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

3

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5 **Abstract**

6 Background

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

11 Methods

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

17 Results

18 We found 494 full papers covering 14 studies. Data were shared by the authors of four RCTs
19 comparing vitamin B12 with placebo (n=343) and of three RCTs comparing folic acid with
20 placebo (n=929). We found no effect of vitamin B12 supplementation on haemoglobin
21 (change 0.00 g/dL, 95% CI -0.19;0.18), and no effect of folic acid supplementation (change -
22 0.09 g/dL, 95% CI -0.19;0.01). The effects of supplementation on other haematological
23 parameters were similar. The effects did not differ by sex or by age group. Also, no effect
24 was found in a subgroup of patients with anaemia and a subgroup of patients who were
25 treated >4 weeks.

26 Conclusions

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

29 research is needed before firm recommendations can be made concerning the

30 supplementation of vitamin B12 and folate.

31

32 **Background**

33 The prevalence of anaemia in older persons is high (around 10% among people aged
34 ≥ 65 years) and rises with advancing age (1, 2). In about one third of older persons with
35 anaemia co-incident nutritional deficiencies, such as iron, vitamin B12 and folate
36 deficiency, exist [(2). Among people aged ≥ 75 years, the prevalence of both vitamin B12 and
37 folate deficiency is $>10\%$ (3-7). These deficiencies are not only associated with macrocytic
38 anaemia but also with dementia, peripheral neuropathy, combined degeneration of the spinal
39 cord and cardiovascular disease (8-11).

40 As the prevalence of deficiencies in vitamin B12 and folate are high, screening for
41 deficiencies in vitamin B12 and folate has been recommended as part of a geriatric work-up
42 (12, 13). Guidelines recommend vitamin B12 supplementation in patients with very low
43 serum vitamin B12 concentrations due to lack of intrinsic factor (IF) (pernicious anaemia) or
44 food-vitamin B12 malabsorption. Several studies have shown significant increases in
45 haemoglobin after vitamin B12 administration in these patients (14-16). Thus, when low
46 levels of vitamin B12 and folate are found, patients are often treated with injections or oral
47 supplements (9, 11, 17).

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

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

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

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

65

66 **Methods**

67 *Criteria for considering studies for this meta-analysis*

68 We considered all randomized controlled trials (RCTs) where community-dwelling elderly
69 (60+ years) were treated with vitamin B12 or folic acid (all dosages and all forms of
70 administration) and were compared with elderly who were given a placebo. The presence of
71 anaemia or a vitamin B12/folate deficiency was not required for inclusion. Exclusion criteria
72 were: mean or median age less than 60 years, no availability of haemoglobin concentrations
73 or haematocrit at baseline and during follow-up, combinations of vitamin B12 and folic acid
74 supplementation, and specific populations (e.g. studies in diabetes patients or patients with
75 renal failure).

76 *Search strategy*

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

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

85 *Selection of studies*

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

92 *Data collection*

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

100 *Analyses*

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

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

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

117

118 **Results**

119 *Selection of studies*

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

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

134 *Heterogeneity*

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

141 *Results of analyses*

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

150 In addition, no differences in the mean change in other haematological parameters were
151 observed. For vitamin B12 supplementation, the pooled estimate of the effect was 0.00 g/dL
152 (95% CI -0;0.01) for haematocrit, 0.07 g/dL (95% CI -0.58;0.72) for MCV and 0.00 g/dL
153 (95% CI -0.06;0.06) for RBC. For folic acid supplementation, the pooled estimate of effect
154 was -0.00 g/dL (95% CI -0.01;0) for haematocrit, -0.37 g/dL (95% CI -0.82;0.08) for MCV
155 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

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

189

190 **Discussion**

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

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

206 Other genetic or environmental factors may be involved in the onset of anaemia (19). Also,
207 other causes such as chronic inflammation may play a role in the development of anaemia
208 (35).

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

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

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

232

233 **Conclusions**

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

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

244

245 **Supplementary information**

246 Supplementary information is available at EJCN's website.

247

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252

253 **Conflicts of interest**

254 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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