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Research Article

Clinical Consequences of Metformin-Associated Vitamin B12 Deficiency Among Patients with Type 2 Diabetes

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Keywords

- Type 2 Diabetes
- Metformin
- Vitamin B12
- Homocysteine
- Silent myocardial ischemia

Abstract

Aim: Metformin is the first-line treatment in type 2 diabetic patients. However, it may raise homocystein levels through vitamin B12 deficiency, which might adversly affects its alleged cardiovascular advantages. In this study, we investigated the association between metformin-associated vitamin B12 deficiency and the occurrence of silent myocardial ischemia and hematological abnormalities in type 2 diabetes.

Methods: This hospital-based cross-sectional study evaluated 798 asymptomatic patients with type 2 diabetes and at least one cardiovascular risk factor. Biological and clinical parameters were compared first between the 497 metformin-treated patients and the 301 metformin-free patients, and second between metformin users with and without vitamin B12 deficiency. Outcomes included vitamin B12 deficiency (<150 pmol/l), hyperhomocysteinemia (>15 µmol/l), anemia, macrocytosis and silent myocardial ischemia (positive stress myocardial perfusion imaging).

Results: Mean diabetes duration of the whole sample was 14.3 ± 9.5 years. Crude prevalence of vitamin B12 deficiency was 12.5% in metformin-treated patients and 6.1% in metformin free patients ($p<0.01$). The homocystein level was not increased in metformin users, but among them it was higher in vitamin B12 deficient patients. In both univariate and multivariate analyses, metformin-associated vitamin B12 deficiency was not related to increased risks of hyperhomocysteinemia, silent myocardial ischemia, or to the combined endpoint of macrocytosis and anemia.

Conclusion: This large cross-sectional study confirms that vitamin B12 deficiency is more common among metformin users, but does not suggest a detrimental effect of metformin on silent myocardial ischemia nor on hematological abnormalities, even though such effects cannot be excluded.

ABBREVIATIONS

SMI: Silent Myocardial Ischemia; LDL: Low-density Lipoprotein; HDL: High-density Lipoprotein; HPLC: High Performance Liquid Chromatography; ALT: Alanine Aminotranferase; AST: Aspartate Aminotransferase; GGT: Gamma Glutamyltransferase; MCV: Mean Corpuscular Volume

INTRODUCTION

Metformin is currently the first-line therapy for the treatment of type 2 diabetes [1]. It improves insulin sensitivity and glucose control and has been associated with reduced cardiovascular mortality, the major cause of death among patients with diabetes [2]. Recent data also support metformin use as a means of secondary prevention [3]. Nevertheless, a recent meta-analysis of randomised controlled trials concluded that its benefit-risk ratio remained uncertain, both for all-cause mortality and for cardiovascular mortality [4].

Metformin has a good safety profile and limited side effects although early discontinuations due to gastro-intestinal intolerance occur in up to 20% of cases. Malabsorption of vitamin B12 under metformin treatment has been known for decades [5], and low vitamin B12 levels in metormin-treated patients was confirmed in a recent meta-analysis of randomized clinical trials [6]. However vitamin B12 measurement is not recommended for the follow-up of this treatment.

Vitamin B12 deficiency is associated with a significant increase in homocysteine concentration [7]. This may negatively impact patients health, as elevated homocysteine levels are associated with an increased risk of cardiovascular disease [8], independently of classic cardiovascular risk factors [9]. In type 2 diabetes, homocysteine concentrations higher than 15 µmol/l have been related to an increased cardiovascular risk [10], and

seem to be a higher risk factor for mortality than in subjects without diabetes [11]. Consequently, the fact that metformin treatment may raise homocysteine levels through vitamin B12 deficiency [12,13], particularly in the absence of exogenous supplementation of folic acid or B-group vitamins [14] might question its alleged cardiovascular benefits.

WHO experts predicted over 300 million people with diabetes on earth by the year 2025 [15], a large proportion of which should receive metformin. Without considerable anticipatation, a serious rise in the prevalence of vitamin B12 deficiency has to be expected. However, there is very limited data available concerning clinical outcomes of vitamin B12 deficiency in metformin users.

We therefore undertook an analysis of our cohort of high-risk patients with diabetes to investigate the association of metformin-associated vitamin B12 deficiency with silent myocardial ischemia (SMI) and hematologic abnormalities (anemia and macrocytosis) among type 2 diabetes patients.

MATERIALS AND METHODS

Study population

Between March 2001 and December 2010, all asymptomatic patients with diabetes referred to our clinic were regularly considered for SMI screening according to the Société Francophone du Diabète guidelines that were available at that time [16]. The present report describes a cross-sectional analysis of 798 asymptomatic patients with type 2 diabetes consecutively referred between February 2001 and January 2011 and who had at least one of the following criteria: age ≥ 60 years, active smoker, albuminuria, hypertension (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 80 mmHg or antihypertensive medication), dyslipidemia (low-density lipoprotein (LDL) cholesterol ≥ 1.3 g/L and/or high-density lipoprotein (HDL) cholesterol <0.4 g/L or triglycerides ≥ 1.5 g/L or lipid-lowering therapy), family history of premature coronary artery disease (i.e. occurring before 55 in a male first-degree relative or before 65 in a female first-degree relative), and peripheral arterial disease (absence of one or more peripheral arterial pulses at clinical examination or intermittent claudication or past history of revascularization of the lower limbs). Screening was not performed in the cases of history or symptoms of coronary events, abnormal resting electrocardiogram, alcohol abuse and associated diseases, pregnancy, neoplasia, and contraindications to dipyridamole infusion, such as asthma.

Informed consent was obtained from all patients. Investigations have been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Stress protocol and myocardial perfusion imaging

Stress tests and myocardial perfusion imaging were performed as previously described [17]. Briefly, all subjects underwent a combined dipyridamole (0.75 mg/kg) exercise stress test followed by a same-day stress-rest imaging protocol using ^{99m}Tc-sestamibi (1 mCi/10 kg body weight with a minimum of 7 mCi; 259 MBq). Stress and rest images were acquired, in a prone position, 1 h after injection of ^{99m}Tc-sestamibi. Acquisition was performed using a double-headed gamma camera (GE-SMV DST-XL) with low-energy, high-resolution, parallel-hole collimators. Stress and rest acquisitions were gated (10% R-R interval acceptance window, eight gated intervals). SMI was defined as positive myocardial perfusion imaging (mean activity <70% of the maximal myocardium activity in 3 of the 20 segments).

Laboratory procedures

Biological parameters were evaluated in fasting conditions on the same day as SMI screening. Serum total cholesterol, HDL-cholesterol and triglyceride levels were measured by routine enzymatic tests (Architect C8000, Abbott, Rungis France). LDL-cholesterol was calculated using the Friedewald formula. Glycated hemoglobin (HbA1c) was measured by a routine HPLC-based ion-exchange procedure (HA-8140; Menarini, Rungis, France). The total homocysteine level was measured by HPLC with fluorometric detection (Dionex Corporation, Sunnyvale, CA, USA) using a commercial Recipe HPLC analytical kit (Recipe Chemicals & Instruments GmbH, Munich, Germany). Red blood cells folates and plasma vitamin B12 measurements were determined through radioimmunoassay (Simultrac, Diasorin, Italy). Enzymatic creatinine (Randox reagent, Mauguio, France), high sensitivity C-reactive protein (Randox reagent, Mauguio, France), glucose, alanine aminotranferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) were measured by routine biochemical tests by the use of a AU2700 analyzer (Beckman Coulter, Villepinte, France). The estimated glomerular filtration rate was calculated using the Cockcroft and Gault formula [18]. The urinary albumin excretion rate and proteinuria were measured by 24-h urine collections using a radioimmunoassay (Beckman Coulter, Villepinte, France).

Definitions

Vitamin B12 deficiency was defined as < 150 pmol/L [19]. Raised homocysteine concentrations were defined as values above 15 µmol/l as this limit has been associated with increased cardiovascular events [20]. Macrocytosis was defined as a mean corpuscular volume (MCV) >97 femoliters. Microalbuminuria and proteinuria were defined as urinary albumine excretion above 30 mg/24h and 300 mg/24h, respectively.

Statistical analysis

Clinical and biological parameters were described using means and standard deviations for continuous variables or percentages for categorical variables. The normality of the distributions was checked with a Shapiro-Wilk test. Comparisons between groups were performed using the T-test when the distributions of continuous variables were normal and using the Wilcoxon test if they were skewed. Categorical variables were compared with the Chi2 test.

Association between vitamin B12 deficiency and cardiological and hematological outcomes were evaluated using univariate and multivariate logistic regression models. In the latter, we addressed the issue of missing values by carrying out data imputation on both outcome variables and covariates. We used the multiple imputation by Markov chain Monte Carlo method [21], using MI and MIanalyze Procedures implemented in SAS V9

[22]. The imputed dataset was generated by performing twenty imputation cycles.

Statistical analyses were performed at the conventional two-tailed α level of 0.05 using SAS, version 9.1 (SAS Institute, Cary, North Carolina).

RESULTS AND DISCUSSION

Description of the metformin-treated population

Of the 806 patients with diabetes included, 798 were analyzed, of which 497 patients were treated with metformin (Figure 1). The main clinical and biological parameters in metformin-treated and metformin-free patients are listed in table 1. Metformin-treated patients were characterized by a younger age, a lower duration of diabetes, a greater body mass index, a better renal function, a less frequent use of insulin and a more frequent use of sulfonylureas.

Crude prevalence of vitamin B12 deficiency was twofold higher in metformin-treated patients than in non-metformin-treated patients (p=0.01). After adjustment for tobacco use, gender, body mass index, age, glomerular filtration rate, insulin use and presence of hypertension, metformin use was associated with a significant risk of vitamin B12 deficiency (OR 3.22; 95% CI = 1.44 - 7.19).

In the entire sample, the prevalence of anemia was 21.8%, with a tendency towards a lower prevalence in metformin-treated subjects (p=0.06). Homocystein levels as well as prevalences of macrocytosis, hyperhomocysteinemia and SMI did not differ significantly between metformin-treated and metformin-free patients (Table 1).

Vitamin B12 deficient patients

Among metformin-treated patients, subjects with vitamin B12 deficiency (N=48) were compared to non-deficient patients (N=336) (Table 1). Vitamin B12 deficient patients were older, had a longer duration of diabetes, a higher AST/ALT ratio and a lower creatinine clearance. When analyzing the risk of vitamin B12 deficiency in the metformin-treated population using a multivariate model, the only significant independent predictor of deficiency was age (OR = 1.09, CI95%=1.04-1.14, p <0.001). The two other variables of the model were no longer significant: diabetes duration (OR: 1.02, CI95%= 0.99-1.06, p = 0.16) and creatine clearance (OR = 1.00, CI95%= 0.99-1.01, p=0.21).

Crude prevalences of SMI, macrocytosis, anemia and hyperhomocysteinemia did not differ between the two groups, despite a higher homocysteine level in vitamin B12 deficient patients (Table 1). After adjustment, metformin-associated vitamin B12 deficiency was not linked to higher risks of hyperhomocysteinemia, SMI or hematological abnormalities (Table 2).

DISCUSSION

In this cross-sectional study on patients with type 2 diabetes, vitamin B12 deficiency was twofold higher among metformin users, but was not related to the incidence of anemia or silent myocardial ischemia.

Vitamin B12 deficiency associated with metformin use has been known for a long time. Several mechanisms were proposed to explain how metformin interferes with absrption of vitamin B12, the most recent suggesting that metformin interferes with

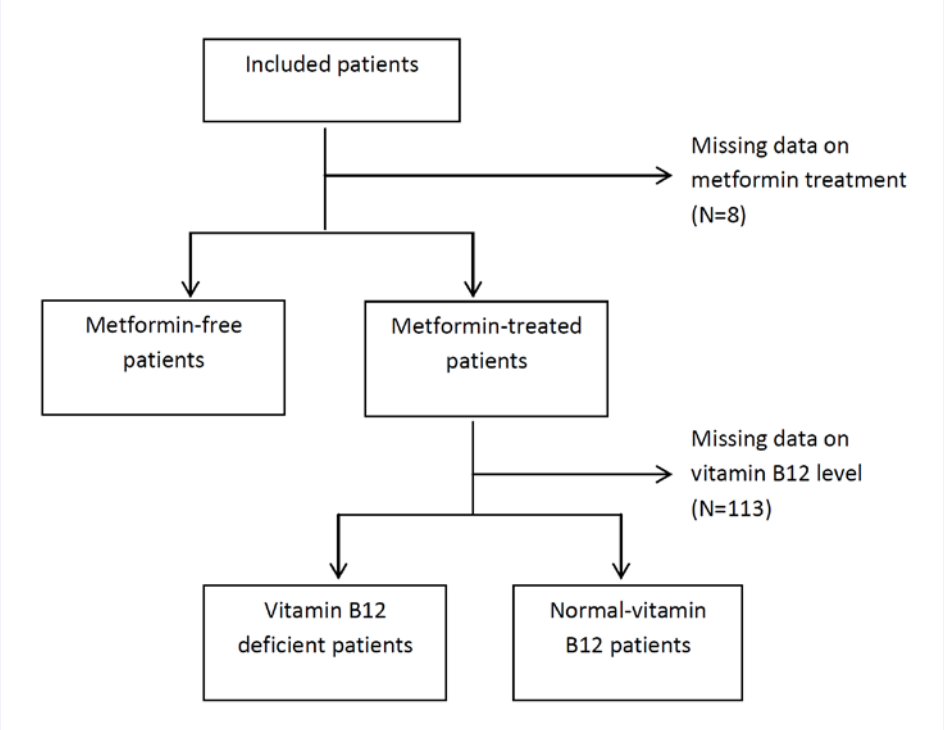

Figure 1 Flow chart of the studied population.

Table 1: Main clinical and biological parameters according to metformin treatment in the entire sample, and according to vitamin B12 deficiency in the metformin-treated population.

Characteristics	Metformin-free (N=301)	Metformin-treated (N=497)	p	Among metformin-treated		
				Vitamin B12 deficiency (N=48)	No vitamin B12 deficiency (N=336)	p
Clinical parameters						
Age *(years)*, mean ± SD	64.7 ± 9.9	61.5 ± 9.2	**<0.001**	66.7 ± 7.7	60.6 ± 9.1	**< 0.001**
Women, %	39.5	44.1	0.20	46.8	41.9	0.53
Diabetes duration *(years)*, mean ± SD	15.4 ± 9.9	13.6 ± 9.1	**0.02**	16.7 ± 11.1	12.9 ± 8.8	**0.03**
BMI *(kg/m²)*, mean ± SD	29.1 ± 5.7	31.2 ± 5.7	**<0.001**	31.5 ± 7.1	30.9 ± 5.1	0.76
Hypertension, %	56.4	49.6	0.09	50.0	45.2	0.56
Treatments						
Insulin, %	70.4	36.0	**<0.001**	35.4	38.1	0.72
Sulfonylureas , %	22.3	56.5	**<0.001**	52.1	55.4	0.67
Alpha-glucosidase inhibitors, %	7.3	10.5	0.13	6.3	10.4	0.36
Glitazones, %	9.3	15.1	**<0.05**	25.0	17.0	0.17
Glinides, %	9.3	8.85	0.83	12.5	8.6	0.41
Antiaggregant, %	33.3	32.5	0.80	33.3	24.5	0.87
Lipid-lowerin therapy, %	56.7	54.8	0.61	64.6	56.3	0.27
Beta blockers, %	13.0	13.9	0.72	25.0	9.9	**<0.01**
Calcium channel blockers, %	29.0	23.9	0.11	29.2	21.1	0.21
ACE inhibitors, %	42.0	36.2	0.11	27.1	39.6	0.09
Angiotensin II receptor blockers, %	21.1	26.6	0.08	31.3	25.0	0.35
Diuretics, %	33.2	32.7	0.87	33.3	32.3	0.77
Biological parameters						
AST/ALT ratio, mean ± SD	1.1 ± 0.5	1.0 ± 0.4	**<0.001**	1.1 ± 0.5	0.9 ± 0.4	**<0.001**
HbA1c *(%; mmol/mol)*, mean ± SD	8.3 ± 1.5	8.3 ± 1.5	0.89	8.0 ± 1.4	8.3 ± 1.6	0.32
Hemoglobin *(g/100mL)*, mean ± SD	13.5 ± 1.6	13.6 ± 1.5	0.39	13.3 ± 1.5	13.6 ± 1.5	0.16
hs-CRP *(mg/L)*, mean ± SD	3.42± 4.0	3.77 ± 5.4	0.85	3.3 ± 4.4	3.7 ± 5.5	0.56
Clearance *(mL/min)*, mean ± SD	86.8 ± 42.2	112.2 ± 44.8	**<0.001**	103.9 ± 59.8	114.0 ± 41.6	**0.004**
Urinary albumin excretion Microalbuminuria, % Proteinuria, %	 26.7 19.9	 28.3 9.8	**<0.001**	 31.9 14.9	 29.0 10.2	0.51
MCV *(fL)*, mean ± SD	90.5 ± 4.5	89.8 ± 5.9	0.21	90.7 ± 6.2	89.8 ± 6.2	0.58
RBC folates *(nmol/L)*, mean ± SD	977.5 ± 416.6	981.1 ± 448.5	0.74	817.2 ± 357.8	1009.0 ± 455.9	**0.002**
Vitamin B12 *(pmol/L)*, mean ± SD	363.5 ± 175.6	297.4 ± 156.3	**<0.001**	102.0 ± 33.5	327.0 ± 146.4	**<0.001**
Homocysteine *(µmol/L)*, mean ± SD	14.9 ± 5.3	14.3 ± 5.2	0.24	17.3 ± 5.9	14.0 ± 5.1	**0.008**
Cardiovascular or hematological abnormalities						
Vitamin B12 deficiency, %	6.1	12.5	**0.01**	100	0	N/A
Silent Myocardial Ischemia, %	11.7	15.3	0.17	11.1	14.9	0.50
Macrocytosis, %	2.2	2.3	1.00	4.7	2.5	0.35
Anemia, %	25.7	19.5	0.06	24.4	20.2	0.51
Hyperhomocysteinemia, %	40.2	35.9	0.41	52.6	34.6	0.12

SD: Standard Deviation; BMI: Body Mass Index; ALT: Alanine Aminotranferase; AST: Aspartate Aminotransferase; HbA1c: Hemoglobin A1c; hs-CRP: high sensitivity C-reactive protein; MCV: Mean Corpuscular Volume; RBC : Red Blood Cells; N/A: Not Applicable

the calcium-dependant Intrinsic factor-vitamin B12 complex binding to the ileal cubilin receptor [23]. Our large hospital-based study confirmed this association, with 12.5% of metformin users being vitamin B12 deficient after a mean diabetes duration of 14 years. We have not precisely recorded the duration of metformin treatment in our series. However, given that metformin has been considered as a first-line therapy for decades, the duration of metformin treatment is probably very close to that of diabetes in current users. A recent double-blind placebo-controlled trial found that vitamin B12 concentrations dropped by nearly 20% in metformin users after a 4.3 years follow-up, with a crude prevalence of vitamin B12 deficiency equal to 13% [24]. Interestingly, they report a prevalence of vitamin B12 deficiency very close to that found in our series, despite different treatment durations and different population characteristics.

In our study, metformin use was not related to higher homocysteine levels in type 2 diabetes: both metformin-treated and metformin-free patients had mean values below 15 µmol/L, a threshold chosen as it indicates a higher cardiovascular risk in type 2 diabetes [10,20]. However, within metformin users, vitamin B12 deficiency was linked to mean homocystein levels above this

Table 2: Main cardiovascular and hematological outcomes in metformin-treated patients according to vitamin B12 deficiency

		OR [95% CI]	p	Adjusted OR [95% CI]	p
Hyperhomo-cysteinemia	No vitamin B12 deficiency	1		1	
	Vitamin B12 deficiency	1.49 [0.76-2.96]	0.24	1.33 [0.65-2.77][a]	0.41
Silent myocardial ischemia	No Vitamin B12 deficiency	1		1	
	Vitamin B12 deficiency	0.79 [0.31-1.93]	0.60	0.77 [0.28-2.07][b]	0.61
Macrocytosis or anemia[c]	No Vitamin B12 deficiency	1		1	
	Vitamin B12 deficiency	1.42 [0.73 -2.78]	0.24	1.03 [0.49-2.15][d]	0.41

[a] adjusted for age, creatinine clearance, AST/ALT ratio and RBC folates
[b] adjusted for age, sex, microalbuminuria, LDL-cholesterol, tobacco, hypertension, BMI and hs-CRP
[c] macrocytosis and anemia were grouped due to a low frequency of macrocytosis
[d] adjusted for age, sex, creatinine clearance, AST/ALT ratio and RBC folates
CI: Confidence Interval; BMI: Body Mass Index; ALT: Alanine Aminotranferase; AST: Aspartate Aminotransferase; hs-CRP : high sensitivity C-reactive protein; RBC : Red Blood Cells.

threshold, although the frequency of hyperhomocysteinemia was not significantly increased, possibly due to a small sample size. Several trials have investigated the relationship between metformin treatment and serum homocystein levels, with conflicting results. The two largest randomized controlled trials published to date concluded that metformin treatment was associated with higher mean serum homocystein values, whether compared with rosiglitazone (165 subjects) [25], or with a placebo (390 sujects) [24]. This last trial was carried out for four years, and demonstrated that homocysteine concentrations did not differ between metformin users and non users after stratification for the end-of-treatment vitamin B12 concentration. A large cross-sectionnal study conducted among 6005 subjects found very close levels of serum homocystein among metformin-treated (9.7 µmol/L), sulfonylurea-treated (9.4 µmol/L) and diet-treated subjects with diabetes (9.2 µmol/L) (26). Although very small, these differences were statistically significant. Our study specifically evaluates serum homocystein values among vitamin B12-deficient metformin-treated subjects. Our results suggest that the increase in homocystein levels concerns this subgroup particularly. Thus, elevated mean homocystein levels are not detected in the entire metformin-treated population because vitamin B12 deficiency prevalence is less than 15% in metformin-treated subjects.

Vitamin B12 deficiency is also known to be associated with hematological abnormalities such as anemia and macrocytosis [27,28], which constitute well recognized cardiovascular risk factors in type 2 diabetes [29]. In the present study, metformin-associated vitamin B12 deficiency was not linked to an increased risk of anemia or macrocytosis, even when taking into account potential counfounding factors. A lack of power might explain this absence of association. However the mean hemoglobin levels and mean corpuscular volumes are very similar between vitamin B12 deficient and non deficient metformin users (for these quantitative variables, we had a 80% statistical power to detect a 0.66g/100mL difference in hemoglobin levels and a 2.7fL difference in mean corpuscular volumes). A trend towards a lower prevalence of anemia was even observed in metformin users, which could be explained by a better renal function. Even if vitamin B12 malabsorption-related anemia has rarely been reported in metformin users [30], such hematological abnormalities may occur, particularly in patients with specific risk factors such as gastrectomy [31]. This may also be the case after bariatric surgery, and such patients should benefit from particular attention.

In this series, we do not report an increased risk of SMI among metformin users with vitamin B12 deficiency. The value of the reported odds-ratio was largely below 1 (OR=0.7) which limits the possibility that a lack of power could be responsible for this result. Two recent meta-analyses of randomized clinical trials (including the UKPDS) evaluated the cardiovascular safety of metformin treatment [4,32]. They remain unconclusive, questionning the beneficial effect of metformin especially when combined with sulfonylurea [32], and underlining the significant heterogeneity observed when the UKPDS was included [4]. Similarly, although our data do not suggest a detrimental effect of metformin treatment on SMI, they do not allow us to exclude it either. A small increase in risk could potentially have important public health consequences given the large number of metformin-treated patients with diabetes worldwide.

The originality of this study relies on the fact that we provide data on the sub-group of vitamin B12 deficient metformin users, and that patients have been studied after having had diabetes for a long duration. Moreover, these results were obtained within a hospital-based population, which mostly consists of patients with severe forms of diabetes where complications would have most likely been detected. Thus we assume that our conclusions on clinical consequences of metformin generally apply to all forms of type 2 diabetes, including less severe forms. One of the limitations is that only vitamin B12 levels were measured, while levels of holotranscobalamin II or methylmalonic acid, which might have been more precise indicators of vitamin B12 status, were not measured. Lastly, the clinical safety of metformin could not be fully assessed as neurological data had not been collected.

CONCLUSION

Routine vitamin B12 screening among metformin users is not currently performed in clinical practice, and our data is insufficient to recommend it. However, we suggest that the cost-effectiveness of a systematic screening should be evaluated on a population basis. In particular, such screenings might be proposed to patients who are at increased risk for vitamin B12 deficiency, such as vegetarians, patients with malabsorption or those who have undergone digestive surgery.

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