# The effect of vitamin B12 and folic acid supplementation on routine haematological parameters in older people: an individual participant data meta-analysis


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**Abstract**

Background

Low vitamin B12 and folate levels in community-dwelling older people are usually corrected with supplements. However, the effect of this supplementation on haematological parameters in older persons is not known. Therefore, we executed a systematic review and individual participant data meta-analysis of randomized placebo-controlled trials (RCTs).

Methods

We performed a systematic search in PubMed, EMBASE, Web of Science, Cochrane and CENTRAL for RCTs published between January 1950 and April 2016 where community-dwelling elderly (60+ years) who were treated with vitamin B12 or folic acid or placebo. The presence of anaemia was not required. We analysed the data on haematological parameters with a 2-stage IPD meta-analysis.

Results

We found 494 full papers covering 14 studies. Data were shared by the authors of four RCTs comparing vitamin B12 with placebo (n=343) and of three RCTs comparing folic acid with placebo (n=929). We found no effect of vitamin B12 supplementation on haemoglobin (change 0.00 g/dL, 95% CI -0.19;0.18), and no effect of folic acid supplementation (change -0.09 g/dL, 95% CI -0.19;0.01). The effects of supplementation on other haematological parameters were similar. The effects did not differ by sex or by age group. Also, no effect was found in a subgroup of patients with anaemia and a subgroup of patients who were treated >4 weeks.

Conclusions

Evidence on the effects of supplementation of low concentrations of vitamin B12 and folate on haematological parameters in community-dwelling older people is inconclusive. Further

research is needed before firm recommendations can be made concerning the supplementation of vitamin B12 and folate.

## Background

The prevalence of anaemia in older persons is high (around 10% among people aged ≥65 years) and rises with advancing age (1, 2). In about one third of older persons with anaemia co-incidental nutritional deficiencies, such as iron, vitamin B12 and folate deficiency, exist [(2). Among people aged ≥75 years, the prevalence of both vitamin B12 and folate deficiency is >10% (3-7).These deficiencies are not only associated with macrocytic anaemia but also with dementia, peripheral neuropathy, combined degeneration of the spinal cord and cardiovascular disease (8-11).

As the prevalence of deficiencies in vitamin B12 and folate are high, screening for deficiencies in vitamin B12 and folate has been recommended as part of a geriatric work-up (12, 13). Guidelines recommend vitamin B12 supplementation in patients with very low serum vitamin B12 concentrations due to lack of intrinsic factor (IF) (pernicious anaemia) or food-vitamin B12 malabsorption. Several studies have shown significant increases in haemoglobin after vitamin B12 administration in these patients (14-16). Thus, when low levels of vitamin B12 and folate are found, patients are often treated with injections or oral supplements (9, 11, 17).

Interestingly, evidence of an association between a low serum vitamin B12 concentration and anaemia in older individuals in the general population is limited and inconclusive. Data from the Leiden 85-plus Study showed that low vitamin B12 concentrations (<150 pmol/L) in 85-year-old persons are not associated with anaemia (18). Also, the effect of vitamin B12 supplementation on haemoglobin and Mean Corpuscular Volume (MCV) in elderly with low levels of vitamin B12 is unclear (19).

In contrast to vitamin B12 deficiency, folate deficiency seems to be associated with the presence and the development of anaemia in older individuals (18). Therefore, early detection of folate deficiency may identify older individuals at risk of anaemia. Preventive

folic acid supplementation or folic acid fortification of grain and cereal products has been recommended and has already been initiated at population level in several countries. However, to date, it is not known if older persons benefit from of prophylactic folic acid supplementation with regards to haemoglobin levels, as no evidence is available to support this assumption.

In order to evaluate the effect of vitamin B12 and folic acid supplementation on haematological parameters in elderly, we executed a systematic review and individual participant data meta-analysis of randomized placebo-controlled trials.

## Methods

*Criteria for considering studies for this meta-analysis*

We considered all randomized controlled trials (RCTs) where community-dwelling elderly (60+ years) were treated with vitamin B12 or folic acid (all dosages and all forms of administration) and were compared with elderly who were given a placebo. The presence of anaemia or a vitamin B12/folate deficiency was not required for inclusion. Exclusion criteria were: mean or median age less than 60 years, no availability of haemoglobin concentrations or haematocrit at baseline and during follow-up, combinations of vitamin B12 and folic acid supplementation, and specific populations (e.g. studies in diabetes patients or patients with renal failure).

*Search strategy*

We performed a search on PubMed, EMBASE, Web of Science, Cochrane and CENTRAL with relevant MeSH-headings and title words for vitamin B12 and folic acid for randomized controlled trials (RCTs) published between January 1950 and April 2016. Case reports and letters were excluded. We also searched ClinicalTrials.gov for (ongoing) trials on vitamin B12 or folic acid supplementation in older persons. We also used backward and forward

citation screening. The electronic search was performed by an information scientist from the Walaeus Library of the Leiden University Medical Center. The exact search strategy can be found in Supplementary file 1.

*Selection of studies*

All titles and abstracts found in the electronic databases were assessed by the last author. Full copies were acquired of articles that were potentially relevant or in case of uncertainty. WPJ den Elzen and LW Bermingham appraised these full copies. Disagreement was resolved by consensus. Investigators from each eligible study were invited to join the project and to share their data. The risk of bias of the included studies was assessed using the Cochrane risk of bias assessment table (see Supplemental File 3).

*Data collection*

We centrally collected data on demographic characteristics, pre- and post-treatment concentrations of serum vitamin B12, serum / red cell folate, homocysteine, methylmalonic acid, haemoglobin, as well as on levels of haematocrit, Mean Corpuscular Volume (MCV) and Red Blood Cell Count (RBC) for all individuals included in the studies. Since vitamin B12 and folate deficiency is defined ideally in terms of serum values of vitamin B12 and folate in combination with homocysteine and methylmalonic acid (8) we also collected data on homocysteine and methylmalonic acid, when available.

*Analyses*

We combined the results of the individual studies into two meta-analyses; one on the effect of vitamin B12 supplementation and one on the effect of folic acid supplementation on haematological parameters. We performed a two-stage individual participant data meta-analysis. First, for all individual studies separately, we calculated the mean change in haematological parameters between baseline and follow-up (i.e. follow-up minus baseline) in the active treatment group, and in the placebo group (IBM SPSS Statistics 20). Subsequently,

we calculated the difference in mean change (together with its standard error) between the active treatment group and placebo group. Then, pooled estimates for each outcome were calculated using a fixed-effects model, using Review Manager version 5.3.3 The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. We measured statistical heterogeneity using the $I^2$ statistic. An $I^2$ value greater than 50% indicates at least moderate statistical heterogeneity (20).

We performed subgroup analyses for sex and age. Interaction between treatment and sex, and treatment and age, was evaluated by linear regression analysis. Further post-hoc analyses were performed in the subgroup of participants with anaemia at baseline, in MCV subgroups, and in studies with a short (≤4 weeks) versus long duration of treatment (>4 weeks).

## Results

*Selection of studies*

The electronic search identified 5691 potentially relevant titles and abstracts of papers (performed 25-3-2016). We identified 494 papers, of which 47 fulfilled our inclusion criteria (Figure 1). Investigators from these studies were invited to join the project and to share their data. Eight authors could not provide haematological data because these were not measured or because individual data were not available anymore. Seven authors did not respond to our request (21-27). The authors of the other 32 papers (n=10 for vitamin B12 and n=22 for folic acid) agreed to participate in this project and to share their data on haemoglobin concentrations or haematocrit fractions after supplementation. These 32 papers comprised 4 unique studies on vitamin B12 supplementation (28-31) and 3 unique studies on folic acid supplementation (Table 1) (32-34). No additional (ongoing) studies were found on ClinicalTrials.gov. The risk of bias of included studies was low (see Supplemental File 1).

Table 1 describes a summary of the characteristics of the included studies. The sample sizes of the included studies varied from 'n=24' (33) to 'n=802'(32). Also, the dosage and the forms of administration and the time to follow-up varied to a great extent (Table 1).

*Heterogeneity*

We found no indications for statistical heterogeneity in the studies on vitamin B12 ($I^2$=0%) for all 4 outcomes, but for the studies on folic acid we found indications for at least moderate statistical heterogeneity ($I^2$=72%) when analysing RBC as an outcome. Also, clinical heterogeneity existed between studies due to different methods of administration, dosage of vitamin B12 and folic acid, outcomes and follow-up time. Therefore, we show both the results of the individual studies and the results of the pooled effect estimates (Table 2).

*Results of analyses*

In table 2 we show the baseline values of haemoglobin, vitamin B12 and folate. The numbers of individuals with anaemia was relatively small. None of the individual studies showed a significant effect of vitamin B12 (total n=343) or folic acid supplementation (total n=929) on changes in haematological parameters, except for the study by Ntaios on RBC (Table 3). The pooled estimate of the effect on haemoglobin was 0.00 g/dL (95% CI -0.19;0.18) for vitamin B12 supplementation and -0.09 g/dL (95% CI -0.19;0.01) for folic acid supplementation, meaning that there was no difference in the mean change in haemoglobin concentrations during follow-up between vitamin B12 and placebo, and folic acid and placebo.

In addition, no differences in the mean change in other haematological parameters were observed. For vitamin B12 supplementation, the pooled estimate of the effect was 0.00 g/dL (95% CI -0;0.01) for haematocrit, 0.07 g/dL (95% CI -0.58;0.72) for MCV and 0.00 g/dL (95% CI -0.06;0.06) for RBC. For folic acid supplementation, the pooled estimate of effect was -0.00 g/dL (95% CI -0.01;0) for haematocrit, -0.37 g/dL (95% CI -0.82;0.08) for MCV and -0.02 g/dL (95% CI -0.05;0.02) for RBC.

156

157 *Subgroup analyses*

158 In none of the subgroups on sex and age a significant effect of vitamin B12 or folic acid
159 supplementation on haemoglobin concentrations was found (Table 4). The effect of vitamin
160 B12 and folic acid on haemoglobin was the same for men and women ($P_{interaction}$=0.577 for
161 vitamin B12 and $P_{interaction}$=0.545 for folic acid) or between different age groups
162 ($P_{interaction}$=0.793 for vitamin B12 and $P_{interaction}$=0.836 for folic acid). Similar results were
163 found when we repeated the analyses for haematocrit, MCV and RBC (data not shown).

164 *Post-hoc analyses*

165 When we repeated the analyses in participants with anaemia, we did not observe differences
166 in the change in haemoglobin levels between those who were treated with vitamin B12 and
167 those treated with placebo (table 4). In those treated with folic acid, a significantly larger
168 decline in haemoglobin levels was observed (table 3). Moreover, when we stratified on MCV
169 (<80 fL, 80-100 fL, >100 fL), in none of the subgroups we observed an effect of vitamin B12
170 or folic acid administration on haemoglobin. In addition, no effect on haemoglobin was found
171 in studies with treatment duration of ≤4 weeks or in studies with a treatment duration of >4
172 weeks, both with vitamin B12 and folic acid (Table 4).

173 *Results from studies without individual participant data*

174 Of the 7 studies that could not be included because of a lack of response from the authors, 4
175 studies did not report having measured haemoglobin and/or haematocrit levels. We here
176 discuss the 3 studies that reported having measured haemoglobin and/or haematocrit levels
177 but of which we did not retrieve data (see Supplemental File 2).

178 The first study, by Smidt et al., measured haemoglobin and haematocrit at baseline
179 (25), but did not report haematological data at follow-up. The second study, by Hughes et al.,
180 measured haemoglobin at baseline and at follow-up (26). In their study, 93 men and 132

women were given intramuscular hydroxocobalamin (1,000 ug.), twice in the first week and then at weekly intervals for a further four weeks. During their study, there was a small fall in haemoglobin level, but the difference between the mean changes in those given B12 and those given placebo was small and not statistically significant (0.01 ± 0.35 g.). In the third study by Rampersaud et al. in thirty-three healthy, postmenopausal women aged 60–85 years, treatment groups received two different folate repletion intakes [ie, ≈200 and ≈400 ug folate/d] (23). In this study haematocrit values did not change significantly over the 14-week study period.

**Discussion**

In this individual participant data meta-analysis we did not observe any significant measurable change in routine haematological parameters after treatment with vitamin B12 or folic acid in older persons with either normal or vitamin B12 or folate concentrations below the reference range.

There are several possible explanations for the lack of effect. First, some studies consisted mostly of participants without vitamin B12 or folate deficiency or just below the cut-off values. As these participants may not have had a true tissue deficiency of vitamin B12 or folate, this may have diluted the effect of supplementation. Second, many of the included studies mainly consisted of participants without anaemia. Perhaps haemoglobin levels in non-anaemic patients are less likely to increase in response to vitamin B12 or folic acid treatment than haemoglobin levels in anaemic patients (29). Unfortunately, the low numbers of people with anaemia refrained us from drawing definite conclusions on the effects of supplementation in an anaemic population with low vitamin B12 or folate concentrations. Third, a low vitamin B12 or folate concentration alone may not be the only reason to develop anaemia, and treatment of these low levels may not be sufficient to raise haemoglobin levels.

Other genetic or environmental factors may be involved in the onset of anaemia (19). Also, other causes such as chronic inflammation may play a role in the development of anaemia (35).

Macrocytic anemia is one of the most well-known consequences of vitamin B12 and folate deficiency. An elevated Mean Corpuscular Volume (MCV) is often seen as an indication to test for the presence of vitamin B12 or folate deficiency. However, both the sensitivity and the specificity of a high MCV for these deficiencies are low (10, 36). In our study, we did not observe an effect of vitamin B12 and folic administration on the change in MCV. Also, we did not observe an effect of vitamin B12 or folic acid on haemoglobin in the MCV subcategories. We have to be cautious in interpreting these results as the groups are small and the heterogeneity between studies is large. However, these results are in line with previous analyses in the Leiden 85-plus Study where no relationship was found between vitamin B12 or folate deficiency and changes in MCV over time (18).

This review has some weaknesses. First, there was substantial clinical heterogeneity between studies, due to differences in methods of administration, dose of vitamin B12 and folic acid, outcome measures, treatment follow-up time and sample size, so the results of the meta-analysis have to be interpreted with caution. However, the fact that the results of the individual studies point in the same direction is reassuring. Second, in the post-hoc analyses in the subgroup with anaemia, we observed a significantly larger decline in haemoglobin levels in those treated with folic acid. This unexpected finding may perhaps be explained by random error due to multiple testing and the low number of participants this subgroup. Third, we cannot exclude the possibility of bias as not all studies that were identified in our systematic search of the literature could be included, because some authors did not respond to our request or individual participant data were no longer available. However, these studies

confirm our findings as they also did not observe significant changes in haemoglobin or haematocrit levels (23, 25, 26).

## Conclusions

We did not observe any significant change in routine haematological parameters after treatment with vitamin B12 or folic acid in older persons with either normal vitamin B12 or folate concentrations, or concentrations of vitamin B12 or folate below the reference range. However, we cannot draw firm conclusions because the amount of studies was low, relatively few individuals with anaemia could be included, and the clinical heterogeneity between studies was substantial. Furthermore, we cannot draw conclusions on other benefits of supplementation of vitamin B12 or folate other than haematological outcomes.

Further well-designed large studies are required to determine whether vitamin B12 and folic acid supplementation are beneficial for older patients with low vitamin B12 or folate concentrations and anaemia or prophylactic effects of these supplements.

## Supplementary information

Supplementary information is available at EJCN's website.

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## Conflicts of interest

The authors have no conflicts of interest to declare.

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353 **Figure legends**

354 Figure 1. Schematic representation of the selection of publications for this meta-analysis

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