomisation

Participants will be enrolled consecutively by their general practitioners when attending a primary healthcare centre in the study area (Figure 1). All patients without reason to be excluded will be invited to participate. Those patients that accept this invitation will provide written, informed consent to be included. A blood sample will then be taken and part of this used to determine the serum vitamin B12 concentration (pg/ml). In those returning a value of <179 pg/ml (defined as vitamin B12 deficiency by the reference analytical laboratory analysing the samples collected), the remaining fraction of the sample will be analysed to provide a haemogram (reticulocyte, erythrocyte, leucocyte and platelets counts), the values of biochemical variables (glucose, creatinine, GOT, GPT, GGT and ferritin), the folic acid concentration, and an anti-IF antibody count. Those who meet all inclusion criteria, and no exclusion criteria, will then be randomly assigned to one arm of the treatment, i.e., oral or intramuscular administration of vitamin B12. This will be performed by means of a simple randomisation process performed by the electronic data collection system. This guarantees that neither researcher nor patient has any choice with respect to the group to which the latter is assigned.

Sample size

The sample size required was determined bearing in mind the results of Kuzminski et al. [2]. In the latter study the parenteral administration of vitamin B12 was associated with an increase in serum concentrations of the vitamin of >200 pg/ml at 4 months in over 70% of patients. For the present trial, the level of non-inferiority of the oral treatment is set at a difference (delta) in response compared to the parenteral treatment of ≤10%. This threshold was set given its importance from a clinical rather than a statistical viewpoint, and since it falls within the range normally accepted for this type of study [37].

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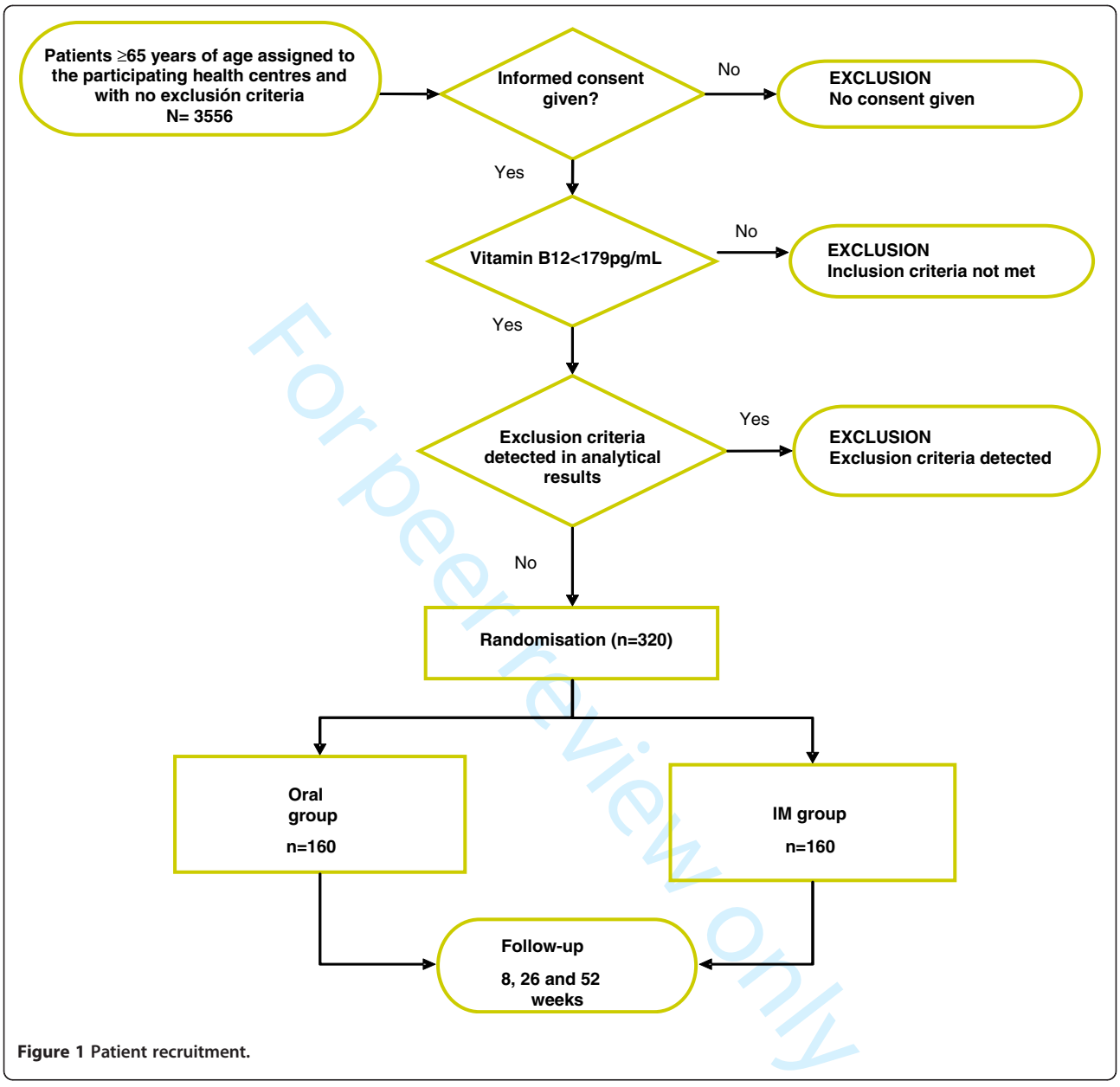

Figure 1 Patient recruitment.

Assuming that the percentage of patients showing an increase in serum vitamin B12 concentration to above 179 pg/ml in both groups is 70%, means the study requires at least 304 patients (152 in each arm) for a threshold of non-inferiority of 10% and a statistical power of 60% with significance set at $p < 0.05$. Given the type of patients to be studied, i.e., patients who have come to the health centres for consultation, plus the fact that their own family doctors are members of the research team, a loss to follow-up of under 5% is expected. The minimum starting sample size for each arm was therefore deemed to be n = 160. With an expected prevalence of vitamin B12 deficiency of 8-10% (a figure of 9% was used in calculations), the original number of patients to be enrolled so that 320 with a vitamin B12 deficiency can be guaranteed is 3556.

Blinding

In studies with the present design it is impossible to blind the patient to the treatment received. However, this limitation is compensated for by the objective measurement of the main outcome variable (the serum vitamin B12 concentration) and the randomisation of the patients to the treatment groups. Further, the persons charged with the statistical analysis of the data will be blind to the identity of the patients in each treatment arm.

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The intervention

The pharmaceutical formulations to be used in the study are commercially available in Spain. The treatments will involve:

- Intramuscular route: 1 mg of vitamin B12 on alternate days during weeks 1 and 2; 1 mg/week over weeks 3–8 (i.e., for 6 weeks); and 1 mg/month from weeks 9–52
- Oral route: 1 mg/day of vitamin B12 for 8 weeks; 1 mg/week from weeks 9–52

Patients in both arms will undergo analytical monitoring in weeks 8, 26 and 52. They will receive appointments for the appropriate dates. The response to treatment will be recorded alongside adherence to treatment and the appearance of any adverse effects.

Work plan

Before work begins, the project will be presented to all the research team members in a special meeting. Training sessions lasting 2–3 h will also be held at each participating health centre. These will involve a review of the inclusion and exclusion criteria, provide instructions regarding the intervention, and examine the ethical requirements to be met for the trial to be held.

The procedures to be followed and information to be recorded at each of a patient's visits to a participating health centre is as follows:

- Selection Visit
  - Signing of informed consent
  - Assessment of inclusion/exclusion criteria
  - Recording of demographic data (age and sex)
  - Analysis: serum vitamin B12. If concentration is <179 pg/ml the following analyses are to be requested: haemogram, biochemical analysis (glucose, creatinine, GOT/GPT/GGT), ferritin, folic acid, anti-IF antibody level. If serum vitamin B12 concentration is >179 pg/ml: patient preference questionnaire
  - Randomisation of patients to treatment group
- Visit 1 (start of treatment)
  - Anamnesis: record whether the patient lives alone or with others, lifestyle habits, use of alcohol, whether a vegan diet is followed, whether the patient has undergone gastrectomy
  - Symptoms: record paresthesia, asthenia, loss or reduction of appetite, sadness or change in state of mind, concomitant pharmacological treatment
  - Physical examination: for Hunter's glositis, positional and vibrational sensitivity
  - Questionnaires: Lobo cognitive mini-exam, EuroQoL-5D
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
- Visit 2 (week 8)
  - Anamnesis: record lifestyle habits and use of alcohol
  - Symptoms: if pathological at the first visit, record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
  - Physical examination: if pathological at the first visit examine for Hunter's glositis, positional and vibrational sensitivity
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Questionnaires: EuroQoL-5D
  - Assessment of adverse effects
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
  - Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given
- Visit 3 (week 26)
  - Anamnesis: record lifestyle habits and use of alcohol
  - Symptoms: if pathological at the first visit, record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
  - Physical examination: if pathological at the first visit examine for Hunter's glositis, positional and vibrational sensitivity
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Questionnaire: EuroQoL-5D
  - Assessment of adverse effects
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
  - Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given
- Visit 4 (week 52)
  - Anamnesis: record lifestyle habits and use of alcohol

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- Symptoms: record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
- Physical examination: for Hunter's glositis, positional and vibrational sensitivity
- Record concomitant treatment
- Questionnaires: EuroQoL-5D, satisfaction and preferences
- Assessment of haemogram and serum vitamin B12 concentration
- Assessment of adverse effects
- Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given

Variables

Outcome variables

The main outcome to be measured is the normalisation of the serum vitamin B12 concentration (>179 pg/ml) at 8, 26 and 52 weeks. The secondary outcomes will be the serum vitamin B12 concentration (pg/ml), adverse events (description, moment of onset and resolution, intensity, cause, steps taken), adherence to treatment (measured at each patient visit via the number of vials used for patients in the oral arm, and the number of injections given in the intramuscular arm), quality of life (measured using the EuroQoL-5D questionnaire), and patient satisfaction and preferences.

Anamnesis, demographic and lifestyle information

Including age, sex, whether the patient lives alone or with others, whether a vegan diet is followed, and the use of alcohol (g/week).

Clinical variables

Symptoms such as paresthesia, asthenia, loss or reduction of appetite, sadness or change in state of mind (anamnesis), Hunter's glositis, positional and vibrational sensitivity (all via physical examination), and cognitive decline (Lobo test).

Analytical variables

Haemogram (complete blood cell and platelet count) and biochemical analysis (folic acid, glucose, creatinine, GOT, GPT, GGT, ferritin, anti-IF antibodies). Blood analyses will be performed in plasma or serum as required and under standard conditions.

Concomitant treatment

Recording of the taking of protein pump inhibitors, H2 receptor antagonists, antacids, potassium, metformin, colchicine, neomycin, p-aminosalicylic acid, parenteral chloramphenicol, Fe, vitamin C and other vitamin supplements.

Losses and withdrawals

Patients will be removed from the trial if any of the following conditions are met:

- Serum vitamin B12 concentration still <179 pg/ml after 8 weeks of treatment. Treatment will be deemed to have failed in these patients, and they will be further studied and treated outside the trial according to normal clinical practice.
- Serious adverse events.
- Voluntary withdrawal or violation of the protocol.

At least two attempts will be made to contact by telephone those patients who do not come for their scheduled visits. All patients will be informed that they can abandon the study at any time without this affecting their future medical treatment in any way.

Analysis

Descriptive analysis of the patients

The trial will involve a descriptive statistical analysis of the baseline characteristics of patients in both treatment arms. Quantitative variables will be described in terms of their measure of central tendency, mean or median (for those showing asymmetric distributions), and the corresponding dispersion, standard deviation or interquartile range. Qualitative variables will be described in terms of proportions and their corresponding confidence intervals.

Baseline comparisons

The Student t test or Mann–Whitney U test (when the normal hypothesis is rejected) will be used to determine whether the two treatment arms are comparable based on their quantitative baseline characteristics and known prognostic factors. Comparisons on qualitative variables will be undertaken using the Pearson Chi-squared test or Fisher's Exact test as required. If cases of inequality are detected, the confounding factors will be defined and appropriate adjustments made.

Analysis of effectiveness of treatment (main outcome) at the three monitoring points

Intention-to-treat and per-protocol analyses will both be performed, as is recommended for non-inferiority studies [38].

The effectiveness of treatment will be analysed by examining the therapeutic success achieved in each arm at 8, 26 and 52 weeks, determining the 95% confidence interval for the percentage of patients in each treatment arm whose serum vitamin B12 concentrations become normalised. If the confidence intervals do not fall outside the non-inferiority limit (10%), it can be concluded that

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the oral treatment is not inferior to the intramuscular treatment. The within-patient percentage change in serum vitamin B12 concentration at each monitoring point will be determined, and the confidence intervals for the difference in the mean values for each arm calculated.

If the distribution of confounding factors differs in the two arms, explicative regression analysis will be performed in which the dependent variable will be the normalisation of the serum vitamin B12 concentration, and the independent variable will be the treatment group.

Repeated measures ANOVA will be used to examine the change in serum vitamin B12 concentration in each group at each monitoring point.

Safety analysis

The incidence of adverse events in the two arms will be compared using the Pearson Chi-squared test or Fisher's Exact test as required.

Quality of life analysis

The perception of quality of life by the patients of each arm will be assessed by comparing the EuroQol 5D scores (determined using a visual analogue scale) and the transformation of these scores into utility-based quality of life values.

Analysis of adherence to treatment

Adherence to treatment will be examined via the counting of oral doses taken in the oral arm, and the number of injections given in the intramuscular arm. An operative indicator variable will then be defined to describe the degree of adherence.

Ethics

The trial has been approved by the Madrid Region Clinical Research Ethics Committee (February 8th 2011). It will be performed by qualified medical and scientific staff. The rights and welfare of the patients will be respected at all times. All patients will be adequately informed, both verbally and in writing, of the nature of the trial, its aim, and its risks and possible benefits. Given that the study is a non-inferiority trial, all patients will be informed that the oral treatment is expected to be as effective as the standard intramuscular treatment. Signed, dated consent to be included will be required from each patient.

Spanish law regarding the use of human subjects in clinical trials will be adhered to. The trial will respect all basic ethical principles of autonomy, justice, goodness of intent and absence of malintent according to the standards of good clinical practice enshrined in the Declaration of Helsinki (Seoul, 2008) and the Oviedo Agreement (*Convenio de Oviedo*) (1997).

Discussion

From a clinical point of view, the results obtained will help establish whether the oral administration of vitamin B12 is as effective as intramuscular treatment in the normalisation of serum vitamin B12 concentrations in patients ≥65 years of age with a deficiency. Knowledge in this respect is important since oral administration should provide these patients with greater autonomy, improve patient satisfaction with treatment, and reduce treatment costs. Patients receiving anti-coagulation treatment, for whom intramuscular treatment may be contraindicated, should also benefit. The possibility of taking an oral preparation would also allow patient preferences to be taken into account when deciding on what treatment to prescribe; indeed, patient preference is a factor of prime importance in clinical decision-taking. The possibility of providing treatment options in normal clinical practice rests on two conditions being met: 1) that quality scientific information supports the effectiveness of the therapeutic options on offer, and 2) that heterogeneous groups of patients have recorded their satisfaction with these options. The present trial provides for information in this respect to be gathered [39] and therefore treatment preferences to be taken into account at the time of prescription.

The trial is also designed to provide information on the effect of the normalisation of serum vitamin B12 concentrations by both treatments on patient-perceived quality of life. Physicians commonly assume that taking oral supplements will be associated with a feeling of greater well-being, although this has never been proven [40]. The present trial should also throw light on this.

The trial suffers from the practical limitation of having to enrol a large number of patients to meet its sample size requirements. However, a high degree of motivation is expected of the research team since its clinical assistance members are those involved in the enrolment process. Further, the fact that the patients to be enrolled will be seeking medical help (although not necessarily for vitamin B12 deficiency) suggests few will be lost to follow-up. A further possible limitation is the low statistical power used in the calculation of the sample size. The 60% power contemplated requires a sample size of 304 patients (152 in each arm) – higher powers would increase the sample size required and the enrolment of such numbers cannot be guaranteed. However, given the results reported in previous studies (2,25,31-33) that used moderate/high doses of vitamin B12, it should be possible to demonstrate the non-inferiority of the oral treatment with this power level. If the 95% confidence interval were to cross the non-inferiority threshold, i.e., showing the results to be inconclusive, the intramuscular treatment would remain the treatment of choice. To determine the degree of adherence to treatment (and thus avoid outcome dilution effects) [41], the

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number of doses taken orally and received by injection will be recorded. The characteristics of all the original 320 patients will be recorded to provide insight into the type of patient left in the study after any withdrawals, as recommended by the CONSORT group [41,42]. Basic information (age, sex, etc.) on potentially eligible patients who decline to take part will also be recorded. This type of information is of use when assessing the possible extrapolation of the trial results to more general populations.

The decision not to take serum methylnalonic acid and homocysteine concentrations into account as diagnostic markers and outcome variables was made bearing in mind that these are not normally determined, either at diagnosis or during follow-up, in patients with a vitamin B12 deficiency.

Finally, given the pragmatic nature of the proposed trial, the decision was taken to include consecutive patients seeking medical help at the participating centres, thus ensuring the enrolment of subjects similar to those that would be seen in normal clinical practice.

Abbreviations

Fe: Ferrum; g: Gram; GFR: Glomerular filtration rate; GGT: Gamma-glutamyl transpeptidase; GOT: Glutamic oxaloacetic transaminase; GP: General practitioner; GPT: Glutamic-pyruvic transaminase; HIV: Human immunodeficiency virus; HVB: Hepatitis B virus; HVC: Hepatitis C virus; IF: Intrinsic factor; µg: Microgram; MMA: Methylmalonic acid; mg: Milligrams; ng: Nanograms; pg: Picograms.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PGE y RRF conceived of the study and participated in its design. TSC; RRF; SGE; ldCG; JMF; EEM; participated in the design and coordination of the study. FRS; MGS; RGG; MAMS; COL; MLSP; CMR; BMB; AVP; FGBG; JEMS; RRB; GAC; LMCB; EPC; MRB; MTRM; SSD; SMI; RRG; IBL; MVN; JSD; TGG; MDC; AAB participated in different phases of the design. TSC; RRF; SGE; ldCG; JMF; EEM directed the writing of the manuscript. All authors OB12 Group read and approved the final manuscript.

Funding

This study was funded by the *Ministerio de Sanidad, Política Social e Igualdad*, Spain (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122) and by CAIBER - Spanish Clinical Research Network. The authors thank the following persons for their contributions to this work: Dolores Otero-Criado, Carlos Carvajales Fernández, Rosa Zurdo-Baz and Raisa González-Pérez.

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Received: 4 May 2012 Accepted: 31 May 2012
Published: 31 May 2012

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doi:10.1186/1471-2458-12-394
Cite this article as: Sanz-Cuesta *et al.*: Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). *BMC Public Health* 2012 **12**:394.

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Supplement 2: Bayesian Analysis

Bayesian analysis is a highly appropriate analysis strategy when working with small sample sizes. Previous knowledge about the studied item can be taken advantage of by means of the assessment of the plausibility of a given hypothesis after incorporating the new observed data.[1]

The noninferiority hypothesis, formally $\Delta < -10\%$, was tested, taking into account the observed results but also taking into account the results of the trials by Kuzminski et al.[2] and Saraswathy et al.[3]

P1 denotes the percentage of patients who responded to VB12 oral administration, and P0 represents the percentage of those responding to VB12 intramuscular administration. Bayesian analysis allows for calculating the probability of P1 being equal to or smaller than P0 by a specified magnitude, the noninferiority limit ($\Delta < -10\%$). For each of the parameters P1 and P0, both measured at 8, 26 and 52 weeks, we selected *a priori* distributions from the family of beta distributions with parameters **a** and **b**, which are related to the proportions of those responding in each trial arm. The gamma distribution represents the *a priori* hypothesis of the distribution of differences. According to the results of both trials by Kuzminski et al.[2] and Saraswathy et al.,[3] included in the review by Wang et al.,[4] 79.1% and 84.1% of patients normalized their VB12 levels in the oral and IM treatment groups, respectively.[4] The respective CIs associated with these prior data were calculated, and parameters were chosen (**a** and **b** in the beta distribution) such that the maximum density intervals of these distributions approximately coincided with the CI previously obtained (see Figure 1). Beta distributions for the success rate in each arm of the trial were obtained using binomial data. A total of 10000 simulations were made from these *a posteriori* distributions, and the corresponding differences, P1-P0, were calculated yielding an *a posteriori* distribution of differences. This distribution was used to derive simulation-based estimates of the probability of relevant magnitudes concerning Δ: P1-P0>0.10 at weeks 8, 26, and 52. Both PPT and ITT analyses were performed. EPIDAT 4.2 software was used for all computations.

Table 1 shows the *a posteriori* probability of differences in treatment effectiveness between oral and IM routes at different weeks (8, 26 and 52). The probabilities of the differences in treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively (per protocol analysis). In the intention-to-treat (ITT) analysis, these values were 0.000, 0.015, and 0.060 at weeks 8, 26, and 52, respectively.

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Figure 1. A priori distributions of the differences between oral and intramuscular treatment

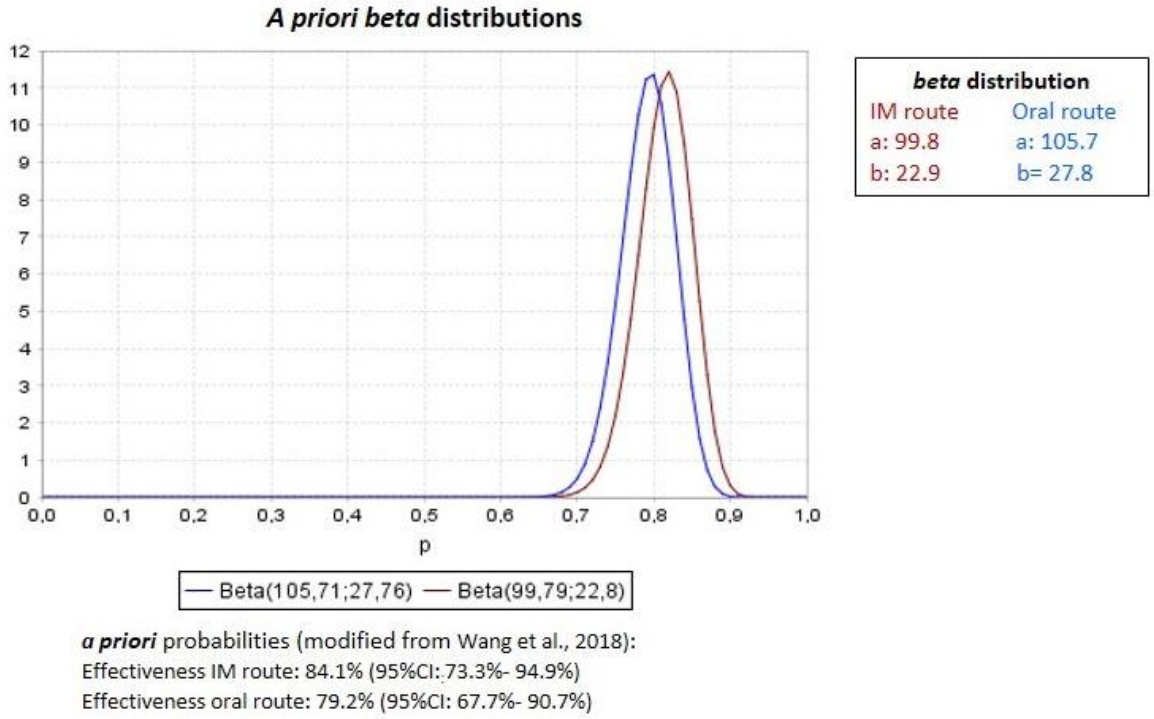

Table 1. *A posteriori* probability of differences in treatment effectiveness between oral and IM routes at 8, 26, and 52 weeks.

A posteriori **probability (Δ < -10%)**	**Week 8**	**Week 26**	**Week 52**
Per-protocol analysis	0.001	0.201	0.036
Intention-to-treat analysis	0.000	0.015	0.060

Δ: threshold of non-inferiority

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Supplement 3: Receiver Operating Characteristic (ROC) Curve

To explore factors affecting the normalization of serum VB12 concentration (yes/no) at 52 weeks, serum VB12 levels were studied at 8 weeks (at the end of the "charging period"). An ROC curve was built to determine the likelihood ratios for each cutpoint after the charging period to "predict" the normalization of levels (serum VB12 levels ≥211 pg/mL) at the end of the study.[1]

Table 1 shows the results of the likelihood ratios for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 ("charging period") to predict normalized VB12 serum levels at the end of the study. In Figure 1, the ROC curve is plotted. The level at the 5th percentile of the distribution was selected as the most useful value as it showed best classification ability and because when patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they did reach levels over 281 pg at week 8 (12~1/negative likelihood ratio).

References

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Table 1. Exploring the value of several cutpoints of OB12 serum levels at week 8 to "predict" normalization of values of Vit B12 at the end of the study

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-	Percentil
≥ 281	0.977	0.273	94.30%	1.3435	0.0841	5
≥ 328	0.963	0.546	94.30%	2.1193	0.0673	10
≥ 353	0.931	0.636	91.70%	2.5608	0.1081	15
≥ 389	0.895	0.818	89.10%	4.9197	0.129	20
≥ 421	0.839	0.818	83.80%	4.617	0.1962	25

LR+: Positive Likelihood ratio. LR-: Negative Likelihood ratio

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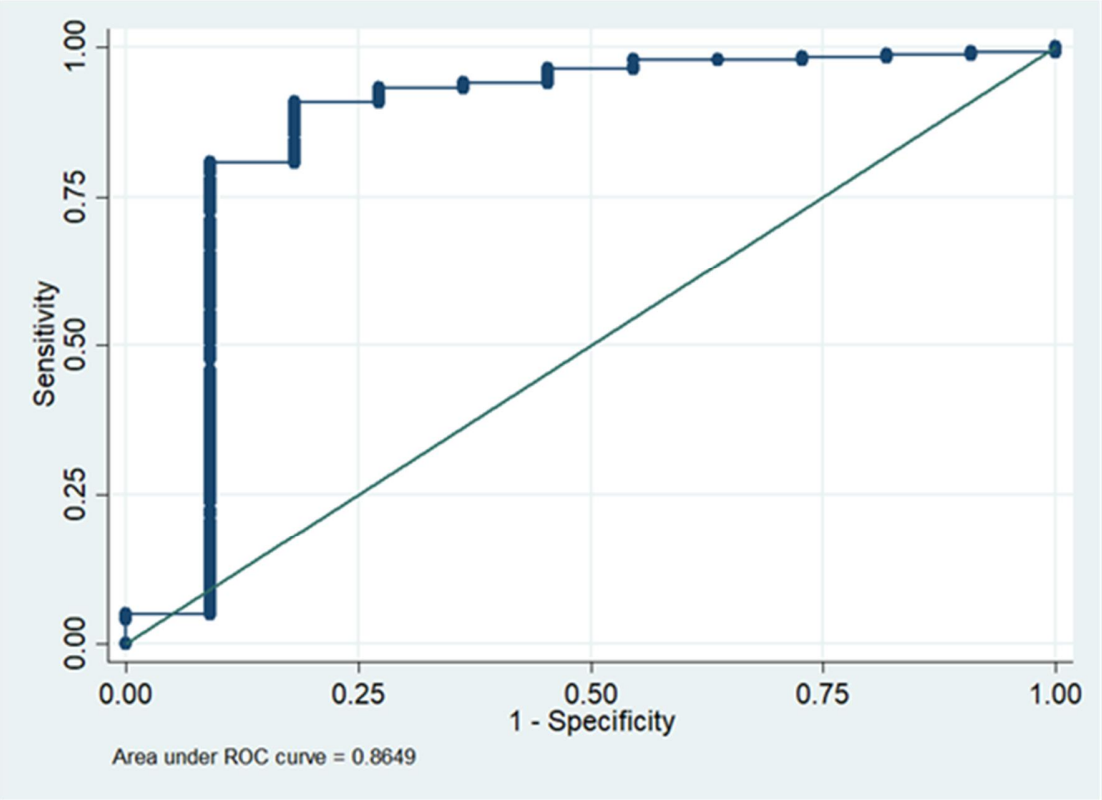
Figure 1. ROC curve



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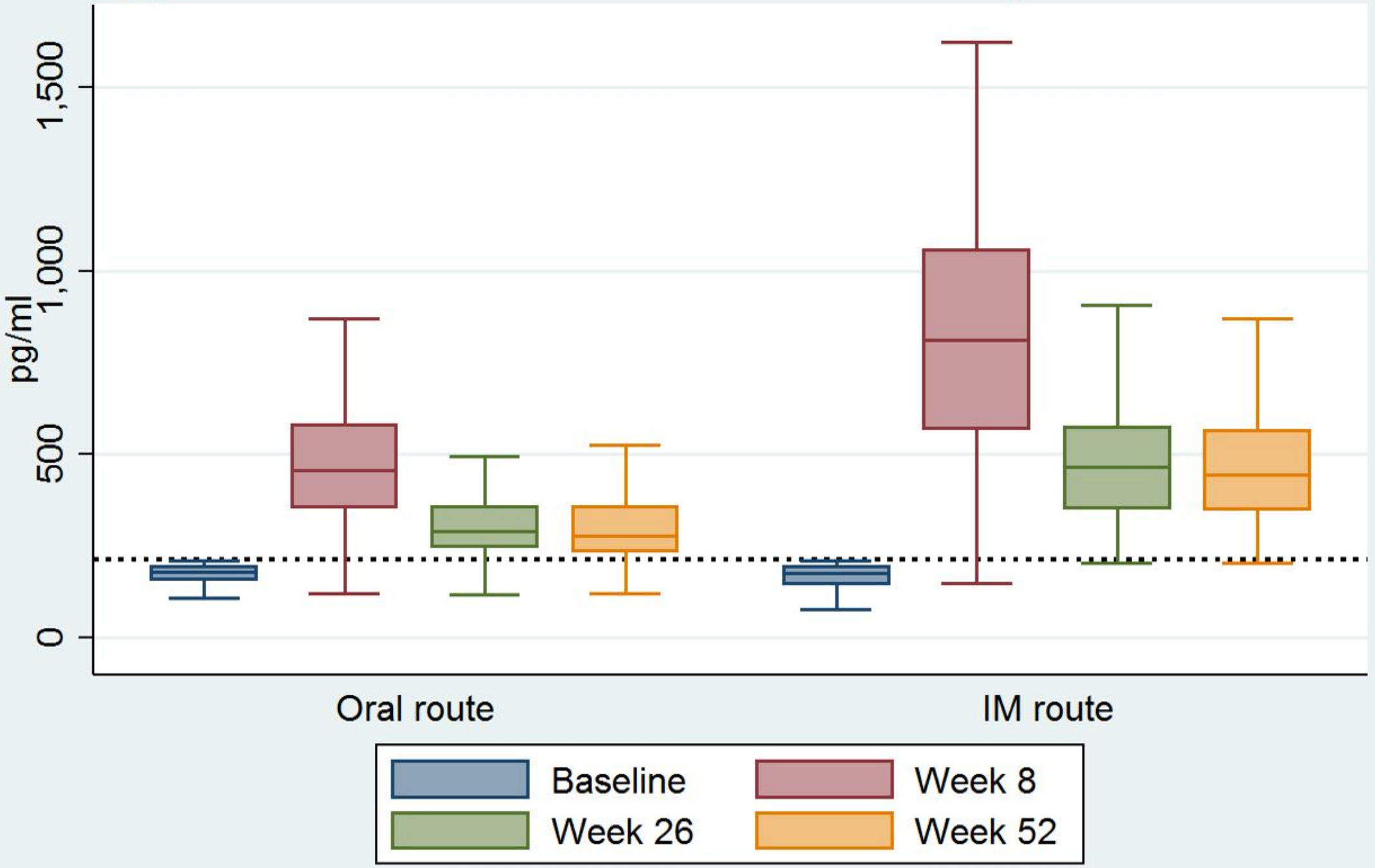
Supplement 4: Vitamin B12 mean levels by treatment route



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CONSORT Statement 2006 - Checklist for Non-inferiority and Equivalence Trials

Items to include when reporting a non-inferiority or equivalence randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned"), *specifying that the trial is a non-inferiority or equivalence trial.*	Page 1 and 2
INTRODUCTION Background	2	Scientific background and explanation of rationale, *including the rationale for using a non-inferiority or equivalence design.*	Page 5 and 6
METHODS Participants	3	Eligibility criteria for participants *(detailing whether participants in the non-inferiority or equivalence trial are similar to those in any trial(s) that established efficacy of the reference treatment)* and the settings and locations where the data were collected.	Page 7 Supplement 1
Interventions	4	Precise details of the interventions intended for each group *detailing whether the reference treatment in the non-inferiority or equivalence trial is identical (or very similar) to that in any trial(s) that established efficacy,* and how and when they were actually administered.	Page 7
Objectives	5	Specific objectives and hypotheses, *including the hypothesis concerning non-inferiority or equivalence.*	Page 6
Outcomes	6	Clearly defined primary and secondary outcome measures *detailing whether the outcomes in the non-inferiority or equivalence trial are identical (or very similar) to those in any trial(s) that established efficacy of the reference treatment* and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	Page 8
Sample size	7	How sample size was determined *detailing whether it was calculated using a non-inferiority or equivalence criterion and specifying the margin of equivalence with the rationale for its choice*. When applicable, explanation of any interim analyses and stopping rules (*and whether related to a non-inferiority or equivalence hypothesis*).	Page 8
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	Page 7 Supplement 1
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	Page 7 Supplement 1

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Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	Page 7 and 8
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	Not blinded
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s), *specifying whether a one or two-sided confidence interval approach was used.* Methods for additional analyses, such as subgroup analyses and adjusted analyses.	Page 8 and 9 Supplement 2 Supplement 3
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	Page 7 and 8 Figure 1
Baseline data	15	Baseline demographic and clinical characteristics of each group.	Page 9 and 10 Table 1
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was *"intention-to-treat" and/or alternative analyses were conducted*. State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	Figure 1 and Figure 2
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval). *For the outcome(s) for which non-inferiority or equivalence is hypothesized, a figure showing confidence intervals and margins of equivalence may be useful.*	Page 11 to 13 Table 2 Table 3 Figure 2 Supplement 2
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	Page 12
Adverse events	19	All important adverse events or side effects in each intervention group.	Page 13
DISCUSSION Interpretation	20	Interpretation of the results, taking into account the *non-inferiority or equivalence hypothesis and any other* study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	Page 14 to16
Generalizability	21	Generalizability (external validity) of the trial findings.	Page 14 to 16
Overall evidence	22	General interpretation of the results in the context of current evidence.	Page 16 and 17

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033687.R3
Article Type:	Research
Date Submitted by the Author:	02-Apr-2020
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Primary Subject Heading:	General practice / Family practice
Secondary Subject Heading:	Geriatric medicine, Evidence based practice, Health services research
Keywords:	Vitamin B 12 Deficiency, Administration Oral, Equivalence Trial,, Injections Intramuscular, primary health care, Patient preference

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Oral versus intramuscular administration of vitamin B12 for vitamin B12 deficiency in primary care: a pragmatic, randomized, noninferiority clinical trial (OB12)

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Abstract

Objectives: To compare the effectiveness of oral versus intramuscular vitamin B12 (VB12) in patients aged ≥65 years with VB12 deficiency.

Design: Pragmatic, randomized, noninferiority, multicenter trial in patients ≥65 years in 22 primary healthcare centres in Madrid (Spain). **Participants**: 283 adults with VB12 deficiency were randomly assigned to oral (n=140) or intramuscular (n=143) treatment arm. **Interventions:** The intramuscular arm received 1mg VB12 on alternate days in weeks 1–2, 1mg/week in weeks 3–8, and 1mg/month in weeks 9–52. The oral arm received 1mg/day in weeks 1–8 and 1 mg/week in weeks 9–52.

Main outcomes: Serum VB12 concentration normalization (≥ 211 pg/mL) at 8, 26, and 52 weeks. Noninferiority would be declared if the difference between arms is 10% or less. Secondary outcomes included symptoms, adverse events, adherence to treatment, quality of life, patient preferences and satisfaction.

Results: The follow-up period (52 weeks) was completed by 229 patients (80.9%). At week 8, the percentage of patients in each arm who achieved normal B12 levels was well above 90%; the differences in this percentage between the oral and intramuscular arm were -0.7% (133 out of 135 vs 129 out of 130; 95% CI: -3.2 to 1.8; p>0.999) by per-protocol (PPT) analysis and 4.8% (133 out of 140 vs 129 out of 143; 95% CI: -1.3 to 10.9; p=0.124) by intention-to-treat (ITT) analysis. At week 52, the percentage of patients who achieved normal B12 levels was 73.6% in the oral arm and 80.4% in the intramuscular (IM) arm; these differences were -6.3% (103 out of 112 vs 115 out of 117; 95% CI: -11.9 to -0.1; p=0.025) and -6.8% (103 out of 140 vs 115 out of 143; 95% CI: -16.6 to 2.9; p=0.171), respectively. Factors affecting the success rate at week 52 were age, OR=0.95 (95% CI: 0.91 to 0.99), and having reached VB12 levels ≥281 pg/mL at week 8, OR=

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8.1 (95% CI: 2.4 to 27.3). Under a Bayesian framework, noninferiority probabilities (Δ>-10%) at week 52 were 0.036 (PPT) and 0.060 (ITT). Quality of life and adverse effects were comparable across groups. 83.4% of patients preferred the oral route.

Conclusions: Oral administration was no less effective than intramuscular administration at 8 weeks. Although differences were found between administration routes at week 52, the probability that the differences were below the noninferiority threshold was very low.

Trial registration: ClinicalTrials.gov (NCT 01476007) and EUDRACT (2010-024129-20).

Funding: Ministerio de Sanidad y Consumo Español. Instituto de Salud Carlos III (ISCIII). European Regional Development Fund.

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Strengths and limitations of this study

- This is the largest and longest follow-up randomized clinical trial in patients aged ≥65 years with VB12 deficiency.
- In addition to VB12 levels, this study incorporates patient-reported outcomes such as symptoms, quality of life, and patient preferences.
- The study design did not allow patient blinding; however, the main outcome measurement was objective.
- The rates of loss to follow-up were low at week 8 and week 26 and higher at week 52, consistent with pragmatically designed clinical trials.

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INTRODUCTION

Vitamin B12 (VB12) is an essential nutrient for the synthesis of cellular DNA. It is generally accepted that daily needs in adults range from 1 to 2 μg/day, [1] but other standards recently recommend 3-4 μg per day.[2] The Western diet is estimated to contain 7–30 μg/day of cobalamin, of which 1–5 μg is absorbed and stored (estimated reserves of 2–5 mg); therefore, symptoms resulting from a VB12 deficit would not appear until 3–5 years after establishing a low-ingestion or poor-absorption regimen.[1] VB12 deficiency can lead to hematological and neuropsychiatric disorders,[3] as well as cardiovascular risk factors.[4] The prevalence of VB12 deficiency in the elderly is highly variable across studies, which report values of 1.5% to 15%.[5–8]

In primary care, the most commonly observed causes of VB12 deficiency are related to abnormalities in digestion (atrophic gastritis, achlorhydria) or absorption (autoimmune pernicious anaemia, chronic pancreatitis, Crohn's disease, the effect of medications that alter the mucosa of the ileum such as metformin, antacids -proton-pump inhibitors and H2-receptor antagonists-, antibiotics, and colchicine)[9] or the consequences of surgical resection.[10] A deficiency stemming solely from dietary habits is rare and usually affects strict vegans.[11] In the elderly, different alterations in the processes involved in VB12 absorption increase the prevalence of this deficit, which can appear in the absence of specific symptoms, thereby hindering its diagnosis. [12]

The traditional treatment for VB12 deficiency consists of intramuscular (IM) injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum*.[10,13,14]. The vitamin may, however, be administered orally. Several studies have shown serum VB12 concentrations to normalize after taking large oral doses.[15,16] Studies taking into consideration the patients´ preferences have found differences in

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favour of the oral route.[17,18] Furthermore, oral treatment could avoid injection nuisances, reduce unnecessary travel for the patients or nurses, and minimize treatment costs.[19]

Some authors have questioned the use of oral administration while others favour it, although no firm conclusions can be drawn due to the methodological limitations of the evidence the authors provide.[10,20–22] The 2018 Cochrane Review[5] includes three randomized clinical trials comparing the effectiveness of oral and IM administration. There are differences among the trials in terms of treatment regimens and follow-up duration, ranging from 3 to 4 months, and average age of the patients, as well as the frequency and VB12 daily dose for both routes. In terms of outcomes, adverse events, and cost, the overall quality of the evidence was low due to the small number of studies and limited sample sizes.[23–25] In their conclusions, the authors state the need for trials with improved methods for random allocation and masking, larger sample sizes, and information on other relevant outcome variables that are preferably conducted in the primary care setting.

The aim of this study was to compare the effectiveness of oral- and IM-administered VB12 in the normalization of serum VB12 concentrations at 8, 26, and 52 weeks in patients aged ≥65 years with VB12 deficiency treated at primary healthcare centres (PHC). Secondary outcomes included safety (adverse events), quality of life, and adherence to treatment. Additional aims were to describe patient preferences and satisfaction with treatment and to explore the immediate response (8 weeks) as a normalization predictor of one-year outcomes to propose clinical recommendations.

METHODS

Study design and participants

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A pragmatic, randomized, multicenter, noninferiority clinical trial with a duration of 12 months was conducted in a PHC. On ethical grounds, a placebo-controlled trial was not appropriate.[26] Methodological issues of this trial have been published elsewhere (Supplement 1).[27]

Competitive recruitment was performed in 22 PHC in Madrid (Spain) from July 2014 to November 2016. Eligible patients were 65 years of age or older and had been attending a PHC for consultation on any medical matter. Patients were assessed for eligibility and invited to participate consecutively by their general practitioners. Written informed consent was obtained from all participants. A blood test was performed, and in patients with a serum concentration of VB12 of <211 pg/mL, the remaining inclusion and exclusion criteria were evaluated. The cut-off value selected in the trial register/ trial protocol was <179 pg/mL; this value was modified by the laboratory following the recommendations of the provider. This change took place prior to the beginning of the recruitment. Patient recruitment was always performed using the same methodology and cut-off point. The procedures for measurement of the biomarkers were ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY, USA).

Randomization and masking

Patients were allocated by simple randomization at a 1:1 ratio to oral or intramuscular administration of vitamin B12. The randomization system was incorporated into the electronic data collection system to assure allocation concealment. Because of the nature of the intervention, patients and general practitioners were aware of their treatment allocation. Analysis was performed by the trial statistician, who was blinded to allocation.

Intervention

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The pharmaceutical formulations used in the study are commercially available in Spain (Optovite® vials). Its pharmaceutical presentation is in silk-screen-printed clear glass ampoules that are presented in PVC blister support. The treatment regimen was : a) IM route: 1 mg of cyanocobalamin on alternate days during weeks 1–2, 1 mg/week during weeks 3–8 and 1 mg/month during weeks 9–52; b) oral route: 1 mg/day of cyanocobalamin for 8 weeks and 1 mg/week during weeks 9–52. The period between 1-8 weeks was considered the charging period. In the oral route, the medication was provided to the patient at the health centre, along with instructions for self-administration at home. The information sheet explained to the patient the procedure for oral administration, i.e., how to open the ampoule and dilute its contents in a glass, then drink it.

In the IM route, the medication was administered by the nurse at the health centre.

Outcomes

The main outcome was the normalization of serum VB12 concentrations (≥211 pg/mL) at 8, 26, and 52 weeks. The secondary outcomes were the serum VB12 concentrations (pg/mL), adverse events, adherence to treatment (number of vials for the oral arm and the number of injections for the IM arm during each visit; good adherence was considered greater than 80%), quality of life (EQ-5D-3L) [28] and patient preferences and satisfaction were assessed. Anamnesis, demographic and lifestyle information, clinical variables, analytical variables, and concomitant treatment were recorded.[27]

Procedures

After signing the consent form, those who agreed to participate had serum VB12 concentrations determined. If the VB12 value was <211 pg/mL, a hemogram, biochemical analysis, and anti-

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intrinsic factor antibody levels were assessed.[27] The patients also received a medication diary to be filled out daily. Baseline data were collected by the family physician and/or a nurse. IM treatments were administered by nurses in the health centres. The follow-up visits were conducted during weeks 8, 26 and 52.[27]

Statistical analysis

Sample size. Assuming that 70% of patients reach a serum VB12 concentration of ≥ 211 pg/mL in both groups, for a threshold of noninferiority of 10%, statistical power of 60% with significance set at $p<0.05$ and a 5% loss to follow-up, the final sample size was word 320 (160 in each arm).

As recommended for noninferiority studies, both PPT and ITT analyses were performed, with the null hypothesis being that there were differences between treatments at the three monitoring points. Comparing both arms, we calculated the difference between the percentage of patients in each treatment arm whose serum VB12 concentrations became normalized at 8, 26, and 52 weeks, with their 95% CI. If the confidence intervals do not fall outside the noninferiority limit (10%), it can be concluded that the oral treatment is not inferior to the intramuscular treatment.[29,30] In ITT analyses, missing values for the main outcome variable were added using the 'last observation carried forward' (LOCF) method.[31]

To explore factors affecting the normalization of serum VB12 concentration at 52 weeks, serum VB12 levels were studied at 8 weeks. A receiver operating characteristic (ROC) curve was built to determine the likelihood ratios of each cutpoint after the charging period to “predict” the normalization of levels at the end of the study. After this, a generalized linear model (GLM) was built (function logit). [32,33] The normalization of serum VB12 levels at 52 weeks was the dependent variable, and the treatment group was the independent variable. Variables considered

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significant by the researchers from a clinical perspective were included in the model. To test the noninferiority hypothesis, adding the information contained in these data to previous knowledge, additional statistical analyses were performed using a Bayesian approach. Secondary outcome variables were analyzed using the appropriate statistical tests, and their means or proportions were used to estimate differences between groups. All analyses were performed using STATA 14 and EPIDAT 4.2 software.

Patient involvement

Patients were not involved in the development of plans for recruitment, design, outcome measures, or implementation of the study conduct. No patients were asked to advise on the interpretation or writing of the results. Patients explained the experience of participating in the study on the occasion of International Clinical Trial´s day in *Radio Nacional de España (RNE)*. We will pursue patient and public involvement in the development of an appropriate method for further dissemination.

RESULTS

Characteristics of the study participants

A total of 2342 patients were offered participation, and 2152 provided informed consent. A total of 307 patients showed a VB12 deficit (14.3%), 283 of whom were allocated to receive VB12 treatment via the IM route (n=143) or orally (n=140). The follow-up period (52 weeks) was completed by 229 patients (80.9%). Losses to follow-up were similar in both regimens, 28 out of 140 and 26 out of 143 losses in the oral and intramuscular arms respectively (p=0.697). (Figure 1).

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The average age was 75.2 (6.34), and 58.3% of the patients were women. Table 1 describes the baseline characteristics of the patients included in the trial. No relevant differences were found between groups at baseline for demographic and medical characteristics or for the study endpoints.

Table 1. Baseline characteristics at baseline by group

Variable	No. (%)		
	Oral route (n=140)	**IM route (n=143)**	**Total (n=283)**
Sociodemographic data			
Women	87 (62.1)	78 (54.5)	165 (58.3)
Age (years), mean (SD)	74.2 (5.8)	76.2 (6.7)	75.2 (6.3)
Educational level			
Illiteracy	4 (2.9)	7 (5.1)	11 (4.0)
Incomplete education	48 (34.5)	46 (33.6)	94 (34.1)
Primary education	58 (41.7)	63 (46.0)	121 (43.8)
Secondary education	16 (11.5)	10 (7.3)	26 (9.4)
Tertiary education	4 (2.9)	4 (2.9)	8 (2.9)
Higher education	9 (6.5)	7 (5.1)	16 (5.8)
Social occupational class [a]			
Class I - IV	31 (27.7)	33 (27.3)	64 (27.5)
Class V - VI	81 (72.3)	88 (72.7)	169 (72.5)
Living alone	32 (21.4)	30 (22.2)	62 (21.9)
Clinical data			
Tobacco habit			
Ex-smoker	27 (19.7)	25 (18.4)	52 (19.0)
Smoker	9 (6.6)	10 (7.4)	19 (7.0)
Nonsmoker	101 (73.7)	101 (74.3)	202 (74.0)
Vegetarian	2 (1.4)	0 (0)	2 (0.7)
Having undergone gastrectomy	1 (0.7)	2 (1.4)	3 (1.1)
Symptoms			
Paresthesia	33 (23.6)	45 (31.5)	78 (27.6)
Asthenia	43 (30.7)	54 (37.8)	97 (34.3)
Loss of appetite	12 (8.6)	30 (21.0)	42 (14.8)
Sadness	37 (26.4)	53 (37.1)	90 (31.8)
Showing ≥1 symptom	70 (50.0)	83 (58.0)	153 (54.1)
Signs			

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Glossitis	2 (1.4)	9 (6.3)	11 (3.9)
Position sensitivity	2 (1.4)	1 (0.7)	3 (1.1)
Vibration sensitivity	15 (10.7)	13 (9.1)	28 (9.9)
Showing ≥1 altered sign	16 (11.4)	21 (14.7)	37 (13.1)
Hemogram-Clinical Biochemistry			
Vitamin B12 (pg/mL), mean (SD)	173.1 (27.3)	166.4 (32.6)	169.7 (6.3)
Anemia [b]	16 (11.4)	27 (18.9)	43 (15.2)
Hematocrit (%), mean (SD)	42.4 (4.0)	41.9 (4.2)	442.1 (4.1)
MCV (fL), mean (SD)	92.1 (6.7)	94.3 (7.4)	93.2 (7.1)
Anti-intrinsic factor antibody	15 (11.0)	15 (10.5)	30 (10.8)
Medication			
Proton-pump inhibitors (PPI)	57 (40.7)	64 (44.8)	121 (42.8)
Metformin PPI and metformin	69 (49.3) 33 (23.6)	56 (39.2) 30 (21.0)	125 (44.2) 63 (22.3)
Scales			
MMSE [c], mean (SD)	30.8 (4.6)	30.2 (4.8)	30.5 (4.7)
EQ-5D-Utilities, mean (SD)	0,817 (0,169)	0,855 (0,139)	0,836 (0,171)

[a]Neoweberian occupational social class (CSO-SEE12). Gac Sanit. 2013;27(3):263–272.
[b]Anaemia was defined by the World Health Organization criteria (haemoglobin <12 g/dL in women and <13 g/dL in men). https://www.who.int/vmnis/indicators/haemoglobin
[c]Mini Mental State Examination. Maximum score= 35 points. Normal score= 30–35. Borderline score= 24–29 points. Scores < 24 points in patients aged >65 years and scores < 29 points in patients aged <65 years suggest cognitive impairment.

Primary outcomes

At week 8, the difference in the success rate between the oral and IM routes was -0.7% (95%CI: -3.2% to 1.8%; p>0,999) and 4.8% (95%CI: -1.3% to 10.9%; p=0.124) with the PPT and ITT analyses, respectively. At week 26, these differences were -12.9% (95%CI: -17.9% to -6.1%; p<0.001) and -3.2% (95%CI: -11.8% to 5.4%; p=0.470), respectively. At week 52, these differences were -6.3% (95%CI: -11.9% to -0.07%; p=0.025) and -6.8% (95%CI: -16.6% to 2.9%; p=0.171), respectively (Figure 2).

In the PPT analysis under a Bayesian approach, the probabilities of differences in the treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively. In the ITT analysis, these values were 0.000, 0.015,

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and 0.060 at weeks 8, 26, and 52, respectively (Supplement 2). The result of the likelihood ratio for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 to predict normalization at the end of the study is shown in Supplement 3. The level at the 5th percentile of the distribution was selected as the most useful value because it showed the best classification ability. When patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they had reached levels over 281 pg/mL (12~1/negative likelihood ratio).

In the ITT analysis, the factors affecting the success rate at week 52 were age, for each year of increase in age, the success rate decreased by 5%, and having attained VB12 levels of ≥281 pg/mL at week 8, which yielded a success rate 8.1 times higher (Table 2).

Table 2. Factors associated with VB12 concentrations ≥ 211 pg/ml at week 52

Variable	Odds ratio	Robust std. error	P>z	95% CI
IM vs. oral route	1.10	0.370	0.776	(0.57 to 2.13)
Age	0.95	0.022	0.025	(0.91 to 0.99)
VB12 concentration >281 pg/ml at week 8	8.10	5.014	0.001	(2.41 to 27.25)
Constant	0.78	0.622	0.755	(0.16 to 3.72)

GLM, N=265. Variance function: V(u) = u*(1-u/1) [Binomial]. Link function: g(u) = ln(u/(1-u)) [Logit]. AIC= 0.89967. BIC = -1225.89.

The mean levels of VB12 for each follow-up visit were above the normalization threshold in both groups, although these values were much greater in the IM group (Supplement 4). In 51 patients (36 IM and 5 oral), the levels of VB12 in week 8 were above the normal range limit of the laboratory (≥911 pg/mL), so the treatment regimen was changed from the initial planned pattern.

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Secondary outcomes

In terms of quality of life and the presence of signs related to VB12 deficiency, no significant differences were found between treatment arms at any of the follow-up visits (Table 3).

Table 3. Secondary outcomes (Quality of life and exploratory findings) at weeks 8, 26 and 52

Visit	Oral route		IM route		p	Mean difference (95% CI)
	N	Mean (SD)	N	Mean (SD)		
Quality of life (EQ-5D-5L Index)						
Baseline	139	0.855 (0.139)	137	0.817 (0.197)	0.066	0.038 (-0.002 to 0.078)
Week 8	134	0.853 (0.158)	130	0.822 (0.204)	0.173	0.031 (-0.013 to 0.075)
Week 26	128	0.853 (0.153)	122	0.826 (0.191)	0.219	0.027 (-0.016 to 0.070)
Week 52	112	0.824 (0.179)	117	0.823 (0.194)	0.958	0.001 (-0.047 to 0.049)
At least one altered sign (glossitis and/or altered vibration sensitivity and/or altered position sensitivity)						
Visit	**N**	**n (%)**	**N**	**n (%)**	**p**	**Proportion difference (95% CI)**
Baseline	140	16 (11.4%)	143	21 (14.7)	0.416	-3.3 (-11.1% to 4.6)
Week 8	135	15 (11.1%)	130	13 (10.0)	0.769	1.1 (-6.3% to 8.5)
Week 26	131	14 (10.7%)	122	12 (9.8)	0.824	0.9 (-6.6% to 8.3)
Week 52	122	14 (12.5%)	117	9 (7.7)	0.226	3.8 (-3.7% to 11.2)

Eleven adverse events were reported and none of them were severe; five (3.57%) occurred with patients in the oral arm and six (4.20%) with patients in the IM arm, yielding a difference of -0.63% (95%CI: -5.12% to 3.87%, p=0.786). Three patients withdrew from the study: one patient in the oral group due to urticaria, and two in the IM group due to reddening and pruritic facial erythema and generalized itching (mainly in the cheeks with scarce urticariform lesions). In three other cases, treatment for the adverse events was prescribed (constipation and erythema), and in five cases, it was not necessary to take further measures (Table 4).

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Table 4. Description of adverse events by patient and route of administration

Route	Adverse event	Action
IM route	Constipation	Administration of specific treatment
	Generalized itching and hives on the cheeks	Withdrawal
	Dyspepsia	Treatment not required
	Constipation	Administration of specific treatment
	Redness and pruritic facial erythema	Withdrawal
	Erythema on forearms	Administration of specific treatment
Oral route	Urticaria on the neck and arms	Treatment not required
	Occasional postprandial dyspepsia	Treatment not required
	Occasional postprandial dyspepsia	Treatment not required
	Urticaria	Withdrawal
	Increased irritability and nervousness	Treatment not required

At week 8, adherence to treatment was evaluated in 265 patients, of whom 95.5% were adherent (97.8% oral and 93.8% IM); the difference between the groups was 4% (95%CI: -0. 1% to 8.7%; p=0.109). At week 52, adherence was evaluated in 229 patients, of whom 220 (96.1%) were adherent (98.2% oral and 94.0% IM); the difference was 4.2% (95%CI: -0.7% to 9.1%; p=0.172).

Overall, 89.5% of the patients reported being satisfied or very satisfied with the treatment via the oral route (91.3%) and the IM route (87.6%). The difference was 3.7% (95% CI: -4.0% to 11.3%; p=0.348).

A total of 83.4% of patients preferred the oral route (97.6% among the patients receiving VB12 orally vs. 68.6% of the patients in the IM group); the difference was 29.0% (95%CI: 20.3 to 37.7;p<0.001). The preferences expressed by the patients referred to their potential choice regardless of the arm to which they were assigned

DISCUSSION

Main findings of the study

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Supplementing VB12 in patients with VB12 deficiency, whether orally or intramuscularly, achieves the normalization of VB12 levels in most cases. The oral route was not inferior to the IM route during the charging period. Formally, the pre-established conditions for determining the noninferiority of oral administration were not met for the complete follow-up period, but these results merit a deeper analysis.

Differences between the administration routes were found at 26 and 52 weeks. The IM maintenance treatment of 1 mg/month was effective in maintaining VB12 levels, while oral administration of 1 mg/week had a probability of being inferior (by more than 10%) to the IM route by 20% in the most unfavourable scenario (PPT). However, given that no strategy was superior in the charging period, and in view of the model results showing that when VB12 levels reached ≥ 281 pg/mL during the charging period, the success rate at 12 months was 8 times higher, the probability that the differences between groups would exceed Δ was very low, independent of the administration route. The most plausible explanation for the observed difference between routes might be that in patients below this threshold, the maintenance oral dose should be higher than the dose used in the present study. Some authors have recommended that an oral dose of 2 mg/week be administered as a maintenance dose.[34]

The incidence of adverse events was very low and similar for oral and intramuscular administration, and nonserious adverse events were found. These findings were similar to other studies.[5] Patients' preferences can be a decisive factor for determining the administration route. In this trial, similar to previous studies,[17] there was a clear preference for the oral route, especially among the patients assigned to this group.

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The effect of VB12 supplements on quality of life remains unclear,[35,36] but the present results show that the treatment route does not improve patients´ perception of their health-related quality of life or related symptoms.

We did not find significant differences in adherence. Adherence to the treatment via the IM route was lower than expected. Although drug administration was assured once the patient attended the consultation, the patient could choose not to attend appointments for various reasons. However, in usual practice, adherence with the oral route could be more compromised than with the IM route, and this factor should be taken into consideration to personalize prescription.

Comparison with other studies

The comparison with other studies is difficult, due to the treatment different doses used, but especially because of the follow-up length had been inferior to 4 months and the number of patients included was small.

As far as we know, the present trial is the largest clinical trial with the longest follow-up period, and it is the first to evaluate, in addition to VB12 levels, clinical signs and symptoms, health-related quality of life, and patient preferences. The 3 clinical trials[23–25] described in the 2018 Cochrane Systematic Review[5] had a duration between 3 and 4 months and included a total of 153 patients. In the Saraswathy trial, patients in the oral route at 3 months normalised levels 20/30 (66.7%) vs 27/30 (90%) of the patients in the IM route.[25] In Kuzminski's patients in the oral route at 4 months normalised levels 18/18 (100%) vs 10/14 (71.4%) of the patients in the IM route.[23] These differences were statistically non-significant in both studies.

Two studies have recently been published and add evidence in favour of oral and sublingual administration of VB12.[37,38] The follow-up of Moleiro's study reached 24 months versus 12

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months in our study. However, Moleiro et al performed a prospective uncontrolled study that included 26 patients submitted to total gastrectomy. All patients received oral VB12 supplementation (1 mg/day), and all of them maintained normalization V12 at 6, 12, 18, and 24 months. There was a progressive increase in serum V12 levels within the first 12 months, which remained stable thereafter.[37] The long-term effectiveness of the oral route in absorption-deficient people such as gastrectomized patients would support the results of our study.

Bensky et al. compared the efficacy of sublingual vs. intramuscular administration of vitamin B12 in a retrospective observational study from the computerized pharmacy records of Maccabi Health Service (MHS). Among 4281 patients treated with VB12 supplements (830 (19.3%) with IM and 3451 (80.7%) with sublingual tablets, the IM group achieved a significant increase in VB12 levels compared with the sublingual group, OR 1.85, CI 95% 1.5-2.3. [38] Although this study has a large sample size, the important methodological limitations on its effectiveness (retrospective design; reliance on clinical records; absence of epidemiological information such as patient age and sex or the aetiology of the deficit) should be considered in the interpretation of their results.

Strengths and limitations

Our study was pragmatic[39] in both the inclusion and diagnostic methods criteria. The majority of the patients with deficits included in this study presented no symptomatology or very low-level symptoms, with no anemia, which is the common profile of most patients who present with VB12 deficits in primary care. The study design did not allow for masking the patients to the received treatment. However, these limitations were compensated for by the objective measurement of the main outcome variable.

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As occurs in all pragmatic clinical trials, patient recruitment was complicated, and the sample size reached only 88.4% of the calculated necessary size, which implies that the power of the study was limited. Hence, the analysis was complemented using Bayesian methods that allow for studying *a posteriori* the likelihood of a difference between two outcomes to exceed a certain limit.[40] Under this approach, the *a posteriori* probability for differences to exceed the proposed Δ=-10% was not significant during the charging period, and the probabilities were low but not negligible in the PPT analysis and low in the ITT analysis over the complete follow-up period.

Loss to follow-up was low at 8 and 26 weeks and higher at 52 weeks. This effect has been observed in pragmatic clinical trials with long follow-up periods. Missing data were greater in the IM arm, during the interval between randomization and initiation of treatment (6% IM vs 1% oral), over 8 weeks (9% IM vs 4% oral) and over 26 weeks (15% IM vs 6%). These differences could represent a lower acceptability of the IM route by patients, since the missing data were mostly due to patient dropout. At 52 weeks, the numbers of losses in the two arms were similar (20% oral and 18% IM), and in the case of oral treatment, several of those losses were withdrawals occasioned by not achieving particular levels of VB12.

Implications of the study findings

On the basis of our results and the available evidence, we propose the oral administration of VB12 at 1 mg/day during the charging period. Subsequently, the recommended dose would vary as a function of the VB12 levels reached during the charging period. For VB12 concentrations between the normal levels of 211 pg/mL (in our laboratory) and 281 pg/mL (the 5th percentile of the distribution in this trial), a dose of 2 mg/week is suggested. When the levels reached in the charging period are between 281 and 380 pg/mL (the 20th percentile of the distribution), it may be appropriate to perform an analysis between 8 and 26 weeks to confirm

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that normal levels are maintained. All patients who reach a level of 380 pg/mL by week 8 could be maintained at the initial dosage (1 mg/week) without subsequent analyses during the year of follow-up.

If the IM route is chosen, the proposed dose for this route during the first few weeks may be excessive for patients with VB12 deficiency. The scheduled IM dose should be reconsidered in the first two weeks based on VB12 levels, and the scheduled dose could be limited to 1 mg/week if warranted by the outcome. Nevertheless, these recommendations must be assessed in further research.

Oral administration of VB12 in patients older than 65 years is probably as effective as intramuscular administration, and it also lacks adverse effects and is preferred by patients. We must also highlight the potential benefit of the oral route in terms of safety for patients with coagulation problems, for whom IM-administered medication is often contraindicated. A small number of patients may require additional follow-up after 8 weeks if a certain concentration of VB12 in blood is not reached.

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Authors' Contributions

- Trial Management Committee: TSC; EEM; IDC; JMF; RRF; SGE.
- Healthcare Centres (managers)*: JEMS, TGG,MAV, IGG, PGE,CVMC,MNA, FGBG,RBM,CDL,NCR,AHD, RFG,JHH,BPG study coordination development in each healthcare centre with principal investigator supervision.
- Technical Support Group**: participated in different phases of the design and development of the research. MLSP; CMR; BMB; MAJ; coordinated the pharmaceutical aspects.
- Clinical Investigators: collected the data for the study, which included recruiting patients, obtaining consent, performing blood tests, applying interventions, collecting data, and arranging and performing follow-up for patients.
- Statistical analysis: TSC; JMF; IDC; EEM with the collaboration of the Research Unit (JGM and MMM).
- Writing Committee: TSC; EEM; IDC; JMF, SGE, and RRF wrote the manuscript. All authors in the OB12 Group read and approved the final manuscript.

Acknowledgements

To Margarita Blázquez Herranz, Instituto de Salud Carlos III, for making the impossible possible.

To Juan Carlos Abánades Herranz for trusting us and his contribution to facilitate our work.

To Antonio Jesús Alemany López and Marta Sánchez-Celaya, Managing Director of Primary Healthcare for accepting to sponsor this research after the dissolution of CAIBER.

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To the Fundación para la Investigación e Innovación Biomédica de Atención Primaria (FIIBAP) for the grant awarded for the publishing of the research.

To Fernando Cava Valencia and Raquel Guillén Santos from the Clinical Laboratory BRSALUD.

To Elena Tolosa Oropesa from the Pharmacy Department, Gerencia Asistencial de Atención Primaria de Madrid.

To our colleagues from the Research Unit, including the assistants Marcial Caboblanco Muñoz, Juan Carlos Gil and Isabel Morales Ropero for their unconditional support; project manager Pilar Pamplona-Gardeta, researchers Juan A. López Rodríguez, Jaime Barrio Cortes, Cristina Lozano Hernandez and Medical Residents Daniel Toledo Bartolomé, Irene Wijers and Palmira Jurado Macías for their technical advice in finalizing this paper.

To Javier Zamora Romero, head of the Unit of Biostatistics, Hospital Ramón y Cajal of Madrid, for revising the manuscript.

To all the professionals from the participating healthcare centers.

To all patients who contributed to this research.

Competing Interests

The authors declare that they have no competing interests.

Funding

The trial was financed by Ministerio de Sanidad y Consumo Español through their call for independent clinical research, Orden Ministerial SAS/2377, 2010 (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122); CAIBER – Spanish Clinical Research Network, Instituto de Salud

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Carlos III (ISCIII) (CAI08/010044); and Gerencia Asistencial de Atención Primaria de Madrid. This study is also supported by the Spanish Clinical Research Network (SCReN), funded by ISCIII-Subdirección General de Evaluación y Fomento de la Investigación, project number PT13/0002/0007, within the National Research Program I+D+I 2013-2016 and co-funded with European Union ERDF funds (European Regional Development Fund).

The funders of the study had no role in the study design, data collection, onsite monitoring, data analysis, data interpretation, or writing of the manuscript.

Ethics approval

Madrid Region Clinical Research Ethics Committee on February 8th, 2011.

Data sharing statement

Individual de-identified participant data will be shared upon reasonable request. These data will include every variable used in the analysis shown in this report, and they will be available for five years upon request to corresponding author.

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Figure legends

Figure 1. Trial profile

Figure 2. Difference between the oral and intramuscular routes in the proportion of patients whose VB12 levels returned to normal (≥211 pg/ml)

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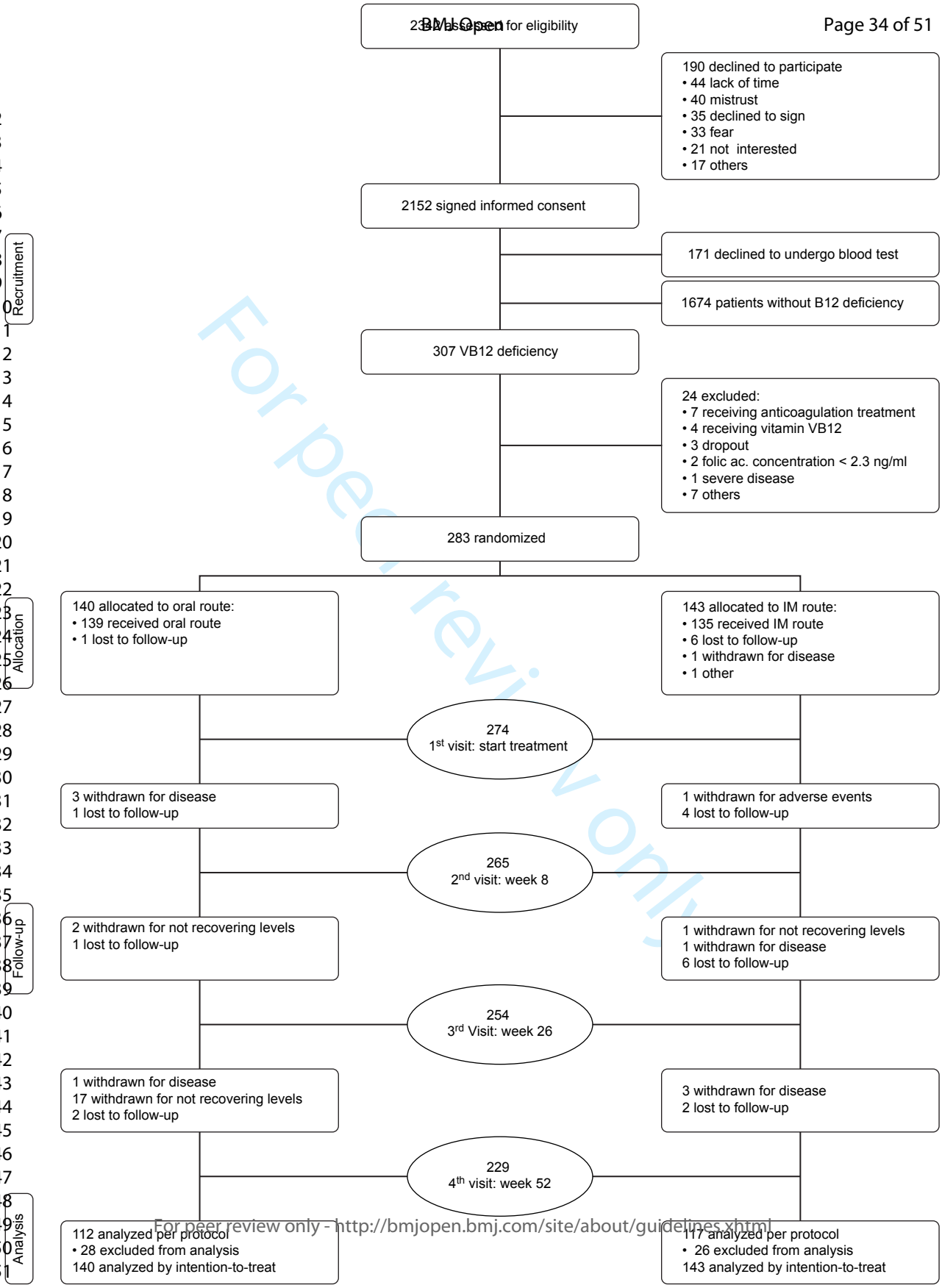

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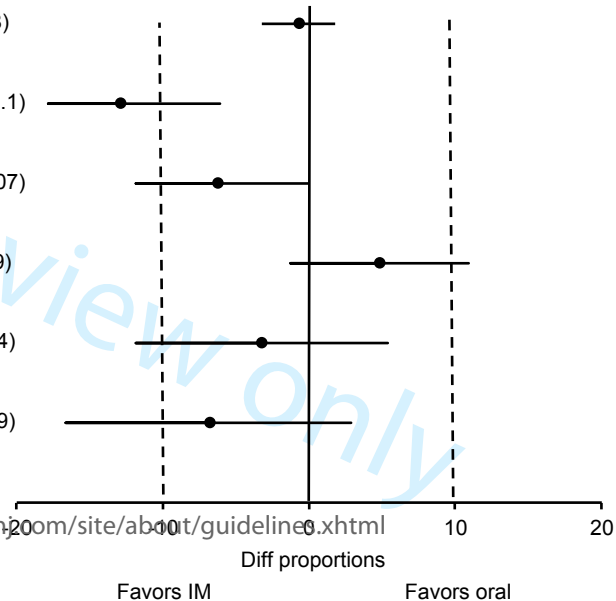
	Visit	Oral route n/N (%)	IM route n/N (%)	Difference in proportions (95% CI)
BPT	Week 8	133/135 (98.5)	129/130 (99.2)	-0.7 (-3.2 to 1.8)
	Week 26	115/132 (87.1)	122/122 (100.0)	-12.9 (-17.9 to -6.1)
	Week 52	103/112 (92.0)	115/117 (98.3)	-6.3 (-11.9 to -0.07)
ITT	Week 8	133/140 (95.0)	129/143 (90.2)	4.8 (-1.3 to 10.9)
	Week 26	115/140 (82.1)	122/143 (85.3)	-3.2 (-11.8 to 5.4)
	Week 52	103/140 (73.6)	115/143 (80.4)	-6.8 (-16.6 to 2.9)



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Sanz-Cuesta *et al. BMC Public Health* 2012, **12**:394
http://www.biomedcentral.com/1471-2458/12/394

BMC Public Health

STUDY PROTOCOL

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Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12)

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Abstract

Background: The oral administration of vitamin B12 offers a potentially simpler and cheaper alternative to parenteral administration, but its effectiveness has not been definitively demonstrated. The following protocol was designed to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the treatment of patients ≥65 years of age with vitamin B12 deficiency.

Methods/design: The proposed study involves a controlled, randomised, multicentre, parallel, non-inferiority clinical trial lasting one year, involving 23 primary healthcare centres in the Madrid region (Spain), and patients ≥65 years of age. The minimum number of patients required for the study was calculated as 320 (160 in each arm). Bearing in mind an estimated 8-10% prevalence of vitamin B12 deficiency among the population of this age group, an initial sample of 3556 patients will need to be recruited.

Eligible patients will be randomly assigned to one of the two treatment arms. In the intramuscular treatment arm, vitamin B12 will be administered as follows: 1 mg on alternate days in weeks 1 and 2, 1 mg/week in weeks 3–8,and 1 mg/month in weeks 9–52. In the oral arm, the vitamin will be administered as: 1 mg/day in weeks 1–8 and 1 mg/week in weeks 9–52. The main outcome variable to be monitored in both treatment arms is the normalisation of the serum vitamin B12 concentration at weeks 8, 26 and 52; the secondary outcome variables include the serum concentration of vitamin B12 (in pg/ml), adherence to treatment, quality of life (EuroQoL-5D questionnaire), patient

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3satisfaction and patient preferences. All statistical tests will be performed with intention to treat and per protocol. Logistic regression with random effects will be used to adjust for prognostic factors. Confounding factors or factors that might alter the effect recorded will be taken into account in analyses.

Discussion: The results of this study should help establish, taking quality of life into account, whether the oral administration of vitamin B12 is an effective alternative to its intramuscular administration. If this administration route is effective, it should provide a cheaper means of treating vitamin B12 deficiency while inducing fewer adverse effects. Having such an alternative would also allow patient preferences to be taken into consideration at the time of prescribing treatment.

Trial registration: This trial has been registered with ClinicalTrials.gov, number NCT 01476007, and under EUDRACT number 2010-024129-20.

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Background

Vitamin B12 (cyanocobalamin), along with other derivatives of folic acid, is a nutrient essential for the synthesis of DNA. Its deficiency is manifested through changes in the number and morphology of erythrocytes, leucocytes and platelets, and by neurological alterations owed to the progressive demineralisation of the nervous system (a consequence of defective myelin synthesis). Vitamin B12 is found mostly in food of animal origin. It is separated from ingested food through the action of the gastric acid, and in the duodenum the vast majority binds to intrinsic factor (IF). The vitamin B12/IF complex formed, which is very resistant to digestion, is then absorbed by endocytosis in the terminal ileum. Only 1-2% of vitamin B12 absorption occurs independent of IF [1]. Daily vitamin B12 requirements vary between 1 and 2 μg/day in adults [2]. A balanced diet, however, provides somewhere between 7 and 30 μg/day. Some of this excess can be stored (some 2–5 mg), meaning that deficiency symptoms may not occur until 3–5 years after the diet fails to provide sufficient vitamin B12 or its absorption becomes inadequate [3].

In the primary healthcare setting, the most commonly seen causes of vitamin B12 deficiency are related to abnormalities of digestion (atrophic gastritis, achlorhydria or the consequences of gastrectomy) or absorption (autoimmune pernicious anaemia, chronic pancreatitis, Crohn's disease, the effect of medications that alter the mucosa of the ileum, or the consequences of surgical resection), and, to a lesser extent, a lack of exogenous supply. The exact prevalence of vitamin B12 deficiency in industrialised countries is unknown; indeed, different studies using different definitions have reported it as between 5% and 60% [4]. Results have even differed widely between similar studies using an identical definition of deficiency, and after stratifying by age [5]. In Spain, the prevalence of vitamin B12 deficiency may reach 18% according to a meta-analysis of the studies undertaken up to 1999 [6]. However, population-based studies performed in Catalonia and the Canary Islands [7,8], both of which used a serum vitamin B12 cut-off of 200 pg/ml, returned values of 1.9% and 3.4% respectively. What does appear to be constant in all studies reviewed for the present work is that the prevalence of deficiency is greater among people aged 65–76 years. For example, the above Catalonian and Canary Island studies returned values of 3.8% and 8.5% for these age groups. Among elderly patients belonging to the Framingham cohort, Lidenbaun [9] observed a prevalence of over 5.3%. Other authors [10,11], however, report figures of 30-40% in elderly people with degenerative neuropsychiatric disorders and those receiving institutionalised care.

In the elderly, the symptoms of vitamin B12 deficiency caused by deficient diets and/or digestive and/or absorption problems can be nonspecific, making a diagnosis of deficiency more difficult. For example, up to 40% of elderly people show no haematological alterations. Further, neurological symptoms may appear before those of anaemia; indeed, only about 60% of elderly people with vitamin B12 deficiency are anaemic [12].

In primary healthcare in Spain, vitamin B12 deficiency is diagnosed via the determination of the serum concentration of the vitamin. Some studies [13-17] have described the limitations of trying to diagnose vitamin B12 deficiency exclusively via the measurement of this concentration, and report blood methylmalonic acid (MMA) and homocysteine concentrations to be more sensitive markers capable of detecting subclinical deficiency.

The traditional treatment of vitamin B12 deficiency is the intramuscular injection of cyanocobalamin, generally 1 mg/day for one week, followed by 1 mg/week for one month, and then 1 mg every 1 or 2 months *ad perpetuum* [4,18,19]. The vitamin may, however, be offered orally. In some circles this route has been regarded as an effective alternative to parenteral administration since the 1950s, during which time several studies showed serum vitamin B12 concentration to normalise after taking large oral doses. These results prompted the spread of oral administration in Sweden and Canada [3]. In the former country, 13% of the population over 70 years of age

now receives treatment for vitamin B12 deficiency, with two of every three patients treated via the oral route [20]. However, in the rest of the world, the parenteral route remains the most used. Indeed, controversy still surrounds the advantages and effectiveness of the oral route. Some authors question its use [21] while others favour it, although the methodological limitations of the evidence they provide means no firm conclusions can be drawn. In reviews of the literature published between 1999 and 2007, Daly-Youcef [4] and Andrés E [19] concluded that orally administered vitamin B12 provided effective treatment for adult and elderly patients with deficiencies, although they highlighted that further studies were needed to determine its effectiveness in patients with severe neurological symptoms. Federicia [22], who reviewed the treatment criteria followed in different studies, concluded oral administration to be effective, but recommended further work to confirm this. Shatsky[23], who examined evidence derived from the use of oral and intramuscular administration, indicated that high dose oral administration appeared to be safe, effective and cost-effective, although long term clinical trials were required to confirm this. In a prospective study performed in Spain involving commercially available multi-vitamin supplements, Rabuñal et al. [24] reported the effectiveness and tolerance of oral vitamin B12 to be excellent, but also indicated that the dosage to be used was yet to fully established. In 2005, a Cochrane review [3] was published that examined two randomised clinical trials - those reported by Kuzminski [2] and Bolaman [25] - that studied the effectiveness of oral vs. intramuscular administration of vitamin B12 for the treatment of its deficiency. The Kuzminski trial involved 33 patients (18 in the oral arm and 15 in the intramuscular arm), while the Bolaman trial involved 60 (26 in the oral arm and 15 in the intramuscular arm). The Cochrane concluded that orally administered vitamin B12 appeared to be as effective as the intramuscular route with respect to the short-term haematological and neurological responses observed in patients with deficiencies, but highlighted methodological limitations in both trials. A large clinical trial was called for in the primary healthcare setting, where a high percentage of patients with vitamin B12 deficiency is seen. The Cochrane review also underscored the need to include a measurement of the quality of life as an outcome, and patient preference at the time of prescribing treatment. Among other variables, three studies [24,26,27] have recorded patient views on the administration route, and record a high level of acceptance of the oral route, the advantages of which include avoiding the displacement of patients to receive injections, avoiding the discomfort of injection, and a reduction in treatment costs [28,29].

A further question still to be answered is that of the optimum dose when using the oral route [3].

In summary, despite many studies indicating the oral administration of vitamin B12 to be easy, effective and less costly than intramuscular administration, their designs, and in some cases their methodological limitations, mean that debate still surrounds the effectiveness of the oral route. This may help explain why it is little used by health professionals [30].

Although some authors [31,32] recommend the use of moderately high doses (which have obtained the best results), studies are still being performed to investigate this. In a randomised clinical trial involving five treatment arms with doses of between 2.5 μg/day and 1000 μg/day, Eussen [33] concluded that a dose of at least 600 μg/day was required to obtain adequate results. However, in guidelines published in 2012, the British Columbia Medical Association (Canadian Ministry of Health) recommended a dose of 1000 μg/day for pernicious anemia or food-bound cobalamin malabsorption [34].

The proposed study examines the questions that, according to the Cochrane review mentioned above [3], are still to be answered, via a clinical trial (of ample duration and with a large number of patients) in the primary healthcare setting. As recommended, one of the outcomes examined is quality of life. The results obtained should provide high quality scientific evidence of use when taking treatment decisions in the primary healthcare centres, while allowing patient preference of administration route to be taken into consideration. The results may reveal oral treatment with vitamin B12 to be, as Lederle [35] put it, “medicine’s best kept secret”.

Aim

The aim of the proposed protocol is to compare the effectiveness of orally and intramuscularly administered vitamin B12 in the normalisation of serum vitamin B12 concentrations at 8, 26 and 52 weeks of treatment, in patients aged ≥65 years with vitamin B12 deficiency treated at primary healthcare centres in the Madrid region, Spain. The secondary outcomes to be measured include the safety of both administration routes, quality of life (measured using the EuroQoL-5D questionnaire) and adherence to treatment. Patient preferences and satisfaction with treatment will also be recorded, along with patient sociodemographic profiles, lifestyle habits, and the clinical manifestation of each patient’s deficiency.

Methods/design

Study type

This study takes the form a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the

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primary healthcare setting, with a duration of one year. For ethical reasons, a placebo controlled trial would not be appropriate [36].

The study involves 23 primary healthcare centres in the Madrid region of Spain. The research team is composed of a clinical assistance group of 169 general practitioners and nurses, and a technical group of 22 health professionals including doctors of different specialities, nurses and pharmacists. For the undertaking of fieldwork, these 191 team members are divided into smaller groups (with similar numbers of clinical and technical personnel), each in charge of one of five subprojects. Each subproject is led by a member of the technical personnel. Together, these five leaders form the coordination group for the trial as a whole.

The trial protocol was approved by the Madrid Region Clinical Research Ethics Committee (*Comité Ético de Investigación Clínica Regional de la Comunidad de Madrid*) on February 8th 2011, and has been registered with ClinicalTrials.gov number NCT 01476007, and under EUDRACT number 2010-024129-20 [Oral Versus Intramuscular Cobalamin to treat Cobalamin Deficiency: Noninferiority randomised controlled trial, pragmatic and multi-center in the primary healthcare setting (OB12 project)].

Patients

1. Inclusion criteria: all participants must:
   - be ≥65 years of age
   - be attending a primary healthcare centre for consultation on some medical matter
   - provide their informed consent to be included
   - have a serum B12 concentration of <179 pg/ml.
2. Exclusion criteria: patients meeting any of the following conditions will be excluded:
   - having been treated (under medical prescription) in the last five years for vitamin B12 deficiency
   - serious neurological or psychiatric symptoms, including psychotic problems
   - dementia preventing the giving of informed consent to take part
   - atrophy of the optic nerve
   - serum folic acid concentration of <2.3 ng/ml
   - stage 4 kidney disease 4 (estimated glomerular filtration rate [GFR] 15–29 ml/min)
   - having received/suffering malabsorption-related:
     - surgery or diseases affecting the jejunum-ileum
     - inflammatory-intestinal disease, e.g., Crohn's disease, ulcerative colitis
     - celiac disease
   - chronic pancreatitis
   - myelodisplasia or malignant blood disease
   - haemophilia or other coagulation problems contraindicating parenteral administration
   - severe systemic disease
   - having been involved in any other trial involving the administration of any experimental treatment in the 28 days prior to the start of the present study
   - being treated for HIV, HVB or HVC infection
   - hypersensitivity to vitamin B12, or any of the vitamin preparation's excipients
   - receiving anticoagulation treatment
   - being away from home and with no intention of residing for the following year in the health district where consultation was made
   - failing to meet any inclusion criterion
   - limitations regarding oral treatment

Randomisation

Participants will be enrolled consecutively by their general practitioners when attending a primary healthcare centre in the study area (Figure 1). All patients without reason to be excluded will be invited to participate. Those patients that accept this invitation will provide written, informed consent to be included. A blood sample will then be taken and part of this used to determine the serum vitamin B12 concentration (pg/ml). In those returning a value of <179 pg/ml (defined as vitamin B12 deficiency by the reference analytical laboratory analysing the samples collected), the remaining fraction of the sample will be analysed to provide a haemogram (reticulocyte, erythrocyte, leucocyte and platelets counts), the values of biochemical variables (glucose, creatinine, GOT, GPT, GGT and ferritin), the folic acid concentration, and an anti-IF antibody count. Those who meet all inclusion criteria, and no exclusion criteria, will then be randomly assigned to one arm of the treatment, i.e., oral or intramuscular administration of vitamin B12. This will be performed by means of a simple randomisation process performed by the electronic data collection system. This guarantees that neither researcher nor patient has any choice with respect to the group to which the latter is assigned.

Sample size

The sample size required was determined bearing in mind the results of Kuzminski et al. [2]. In the latter study the parenteral administration of vitamin B12 was associated with an increase in serum concentrations of the vitamin of >200 pg/ml at 4 months in over 70% of patients. For the present trial, the level of non-inferiority of the oral treatment is set at a difference (delta) in response compared to the parenteral treatment of ≤10%. This threshold was set given its importance from a clinical rather than a statistical viewpoint, and since it falls within the range normally accepted for this type of study [37].

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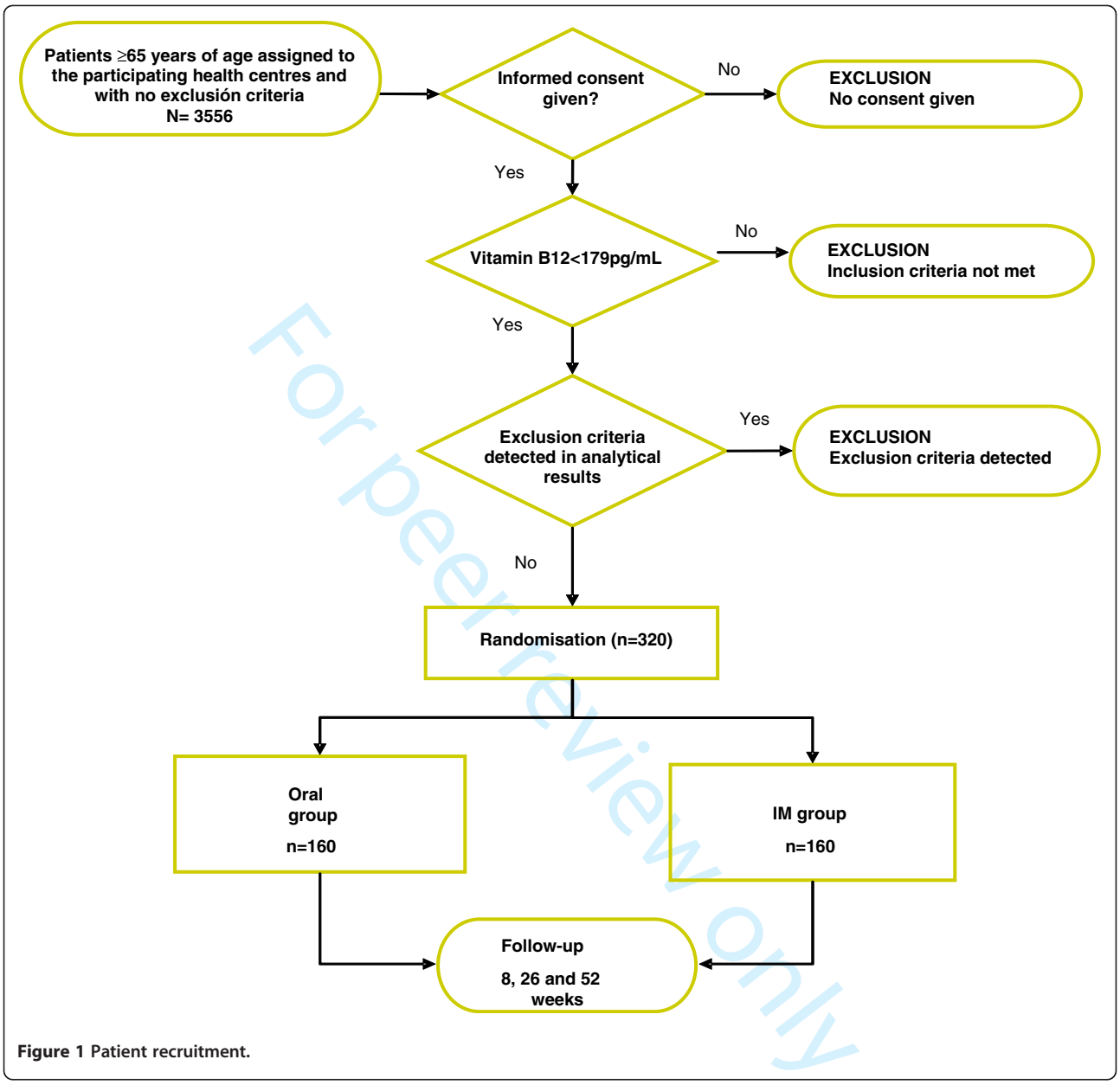

Figure 1 Patient recruitment.

Assuming that the percentage of patients showing an increase in serum vitamin B12 concentration to above 179 pg/ml in both groups is 70%, means the study requires at least 304 patients (152 in each arm) for a threshold of non-inferiority of 10% and a statistical power of 60% with significance set at $p < 0.05$. Given the type of patients to be studied, i.e., patients who have come to the health centres for consultation, plus the fact that their own family doctors are members of the research team, a loss to follow-up of under 5% is expected. The minimum starting sample size for each arm was therefore deemed to be n = 160. With an expected prevalence of vitamin B12 deficiency of 8-10% (a figure of 9% was used in calculations), the original number of patients to be enrolled so that 320 with a vitamin B12 deficiency can be guaranteed is 3556.

Blinding

In studies with the present design it is impossible to blind the patient to the treatment received. However, this limitation is compensated for by the objective measurement of the main outcome variable (the serum vitamin B12 concentration) and the randomisation of the patients to the treatment groups. Further, the persons charged with the statistical analysis of the data will be blind to the identity of the patients in each treatment arm.

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The intervention

The pharmaceutical formulations to be used in the study are commercially available in Spain. The treatments will involve:

- Intramuscular route: 1 mg of vitamin B12 on alternate days during weeks 1 and 2; 1 mg/week over weeks 3–8 (i.e., for 6 weeks); and 1 mg/month from weeks 9–52
- Oral route: 1 mg/day of vitamin B12 for 8 weeks; 1 mg/week from weeks 9–52

Patients in both arms will undergo analytical monitoring in weeks 8, 26 and 52. They will receive appointments for the appropriate dates. The response to treatment will be recorded alongside adherence to treatment and the appearance of any adverse effects.

Work plan

Before work begins, the project will be presented to all the research team members in a special meeting. Training sessions lasting 2–3 h will also be held at each participating health centre. These will involve a review of the inclusion and exclusion criteria, provide instructions regarding the intervention, and examine the ethical requirements to be met for the trial to be held.

The procedures to be followed and information to be recorded at each of a patient's visits to a participating health centre is as follows:

- Selection Visit
  - Signing of informed consent
  - Assessment of inclusion/exclusion criteria
  - Recording of demographic data (age and sex)
  - Analysis: serum vitamin B12. If concentration is <179 pg/ml the following analyses are to be requested: haemogram, biochemical analysis (glucose, creatinine, GOT/GPT/GGT), ferritin, folic acid, anti-IF antibody level. If serum vitamin B12 concentration is >179 pg/ml: patient preference questionnaire
  - Randomisation of patients to treatment group
- Visit 1 (start of treatment)
  - Anamnesis: record whether the patient lives alone or with others, lifestyle habits, use of alcohol, whether a vegan diet is followed, whether the patient has undergone gastrectomy
  - Symptoms: record paresthesia, asthenia, loss or reduction of appetite, sadness or change in state of mind, concomitant pharmacological treatment
  - Physical examination: for Hunter's glositis, positional and vibrational sensitivity
  - Questionnaires: Lobo cognitive mini-exam, EuroQoL-5D
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
- Visit 2 (week 8)
  - Anamnesis: record lifestyle habits and use of alcohol
  - Symptoms: if pathological at the first visit, record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
  - Physical examination: if pathological at the first visit examine for Hunter's glositis, positional and vibrational sensitivity
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Questionnaires: EuroQoL-5D
  - Assessment of adverse effects
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
  - Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given
- Visit 3 (week 26)
  - Anamnesis: record lifestyle habits and use of alcohol
  - Symptoms: if pathological at the first visit, record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
  - Physical examination: if pathological at the first visit examine for Hunter's glositis, positional and vibrational sensitivity
  - Record concomitant treatment
  - Request analyses to be performed one week before next visit: haemogram and serum vitamin B12
  - Questionnaire: EuroQoL-5D
  - Assessment of adverse effects
  - Therapeutic plan: patient in oral arm – provision of medication; patient in intramuscular arm – provide appointments for injections
  - Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given
- Visit 4 (week 52)
  - Anamnesis: record lifestyle habits and use of alcohol

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- Symptoms: record paresthesia, asthenia, loss or reduction of appetite, sadness or change in level of happiness, and concomitant pharmacological treatment
- Physical examination: for Hunter's glositis, positional and vibrational sensitivity
- Record concomitant treatment
- Questionnaires: EuroQoL-5D, satisfaction and preferences
- Assessment of haemogram and serum vitamin B12 concentration
- Assessment of adverse effects
- Assess adherence to treatment: oral route – count number of vials used; intramuscular route: count injections given

Variables

Outcome variables

The main outcome to be measured is the normalisation of the serum vitamin B12 concentration (>179 pg/ml) at 8, 26 and 52 weeks. The secondary outcomes will be the serum vitamin B12 concentration (pg/ml), adverse events (description, moment of onset and resolution, intensity, cause, steps taken), adherence to treatment (measured at each patient visit via the number of vials used for patients in the oral arm, and the number of injections given in the intramuscular arm), quality of life (measured using the EuroQoL-5D questionnaire), and patient satisfaction and preferences.

Anamnesis, demographic and lifestyle information

Including age, sex, whether the patient lives alone or with others, whether a vegan diet is followed, and the use of alcohol (g/week).

Clinical variables

Symptoms such as paresthesia, asthenia, loss or reduction of appetite, sadness or change in state of mind (anamnesis), Hunter's glositis, positional and vibrational sensitivity (all via physical examination), and cognitive decline (Lobo test).

Analytical variables

Haemogram (complete blood cell and platelet count) and biochemical analysis (folic acid, glucose, creatinine, GOT, GPT, GGT, ferritin, anti-IF antibodies). Blood analyses will be performed in plasma or serum as required and under standard conditions.

Concomitant treatment

Recording of the taking of protein pump inhibitors, H2 receptor antagonists, antacids, potassium, metformin, colchicine, neomycin, p-aminosalicylic acid, parenteral chloramphenicol, Fe, vitamin C and other vitamin supplements.

Losses and withdrawals

Patients will be removed from the trial if any of the following conditions are met:

- Serum vitamin B12 concentration still <179 pg/ml after 8 weeks of treatment. Treatment will be deemed to have failed in these patients, and they will be further studied and treated outside the trial according to normal clinical practice.
- Serious adverse events.
- Voluntary withdrawal or violation of the protocol.

At least two attempts will be made to contact by telephone those patients who do not come for their scheduled visits. All patients will be informed that they can abandon the study at any time without this affecting their future medical treatment in any way.

Analysis

Descriptive analysis of the patients

The trial will involve a descriptive statistical analysis of the baseline characteristics of patients in both treatment arms. Quantitative variables will be described in terms of their measure of central tendency, mean or median (for those showing asymmetric distributions), and the corresponding dispersion, standard deviation or interquartile range. Qualitative variables will be described in terms of proportions and their corresponding confidence intervals.

Baseline comparisons

The Student t test or Mann–Whitney U test (when the normal hypothesis is rejected) will be used to determine whether the two treatment arms are comparable based on their quantitative baseline characteristics and known prognostic factors. Comparisons on qualitative variables will be undertaken using the Pearson Chi-squared test or Fisher's Exact test as required. If cases of inequality are detected, the confounding factors will be defined and appropriate adjustments made.

Analysis of effectiveness of treatment (main outcome) at the three monitoring points

Intention-to-treat and per-protocol analyses will both be performed, as is recommended for non-inferiority studies [38].

The effectiveness of treatment will be analysed by examining the therapeutic success achieved in each arm at 8, 26 and 52 weeks, determining the 95% confidence interval for the percentage of patients in each treatment arm whose serum vitamin B12 concentrations become normalised. If the confidence intervals do not fall outside the non-inferiority limit (10%), it can be concluded that

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the oral treatment is not inferior to the intramuscular treatment. The within-patient percentage change in serum vitamin B12 concentration at each monitoring point will be determined, and the confidence intervals for the difference in the mean values for each arm calculated.

If the distribution of confounding factors differs in the two arms, explicative regression analysis will be performed in which the dependent variable will be the normalisation of the serum vitamin B12 concentration, and the independent variable will be the treatment group.

Repeated measures ANOVA will be used to examine the change in serum vitamin B12 concentration in each group at each monitoring point.

Safety analysis

The incidence of adverse events in the two arms will be compared using the Pearson Chi-squared test or Fisher's Exact test as required.

Quality of life analysis

The perception of quality of life by the patients of each arm will be assessed by comparing the EuroQol 5D scores (determined using a visual analogue scale) and the transformation of these scores into utility-based quality of life values.

Analysis of adherence to treatment

Adherence to treatment will be examined via the counting of oral doses taken in the oral arm, and the number of injections given in the intramuscular arm. An operative indicator variable will then be defined to describe the degree of adherence.

Ethics

The trial has been approved by the Madrid Region Clinical Research Ethics Committee (February 8th 2011). It will be performed by qualified medical and scientific staff. The rights and welfare of the patients will be respected at all times. All patients will be adequately informed, both verbally and in writing, of the nature of the trial, its aim, and its risks and possible benefits. Given that the study is a non-inferiority trial, all patients will be informed that the oral treatment is expected to be as effective as the standard intramuscular treatment. Signed, dated consent to be included will be required from each patient.

Spanish law regarding the use of human subjects in clinical trials will be adhered to. The trial will respect all basic ethical principles of autonomy, justice, goodness of intent and absence of malintent according to the standards of good clinical practice enshrined in the Declaration of Helsinki (Seoul, 2008) and the Oviedo Agreement (*Convenio de Oviedo*) (1997).

Discussion

From a clinical point of view, the results obtained will help establish whether the oral administration of vitamin B12 is as effective as intramuscular treatment in the normalisation of serum vitamin B12 concentrations in patients ≥65 years of age with a deficiency. Knowledge in this respect is important since oral administration should provide these patients with greater autonomy, improve patient satisfaction with treatment, and reduce treatment costs. Patients receiving anti-coagulation treatment, for whom intramuscular treatment may be contraindicated, should also benefit. The possibility of taking an oral preparation would also allow patient preferences to be taken into account when deciding on what treatment to prescribe; indeed, patient preference is a factor of prime importance in clinical decision-taking. The possibility of providing treatment options in normal clinical practice rests on two conditions being met: 1) that quality scientific information supports the effectiveness of the therapeutic options on offer, and 2) that heterogeneous groups of patients have recorded their satisfaction with these options. The present trial provides for information in this respect to be gathered [39] and therefore treatment preferences to be taken into account at the time of prescription.

The trial is also designed to provide information on the effect of the normalisation of serum vitamin B12 concentrations by both treatments on patient-perceived quality of life. Physicians commonly assume that taking oral supplements will be associated with a feeling of greater well-being, although this has never been proven [40]. The present trial should also throw light on this.

The trial suffers from the practical limitation of having to enrol a large number of patients to meet its sample size requirements. However, a high degree of motivation is expected of the research team since its clinical assistance members are those involved in the enrolment process. Further, the fact that the patients to be enrolled will be seeking medical help (although not necessarily for vitamin B12 deficiency) suggests few will be lost to follow-up. A further possible limitation is the low statistical power used in the calculation of the sample size. The 60% power contemplated requires a sample size of 304 patients (152 in each arm) – higher powers would increase the sample size required and the enrolment of such numbers cannot be guaranteed. However, given the results reported in previous studies (2,25,31-33) that used moderate/high doses of vitamin B12, it should be possible to demonstrate the non-inferiority of the oral treatment with this power level. If the 95% confidence interval were to cross the non-inferiority threshold, i.e., showing the results to be inconclusive, the intramuscular treatment would remain the treatment of choice. To determine the degree of adherence to treatment (and thus avoid outcome dilution effects) [41], the

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number of doses taken orally and received by injection will be recorded. The characteristics of all the original 320 patients will be recorded to provide insight into the type of patient left in the study after any withdrawals, as recommended by the CONSORT group [41,42]. Basic information (age, sex, etc.) on potentially eligible patients who decline to take part will also be recorded. This type of information is of use when assessing the possible extrapolation of the trial results to more general populations.

The decision not to take serum methylnalonic acid and homocysteine concentrations into account as diagnostic markers and outcome variables was made bearing in mind that these are not normally determined, either at diagnosis or during follow-up, in patients with a vitamin B12 deficiency.

Finally, given the pragmatic nature of the proposed trial, the decision was taken to include consecutive patients seeking medical help at the participating centres, thus ensuring the enrolment of subjects similar to those that would be seen in normal clinical practice.

Abbreviations

Fe: Ferrum; g: Gram; GFR: Glomerular filtration rate; GGT: Gamma-glutamyl transpeptidase; GOT: Glutamic oxaloacetic transaminase; GP: General practitioner; GPT: Glutamic-pyruvic transaminase; HIV: Human immunodeficiency virus; HVB: Hepatitis B virus; HVC: Hepatitis C virus; IF: Intrinsic factor; µg: Microgram; MMA: Methylmalonic acid; mg: Milligrams; ng: Nanograms; pg: Picograms.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PGE y RRF conceived of the study and participated in its design. TSC; RRF; SGE; ldCG; JMF; EEM; participated in the design and coordination of the study. FRS; MGS; RGG; MAMS; COL; MLSP; CMR; BMB; AVP; FGBG; JEMS; RRB; GAC; LMCB; EPC; MRB; MTRM; SSD; SMI; RRG; IBL; MVN; JSD; TGG; MDC; AAB participated in different phases of the design. TSC; RRF; SGE; ldCG; JMF; EEM directed the writing of the manuscript. All authors OB12 Group read and approved the final manuscript.

Funding

This study was funded by the *Ministerio de Sanidad, Política Social e Igualdad*, Spain (EC10-115, EC10-116, EC10-117, EC10-119, EC10-122) and by CAIBER - Spanish Clinical Research Network. The authors thank the following persons for their contributions to this work: Dolores Otero-Criado, Carlos Carvajales Fernández, Rosa Zurdo-Baz and Raisa González-Pérez.

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Received: 4 May 2012 Accepted: 31 May 2012
Published: 31 May 2012

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doi:10.1186/1471-2458-12-394
Cite this article as: Sanz-Cuesta *et al.*: Oral versus intramuscular administration of vitamin B12 for the treatment of patients with vitamin B12 deficiency: a pragmatic, randomised, multicentre, non-inferiority clinical trial undertaken in the primary healthcare setting (Project OB12). *BMC Public Health* 2012 **12**:394.

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Supplement 2: Bayesian Analysis

Bayesian analysis is a highly appropriate analysis strategy when working with small sample sizes. Previous knowledge about the studied item can be taken advantage of by means of the assessment of the plausibility of a given hypothesis after incorporating the new observed data.[1]

The noninferiority hypothesis, formally $\Delta < -10\%$, was tested, taking into account the observed results but also taking into account the results of the trials by Kuzminski et al.[2] and Saraswathy et al.[3]

P1 denotes the percentage of patients who responded to VB12 oral administration, and P0 represents the percentage of those responding to VB12 intramuscular administration. Bayesian analysis allows for calculating the probability of P1 being equal to or smaller than P0 by a specified magnitude, the noninferiority limit ($\Delta < -10\%$). For each of the parameters P1 and P0, both measured at 8, 26 and 52 weeks, we selected *a priori* distributions from the family of beta distributions with parameters **a** and **b**, which are related to the proportions of those responding in each trial arm. The gamma distribution represents the *a priori* hypothesis of the distribution of differences. According to the results of both trials by Kuzminski et al.[2] and Saraswathy et al.,[3] included in the review by Wang et al.,[4] 79.1% and 84.1% of patients normalized their VB12 levels in the oral and IM treatment groups, respectively.[4] The respective CIs associated with these prior data were calculated, and parameters were chosen (**a** and **b** in the beta distribution) such that the maximum density intervals of these distributions approximately coincided with the CI previously obtained (see Figure 1). Beta distributions for the success rate in each arm of the trial were obtained using binomial data. A total of 10000 simulations were made from these *a posteriori* distributions, and the corresponding differences, P1-P0, were calculated yielding an *a posteriori* distribution of differences. This distribution was used to derive simulation-based estimates of the probability of relevant magnitudes concerning Δ: P1-P0>0.10 at weeks 8, 26, and 52. Both PPT and ITT analyses were performed. EPIDAT 4.2 software was used for all computations.

Table 1 shows the *a posteriori* probability of differences in treatment effectiveness between oral and IM routes at different weeks (8, 26 and 52). The probabilities of the differences in treatment effectiveness being >10% between the oral and IM groups were 0.001, 0.201, and 0.036 at weeks 8, 26, and 52, respectively (per protocol analysis). In the intention-to-treat (ITT) analysis, these values were 0.000, 0.015, and 0.060 at weeks 8, 26, and 52, respectively.

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Figure 1. A priori distributions of the differences between oral and intramuscular treatment

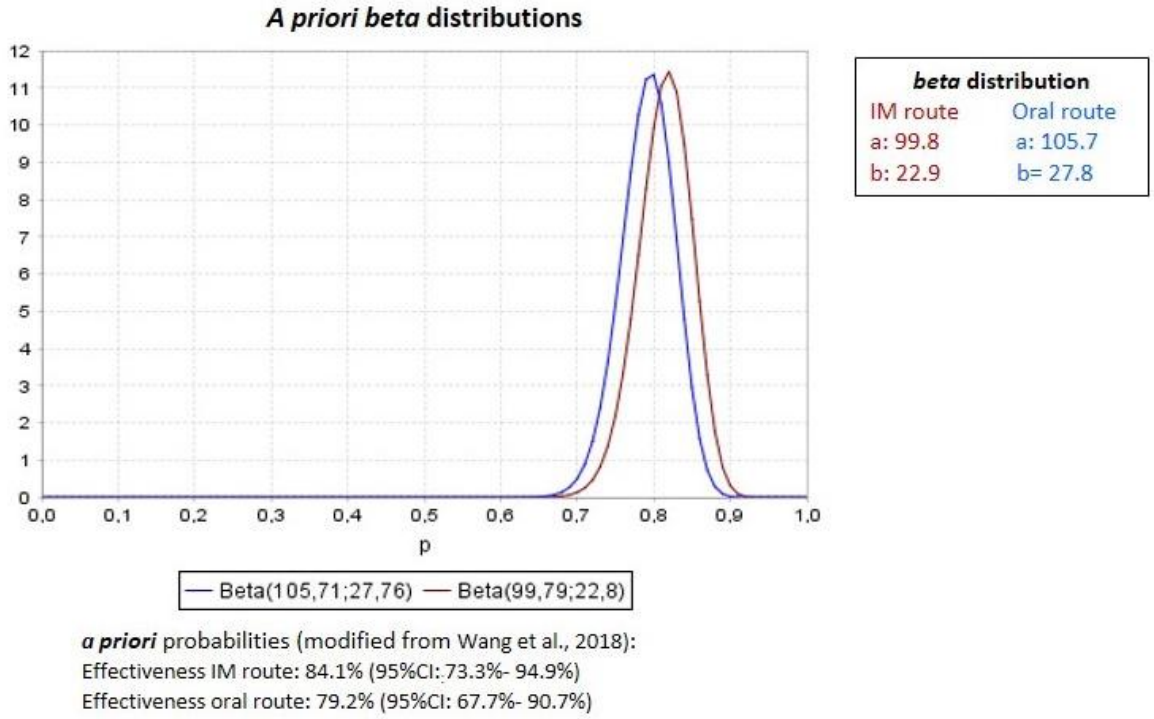

Table 1. *A posteriori* probability of differences in treatment effectiveness between oral and IM routes at 8, 26, and 52 weeks.

A posteriori **probability (Δ < -10%)**	**Week 8**	**Week 26**	**Week 52**
Per-protocol analysis	0.001	0.201	0.036
Intention-to-treat analysis	0.000	0.015	0.060

Δ: threshold of non-inferiority

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Supplement 3: Receiver Operating Characteristic (ROC) Curve

To explore factors affecting the normalization of serum VB12 concentration (yes/no) at 52 weeks, serum VB12 levels were studied at 8 weeks (at the end of the "charging period"). An ROC curve was built to determine the likelihood ratios for each cutpoint after the charging period to "predict" the normalization of levels (serum VB12 levels ≥211 pg/mL) at the end of the study.[1]

Table 1 shows the results of the likelihood ratios for the cutpoints at the main percentiles of the distribution of VB12 serum levels at week 8 ("charging period") to predict normalized VB12 serum levels at the end of the study. In Figure 1, the ROC curve is plotted. The level at the 5th percentile of the distribution was selected as the most useful value as it showed best classification ability and because when patients did not reach this level at week 8, they were almost twelve times more likely to not reach suitable VB12 levels at the end of the study than if they did reach levels over 281 pg at week 8 (12~1/negative likelihood ratio).

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Table 1. Exploring the value of several cutpoints of OB12 serum levels at week 8 to "predict" normalization of values of Vit B12 at the end of the study

Cutpoint	Sensitivity	Specificity	Correctly Classified	LR+	LR-	Percentil
≥ 281	0.977	0.273	94.30%	1.3435	0.0841	5
≥ 328	0.963	0.546	94.30%	2.1193	0.0673	10
≥ 353	0.931	0.636	91.70%	2.5608	0.1081	15
≥ 389	0.895	0.818	89.10%	4.9197	0.129	20
≥ 421	0.839	0.818	83.80%	4.617	0.1962	25

LR+: Positive Likelihood ratio. LR-: Negative Likelihood ratio

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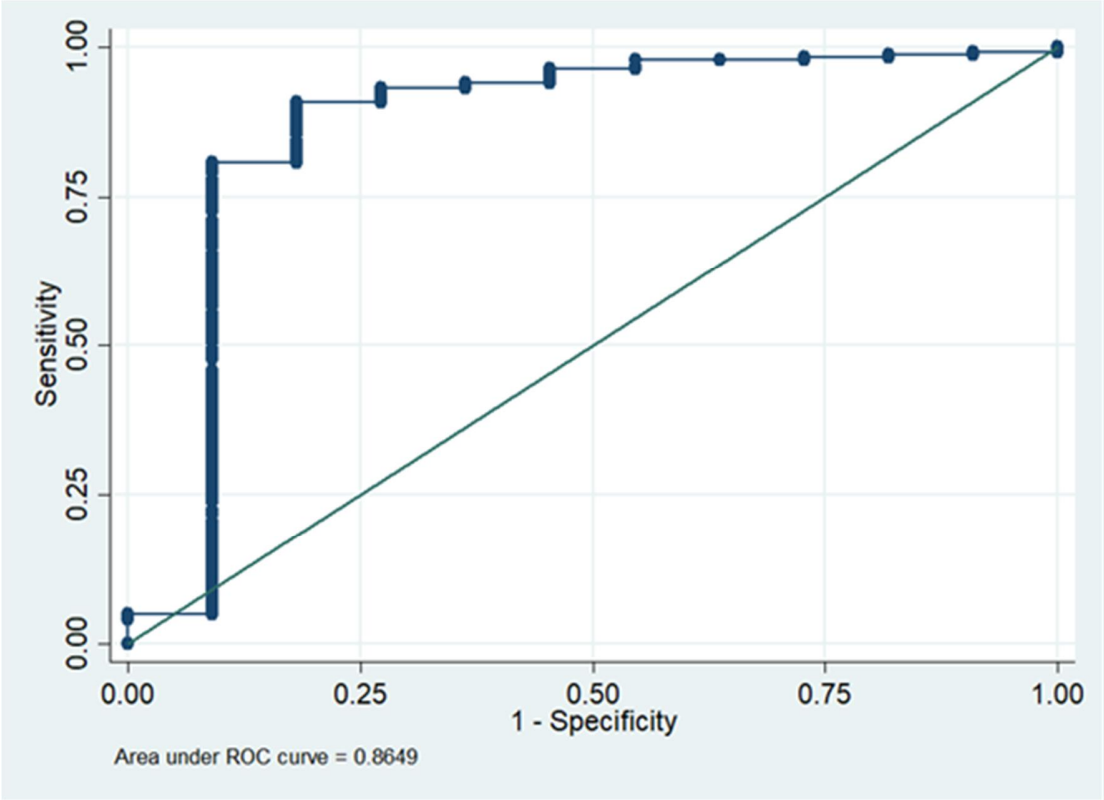
Figure 1. ROC curve



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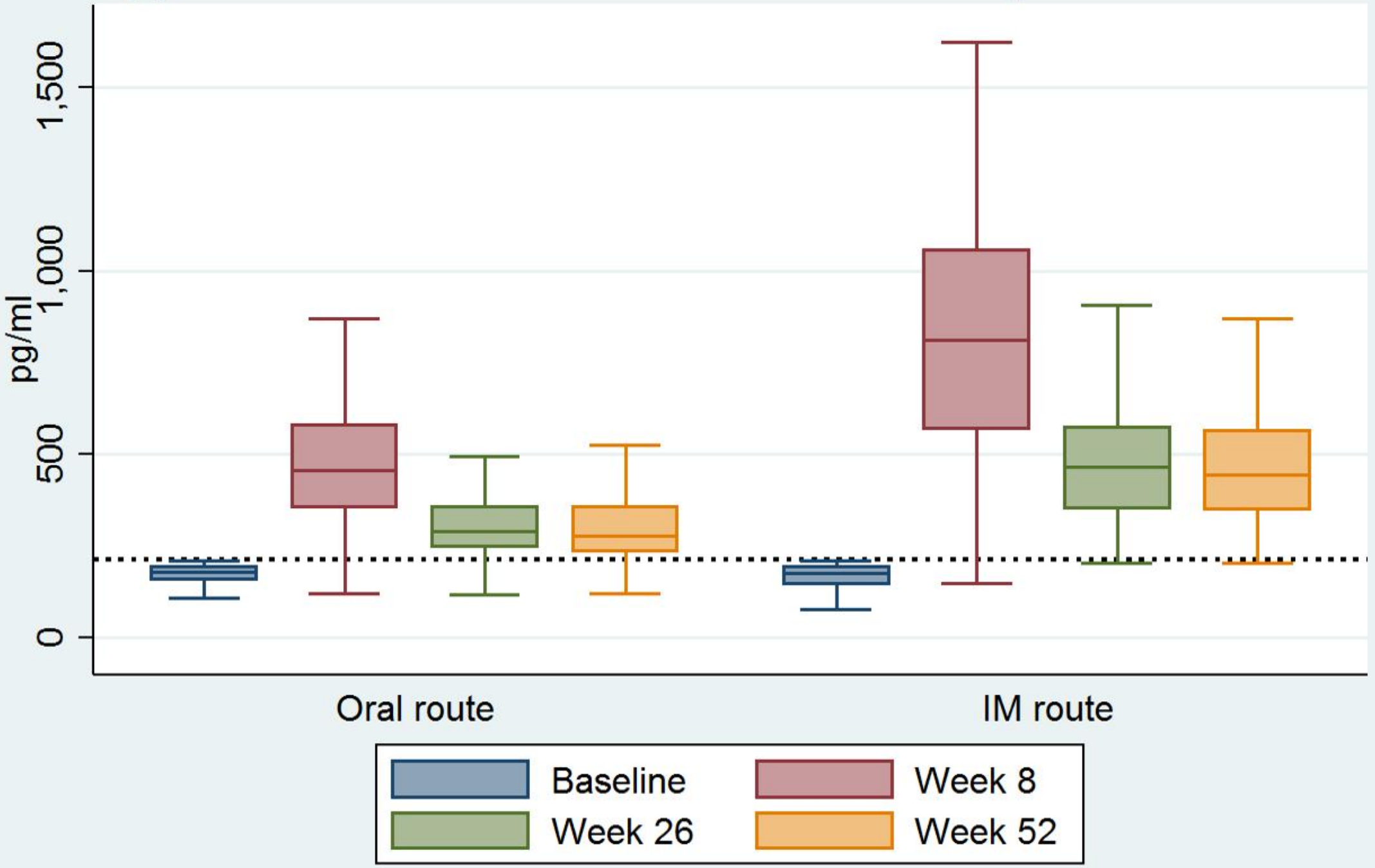
Supplement 4: Vitamin B12 mean levels by treatment route



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CONSORT Statement 2006 - Checklist for Non-inferiority and Equivalence Trials

Items to include when reporting a non-inferiority or equivalence randomized trial

PAPER SECTION And topic	Item	Descriptor	Reported on Page #
TITLE & ABSTRACT	1	How participants were allocated to interventions (*e.g.*, "random allocation", "randomized", or "randomly assigned"), *specifying that the trial is a non-inferiority or equivalence trial.*	Page 1 and 2
INTRODUCTION Background	2	Scientific background and explanation of rationale, *including the rationale for using a non-inferiority or equivalence design.*	Page 5 and 6
METHODS Participants	3	Eligibility criteria for participants *(detailing whether participants in the non-inferiority or equivalence trial are similar to those in any trial(s) that established efficacy of the reference treatment)* and the settings and locations where the data were collected.	Page 7 Supplement 1
Interventions	4	Precise details of the interventions intended for each group *detailing whether the reference treatment in the non-inferiority or equivalence trial is identical (or very similar) to that in any trial(s) that established efficacy,* and how and when they were actually administered.	Page 7
Objectives	5	Specific objectives and hypotheses, *including the hypothesis concerning non-inferiority or equivalence.*	Page 6
Outcomes	6	Clearly defined primary and secondary outcome measures *detailing whether the outcomes in the non-inferiority or equivalence trial are identical (or very similar) to those in any trial(s) that established efficacy of the reference treatment* and, when applicable, any methods used to enhance the quality of measurements (*e.g.*, multiple observations, training of assessors).	Page 8
Sample size	7	How sample size was determined *detailing whether it was calculated using a non-inferiority or equivalence criterion and specifying the margin of equivalence with the rationale for its choice*. When applicable, explanation of any interim analyses and stopping rules (*and whether related to a non-inferiority or equivalence hypothesis*).	Page 8
Randomization -- Sequence generation	8	Method used to generate the random allocation sequence, including details of any restrictions (*e.g.*, blocking, stratification)	Page 7 Supplement 1
Randomization -- Allocation concealment	9	Method used to implement the random allocation sequence (*e.g.*, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	Page 7 Supplement 1

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Randomization -- Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	Page 7 and 8
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	Not blinded
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s), *specifying whether a one or two-sided confidence interval approach was used.* Methods for additional analyses, such as subgroup analyses and adjusted analyses.	Page 8 and 9 Supplement 2 Supplement 3
RESULTS Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	Figure 1
Recruitment	14	Dates defining the periods of recruitment and follow-up.	Page 7 and 8 Figure 1
Baseline data	15	Baseline demographic and clinical characteristics of each group.	Page 9 and 10 Table 1
Numbers analyzed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was *"intention-to-treat" and/or alternative analyses were conducted*. State the results in absolute numbers when feasible (*e.g.*, 10/20, not 50%).	Figure 1 and Figure 2
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group, and the estimated effect size and its precision (*e.g.*, 95% confidence interval). *For the outcome(s) for which non-inferiority or equivalence is hypothesized, a figure showing confidence intervals and margins of equivalence may be useful.*	Page 11 to 13 Table 2 Table 3 Figure 2 Supplement 2
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those pre-specified and those exploratory.	Page 12
Adverse events	19	All important adverse events or side effects in each intervention group.	Page 13
DISCUSSION Interpretation	20	Interpretation of the results, taking into account the *non-inferiority or equivalence hypothesis and any other* study hypotheses, sources of potential bias or imprecision and the dangers associated with multiplicity of analyses and outcomes.	Page 14 to16
Generalizability	21	Generalizability (external validity) of the trial findings.	Page 14 to 16
Overall evidence	22	General interpretation of the results in the context of current evidence.	Page 16 and 17

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