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ORIGINAL ARTICLE

Intranasal vitamin B_{12} administration in elderly patients: A randomized controlled comparison of two dosage regimens

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Aim: Vitamin B_{12} deficiency is common in the elderly population. Standard treatment via intramuscular injections, however, has several disadvantages. Safer and more convenient dosage forms such as intranasal are therefore being explored. This study compares the effects of two intranasal vitamin B_{12} dosage regimens in elderly vitamin B_{12}-deficient patients.

Methods: Sixty patients ≥65 years were randomly assigned to either a loading dose (daily administration for 14 days followed by weekly administration) or a no loading dose (administration every 3 days) regimen for 90 days. Each dose contained 1000 μg cobalamin. Total vitamin B_{12}, holotranscoblamin (holoTC), methylmalonic acid (MMA) and total homocysteine (tHcy) levels in serum were measured on days 0, 7, 14, 30, 60 and 90.

Results: Both dosage regimens resulted in a rapid increase of vitamin B_{12} and holoTC concentrations and normalization of initial high, MMA and tHcy concentrations. The loading dose regimen resulted in the fastest and greatest increase to a median vitamin B_{12} of 1090 pmol/L (reference 350-650 pmol/L) concentration after 14 days. Following weekly administration, B_{12} rapidly decreased to a median concentration of 530 pmol/L after 90 days. The no loading dose regimen resulted in a steady increase to a median vitamin B_{12} of 717 pmol/L after 90 days.

Conclusions: Intranasal vitamin B_{12} administration is an effective and suitable way to replenish and sustain vitamin B_{12} levels in elderly patients.

KEYWORDS
cobalamin, deficiency, dosage regimen, elderly, intranasal, vitamin B_{12}

1 | INTRODUCTION

Vitamin B_{12} (cobalamin) deficiency is common in the elderly population. It has been estimated that about 20% of the population aged 60 years or over and 23-35% of the population of 80 years or over has a vitamin B_{12} deficiency.[1,2] The lower vitamin B_{12} status in the elderly is caused by impairment of the absorption mechanism of food-bound vitamin B_{12}, less dietary intake and the use of certain drugs. Through a complex active absorption mechanism involving the acidic stomach environment, pepsin and intrinsic factor food-bound vitamin B_{12} are absorbed in the intestines and bound to transcobalamin II, transporting the vitamin B_{12} to the liver and other tissues.[3] Inside

drs MPH Tillemans and Dr K.J. Kaliswart are the principal investigators of this study.

these tissue cells, vitamin B_{12} serves as a coenzyme for several important biochemical reactions in the formation of blood and the functioning of the brain and nervous system. Consequently, deficiency may cause anaemia and neurological damage such as muscle weakness, cognitive decline, depression, fatigue, neuropathy and stroke.[4–10]

A vitamin B_{12} deficiency may take 3-4 years to develop due to the large cobalamin stores in the liver. Before a vitamin B_{12}-deficient state with classical symptoms such as pernicious anaemia is reached, a patient passes through a stage of inadequacy. During this stage the levels of biomarkers related to vitamin B_{12} deficiency, such as holotranscobalamin, methylmalonic acid and/or homocysteine, are already deviant. This stage is referred to as subclinical vitamin B_{12} deficiency and is associated with low or marginal vitamin B_{12} concentrations.[11–13]

The standard treatment for vitamin B_{12} deficiency is administration of cobalamin (hydroxo- or cyanocobalamin) via intramuscular injection. These injections, however, are painful, injection-related adverse events such as infections and bruises may occur, and self-administration by elderly patients is difficult. Safer and more convenient ways of administration such as oral and intranasal administration have therefore been explored. In recent years it has been shown that daily oral administration of crystalline cyanocobalamin is effective in normalizing serum cobalamin levels in deficient patients. As oral supplementation is limited by the amount which can be passively absorbed, it is not suitable for rapid supplementation in patients with very low vitamin B_{12} levels.[14,15] For the intranasal route, rapid and reproducible hydroxocobalamin and cyanocobalamin absorption has been demonstrated.[16,17] In a previous study we explored the pharmacokinetics of intranasally administered cyanocobalamin compared to intramuscularly administered hydroxocobalamin in cobalamin-deficient elderly people.[18] This study showed a rapid increase in serum cobalamin concentration after intranasal cobalamin administration. However, it did not provide insight into the pharmacokinetics and effects on vitamin B_{12} and related biomarkers after repeated intranasal administration. In the case of vitamin B_{12} deficiency due to dietary deficiency, intramuscular administration is usually initiated by a loading dose followed by maintenance treatment. This is contrary to oral treatment in which daily steady dose regimens are used due to the limited amount which can be passively absorbed. Whether or not a loading dose is required to rapidly treat vitamin B_{12} deficiency by way of intranasal administration is unknown, therefore the goal of this study was to compare two intranasal vitamin B_{12} dosage regimens (with and without loading dose) in elderly vitamin B_{12}-deficient patients on vitamin B_{12} levels and three biomarkers (holotranscoblamin [holoTC], methylmalonic acid [MMA] and total homocysteine [tHcy]) indicative for vitamin B_{12} deficiency. The effects of these two dosage regimens on cognition and depression were also explored.

What is already known about this subject

- Vitamin B_{12} deficiency and related complications are common in the elderly population.
- Intramuscular administration of vitamin B_{12} is painful, injection-related adverse events may occur and self-administration by elderly patients is difficult.
- Intranasal administration of vitamin B_{12} might be an alternative, and absorption in the elderly after intranasal administration has been shown. Knowledge of the pharmacokinetics, optimal dosing strategy and effects on vitamin B_{12} and related biomarkers after repeated intranasal administration is limited.

What this study adds

- This study shows that repeated intranasal administration of vitamin B_{12} is an effective way to rapidly replenish and sustain vitamin B_{12} levels and has a positive effect on vitamin B_{12}-related biomarkers.
- Elderly patients are able to properly administer the intranasal spray, and intranasal administration is well tolerated over time.
- Based on the results of this study, a dosage regimen for intranasal vitamin B_{12} administration has been proposed.

2 | METHODS

2.1 | Design, setting and study population

This randomized, open label, intervention study was carried out at the geriatric department of the Spaarne Gasthuis, a 626-bed teaching hospital situated in Haarlem and Hoofddorp in the Netherlands. The geriatric department consists of a geriatric acute ward and outpatient department for multi-problem patients, patients with cognitive dysfunction and patients from the fall clinic. All patients aged 65 years or over that were visiting the geriatric outpatient clinic or were admitted to the geriatric ward were eligible for inclusion. Inclusion criteria were a total vitamin B_{12} < 250 pmol/L measured in the past 3 months combined with either hyperhomocysteinemia (>15 μmol/L) or two or more symptoms possibly related to cobalamin deficiency, for example haemoglobin (Hb) below reference value, red blood cell count below reference value, fatigue, memory impairment, irritability, personality changes, muscle weakness, depression, poor appetite, and weight loss. Exclusion criteria were no ability or inability to give informed consent, unable to understand the study information or a Mini Mental State Examination (MMSE) score ≤19, inability to self-administer the intranasal spray (with the exception of help from a spouse or a home care nurse), concomitant use of other nasally administered medication, chronic rhinitis, the use of vitamin B_{12}-containing dietary supplements, severe renal impairment (glomerular filtration rate [GFR] < 20 mL/min), and ethical or medical reasons at the discretion of the investigators.

Informed consent was obtained at the beginning of the first visit by the research assistant. Before this study started a randomization

list following block randomization in blocks of six was created in Excel by the trial bureau. Based on the randomization list patients were allocated to either one of the two treatment arms by the pharmacist (M.T.), who labelled the intranasal spray and provided an administration schedule. Blood samples were collected at the outpatient department or at the patient's home by the research assistant on the day the patient was included ($t = 0$), after 7 days ($t = 7$), 14 days ($t = 14$), 30 days ($t = 30$) and 60 days ($t = 60$), and at the end of the study ($t = 90$). The Dutch version of the MMSE, the clock test and the Dutch version of the 15-item Geriatric Depression Scale (GDS) were executed by a trained research assistant, geriatric resident or geriatrician at the beginning ($t = 0$) and the end of the study ($t = 90$). The collection of blood samples, MMSE and GDS during the 90-day study period is displayed in Figure 1.

Sample size was set at 60 patients, with 30 patients per treatment arm. As there were no data on effect sizes prior to this study a proper power analysis could not be performed. If a patient was withdrawn from the study, this patient was replaced by another patient to complete the sample size. Patients could withdraw from the study at any time. The principal investigators (M.T. and K.K.) could decide to withdraw a subject from the study for lack of compliance, which was defined as missing more than two consecutive administrations, missing two consecutive blood samples collections or missing blood sample collections of all four biomarkers at $t = 0$ and/or $t = 90$.

The study was approved by the regional research ethics committee (METC Noord-Holland).

2.2 | Intervention

Patients were randomly assigned to one of two intranasal dosage regimens. Each dosage regimen lasted 90 days. The intranasal vitamin B_{12} spray was produced by the Pharmacy Foundation of Haarlem Hospitals (SAHZ).[18] A dose of 1000 μg of cyanocobalamin was administered by administering one spray of 500 μg in each nostril. The dosage regimen of the loading dose treatment arm was based on our previous study and pharmacokinetic data in healthy volunteers using Nascobal® intranasal cobalamin spray.[19] Nascobal® is available in the United States and is indicated for maintenance therapy for pernicious anaemia patients and as a supplement for vitamin B_{12} deficiency. Nascobal® contains 500 μg of cyanocobalamin and is administered once weekly. The dosage regimen for the loading dose treatment arm was set at 1000 μg daily for 14 days, followed by weekly administration of 1000 μg. The dosage regimen in the no loading dose treatment arm was based on the registered intramuscular cobalamin dosage regimen. Initially, intramuscular cobalamin 1000 μg injection is administered every 3 days, therefore an intranasal dosage regimen of 1000 μg every 3 days was used in the no loading dose treatment arm.

2.3 | Outcome measures

The primary outcome measures were changes in total vitamin B_{12} and holoTC serum concentrations over time and compared between the two treatment arms. Since total serum vitamin B_{12} is a relative unspecific and insensitive biomarker for vitamin B_{12} deficiency, serum concentrations of three other biomarkers, holoTC, tHcy and MMA, were measured in addition to the total vitamin B_{12} levels in this study. HoloTC or active vitamin B_{12} is the amount of vitamin B_{12} bound to transcobalamin II. Transcobalamin II is a transport protein that delivers vitamin B_{12} to the cells and therefore represents the biologically active amount of vitamin B_{12}. Both MMA and tHcy levels are functional markers of vitamin B_{12} deficiency. Vitamin B_{12} is an essential co-factor in the conversion of methylmalonyl-coenzyme A (CoA) from MMA to succinyl-CoA and the conversion of Hcy to Methionine. As a result, MMA and tHcy levels will rise in cases of vitamin B_{12}

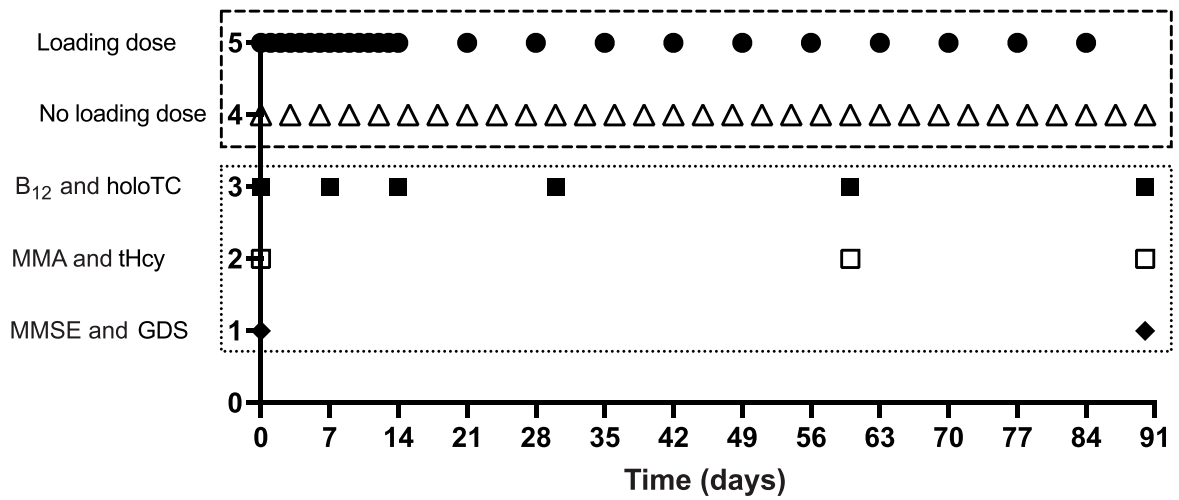

FIGURE 1 Administration schedules and measurements over time. Loading dose (5): 25 doses of 1000 = 25.000 μg over 90 days. No loading dose (4): 31 doses of 1000 = 31.000 μg over 90 days. Measurements B_{12} and holoTC (3) on $t = 0$, 7, 14, 30, 60 and 90 days. MMA and tHcy (2) on $t = 0$, 30 and 90 days. MMSE and GDS (1) on $t = 0$ and 90 days.

deficiency. Changes in MMA and tHcy over time were therefore secondary outcome measures in this study.[20]

A total vitamin B_{12} concentration in serum within the reference range of 350 and 650 pmol/L was considered appropriate for these patients. A mean difference of 100 pmol/L within this range between the two treatment arms was considered relevant. In addition, a holoTC concentration >35 pmol/L was considered adequate. A MMA concentration <0.27 μmol/L and a tHcy concentration <15 μmol/L were considered appropriate.

The collected serum samples were stored in a freezer at −80°C at the clinical chemical laboratory Atalmedial of the Spaarne Gasthuis until they were analysed. Sample analysis was carried out at the clinical chemical laboratory of the Erasmus Medical Centre in Rotterdam, the Netherlands. Total serum vitamin B_{12} concentrations were determined using an electrochemiluminiscent immunoassay E602 Cobas 8000 (Roche) with a measuring range of 22-1476 pmol/L. HoloTC concentrations were determined using a chemiluminiscent microparticle immunoassay on the Architect i system (Abbott) with a measuring range of 5-300 pmol/L. Secondary outcome measures were changes in MMA and tHcy serum concentrations over time. Both the MMA and Hcy concentrations were determined by liquid chromatography tandem mass spectrometry. The measuring ranges for MMA were 0.05-50 μmol/L and for Hcy 0.2-650 μmol/L.

For the exploratory outcome measures of cognitive performance and mood changes, the MMSE + clock test and 15-item GDS were executed at the beginning ($t = 0$) and at the end of the study ($t = 90$) by a trained research assistant, geriatric resident or geriatrician. A MMSE score <24 was used as the cut-off point for dementia. For the GDS scores the following indications for depression were used: 0-4 no depression, 5-8 mild depression, 9-11 moderate depression,12-15 severe depression.[21,22]

2.4 | Statistical analyses

Statistical analyses were carried out using IBM SPSS statistics software version 28.0.1.0 for Mac. Graphs were made with GraphPad Prism version 9.5.0 for MacOS. The Kolmogorov-Smirnov test was used to test whether the data followed a normal distribution. An independent sample *t*-test was used to test differences between normally distributed continuous variables. The Mann-Whitney U test was used to compare differences between not normally distributed variables. In case the variable was not normally distributed, the median and corresponding 25% and 75% interquartile ranges were calculated.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[23]

3 | RESULTS

Over time, a total of 100 patients were included in the study. Of these, 24 patients did not complete the 90-day study period, two patients were excluded for starting other intranasal administered medication, five patients ended study participation at their own request, nine patients were excluded by the researcher due to lack of compliance or because of a Covid-19 lockdown, seven patients were lost in follow up and one patient died. Of the 76 patients that completed the 90-day study period, 16 patients had to be excluded from the final analysis because of missing samples or incorrect handling of the samples. The baseline characteristics of the total study population and both treatment arms are shown in Table 1.

The median changes in total vitamin B_{12} and holoTC concentrations over time are shown in Figure 2A,B. Within the loading dose regimen, daily intranasal cobalamin administration resulted in a rapid increase in total serum vitamin B_{12} concentrations from a median of 230 to 1090 pmol/L after 2 weeks. This was followed by a rapid decrease in vitamin B_{12} concentrations after switching to weekly administration, resulting in a median vitamin B_{12} concentration of 530 pmol/L after 90 days. Contrary to the findings for the loading dose regimen, the no loading dose regimen resulted in a more gradual increase of vitamin B_{12} concentrations over time from 224 to 717 pmol/L after 90 days. The difference in median vitamin B_{12} concentration between the two dosage regimens was significant ($P < 0.001$) at seven and 14 days, but not at 90 days. Overall, the holoTC concentrations showed a similar pattern in both dosage regimens compared to the vitamin B_{12} concentrations. Daily administration in the loading dose regimen resulted in a median holoTC concentration of 300 pmol/L, the upper limit of the measuring range. Weekly administration led to a gradual decrease to a median holoTC concentration of 184 pmol/L after 90 days. Also, the no loading dose regimen showed a more gradual increase to a median holoTC concentration of 260 pmol/L after 90 days.

The median MMA and tHcy concentrations over time are shown in Figure 3A,B. The MMA and tHcy concentrations showed a similar pattern in both dosage regimens. Both MMA and tHcy decreased in the first 30 days of intranasal B_{12} supplementation and remained at this level until the end of the study. The MMA concentrations decreased from 0.27 μmol/L in the loading dose regimen and 0.30 in the no loading dose regimen to approximately 0.20 μmol/L at 30 and 90 days in both treatment arms. The tHcy concentration decreased from 19 μmol/L in the loading dose regimen and 18 μmol/L in the no loading dose regimen to approximately 15 μmol/L at 30 and 90 days in both treatment arms.

For various reasons the MMSE and clock test were not carried out on all patients at the beginning and end of the study. In 39 patients, 19 in the loading dose treatment arm and 20 in the no loading dose treatment arm, the MMSE was executed on both days. The median MMSE score was 27 in the loading dose regimen *vs.* 28 in the no loading dose regimen at the beginning and 27 in both dosage

TABLE 1 Baseline characteristics.

	Total (n = 60)	Loading dose (n = 30)	No loading dose (n = 30)
Social demographic factors			
Sex, male % (n)	70 (42)	70 (21)	70 (21)
Age, years mean (range)	78 (65-93)	77.4 (68-87)	78.6 (65-93)
Body mass index, mean (range)	26.3 (18.8-36.2)	26.2 (18.8-36.2)	26.4 (19.5-35.1)
Medication use potentially associated with B_{12} deficiency			
metformin,% (n)	23.3 (14)	20 (6)	26.7 (8)
Proton pump inhibitor, % (n)	53.3 (32)	50 (15)	56.7 (17)
Symptoms potentially associated with B_{12} deficiency			
Low Hb, % (n)	30 (18)	30 (9)	30 (9)
Low RBC count, % (n)	41.7(25)	33.3 (10)	50 (15)
Fatigue, % (n)	20 (12)	23.3 (7)	16.7 (5)
Memory impairment, % (n)	80 (48)	80 (24)	80 (24)
Irritability, % (n)	3.3 (2)	3.3 (1)	3.3 (1)
Personality changes, % (n)	20 (12)	16.7 (5)	23.3 (7)
Muscle weakness, % (n)	50 (30)	50 (15)	50 (15)
Depression, % (n)	20 (12)	23.3 (7)	16.7 (5)
Poor appetite, % (n)	3.3 (2)	3.3 (1)	3.3 (1)
Weight loss, % (n)	8.3 (5)	6.7 (2)	10 (3)
Biomarkers for vitamin B_{12} deficiency			
Vitamin B_{12} (pmol/L), median (IQR 25%-75%)	224 (183-252)	230 (176-277)	224 (180-239)
HoloTC (pmol/L), median (IQR 25-75%)	67.5 (54.5-86.3)	74.0 (52-95.8)	66.5 (54.5-79.3)
MMA (μmol/L), median (IQR 25-75%)	0.30 (0.2-0.44)	0.27 (0.23-0.58)	0.30 (0.25-0.40)
tHcy (μmol/L), median (IQR 25-75%)	18.0 (15.2-22.3)	18.0 (15.0-23.7)	18.0 (15.4-22.0)

Abbreviations: holoTC, holotranscobalamin; low Hb, haemoglobin below reference value; low RBC, red blood cell count below reference value; MMA, methylmalonic acid; tHcy, total homocysteine.

regimens at the end of the study. The clock test was performed in only 26 patients, 12 in the loading dose treatment arm and 14 in the no loading dose treatment arm, at the beginning and end of the study. There were also no differences in the clock test performance between the treatment arms.

The GDS-15 was carried out in 34 patients, 17 in each treatment arm, at the beginning and at the end of the study. At the beginning the median GDS score was 2.0 in the loading dose treatment arm *vs* 3.5 in the no loading dose treatment arm and it was 2.5 *vs* 2.0 after 90 days. Three patients in the loading dose treatment arm and seven in the no loading dose treatment arm had a GDS score ≥5 at the beginning of the study, which decreased to two patients with a GDS score ≥5 after 90 days in both treatment arms.

One patient died and one patient was hospitalized during study participation. Both events were reported as serious adverse events. A relationship between study participation and these events was considered unlikely. Adverse events were reported in 10 patients. Of these, six patients were assigned to the loading dose treatment arm and four patients to the no loading dose treatment arm. The reported adverse events consisted of rash, headache, nosebleed, fatigue, (nose) cold and (stomach) flu.

4 | DISCUSSION

To our knowledge, this is the first study in which the effect of two dosage regimens of intranasal cobalamin, with and without loading dose, were compared in elderly vitamin B_{12}-deficient patients. Both dosage regimens resulted in a rapid increase in vitamin B_{12} and holoTC concentrations, and a decrease in MMA and tHcy concentrations. Patients in the loading dose regimen showed the fastest and greatest increase in vitamin B_{12} and holoTC concentrations. After switching to weekly administration, the vitamin B_{12} concentration rapidly decreased to a concentration within the reference range (350-650 pmol/L). The no loading dose regimen resulted in a steady increase in vitamin B_{12} and holoTC concentrations over time. No substantial differences were observed between the two treatment arms.

Currently, intramuscular administration of vitamin B_{12} is the treatment of choice in patients with low vitamin B_{12} levels. As injections are painful, injection-related adverse events may occur and injections cannot be self-administered, therefore there is a need for safer and more convenient ways to treat vitamin B_{12} deficiency in elderly patients. Intranasal administration might be a safe and convenient alternative, but there is limited knowledge on the most suitable

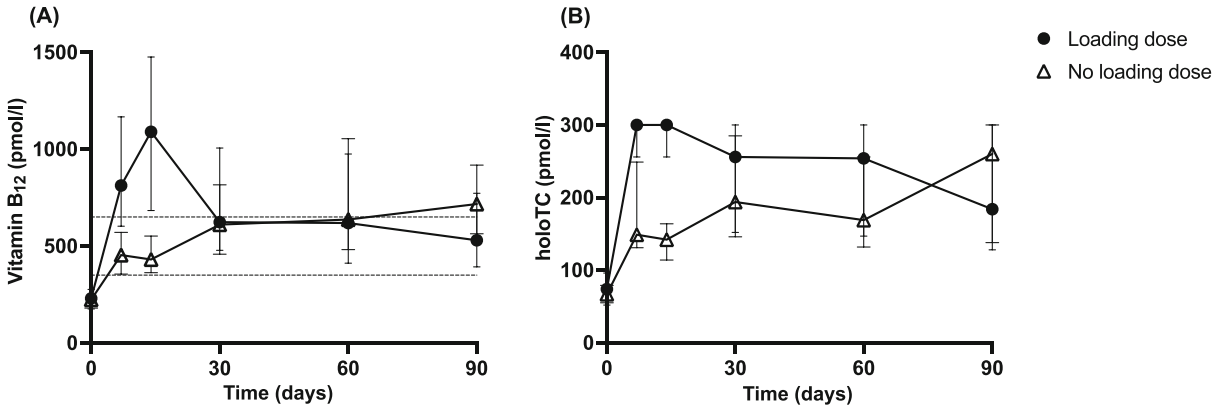

FIGURE 2 Vitamin B_{12} and holoTC concentrations over time. (A) Median vitamin B_{12} concentrations (± interquartile range 25-75%) over time. The dotted lines represent the appropriate range (350-650 pmol/L). (B) Median holotranscobalamin (holoTC) concentrations (± interquartile range 25-75%) over time.

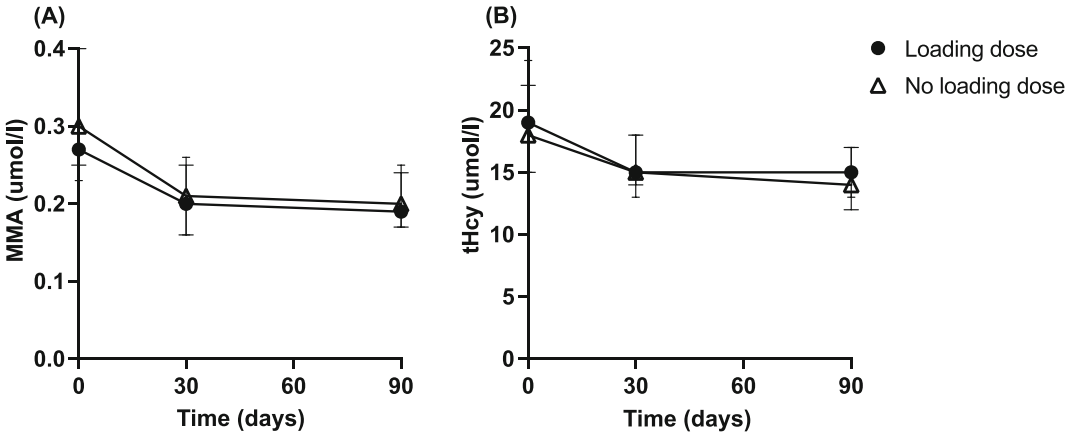

FIGURE 3 MMA and tHcy concentrations over time. (A) Median methylmalonic acid (MMA) concentrations (± interquartile range 25-75%) over time. (B) Median total homocystein (tHcy) concentrations (± interquartile range 25-75%) over time.

dosage regimen. The primary aim of this study was therefore to compare two dosage regimens to determine an appropriate dosage regimen for intranasal vitamin B_{12} administration in elderly vitamin B_{12}-deficient patients. The loading dose regimen resulted in a rapid increase in vitamin B_{12} concentration, exceeding the upper limit of the reference range after 7 days. This was followed by a rapid decrease in vitamin B_{12} concentration on switching to the weekly maintenance dose. The holoTC concentrations, however, showed a more or less gradual decrease on switching from daily to weekly administration. This indicates that the retention of vitamin B_{12} in the body is limited by the transcobalamin II binding capacity resulting in a rapid elimination of unbound vitamin B_{12}. Daily intranasal cobalamin administration of 1000 μg therefore does not seem to be necessary. Intranasal administration of cobalamin every 3 days (the no loading dose regimen) resulted in a more gradual increase in vitamin B_{12} compared to daily administration. After 1 month of treatment, however, the vitamin B_{12} concentration also started to exceed the upper limit of the reference range. Based on these results a combination of both dosage regimens, for instance 2 weeks of intranasal cobalamin administration every 3 days followed by weekly administration, seems appropriate for the treatment and maintenance of vitamin B_{12} deficiency in older patients. This dosage regimen has not been tested in the current study.

Over recent years oral administration of at least 1000 μg of crystalline cyanocobalamin per day has been shown to be effective in normalizing serum cobalamin levels in deficient patients. Absorption of crystalline cyanocobalamin, however, is limited as only 1-5% is estimated to be absorbed by passive diffusion.[24] Rapid replenishment of the depleted vitamin B_{12} stock is considered necessary in patients with critically low vitamin B_{12} concentrations (<150 pmol/L) and/or clinical symptoms to prevent anaemia and irreversible damage to the brain and nervous system. This study showed rapid increases in both vitamin B_{12} and holoTC concentrations after intranasal administration, therefore intranasal administration is preferred over oral administration in patients with critically low vitamin B_{12} or holoTC concentrations and/or elevated levels of MMA or tHcy.

This study was specifically performed in the elderly population, in which vitamin B_{12} deficiency is most common. Repeated intranasal administration over a 90-day study period not only provided insight into vitamin B_{12} and its related biomarkers, but also the applicability and suitability of intranasal administration of vitamin B_{12}. This study showed that elderly patients are able to properly administer the spray and that it is well tolerated over time which is, in combination with the positive effect on vitamin B_{12} and related biomarkers, supportive for the use of intranasal administration of vitamin B_{12}.

Although vitamin B_{12} deficiency is more prevalent in the elderly population, it also occurs in the younger population, for example in intrinsic factor deficiency, malnutrition or strict vegan/vegetarian diets. The absorption and pharmacokinetics of our intranasal vitamin B_{12} spray have not been studied in a younger population. Absorption data for Nascobal® in healthy subjects and application of a cobalamin nasal gel in pernicious anaemia patients indicate that intranasal administered cobalamin might also be a suitable alternative in a younger population. In clinical practice, pernicious anaemia patients with neurological symptoms require high doses of intramuscular administered vitamin B_{12} (eg, 1000 µg twice a week) to alleviate their symptoms. Intranasal administration showed rapid increases in vitamin B_{12} and holoTC levels, which might be sufficient to treat these patients, but this needs to be examined in further research.

Vitamin B_{12} deficiency has been associated with cognitive impairment and depression as vitamin B_{12} plays a major role in the functioning of the brain and nervous system. The effect of vitamin B_{12} treatment on cognitive function or depression is not yet clear. In a recent systematic review, the effects of 1000 µg of vitamin B_{12} by daily oral or weekly intramuscular injection on cognitive function and depression were assessed.[25] In this review four randomized controlled trials assessing the effects of vitamin B_{12} on cognitive function and only one assessing the effects on depression were included. Overall, no significant treatment effects on cognitive function or depression in elderly patients with no or mild cognitive impairment (MCI) and mild vitamin B_{12} deficiency were shown. The patients in our study had comparable cognitive function and vitamin B_{12} status. We also did not find any differences in cognitive performances or depression over time or between the two dosage regimens. Most patients in our study had GDS scores <5, not indicative of depression, at the beginning of the study. However, in the no loading dose treatment arm the number of patients with a GDS score ≥5 decreased from seven at the beginning to two at the end of the study period. This could be indicative for a relationship between vitamin B_{12} and depression. Our study period comprised only 90 days, which might not be long enough to establish the beneficial effects of B_{12} treatment on cognitive function or depression.

One of the strengths of this study is that it was performed in elderly patients, a population in which vitamin B_{12} deficiency is common. A potential limitation of this study is that there currently is no consensus on the cut-off values for total serum vitamin B_{12} concentrations or other biomarkers related to vitamin B_{12} deficiency. As a result, a wide variety of cut-off values have been used in studies exploring vitamin B_{12} deficiency. A cut-off value of 250 pmol/L for total vitamin B_{12} was used in this study as there are patients with a vitamin B_{12} deficiency at the cellular level who have low or marginal (150-249 pmol/L) vitamin B_{12} concentrations. HoloTC is the least-studied biomarker and cut-off levels range between 20 and 50 pmol/L. Based on the study by Herrmann et al, the cut-off for holoTC in the present study was set at 35 pmol/L. In most studies the MMA cut-off values ranged from 0.26 to 0.28 µmol/L, hence we used a cut-off value of 0.27 µmol/L. The most commonly used cut-off value for tHcy in studies is 15 µmol/L. For that reason, a cut-off value of 15 µmol/L for tHcy was also used in this study.[20,26–29] As holoTC and MMA concentrations could not be measured at the clinical laboratory of the Spaarne Gasthuis, we were unable to use these biomarkers as inclusion criteria, therefore in this study a vitamin B_{12} deficiency at the moment of inclusion was defined as a total vitamin B_{12} concentration <250 pmol/L combined with a tHcy >15 µmol/L or at least two clinical symptoms potentially related to vitamin B_{12} deficiency.

At the start of the study both the median MMA and tHcy were elevated, supporting vitamin B_{12} deficiency. However, based on the reference values described by Hermann et al, median holoTC levels did not indicate vitamin B_{12} deficiency at the beginning of the study. Currently, little is known about the association between these four biomarkers. Van Wijngaarden et al. studied the relationship between dietary intake of vitamin B_{12} and the related biomarkers in an elderly population in the Netherlands.[30] It was shown that all four biomarkers were significantly associated with one another. In addition, MMA and tHcy levels started to rise when total vitamin B_{12} serum concentrations dropped below 330 pmoL/and holoTC dropped below 100 pmol/L. Furthermore, MMA and tHcy showed a steeper rise when vitamin B_{12} levels dropped below 220 pmol/L and holoTC below 50 pmol/L. In this study the holoTC levels on the first day of the study were approximately 70 pmol/L in both treatment arms, which is, in combination with elevated MMA and/or tHcy levels, indicative for vitamin B_{12} deficiency according to the study by van Wijngaarden et al.

It was more difficult than expected in this study population to not only include the required number of patients but also to complete the 90-day study period. As a result, some of the samples were stored in a freezer at −80°C for several years before batch analysis took place. Long-term storage in a freezer might have influenced the stability of the samples. In one study a significant decrease in vitamin B_{12} concentrations was shown after long-term storage of samples at −80°C.[31] As a decrease in vitamin B_{12} concentrations in the older samples could not be ruled out, we compared the vitamin B_{12} concentrations of the first 10 patients with the last 10 patients in both dosage regimens. The range of the vitamin B_{12} concentrations measured on the first and last days of the study was comparable between the first 10 patients and the last 10 patients and the median vitamin B_{12} concentrations did not differ significantly. We therefore have no indication that the vitamin B_{12} concentration had decreased during storage in the oldest samples compared to the concentrations in the samples of the last patients included in the study.

5 | CONCLUSION

Intranasal vitamin B_{12} administration is an effective and suitable way to replenish and sustain vitamin B_{12} levels in vitamin B_{12}-deficient elderly patients. No substantial differences were observed between the two treatment arms in this study. A combination of the loading dose and no loading dose regimen, with administration every 3 days for 2 weeks followed by weekly administration, seems the most appropriate dosage regimen for the treatment of vitamin B_{12} deficiency in elderly patients.

AUTHOR CONTRIBUTIONS

M.P.H. Tillemans, K.J. Kalisvaart and T.C.G. Egberts designed the research. J.H. Hooijberg provided essential materials. M.P.H. Tillemans conducted the research and analysed the data. M.P.H. Tillemans, T.J. Giezen and T.C.G. Egberts wrote the first draft of the manuscript. K.J. Kalisvaart had primary responsibility for final content. All authors interpreted the data, critically revised the manuscript, and read and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to report related to the work presented in this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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