A Randomized, Double-Blind, Placebo-Controlled Study of Oral Vitamin B_{12} Supplementation in Older Patients with Subnormal or Borderline Serum Vitamin B_{12} Concentrations

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OBJECTIVES: To determine the effect of small doses of oral cyanocobalamin supplements in older patients with low or borderline serum vitamin B_{12} concentrations but no other evidence of pernicious anemia (PA).

DESIGN: Randomized, double-blind, placebo-controlled study assessing the efficacy of oral cyanocobalamin 10 μg and 50 μg daily for 1 month.

SETTING: Two geriatric hospitals in the North Western Health Care Network, Melbourne, Australia.

PARTICIPANTS: Thirty-one inpatients with serum vitamin B_{12} levels between 100 and 150 pmol/L, without PA, other malabsorption disorders, or progressive neurological or terminal illness. The mean age was 81.4 years.

INTERVENTION: After informed consent, a medical and drug history was taken and the Mini-Mental State Examination (MMSE) completed. A dietitian made assessment of oral cobalamin intake. Blood was taken for serum vitamin B_{12}, serum and red cell folate assay, full blood examination, fasting serum gastrin, parietal and intrinsic factor antibodies, fasting serum homocysteine, and creatinine. Patients were then randomized to receive 10 μg oral cyanocobalamin, 50 μg oral cyanocobalamin, or placebo treatment for 1 month, after which the investigations and clinical examinations were repeated.

MEASUREMENTS: Percentage change in the level of vitamin B_{12}, homocysteine, folate, and red cell parameters and absolute changes in MMSE were calculated and compared between groups. The groups were compared on the number of responders who improved their level of B_{12} by 20%. Chi-square calculations on changes in serum vitamin B_{12} concentration were also performed.

RESULTS: Mean serum vitamin B_{12} ± standard deviation improved by 51.7 ± 47.1% in the 50-μg group, 40.2 ± 34.4% in the 10-μg group, and 11.7 ± 24.5% in the placebo group. The change in the 50-μg cyanocobalamin group was significantly greater than that in the placebo group (P = .044). The change in the 10-μg cyanocobalamin group was not significantly different from that in the placebo group (P = .186). Eight of 10 subjects in each treatment group were classified as responders, compared with two of 11 in the placebo group (P = .004). Homocysteine levels fell in patients receiving cyanocobalamin, but this fall failed to reach statistical significance. There were no significant changes in the other parameters measured.

CONCLUSION: Cyanocobalamin supplementation of 50 μg but not 10 μg daily produced a significant increase in serum vitamin B_{12}. This result has implications for the management of patients with subnormal or borderline serum vitamin B_{12} concentrations and for food fortification with vitamin B_{12}.

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Key words: vitamin B_{12}; oral vitamin B_{12} supplementation; homocysteine; older

Fortification of cereals with folate has been mandatory in the United States since January 1998 and has been recommended in other countries such as Australia. Although the value of increasing dietary folate intake to reduce the incidence of neural tube defects is undisputed, it has been suggested that folate food fortification may be harmful unless all supplements containing folic acid also include vitamin B_{12}, to prevent nerve damage from folate-induced cobalamin deficiency.[1] Because the incidence of subnormal serum vitamin B_{12} levels rises with age, to about 10% in older people, this group would be most susceptible to the possible harmful effect of supplemental folic acid.

The present study was designed to establish the minimum daily dose of vitamin B_{12} required to restore to nor-

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mal the low serum vitamin B_{12} concentration in older people. Herbert et al.[1] initially suggested 25 μg/day as the minimum safe dose but subsequently recommended that all older people should take 25 to 100 μg/day.[2] We present the results of a study of the effect of two dose levels, 10 and 50 μg/day, compared with placebo. These results have implications for the management of older patients with low serum B_{12} concentrations and for food fortification with vitamin B_{12}.

METHODS

Cyanocobalamin Formulation

Oral cyanocobalamin mixture (10 μg/5 ml or 50 μg/5 ml) and placebo were prepared by the hospital pharmacy at the Melbourne Extended Care and Rehabilitation Service (MECRS). All formulations were matched for color and taste. Placebo contained 40 ml Australian Pharmaceutical Formulary (APF) red mixture and 2 ml APF hydrobenzoate compound as preservative. Each bottle of 10 μg/5 ml and 50 μg/5 ml cobalamin formulation contained, in addition, 0.4 mg of cyanocobalamin and 2.0 mg cyanocobalamin, respectively. All bottles were then diluted to 200 ml with distilled water.

Vitamin B_{12} assays were performed on samples of the formulation before the trial. Storage of the medication for a 3-month period either at room temperature or at 4°C did not result in a fall of more than 5% in vitamin B_{12} concentration. The medications were dispensed allowing for a shelf life of 3 months.

On completion of the study, medicine returned from patients was measured and compared with the expected dose consumption, as a measure of compliance. There was no difference in compliance between the treatment groups ($F^{2,18} = 2.53$, $P = .11$).

Participants

Thirty-one inpatients at MECRS and Greenvale Geriatric Centre (two geriatric hospitals in the North West Health Care Network, Melbourne, Australia) with subnormal serum vitamin B_{12} concentrations discovered as part of their clinical assessment were randomized to receive placebo or cyanocobalamin (10 μg or 50 μg daily). There were 11 patients in the placebo arm: five male and six female, with a mean age of 77.6. Ten patients were included in each active treatment arm. In the 10-μg group there were four men and six women, with a mean age of 82.0, whereas in the 50-μg group there were five men and five women, with mean age of 84.9. The overall mean age was 81.4. The differences between groups in mean age and sex were not significant. One additional subject in the placebo group was included because it had been incorrectly reported that one consented subject had already been given unblinded treatment with vitamin B_{12}.

The majority of patients were living in the community before admission. Two of the 11 patients in the placebo group, two in the cyanocobalamin 10-μg arm, and three from the 50-μg arm were admitted from specialized nursing facilities. None had a previous history of gastric or bowel surgery or symptoms to suggest malabsorption. The diagnoses across the groups were similar, with approximately one-third suffering from dementia and almost half having vascular problems in the form of cerebrovascular or cardiovascular disease. Three subjects in the 10-μg group had type II diabetes mellitus, with no patients from the other treatments having this problem.

The Human Research Ethics Committee (MECRS and Greenvale Geriatric Centre) approved the study.

Procedure

The results of all vitamin B_{12} assays of patients from MECRS and Greenvale Geriatric Centre were monitored from August 1996 until September 1999, and patients were considered eligible if their serum B_{12} concentration ranged between 100 and 150 pmol/L. They were then approached to determine their suitability to enter the trial.

After informed consent, a medical and drug history was taken and clinical examination performed. Exclusion criteria included known neoplasm (other than basal cell or squamous cell carcinoma), a life-threatening or terminal illness, a history of malabsorption, pernicious anemia (PA) or anemia from other cause, prior vitamin B_{12} treatment or vitamin supplement, or a neurological disorder other than stroke. A baseline fasting blood sample was taken for full blood examination; serum vitamin B_{12}, serum folate, and red cell folate assays; serum creatinine; fasting homocysteine (Hcy); fasting serum gastrin; and tests for the detection of gastric parietal cell and intrinsic factor antibodies. All tests other than for vitamin B_{12}, folate, and Hcy assays were performed immediately. Sera for vitamin B_{12}, folate, and Hcy assays were stored at −20°C until assayed. PA was suspected if intrinsic factor antibody was detected or serum gastrin was elevated in conjunction with the presence of parietal cell antibodies. Six such subjects were excluded because we considered it unethical to include patients who could not potentially benefit from small doses of oral vitamin B_{12}.

A dietitian estimated the daily vitamin B_{12} intake on the basis of the reported dietary history.[3]

After baseline testing, patients were randomized in a double-blind manner to receive either placebo or vitamin B_{12} 10 μg or 50 μg daily for 4 weeks (average 30.9 days, range 27–39). At the end of the treatment period, patients underwent clinical assessment and repeat blood testing. A recommendation was then made to the patients' doctors as to whether to continue B_{12} replacement at the cessation of the study.

Baseline serum B_{12}, folate, and Hcy samples were batched and assayed later with the corresponding posttreatment samples to minimize the effect of interassay variation. The baseline serum vitamin B_{12} concentration was not known before treatment, but, in the absence of specific treatment, it was assumed to be similar to the screening values. This was not always the case, because the baseline value was found to be slightly higher than 150 pmol/L in 12 subjects.

Clinical Assessment

Clinical assessment included recording all pertinent medical history and medications. All subjects had a neurological examination and a Mini-Mental State Examination (MMSE). This is a screening cognitive assessment designed to test orientation, memory, and other cognitive abilities (0–30 points).[4] It was repeated at the conclusion of the trial.

Laboratory Methods

The hematological methods used were standard. Serum vitamin B_{12}, folate, and red cell folate were determined by solid-phase, no-boil, dual-count radioassay (Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's instructions. The manufacturer's reference ranges were serum vitamin B_{12}, 150 to 600 pmol/L; serum folate, 7 to 39 nmol/L; and red cell folate, 390 to 1600 nmol/L. Parietal cell antibody (PCA) was detected using indirect immunofluorescent assay with rat stomach substrate (Medical Diagnostics, Encinatas, CA), and intrinsic factor antibody (IFA) as blocking antibody using a solid phase kit (Diagnostic Products Corporation). Known positive and negative controls were added to each batch for the PCA and IFA detection. The total serum Hcy was measured by high-performance liquid chromatography. Hcy values >16 μmol/L were considered elevated. Serum gastrin was determined by radioimmunoassay kit (Becton Dickson, Franklin Lakes, NJ). The upper limit reference value is 55 pmol/L. Plasma creatinine was measured using an Olympus 5000 Automatic Biochemical Analyzer (Olympus Optical Co., Ltd., Tokyo, Japan). Renal function was considered impaired at creatinine >0.11 mmol/L. Impairment of renal function may elevate the serum Hcy level independent of vitamin B_{12} status,[5] and renal function is important in a study of the relationship between serum Hcy and vitamin B_{12} nutrition.

Statistical Methods

Data were analyzed using SPSS Version 9.0 for Windows (SPSS, Inc., Chicago, IL). Mean, standard deviation, and range were calculated for all baseline data from each group of patients, and the results after treatment were normalized by calculating a percentage change from the baseline. Absolute changes for baseline were calculated for MMSE.

Differences in changes between groups were determined using one-way analysis of variance with post hoc Tukey honestly significant difference (HSD) for all baseline data and changes in vitamin B_{12}, folate, Hcy, hemoglobin, mean cell volume, and MMSE. Chi-square testing was performed to detect differences in the number of responders as defined by a 20% increase in vitamin B_{12}. Baseline correlations were calculated using the linear correlation coefficient Pearson r.

RESULTS

The results of the baseline data for each treatment group, and testing for differences between groups, are shown in Table 1. There were no significant differences between the baseline parameters in the different groups, with the exception of mean cell volume, which was higher in the cyanocobalamin treatment groups ($P = .024$).

The changes from baseline, calculated as percentage change, with the exception of MMSE (where absolute changes were used) are shown in Table 2. The percentage change in serum vitamin B_{12} concentration was the only result to reach statistical significance ($F^{2,28} = 3.442$, $P = .046$). Post hoc Tukey HSD showed a significant difference between placebo and 50-μg treatment ($P = .044$) but not for placebo or 10-μg treatment ($P = .186$). Although there was a tendency for improvement in the mean value for Hcy with cyanocobalamin treatment, it was not statistically significant (Figure 1).

Table 1. Baseline Data with Descriptive Statistics and ANOVAs in the Three Treatment Groups

Group	B_{12} pmol/L	Folate nmol/L	RCF nmol/L	Hcy μmol/L	Creatinine mmol/L	Hemoglobin g/L	MCV fL	Gastrin pmol/L	MMSE	Dietary B_{12} μg/D
Placebo n	11	9	11	11	10	11	11	11	8	6
mean ± SD	137.9 ± 24.0	11.6 ± 6.2	599.5 ± 221.2	26.4 ± 10.4	0.12 ± 0.05	13.1 ± 1.3	88.1 ± 3.2	35.9 ± 41.3	19.6 ± 6.3	2.5 ± 1.1
Range	96–167	5–21.3	387–1149	16–47	0.06–0.26	11.4–15.2	83.5–93.2	10–129	10–28	1.6–4.6
CCbl-10 μg n	10	10	10	10	10	10	10	9	9	6
mean ± SD	140.3 ± 26.6	12.9 ± 3.8	790.0 ± 521.5	28.4 ± 9.6	0.12 ± 0.05	12.4 ± 1.6	92.4 ± 2.2	22.4 ± 6.9	15.4 ± 7.8	2.0 ± 0.7
Range	106–182	8.6–19.5	153–1770	15–43	0.05–0.23	8.8–14.7	89.0–95.7	15–32	6–27	1.1–3.1
CCbl-50 μg n	10	10	8	10	10	10	10	10	10	5
mean ± SD	162.9 ± 39.2	15.8 ± 7.7	751.0 ± 519.7	20.8 ± 5.9	0.10 ± 0.12	13.3 ±11.9	91.0 ± 4.6	19.4 ± 12.9	19.7 ± 5.3	1.4 ± 0.5
Range	110–227	8.2–28.8	383–1962	13–35	0.07–0.15	10.4–16.7	81.5–97.1	10.–48	11.–26	0.8–1.9
F	2.093	1.326	0.57	1.947	0.447	0.902	4.289	1.162	1.256	2.75
df	2, 28	2, 26	2, 26	2, 28	2, 27	2, 28	2, 28	2, 27	2, 24	2, 14
P-value	.142	.283	.573	.162	.644	.417	.024	.328	.303	.098

ANOVA = analysis of variance; CCbl = cyanocobalamin; SD = standard deviation, RCF = red cell folate; Hcy = homocystine; MCV = mean cell volume; MMSE = Mini-Mental State Examination (0–30 points).

Table 2. Change from Baseline—Descriptive Statistics and ANOVAs in the Three Treatment Groups

	B_{12}	Folate	HCy	Hemolglobin	MCV	MMSE
Group			%			
Placebo n	11	9	11	11	11	8
mean ± SD	11.7 ± 24.5	45.2 ± 43.2	−3.6 ± 24.6	2.3 ± 9.3	0.5 ± 1.3	1.6 ± 2.1
Range	−13–75	−30–112	−50–41	−13–19	−2–2	−1–5
CCbl-10 μg n	10	10	10	10	10	9
mean ± SD	40.2 ± 34.4	43.9 ± 65.3	−10.1 ± 27.1	3.3 ± 9.8	1.4 ± 2.2	0 ± 2.9
Range	−28–83	−10–218	−61–35	−12–24	−3–4	−4–5
CCbl-50 μg n	10	10	10	9	9	9
mean ± SD	51.7 ± 47.1	62.3 ± 141.9	−15.6 ± 18.0	0.7 ± 13.5	0.6 ± 1.1	1 ± 3.2
Range	−11–125	−17–449	−43–15	−11–33	−1–2	−3–7
F	3.442	0.115	0.676	0.134	0.897	0.728
df	2, 28	2, 26	2, 28	2, 27	2, 27	2, 23
P-value	.046	.892	.517	.875	.421	.494

ANOVA = analysis of variance; CCbl = cyanocobalamin; SD = standard deviation; Hcy = homocystine; MCV = mean cell volume; MMSE = Mini-Mental State Examination (0–30 points).

Based on the standard errors, retrospective calculations showed that this study had 80% power to detect a 36% difference in the changes in vitamin B_{12} from baseline between the placebo and cyanocobalamin 10-μg groups at a significance level of .05 (two tailed). Similarly, this study had 80% power to detect 31% and 26% changes in homocysteine levels between the placebo and the cyanocobalamin 10-μg and 50-μg groups, respectively.

There was an increase of 20% or greater in serum vitamin B_{12} in eight of 10 subjects receiving the 10-μg formulation, eight of 10 receiving the 50-μg treatment, and two of 11 receiving the placebo medication. Oxygen analysis of these data indicated that the differences between the supplemented and the placebo groups were statistically significant ($\chi^2 = 11.14$, $P = .004$). At the end of the study, vitamin B_{12} concentrations were greater than 150 pmol/L in eight, eight, and four subjects in the 10-μg and 50-μg cyanocobalamin and placebo groups, respectively. Oxygen analyses of the other parameters outlined in Table 2 did not reveal any significant changes.

One patient in the placebo group had a 75% increase in her posttreatment B_{12} level. This response is much greater than expected (5.4 standard deviations above the mean of 5.2% for the placebo group) and raises the possibility of specific treatment from another source. If this patient is omitted, the *P*-value improves substantially in the 50-μg group compared with placebo (.015), but the overall conclusions of the study remain unaltered.

A reliable estimation of daily vitamin B_{12} intake was possible in 17 patients. The mean intake ± standard deviation was 2.02 ± 0.87 μg, with a range of 0.75 to 4.60 μg. In 14, it was less than 2.4 μg daily.

Hcy levels correlated strongly with creatinine ($r = 0.67$, $P = .000$). There was a nonsignificant negative correlation between folate and Hcy ($r = -0.32$, $P = .091$) but no correlation with serum vitamin B_{12} ($r = -0.027$, $P = .886$).

DISCUSSION

The cause of the subnormal or borderline serum vitamin B_{12} concentrations frequently seen in older people has not been established. The PA defect is present in only about 10% of these subjects;[6] in the remainder, either deficient dietary intake or malabsorption of food-bound (but not free) vitamin B_{12} are possible causes. Two recent studies have provided conflicting evidence on the role of deficient dietary intake of vitamin B_{12}. In a study of older subjects, 95 with abnormal or suspicious findings in vitamin B_{12}–related tests and 78 with normal results, Howard et al. were unable to find any association between vitamin B_{12} status and intake and concluded that the high frequency of abnormal vitamin B_{12} status in older persons cannot be attributed to poor intake of vitamin B_{12}.[7] In contrast, a cross-sectional study of plasma vitamin B_{12} concentrations and dietary intake in 2,999 older subjects in the Framingham Offspring Study concluded that plasma vitamin B_{12} concentrations were associated with vitamin B_{12} intake.[8] A rise in serum vitamin B_{12} concentration after dietary supplementation with free vitamin B_{12}, as occurred in the present study, does not assist in differentiating deficient intake from malabsorption of vitamin B_{12} from food, because such a response would occur with both. In the limited number of patients in whom we could reliably establish food intake, and who were not taking any vitamin B_{12} supplements, vitamin B_{12} intake was less than the recommended dietary allowance of 2.4 μg/day in 14 of 17. This suggests that deficient dietary intake of vitamin B_{12} is a significant factor in our population.

Previous reports of trials of small doses of oral vitamin B_{12} supplements in older people have been published in letter[9] or abstract form[10,11] and do not include placebo groups. In a study of older patients with serum vitamin B_{12} concentrations of less than 220 pg/ml (the lower limit of the reference range), who were given a supplement of 100 μg of vitamin B_{12}/day, the serum vitamin B_{12} rose to normal in 88% of the 44 patients in whom results were available after 1 month of supplementation, and the authors concluded that this level of supplementation was efficient in most older people with vitamin B_{12} deficiency.[9] Miller et al. reached a similar conclusion from a trial of 39 individuals who received oral cyanocobalamin 100 μg/day for 3 months.[10] Postsupplementation serum vitamin B_{12} concen-

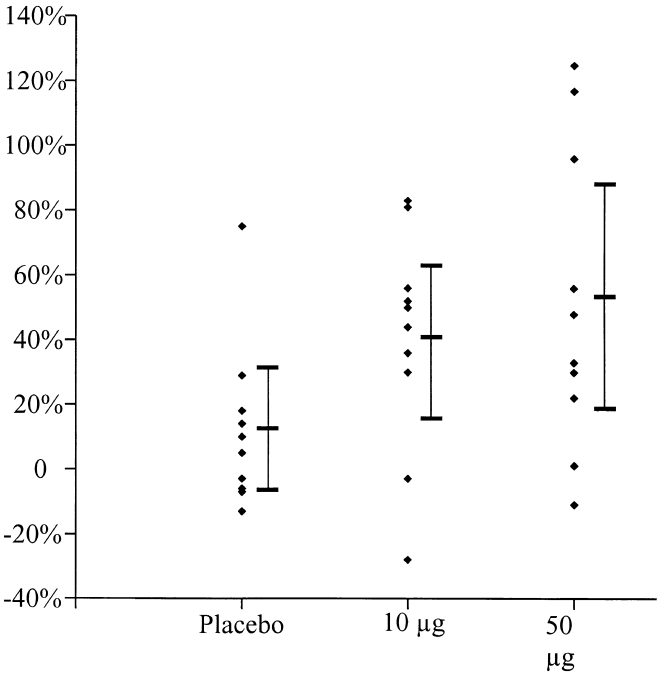

Figure 1. Scatter plots and 95% confidence intervals exploring percentage increase in vitamin B_{12} in patients treated with placebo and 10 μg and 50 μg cyanocobalamin daily.

trations were significantly increased regardless of the baseline vitamin B_{12} level.

In another study reported in abstract form, older patients were screened for vitamin B_{12} deficiency, and no patient regularly taking a source of synthetic vitamin B_{12} equal to or greater than 25 μg/day was found to be vitamin B_{12} deficient.[11]

The results of the present study indicate that, compared with placebo, a daily supplement of 50 μg, but not 10 μg/day, of vitamin B_{12} will significantly raise mean serum vitamin B_{12} concentration in older patients. However, even with the 50-μg supplement, serum vitamin B_{12} did not rise significantly in all patients tested. This finding suggests that a daily supplement greater than 50 μg would be necessary to protect against vitamin B_{12} deficiency, although it is possible that, with a period of supplementation longer than 1 month and/or a larger study group, 50 μg, and possibly 10 μg, could raise the serum vitamin B_{12} significantly in all patients.

The percentage of older adults with subnormal levels of serum vitamin B_{12} reported varies widely, but in most reports the average prevalence is approximately 10% to 15%. Most commercially available multivitamins contain small amounts of vitamin B_{12} (6 μg),[12] which is clearly inadequate to correct subnormal serum B_{12} levels in older people, as shown in this study. Hence, these 10% to 15% would be at risk by taking these supplements.

Vitamin B_{12} deficiency is usually accompanied by hyperhomocystinemia, which is a risk factor for cardiovascular disease. Although there was a fall in Hcy concentrations in patients in the present study who received 50 μg/day of vitamin B_{12}, the fall was not statistically significant. Miller et al. reported that concentrations of Hcy were significantly reduced in older patients with low vitamin B_{12} who received 50 μg/day of oral vitamin B_{12} for 3 months.[10] However, Bjorkegren et al. were able to normalize the serum Hcy concentration in only 15 of 56 older persons with serum B_{12} less than 300 pmol/L after 6 months of treatment with therapeutic doses of vitamin B_{12}.[13] The response to vitamin B_{12} supplementation in older subjects with low vitamin B_{12} concentrations is complicated by other factors that may be associated with raised Hcy levels, such as renal impairment and folate deficiency. Hcy concentrations in plasma rise in parallel with creatinine,[5] and, in the present study, we were able to demonstrate a significant correlation between serum Hcy and creatinine but not between serum Hcy and vitamin B_{12}. It is not unexpected then that, in patients with low or borderline serum vitamin B_{12} concentrations and raised Hcy, Hcy levels may not normalize with vitamin B_{12} supplements only. However, the lowering of Hcy levels in some patients after oral cyanocobalamin supplements might be a further reason to supplement vitamin B_{12} in the diet of older patients with borderline or subnormal serum vitamin B_{12} concentrations.

The results of the present study have implications for the management of older patients with low serum vitamin B_{12} concentrations and for food fortification with vitamin B_{12}. In general, these patients are treated with vitamin B_{12} injections of 1,000 μg at regular intervals. In patients who have hematological or neurological changes that could be attributed to vitamin B_{12} deficiency, or evidence of PA, parenteral vitamin B_{12} therapy is indicated. In those who have hematological changes only, and who can be relied upon to take their medication, oral doses of 2,000 μg/day can be given as an alternative to parenteral vitamin B_{12}.[14] In patients who have isolated low or borderline serum vitamin B_{12} concentrations and no other evidence of PA, oral vitamin B_{12} 100 μg/day may be administered.[2] However, because the dose may not restore serum B_{12} level to normal in all patients, it would be important to monitor them at regular intervals.

With regard to food fortification with vitamin B_{12}, the results of the present study suggest that the supplement should aim to supply more than an additional 50 μg of the vitamin daily.

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