Personalising laboratory medicine in the 'real world': Assessing clinical utility, by clinical indication, of serum total B_{12} and Active-B_{12}® (holotranscobalamin) in the diagnosis of vitamin B_{12} deficiency

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Abstract

Background: Assessing the pre- and post-test probability of disease in the context of routine health care is challenging. We wished to study how test performance parameters relating to clinical utility vary by clinical indication in a 'real-world' setting.

Methods: The diagnostic accuracy of serum total B_{12} and Active-B_{12}® (holotranscobalamin) was evaluated in a primary care population, using serum methylmalonic acid as the reference standard. We used electronic requesting to establish the clinical indication for each request. Routine requests from primary care for serum total B_{12} were included if creatinine was also measured and estimated glomerular filtration rate was at least 60 mL/min/1.73 m^2.

Results: Clinical indications included peripheral neuropathy ($n = 168$), anaemia ($n = 168$), cognitive decline ($n = 125$), suspected dietary deficiency ($n = 76$), other ($n = 362$). For peripheral neuropathy, the area under the receiver operator curve ± 95% confidence interval (AUC ± CI) was 0.63 (0.54–0.71) ($P = 0.002$) for total B_{12} and 0.68 (0.60–0.77) ($P < 0.0001$) for Active-B_{12}®. For anaemia, AUC ± CI was 0.56 (0.47–0.66) ($P = 0.10$) for total B_{12} and 0.69 (0.59–0.78) ($P < 0.0001$) for Active-B_{12}®. For cognitive decline, AUC ± CI was 0.54 (0.43–0.65) ($P = 0.26$) for total B_{12} and 0.69 (0.58–0.80) ($P = 0.0002$) for Active-B_{12}®. The pre–post-test change in probability of disease varied by clinical indication.

Conclusion: Combining diagnostic accuracy studies and electronic testing in a 'real-world' setting allows clinical utility to be assessed by clinical indication. Wider application of this would permit more personalised laboratory medicine. In this study, diagnostic performance of total B_{12} and Active-B_{12}® varied across all indications. Active-B_{12}® provided better discrimination, but this may have reflected the cut-offs used.

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Introduction

Diagnostic investigations are most useful at the point of maximum diagnostic uncertainty, i.e. when the potential gap between pre- and post-test probability of disease is greatest. In the context of routine health care, it is difficult to study the application of this principle to laboratory investigations. Practical challenges include: first, the existence of multiple requests and request sources, each with a potentially different pretest probability of disease; second, the need for information about the clinical indication for the request; third, the need for a validated reference standard. As a result of these difficulties, 'real-world' studies of the clinical utility of tests, and how this varies by clinical indication, are rare.

Separately, accurate assessment of vitamin B_{12} status is problematic.[1] Defining deficiency in terms of tissue stores (e.g. red cell cobalamin)[2] is not always feasible, and no single analytical measurement is ideal, leading some to propose the use of combined indicators of vitamin B_{12} status.[3] Several diagnostic reports[4–8] have used methylmalonic acid (MMA) as reference standard, despite its acknowledged limitations.[9] We adopted this approach in a study of the diagnostic accuracy of serum total B_{12} and Active-$B_{12}^{®}$ (holotranscobalamin, or holoTC) in the assessment of vitamin B_{12} status in an otherwise unselected primary care population. We used electronic requesting to establish the clinical indication for each request. Our study is the first to report how test performance parameters relating to clinical utility vary by clinical indication in a 'real-world' setting.

Methods

General practitioners in 68 practices in Tayside, Scotland (population approximately 400,000) were advised of the intention to perform a study of the diagnostic accuracy of the existing measure of vitamin B_{12} status (i.e. total serum B_{12}), and Active-$B_{12}^{®}$ (see below for details of methods), in the setting of routine health care. Their cooperation was sought in the provision of information about the clinical indication for individual requests. Clinical information was delivered through an electronic order communications system, the Integrated Clinical Environment (ICE) (Sunquest Information Systems, Uxbridge, UK). The study was performed between 12 June 2014 and 31 July 2014.

Participants

The study population consisted of consecutive patients from whom samples were received for routine measurement of total serum vitamin B_{12}. In order to minimise the impact of reduced glomerular filtration rate (GFR)

on serum MMA,[10] patients were excluded if creatinine was not concurrently requested or if estimated glomerular filtration rate (eGFR) was $60\,\text{mL/min/1.73 m}^2$ or less. Only the results of total serum vitamin B_{12} were reported.

Clinical indications

Health-care professionals ordering vitamin B_{12} through the electronic order communications system chose from the following 'drop-down' list of clinical indications: unexplained anaemia; macrocytic anaemia; cognitive decline; suspected dietary deficiency; peripheral neuropathy; other.

Analytical methods

Serum total B_{12} was measured by chemiluminescent microparticle-based competitive immunoassay on an Advia Centaur (Siemens Healthineers, Tarrytown, NY, USA), Active-$B_{12}^{®}$ by chemiluminescent microparticle-based immunoassay on an Abbott Architect i2000 (Abbott Diagnostics, Abbott Park, IL, USA), both at Ninewells Hospital in Dundee. MMA was measured using a MultiPurpose Sampler (Gerstel GmbH, Mülheim an der Ruhr, Germany) coupled to liquid chromatography tandem mass spectrometry (MPS-LC-MS/MS) at the Nutristasis Unit, Viapath, St Thomas Hospital, London.

Cut-offs used to define result categories

The lower limit of the reference interval (200 pg/mL) for serum total B_{12} was used, in line with the manufacturer's recommendations. The cut-off for serum holoTC (Active-$B_{12}^{®}$) was $<35\,\text{pmol/L}$, as used by other groups.[6,11] Two separate cut-offs were used for MMA, reflecting the impact of age-related changes in glomerular function on serum MMA: $>0.28\,\mu\text{mol/L}$ for patients $\leqslant 65$ years and $>0.36\,\mu\text{mol/L}$ for patients >65 years. These were defined using age-specific 97.5th percentiles in vitamin B_{12}-replete patients in a previous study.[12] Total B_{12}, Active-$B_{12}^{®}$ and MMA were measured in isolation from each other; respective assessors of each did not have access to results of the other measurements, and only laboratory staff involved in the routine measurement of total B_{12} had access to the clinical indication for each request.

Statistical methods

Descriptive statistics (mean, 2.5th and 97.5th centiles) were calculated for population characteristics. Test performance parameters and receiver operator curve (ROC) analysis were performed by Analyze-it (Version 2.21; Analyze-it Software Ltd, West

Yorkshire, UK). Youden cut-offs were established for the three commonest indications and test performance recalculated for total B_{12} and Active-$B_{12}^{®}$ by indication.

Results

During the study period, 899 routine requests for serum total B_{12} were received. The breakdown of clinical indications for requests was as follows: unexplained anaemia ($n = 134$), macrocytic anaemia ($n = 34$), cognitive decline ($n = 125$), suspected dietary deficiency ($n = 76$), peripheral neuropathy (168), other ($n = 362$). (The anaemia categories were merged for the purposes of data analysis.) Table 1 summarises group characteristics for the three commonest indications. For the other indications, the characteristics (mean and 2.5–97.5 centiles) were as follows: (1) suspected dietary B_{12} deficiency: age (years) 57 (15–91), haemoglobin (g/L) 137 (109–165), haematocrit 0.42 (0.35–0.50), mean cell volume (fL) 95 (85–110), white cell count ($\times 10^9$/L) 6.4 (3.5–10.9), platelets ($\times 10^9$/L) 254 (108–406), total vitamin B_{12} (pg/mL) 370 (164–1004), Active-$B_{12}^{®}$ (pmol/L) 65 (12–256), MMA (µmol/L) 342 (96–1869); (2) other: age (years) 61 (23–92), haemoglobin (g/L) 134 (99–165), haematocrit 0.41 (0.30–0.49), mean cell volume (fL) 94 (81–111), white cell count ($\times 10^9$/L) 6.8 (3.7–11.9), platelets ($\times 10^9$/L) 256 (130–441), total vitamin B_{12} (pg/mL) 388 (170–974), Active-$B_{12}^{®}$ (pmol/L) 70 (17–256), MMA (µmol/L) 320 (95–1121).

ROC curves for the three commonest specific indications are shown in Figure 1(a) to (c). For other indications, the areas under the curve $\pm$ 95% confidence intervals (AUC $\pm$ CI) were as follows for total B_{12}: suspected dietary deficiency 0.50 (0.36–0.64); other 0.47 (0.41–0.54). For Active-$B_{12}^{®}$, the AUC $\pm$ CI were: suspected dietary deficiency 0.61 (0.47–0.75); other 0.66 (0.60–0.72). For the total population, the AUC $\pm$ CI for total B_{12} was 0.53 (0.49–0.57) ($P = 0.075$), and for Active-$B_{12}^{®}$, AUC $\pm$ CI was 0.68 (0.64–0.72) ($P < 0.0001$). These AUC were significantly different from each other ($P < 0.0001$).

Table 2 summarises the pre- and post-test probabilities of vitamin B_{12} deficiency (as defined by raised serum MMA), before and after measurement of serum total B_{12}, in each of the three commonest specific indications for requesting total B_{12}. Data are presented using the preassigned cut-offs and using Youden cut-offs (designed to optimise differentiating ability).[13] We wished to calculate the post-test probability of disease in the presence of positive and negative results, respectively; therefore, we replaced the negative predictive values (NPV) by [1–NPV]. Table 3 summarises the corresponding data for Active-$B_{12}^{®}$.

Discussion

Optimal targeting of diagnostic tests in the setting of normal care is challenging. Evaluations of diagnostic accuracy are usually performed in well-defined and preselected populations, whereas tests in routine use are usually applied to multiple populations that vary in terms of relevant parameters (e.g. prevalence). In the current study, we have addressed this issue by performing a diagnostic evaluation in the setting of normal care.

We identified several distinct populations, based on the clinical indication for the vitamin B_{12} request. Patients with cognitive decline were, as anticipated, older than other patient groups, and had higher creatinine, reflecting reduced glomerular function. Interestingly, prevalence of vitamin B_{12} deficiency, as defined by raised serum MMA, was broadly similar in the patient groups defined by the three commonest specific indications, ranging from 32.7% in patients with peripheral neuropathy to 36.6% in patients with anaemia. This is important, since any differences in PPV and [1–NPV] across clinical indications are therefore not attributable to large differences in prevalence.

For all categories of indication, measurement of Active-$B_{12}^{®}$ reliably differentiated vitamin B_{12} deficiency from non-deficiency, and for all the AUC for Active-$B_{12}^{®}$ was greater than for total B_{12}, although in the case of peripheral neuropathy, the difference was not statistically significant. However, these findings must be interpreted with caution in the context of the wider literature. The effect on the clinical utility of holoTC of applying different MMA cut-offs,[8] and more widely, the need to base cut-offs (for all vitamin B_{12} biomarkers) on adverse outcomes,[14] have previously been highlighted. The age-specific MMA cut-offs applied here, particularly in patients >65 years, may have affected sensitivity and specificity; other limitations include those of using MMA as a reference standard,[15] and the exclusion of patients where creatinine was not requested, or where eGFR was 60 mL/min/1.73 m^2 or less. On the wider issue, the ability of holoTC to predict, for example, neurological outcomes like cognitive decline in well-designed studies[16–19] has been variable, and its role as a biomarker of clinically meaningful vitamin B_{12} deficiency remains inconclusive. Recent assessments[15,20] acknowledge the limitations of holoTC and other markers and endorse the adoption of algorithm-based approaches that combine more than one measure of vitamin B_{12} status.[3] This seems reasonable, and our findings do not provide a basis for challenging this position.

With these caveats, low Active-$B_{12}^{®}$ results were more useful than normal ones; the increase in probability of vitamin B_{12} deficiency seen with a low result

Table 1. Characteristics of study population.

Group	Parameter		2.5–97.5 percentile	% abnormal results (cut-off value)
Total		*n*		
	Group total	899		
	Female	560		
	>65 years	479		
		Mean		
	Age (years)	64	23–92	
	Haemoglobin (g/L)	133	96–165	27% (<120 female, <130 male)
	Haematocrit	0.41	0.30–0.50	19% (<0.37)
	Mean Cell Volume (fL)	94	81–110	8% (>105)
	White Cell Count ($\times 10^9$/L)	6.8	3.5–11.9	6% (<4×10^9)
	Platelets ($\times 10^9$/L)	257	126–447	7% (<150×10^9)
	Total B_{12} (pg/mL)	408	171–1195	5% (<200)
	Active-B_{12}® (pmol/L)	67	15–256	24% (<35)
	MMA (µmol/L)	345	98–1259	32% (>0.28 ⩽ 65yo; >0.36 > 65yo)
Cognitive decline		*n*		
	Group total	125		
	Female	80		
	>65 years	104		
		Mean		
	Age (y)	77	44–93	
	Haemoglobin (g/L)	132	94–166	30% (<120 female, <130 male)
	Haematocrit	0.41	0.29–0.51	21% (<0.37)
	Mean cell volume (fL)	95	83–107	6% (>105)
	White cell count ($\times 10^9$/L)	6.6	3.4–10.3	6% (<4×10^9)
	Platelets ($\times 10^9$/L)	263	135–479	7% (<150×10^9)
	Total B_{12} (pg/mL)	413	166–1269	7% (<200)yo
	Active-B_{12}® (pmol/L)	62	16–256	34% (<35)yo
	MMA (µmol/L)	346	80–1044	34% (>0.28 ⩽ 65yo; >0.36 > 65yo)
Peripheral neuropathy		*n*		
	Group total	168		
	Female	109		
	>65 years	58		
		Mean		
	Age (y)	58	23–89	
	Haemoglobin (g/L)	142	110–168	7% (<120 female, <130 male)
	Haematocrit	0.43	0.35–0.50	5% (<0.37)
	Mean cell volume (fL)	94	84–106	2% (>105)
	White cell count ($\times 10^9$/L)	7.2	3.8–13.0	4% (<4×10^9)
	Platelets ($\times 10^9$/L)	258	126–434	4% (<150×10^9)
	Total B_{12} (pg/mL)	410	205–838	1% (<200)
	Active-B_{12}® (pmol/L)	66	12–256	27% (<35)
	MMA (µmol/L)	394	119–2011	33% (>0.28 ⩽ 65yo; >0.36 > 65yo)
Unexplained anaemia combined with macrocytic anaemia		*n*		
	Group total	168		
	Female	97		
	>65 years	105		

Table 1. Continued.

Group	Parameter	2.5–97.5 percentile	% abnormal results (cut-off value)
		Mean	
	Age (years)	69	26–91	
	Haemoglobin (g/L)	121	87–159	62% (<120 female, <130 male)
	Haematocrit	0.38	0.28–0.50	43% (<0.37)
	Mean cell volume (fL)	95	79–116	12% (>105)
	White cell count ($\times 10^9$/L)	6.8	3.0–11.9	10% (<4×10^9)
	Platelets ($\times 10^9$/L)	258	110–448	11% (<150×10^9)
	Total B_{12} (pg/mL)	391	144–1541	8% (<200)
	Active-$B_{12}^{®}$ (pmol/L)	67	15–256	21% (<35)
	MMA (μmol/L)	347	84–1149	34% (>0.28 ⩽ 65yo; >0.36 > 65yo)

Note: the upper measuring limit of the Active-$B_{12}^{®}$ assay is 256 pmol/L; all samples ⩾256 pmol/L were recorded for analysis as 256 pmol/L. This does not affect % abnormal results nor the clinical performance calculations. 'Unexplained anaemia' and 'macrocytic anaemia' categories were merged. In the category 'unexplained anaemia', 8 patients from 134 had an elevated MCV (>105 fL). In the category 'macrocytic anaemia', 12 patients from 34 had an elevated MCV.
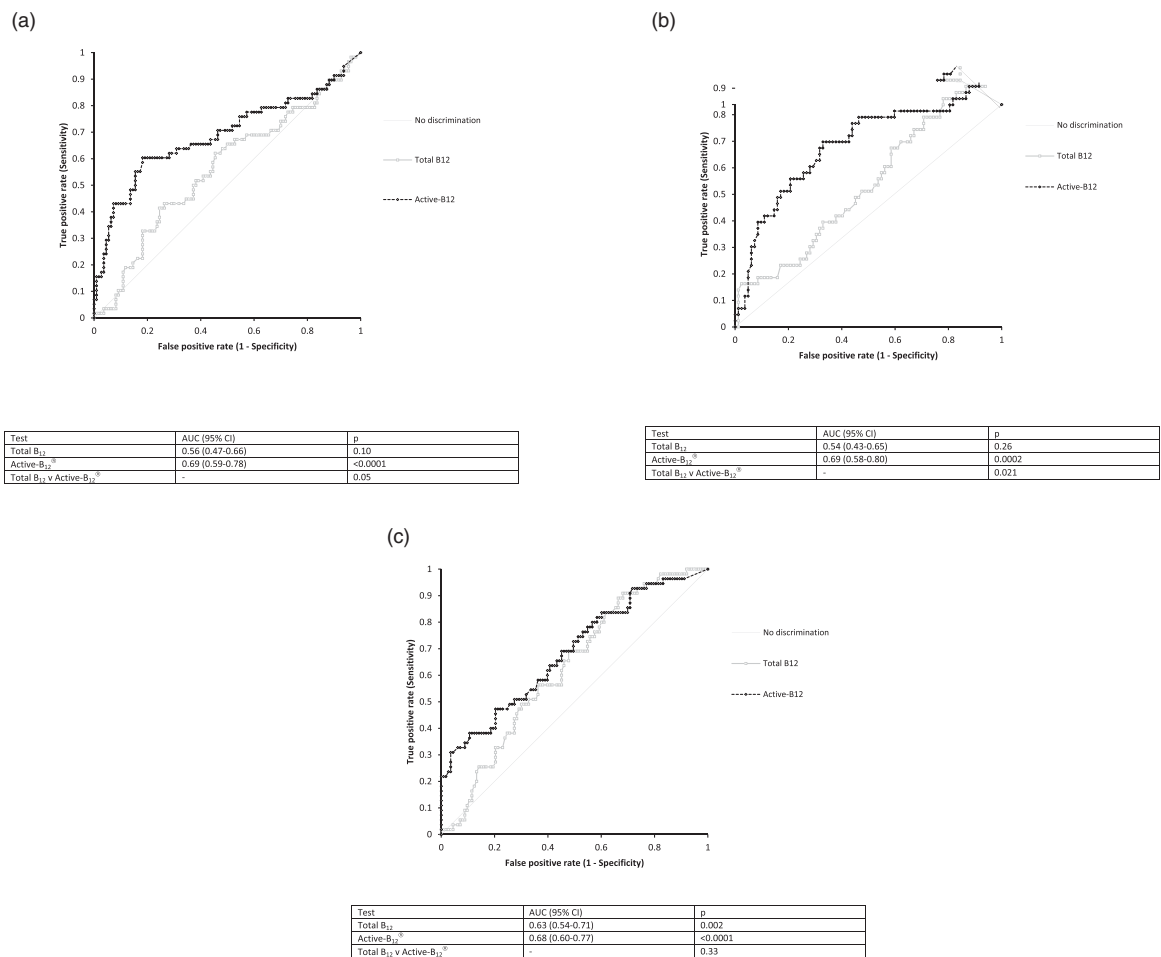
(a)

Test	AUC (95% CI)	p
Total B_{12}	0.56 (0.47-0.66)	0.10
Active-$B_{12}^{®}$	0.69 (0.59-0.78)	<0.0001
Total B_{12} v Active-$B_{12}^{®}$	-	0.05

(b)

Test	AUC (95% CI)	p
Total B_{12}	0.54 (0.43-0.65)	0.26
Active-$B_{12}^{®}$	0.69 (0.58-0.80)	0.0002
Total B_{12} v Active-$B_{12}^{®}$	-	0.021

(c)

Test	AUC (95% CI)	p
Total B_{12}	0.63 (0.54-0.71)	0.002
Active-$B_{12}^{®}$	0.68 (0.60-0.77)	<0.0001
Total B_{12} v Active-$B_{12}^{®}$	-	0.33

Figure 1. (a) ROC curves for total B_{12} and Active-$B_{12}^{®}$ in the diagnosis of vitamin B_{12} deficiency (as defined by raised serum MMA) in anaemia. (b) ROC curves for total B_{12} and Active-$B_{12}^{®}$ in the diagnosis of vitamin B_{12} deficiency (as defined by raised serum MMA) in cognitive decline. (c) ROC curves for total B_{12} and Active-$B_{12}^{®}$ in the diagnosis of vitamin B_{12} deficiency (as defined by raised serum MMA) in peripheral neuropathy.

Table 2. Clinical utility of Total B_{12} by clinical indication.

	Using pre-assigned cut-off (200 pg/mL)					Using Youden cut-offs[a]						
Indication	Abnormal results (n)	PPV (%)	Δ in probability (%) with low result	1 − NPV (%)	Δ in probability (%) with normal result	Youden cut-off (pg/mL)	Abnormal results (n)	PPV (%)	Δ in probability (%) with low result	1−NPV (%)	Δ in probability (%) with normal result	Prevalence (%)
Anaemia n = 168	11	18.2	−18.4	39.0	+2.4	287	46	50.0	+13.4	30.7	−5.9	36.6
Cognitive decline n = 125	9	77.8	+43.4	31.0	−3.4	200	9	77.8	+43.4	31.0	−3.4	34.4
Peripheral neuropathy n = 168	2	50.0	+17.3	32.5	−0.2	481	127	39.4	+6.7	12.2	−20.5	32.7

Δ = change; PPV (positive predictive value) = post-test probability of vitamin B_{12} deficiency in presence of a low total B_{12} result; (1−NPV [negative predictive value]) = post-test probability of vitamin B_{12} deficiency in presence of a normal total B_{12} result.
Note: Probability of vitamin B_{12} deficiency (as defined by raised serum MMA) before and after measurement of total B_{12}, for three clinical indications. Pretest probability (prevalence) is shown, along with the post-test probability associated with low and normal results. For ease of reference, the changes in probability are also shown. Data are presented using preassigned cut-offs, and separately using Youden cut-offs.
[a]The cut-offs that optimise differentiating ability when equal weight is given to sensitivity and specificity.

Table 3. Clinical utility of Active-B_{12}® by clinical indication.

	Using pre-assigned cut-off (35 pmol/L)					Using Youden cut-offs[a]						
Indication	Abnormal results (n)	PPV (%)	Δ in probability (%) with low result	1 − NPV (%)	Δ in probability (%) with normal result	Youden cut-off (pg/mL)	Abnormal results (n)	PPV (%)	Δ in probability (%) with low result	1 − NPV (%)	Δ in probability (%) with normal result	Prevalence (%)
Anaemia n = 168	29	75.9	+39.3	26.7	−9.9	43	44	68.2	+31.6	22.2	−14.4	36.6
Cognitive decline n = 125	43	55.8	+21.4	23.2	−11.2	44	54	52.6	+18.2	19.1	−15.3	34.4
Peripheral neuropathy n = 168	46	50.0	+17.3	26.2	−6.5	29	30	63.6	+30.9	25.2	−7.5	32.7

Δ = change; PPV (positive predictive value) = post-test probability of vitamin B_{12} deficiency in presence of a low Active-B_{12}® result; (1−NPV [negative predictive value]) = post-test probability of vitamin B_{12} deficiency in presence of a normal Active-B_{12}® result.
Note: Probability of vitamin B_{12} deficiency (as defined by raised serum MMA) before and after measurement of Active-B_{12}®, for three clinical indications. Pretest probability (prevalence) is shown, along with the post-test probability associated with low and normal results. For ease of reference, the changes in probability are also shown. Data are presented using pre-assigned cut-offs, and separately using Youden cut-offs.
[a]The cut-offs that optimise differentiating ability when equal weight is given to sensitivity and specificity.

was higher, for all three of the commonest specific indications, than the decrease in probability associated with a normal result (see Table 3). This was true both when the preassigned cut-offs and when Youden cut-offs were used. As anticipated, the PPV ranking for these indications corresponded to the prevalence ranking (when the preassigned cut-off was used), with the highest PPV associated with anaemia and the lowest with peripheral neuropathy.

We have shown in the current study that it is possible to apply the principles of evidence-based medicine to routine requesting. Electronic requesting is now commonplace in health care, and, with the co-operation of clinical colleagues, can readily be harnessed to studies of diagnostic accuracy. In the presence of a validated reference standard, it is possible to establish prevalence by clinical indication and requesting source. The cost of diagnostic studies involving validated reference standards should be offset against the potential savings enabled by greater precision in laboratory requesting, for example through the application of local algorithms.

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References

1. Hughes CF and McNulty H. Assessing biomarker status of vitamin B12 in the laboratory: no simple solution. *Ann Clin Biochem* 2018; 55: 188–189.
2. Valente E, Scott JM, Ueland P-M, et al. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B_{12} status in the elderly. *Clin Chem* 2011; 57: 856–863.
3. Fedosov SN, Brito A, Miller JW, et al. Combined indicator of vitamin B_{12} status: modification for missing biomarkers and folate status and recommendations for revised cut-points. *Clin Chem Lab Med* 2015; 53: 1215–1225.
4. Risch M, Meier DW, Sakem B, et al. Vitamin B_{12} and folate levels in healthy Swiss senior citizens: a prospective study evaluating reference intervals and decision limits. *BMC Geriatr* 2015; 15: 82–91.
5. Clarke R, Sherliker P, Hin H, et al. Detection of vitamin B_{12} deficiency in older people by measuring vitamin B_{12} or the active fraction of vitamin B_{12}, holotranscobalamin. *Clin Chem* 2007; 53: 963–970.
6. Obeid R and Herrmann W. Holotranscobalamin in laboratory diagnosis of cobalamin deficiency compared to total cobalamin and methylmalonic acid. *Clin Chem Lab Med* 2007; 45: 1746–1750.
7. Schrempf W, Eulitz M, Neumeister V, et al. Utility of measuring vitamin B_{12} and its active fraction, holotranscobalamin, in neurological vitamin B_{12} deficiency syndromes. *J Neurol* 2011; 258: 393–401.
8. Heil SG, de Jonge R, de Rotte CFJ, et al. Screening for metabolic vitamin B_{12} deficiency by holotranscobalamin in patients suspected of vitamin B_{12} deficiency: a multicentre study. *Ann Clin Biochem* 2012; 49: 184–189.
9. Green R, Allen LH, Bjørke-Monsen AL, et al. Vitamin B_{12} deficiency. *Nat Rev Dis Primers* 2017; 3: 20.
10. Lewerin C, Ljungman S and Nilsson-Ehle H. Glomerular filtration rate as measured by serum cystatin C is an important determinant of plasma homocysteine and serum methylmalonic acid in the elderly. *J Intern Med* 2007; 261: 65–73.
11. Miller JW, Garrod MG, Rockwood AL, et al. Measurement of total vitamin B_{12} and holotranscobalamin, singly and in combination, in screening for metabolic vitamin B_{12} deficiency. *Clin Chem* 2006; 52: 278–285.
12. Vogiatzoglou A, Oulhaj A, Smith AD, et al. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B_{12} status. *Clin Chem* 2009; 55: 2198–2206.
13. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950; 3: 32–35.
14. Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of vitamin B_{12} status in NHANES: a roundtable summary. *Am J Clin Nutr* 2011; 94: 313S–321S.
15. Wolffenbuttel BHR, Wouters HJCM, Heiner-Fokkema MR, et al. The many faces of cobalamin (vitamin B_{12}) deficiency. *Mayo Clin Proc Innov Qual Outcomes* 2019; 3: 200–214.
16. Clarke R, Birks J, Nexo E, et al. Low vitamin B-12 status and risk of cognitive decline in older adults. *Am J Clin Nutr* 2007; 86: 1384–1391.
17. Kivipelto M, Annerbo S, Hultdin J, et al. Homocysteine and holotranscobalamin and the risk of dementia and Alzheimer's disease: a prospective study. *Eur J Neurol* 2009; 16: 808–813.
18. Hooshmand B, Solomon A, Kareholt I, et al. Homocysteine and holotranscobalamin and the risk of Alzheimer disease: a longitudinal study. *Neurology* 2010; 75: 1408–1414.
19. Miles LM, Allen E, Mills K, et al. Vitamin B_{12} status and neurologic function in older people: a cross-sectional analysis of baseline trial data from the Older People and Enhanced Neurological Function (OPEN) study. *Am J Clin Nutr* 2016; 104: 790–796.
20. Harrington DJ. Laboratory assessment of vitamin B_{12} status. *J Clin Pathol* 2017; 70: 168–173.