Clinical Chemistry 53:5
963–970 (2007)

Nutrition

Detection of Vitamin B_{12} Deficiency in Older People by Measuring Vitamin B_{12} or the Active Fraction of Vitamin B_{12}, Holotranscobalamin

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Background: **Impaired vitamin B_{12} function and decreased vitamin B_{12} status have been associated with neurological and cognitive impairment. Current assays analyze total vitamin B_{12} concentration, only a small percentage of which is metabolically active. Concentrations of this active component, carried on holotranscobalamin (holoTC), may be of greater relevance than total vitamin B_{12}.**

Methods: **We compared the utility of serum holoTC with conventional vitamin B_{12} for detection of vitamin B_{12} deficiency in a population-based study of older people, using increased methylmalonic acid (MMA) concentrations as a marker of metabolic vitamin B_{12} deficiency in the overall population (n = 2403) and in subsets with normal (n = 1651) and abnormal (n = 752) renal function.**

Results: **Among all participants, 6% had definite (MMA >0.75 μmol/L) and 16% had probable (MMA >0.45 μmol/L) metabolic vitamin B_{12} deficiency. In receiver operating characteristic curves for detection of definite vitamin B_{12} deficiency, holoTC had a greater area under the curve (AUC) compared with vitamin B_{12} in all participants (0.85 vs 0.76; P <0.001) and in subsets with normal (AUC: 0.87 vs 0.79; P <0.001) and abnormal (AUC: 0.85 vs 0.74; P = 0.002) renal function. Similar findings were observed for detection of moderate vitamin B_{12} deficiency. Whereas the positive predictive value for both holoTC and vitamin B_{12} was greater for detection of probable than definite vitamin B_{12} deficiency, both tests were associated with more false-positive than true-positive test results.**

Conclusions: **HoloTC has a modestly superior diagnostic accuracy compared with conventional vitamin B_{12} for the detection of vitamin B_{12} deficiency, but neither test can be recommended to screen asymptomatic populations.**

Concerns that older individuals with undiagnosed vitamin B_{12} deficiency may be put at risk by mandatory folic acid fortification have prompted interest in screening older people for undiagnosed vitamin B_{12} deficiency *(1)*. Approximately 5%–20% of older people may have undiagnosed vitamin B_{12} deficiency, defined by low serum concentrations of vitamin B_{12} together with increased concentrations of methylmalonic acid (MMA)[8] or homocysteine (tHcy) *(2–4)*. Population-based studies that screened for anemia, depression, cognitive impairment, and neuropathy in relation to vitamin B_{12} status in older people have indicated that most older individuals with undiagnosed vitamin B_{12} deficiency did not have anemia or neurological disease *(4)*; however, the proportion that

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Received September 9, 2006; accepted February 5, 2007.
Previously published online at DOI: 10.1373/clinchem.2006.080382

[8] Nonstandard abbreviations: MMA, methylmalonic acid; tHcy, homocysteine; holoTC, holotranscobalamin; OHAP, Oxford Healthy Aging Project; OAPR, odds of being affected given a positive result; and AUC, area under the curve.

may subsequently develop signs of vitamin B_{12} deficiency is not known. In clinical practice, vitamin B_{12} deficiency commonly presents as macrocytic anemia, and the neurological signs may occur without anemia in ~20% of cases *(5)*. The administration of folic acid to individuals with vitamin B_{12} deficiency can correct the anemia but may worsen the neurological dysfunction *(6)*. The possibility that mandatory folic acid fortification could result in an insidious progression of neurological disease in individuals with vitamin B_{12} deficiency prompted some to advocate universal screening for vitamin B_{12} deficiency in older people *(2)*.

The diagnosis of vitamin B_{12} deficiency is complicated by poor sensitivity and specificity of the conventional vitamin B_{12} assays *(7–9)*. The advent of novel assays for holotranscobalamin (holoTC), the active fraction of vitamin B_{12}, may improve the detection of vitamin B_{12} deficiency, but the results of previous studies that addressed this question were conflicting *(10–13)*. Approximately 80% of circulating vitamin B_{12} is biologically unavailable, and the rest comprises holoTC, which is the part of vitamin B_{12} bound to transcobalamin that delivers vitamin B_{12} to all cells in the body *(10)*. We compared the diagnostic utility of holoTC with conventional vitamin B_{12} testing to detect definite metabolic vitamin B_{12} deficiency (defined as MMA >0.75 μmol/L) or probable metabolic vitamin B_{12} deficiency (defined as MMA >0.45 μmol/L) in the overall population and in subsets with normal or abnormal renal function in a population-based study of older people living in the UK.

Materials and Methods

STUDY POPULATION

The study population included all participants with data on vitamin status in population-based studies of older people living in Oxford city (Oxford Healthy Aging Project, OHAP) and Oxfordshire (Banbury B_{12} study) *(2)*. OHAP is a longitudinal cohort study of 2741 randomly selected people ages ≥65 years that is a component part of the Medical Research Council Cognitive Function and Aging Study *(14)*. In 1993, we randomly selected the population sample from general practice registers for people living in Oxford city to provide equal numbers of individuals ages 65 to 74 years and ≥75 years. Research nurses visited study participants in their homes and carried out a structured interview. The collected data included medical history, smoking habits, education, and use of medication (including multivitamin supplements or vitamin B_{12} injections). All surviving participants who had not previously refused to be interviewed were invited to provide a blood sample in 1995. Nonfasting blood samples were collected into vacutainers that were allowed to clot at room temperature, and the serum was separated within 2 h and stored at −80 °C until it was shipped on dry ice or thawed for analysis.

Participants in the Banbury B_{12} study were recruited from a random sample of people ages ≥75 years living in their own homes and registered with 3 general practices in Banbury, Oxfordshire *(4)*. Individuals who were known to have a terminal illness or were living in institutions were excluded. Eligible participants (n = 1934) were invited to participate in the study, and those who agreed (n = 1000) were asked to provide written informed consent. Participants were visited in their homes by a research nurse between March 2003 and April 2004, and the data collected included medical history and use of medication. Nonfasting venous blood samples were collected and kept chilled (using a cooling box to ensure that the temperature was maintained <4 °C) until the serum was separated at the local hospital laboratory within 2 h of blood collection and stored at −40 °C until analysis. Participants in both studies provided signed consent, and the protocols (in accordance with the current version of the Helsinki Declaration) were approved by research ethics committees of the participating institutions.

LABORATORY METHODS

Frozen blood samples were thawed for measurements of serum concentrations of creatinine, holoTC, tHcy, MMA, and vitamin B_{12}. We measured serum holoTC concentrations in the OHAP study at Aarhus University Hospital, Aarhus, Denmark, using an ELISA modified for use on an automated analyzer *(13)*. We measured serum holoTC concentrations in the Banbury B_{12} study at the Clinical Trial Service Unit using a RIA (AXIS-Shield ASA) that has been shown to have very good agreement with the ELISA assay *(13, 15)*. We measured serum tHcy concentrations on an Abbott IMx automated analyzer by means of a fluorescence polarization immunoassay in OHAP *(2)* and by gas chromatography-mass spectrometry in the Banbury study *(4)*. We measured serum vitamin B_{12} concentrations on an ACS Centaur with an automated chemiluminescence system (Bayer A/S), using a competitive protein-binding assay at Aarhus University Hospital in both studies. In the Banbury B_{12} study, we also assessed vitamin B_{12} concentrations using a Beckman assay. We measured serum MMA concentrations at the University of Bergen, Bergen, Norway, using stable isotope–dilution capillary gas chromatography-mass spectrometry in both studies *(16)*.

STATISTICAL METHODS

We summarized continuous variables as medians and interquartile ranges. Individuals with extreme increases of vitamin B_{12} (>1000 pmol/L) or holoTC (>400 pmol/L) or who reported use of vitamin B_{12} injections were excluded. We used Spearman correlation coefficients to examine linear associations between the different biochemical markers. We defined metabolic vitamin B_{12} deficiency as definite if serum MMA was >0.75 μmol/L and probable if serum MMA was >0.45 μmol/L. We defined renal function as normal if serum creatinine concentration was <97 μmol/L in women and <124 μmol/L in men. We assessed the performance of specific

cutoff points for either test to detect vitamin B_{12} deficiency using the odds of being affected given a positive result (OAPR), the likelihood ratio, and positive predictive value in addition to the sensitivity and specificity. We used ROC plots to display the sensitivity and specificity of serum concentrations of holoTC and of vitamin B_{12} for detection of definite and probable vitamin B_{12} deficiency. The area under the curve (AUC) of the ROC plots for either test to detect vitamin B_{12} deficiency was estimated in the overall population and in subsets without and with abnormal renal function. Because holoTC was measured using a slightly different method in the OHAP study *(2)* compared with the Banbury study *(4)*, we carried out a sensitivity analysis to compare holoTC with vitamin B_{12} separately in the OHAP and Banbury studies. Because vitamin B_{12} had been measured using both a Centaur (Bayer) method and a Beckman method in the Banbury study, we performed comparisons of holoTC with vitamin B_{12} using both assays for vitamin B_{12} in the Banbury study *(4)*.

Results

CHARACTERISTICS OF THE STUDY POPULATION

Among the 2559 individuals with data on vitamin status in both studies, 70 who reported current use of vitamin B_{12} injections were excluded, as were 13 other individuals with extreme values of vitamin B_{12} (>1000 pmol/L) or holoTC (>400 pmol/L) (indicating likely vitamin B_{12} treatment, but failure to report it), leaving 2476 untreated individuals for analysis. HoloTC or creatinine concentrations were missing for some individuals; complete data were available on 2403 individuals. The mean (SD) age of all participants was 79.2 (6.2) years, and 41% were men. The main analyses were carried out in all 2403 individuals with complete data and in subsets of 1651 individuals with normal renal function and 752 with abnormal renal function.

DISTRIBUTION OF VITAMIN B_{12} STATUS

Table 1 shows the distribution of median (interquartile range) for age and vitamin B_{12}, holoTC, tHcy, MMA, and creatinine concentrations in all participants and in subsets with normal or abnormal renal function. As concentrations of vitamin B_{12} or holoTC in the overall population declined, MMA concentrations increased. Individuals with holoTC concentrations <45 pmol/L or vitamin B_{12} <200 pmol/L tended to have increased MMA concentrations, but most did not have MMA >0.75 μmol/L. Among individuals with normal renal function, those identified with definite metabolic vitamin B_{12} deficiency showed highly significant differences in holoTC, vitamin B_{12}, and tHcy concentrations compared with participants without vitamin B_{12} deficiency, demonstrating the importance of assessing associations before and after stratification for level of renal function (Table 1).

Table 2 shows a shift in the percentage with low concentrations of serum vitamin B_{12} and holoTC with increasing age, with a corresponding shift in the proportion with increased concentrations of MMA with increasing age. Serum holoTC concentrations positively correlated with vitamin B_{12} (correlation coefficient: $r = 0.61$, $P < 0.001$) and inversely correlated with MMA ($r = -0.44$, $P < 0.001$) and tHcy ($r = -0.38$, $P < 0.001$). In contrast, the associations of vitamin B_{12} with both metabolites were less extreme, with inverse correlations with MMA ($r = -0.34$, $P < 0.001$) and tHcy ($r = -0.32$, $P < 0.001$) in the subset with normal renal function. Serum creatinine concentrations positively correlated with MMA ($r = 0.16$, $P < 0.001$) and tHcy ($r = 0.31$, $P < 0.001$) but not with holoTC ($r = 0.002$, $P = 0.92$).

COMPARISON OF HOLOTC WITH VITAMIN B_{12}

There was a tradeoff between the sensitivity and specificity of any particular level of holoTC or vitamin B_{12} concentrations to detect vitamin B_{12} deficiency in all participants and in the subsets with different levels of renal function. Among those with normal renal function, the interval of cutoff points for holoTC concentrations was between 32 pmol/L (90th percentile specificity) and 58 pmol/L (90th percentile sensitivity). The corresponding interval for vitamin B_{12} was between 157 pmol/L (90th percentile specificity) and 272 pmol/L (90th percentile sensitivity). A sensitivity that equaled the specificity was observed for holoTC at 44 pmol/L and for vitamin B_{12} at 199 pmol/L. The value obtained when the sensitivity equaled the specificity was higher for holoTC (77%) than for vitamin B_{12} (73%) for definite vitamin B_{12} deficiency in those with normal renal function. Hence, cutoff

Table 1. Distribution of vitamin status and other covariates by renal function and vitamin B_{12} deficiency categories.

	MMA, μmol/L	Number of Participants	Age, years	Vitamin B_{12}, pmol/L	HoloTC, pmol/L	MMA, μmol/L	tHcy, μmol/L	Creatinine, μmol/L
All		2403	79 (75–83)	244 (191–310)	64 (44–88)	0.28 (0.22–0.38)	13.5 (10.8–17.1)	97 (86–112)
n = 2403								
Normal renal	MMA <0.45	1452	78 (74–82)	250 (199–316)	67 (48–90)	0.24 (0.20–0.30)	12.1 (10.0–14.5)	90 (82–99)
function	MMA 0.45–0.74	129	81 (76–84)	196 (158–243)	40 (27–58)	0.53 (0.48–0.61)	15.1 (12.4–18.6)	93 (86–104)
(n = 1651)	MMA ≥0.75	70	81 (76–84)	151 (110–199)	24 (14–41)	1.12 (0.88–1.52)	19.3 (14.9–24.8)	91 (84–106)
Abnormal renal	MMA <0.45	560	80 (76–84)	267 (208–338)	74 (54–102)	0.29 (0.24–0.35)	15.1 (12.7–18.5)	113 (103–129)
function	MMA 0.45–0.74	121	82 (80–86)	217 (173–261)	51 (36–69)	0.55 (0.50–0.64)	19.4 (15.8–23.7)	126 (108–146)
(*n* = 752)	MMA ≥0.75	71	84 (80–87)	188 (138–239)	31 (22–46)	1.11 (0.82–1.56)	24.9 (20.9–30.8)	134 (111–150)

Data are median (interquartile range).

Table 2. Age-specific distribution of vitamin B_{12}, holoTC, and MMA concentrations.

	Vitamin B_{12}, pmol/L				HoloTC, pmol/L				MMA, μmol/L		
Age, years	<150 n,%	150–199 n, %	200–299 n, %	≥300 n, %	<30 n, %	30–44 n, %	45–59 n, %	≥ 60 n, %	<0.45 n, %	0.45–0.74 n, %	0.75+ n, %
65–69	2 (2)	16 (12)	65 (50)	48 (36)	3 (2)	17 (13)	18 (14)	93 (71)	88 (67)	42 (32)	1 (1)
70–74	28 (6)	79 (16)	208 (43)	171 (35)	31 (6)	67 (14)	89 (18)	299 (62)	312 (64)	162 (33)	12 (3)
75–79	62 (9)	143 (21)	316 (45)	177 (25)	70 (10)	91 (13)	139 (20)	398 (57)	371 (53)	293 (42)	34 (5)
80–84	62 (9)	164 (25)	257 (39)	180 (27)	64 (10)	112 (17)	130 (20)	357 (54)	312 (47)	303 (46)	48 (7)
85–89	35 (11)	73 (23)	129 (42)	73 (24)	41 (13)	72 (23)	66 (21)	131 (42)	117 (38)	164 (53)	29 (9)
≥90	18 (16)	21 (18)	46 (40)	30 (26)	20 (17)	19 (17)	29 (25)	47 (41)	24 (21)	74 (64)	17 (15)
All	207 (9)	496 (21)	1021 (42)	679 (28)	229 (10)	378 (15)	471 (20)	1325 (55)	1224 (51)	1038 (43)	141 (6)
P for trend	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001	*P* <0.001

Data are n (%) in each category (n = 2403).

points of 45 pmol/L for holoTC and 200 pmol/L for vitamin B_{12} were used to compare the utility of both tests for diagnosis of vitamin B_{12} deficiency. Fig. 1A shows a comparison of the ROC plots for holoTC and vitamin B_{12} to detect individuals with definite metabolic vitamin B_{12} deficiency in individuals with normal renal function. Fig. 1B shows a comparison of the ROC plots for holoTC and vitamin B_{12} to detect individuals with probable metabolic vitamin B_{12} deficiency in individuals with normal renal function. Analysis of ROC curves indicated that holoTC had a greater AUC [0.87 (SE 0.02)] compared with vitamin B_{12} [0.79 (0.03)] for the diagnosis of definite vitamin B_{12}

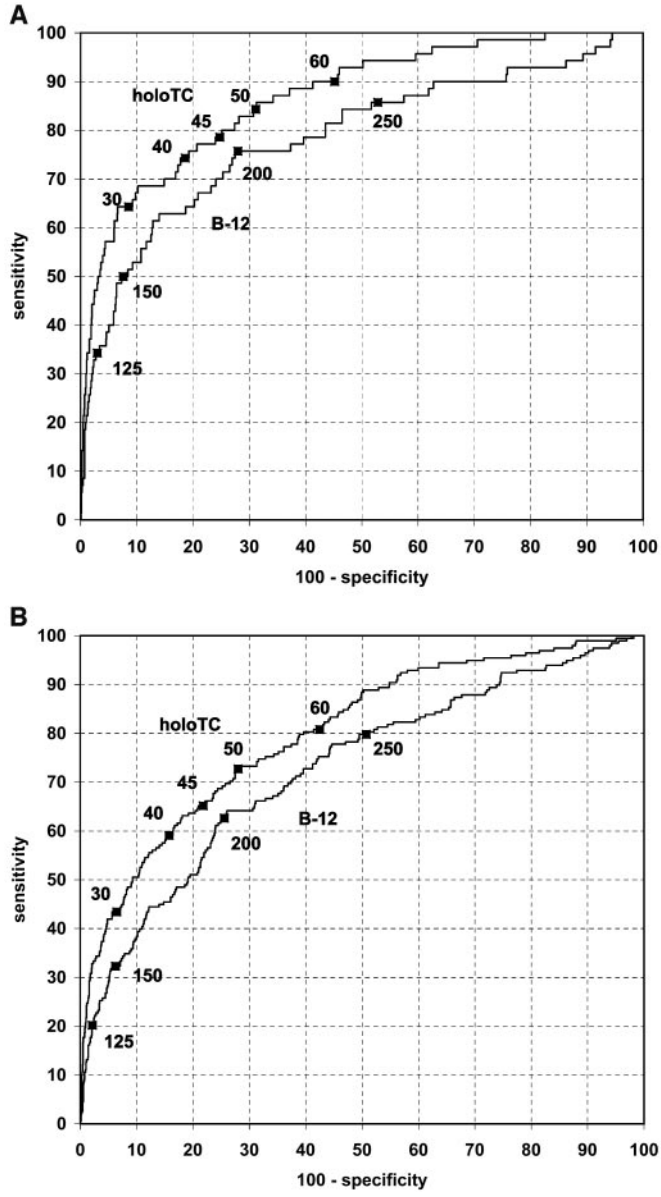

Fig. 1. ROC curves displaying the sensitivity and specificity of holoTC and vitamin B_{12} for diagnosis of definite vitamin B_{12} deficiency (MMA >0.75 μmol/L; *A*) and probable vitamin B_{12} deficiency (MMA >0.45 μmol/L; *B*) in individuals with normal renal function (n = 1651).

deficiency ($P = 0.01$) in individuals with normal renal function.

REPLICATION OF THE FINDINGS IN INDEPENDENT POPULATIONS

The AUCs for holoTC and vitamin B_{12} to detect definite or probable metabolic vitamin B_{12} deficiency in all participants and in the subset with normal renal function are shown in Table 3. Using both definitions of either definite or probable vitamin B_{12} deficiency in each individual population and all participants, holoTC had a modestly superior diagnostic utility for detection of vitamin B_{12} deficiency compared with conventional vitamin B_{12} testing. Although there was some attenuation in the AUC for detection of vitamin B_{12} deficiency in the overall population compared with the subset with normal renal function, the superior diagnostic utility of holoTC compared with vitamin B_{12} was unaffected by the level of renal function. Among the 752 individuals with renal impairment in both studies, the modestly superior diagnostic utility of holoTC compared with vitamin B_{12} for detection of definite metabolic vitamin B_{12} deficiency [AUC (SE): 0.84 (0.03) vs 0.74 (0.03); $P = 0.0002$] was also observed, with a similar finding for probable metabolic vitamin B_{12} deficiency [0.77 (0.02) vs 0.71 (0.02); $P = 0.001$], respectively.

Table 3 also shows comparisons of holoTC with vitamin B_{12} in the individual studies. Despite holoTC being measured using a slightly different method in the OHAP compared with the Banbury study, the modestly superior diagnostic utility of holoTC compared with vitamin B_{12} was observed in both studies. In the Banbury study, vitamin B_{12} was measured using both a Centaur [mean (SD) 246 (93) pmol/L, n = 868] and Beckman method [224 (92) pmol/L], and the AUCs for the 2 vitamin B_{12} assays to detect metabolic vitamin B_{12} deficiency were similar (Table 3). Hence, irrespective of which assay was used to measure vitamin B_{12}, there was a modestly superior diagnostic utility for holoTC compared with vitamin B_{12} to detect metabolic vitamin B_{12} deficiency in the Banbury study, except for the subset with normal renal function using the more extreme cutoff point for metabolic vitamin B_{12} deficiency, where the difference was not statistically significant (Table 3).

UTILITY OF POPULATION SCREENING FOR VITAMIN B_{12} DEFICIENCY

Table 4 compares the utility of holoTC with conventional vitamin B_{12} testing to detect individuals with definite (MMA >0.75 μmol/L) or probable (MMA >0.45 μmol/L) vitamin B_{12} deficiency using cutoff points that provided equal sensitivity and specificity for each assay. A cutoff point of 45 pmol/L for holoTC that had an equal sensitivity and specificity of 79% had a positive predictive value of definite vitamin B_{12} deficiency of 12.5% and an OAPR of 1.14. A cutoff point of 200 pmol/L for vitamin B_{12} that had an equal sensitivity and specificity of 74% had a positive predictive value of 10.8% and an OAPR of 1.12. As expected, the positive predictive value and the OAPR were higher for detection of metabolic vitamin B_{12} deficiency than those for definite vitamin B_{12} deficiency because of the higher prevalence of the outcomes. Nevertheless, use of these cutoff points for both holoTC and vitamin B_{12} was associated with more false positives than

Table 3. Comparison of the AUCs for ROC plots of vitamin B_{12} and holoTC recorded in different populations and with varying levels of renal function for detection of definite (MMA >0.75 μmol/L) or probable (MMA >0.45 μmol/L) vitamin B_{12} deficiency.

	Normal renal function[a]			All participants		
	Banbury	OHAP	Both studies	Banbury	OHAP	Both studies
n	611	1040	1651	939	1464	2403
Definite vitamin B_{12} deficiency						
Vitamin B_{12} (Centaur)	0.82 (0.05)	0.76 (0.04)	0.79 (0.03)	0.78 (0.04)	0.74 (0.03)	0.76 (0.02)
Vitamin B_{12} (Beckman)[b]	0.84 (0.05)			0.79 (0.04)		
HoloTC	0.91 (0.03)	0.84 (0.04)	0.87 (0.02)	0.88 (0.02)	0.82 (0.03)	0.85 (0.02)
P for difference: holoTC vs B_{12} (Centaur)	0.043	0.106	0.010	<0.001	0.009	0.001
P for difference: holoTC vs B_{12} (Beckman)[b]	0.075			0.001		
P for difference: (Centaur vs Beckman)	0.105			0.162		
Probable vitamin B_{12} deficiency						
Vitamin B_{12} (Centaur)	0.75 (0.03)	0.71 (0.027)	0.73 (0.021)	0.73 (0.02)	0.69 (0.02)	0.71 (0.02)
Vitamin B_{12} (Beckman)[b]	0.76 (0.03)			0.74 (0.02)		
HoloTC	0.85 (0.02)	0.77 (0.024)	0.80 (0.018)	0.80 (0.02)	0.75 (0.02)	0.77 (0.01)
P for difference: holoTC vs B_{12} (Centaur)	<0.001	0.026	<0.001	<0.001	<0.001	0.001
P for difference: holoTC vs B_{12} (Beckman)[b]	<0.001			0.001		
P for difference: (Centaur vs Beckman)	0.300			0.049		

Data are AUC (SE).

[a] Serum creatinine <97 μmol/L in women and <124 μmol/L in men.

[b] Vitamin B_{12} was measured by a Centaur assay in both studies and also by a Beckman assay in the Banbury study.

Table 4. Comparison of the utility of holoTC with routine vitamin B_{12} testing for detection of definite (MMA >0.75 μmol/L) or probable (MMA >0.45 μmol/L) vitamin B_{12} deficiency.

	Utility of holoTC (<45 pmol/L)		Utility of vitamin B_{12} (<200 pmol/L)	
Population	Probable vitamin B_{12} deficiency	Definite vitamin B_{12} deficiency	Probable vitamin B_{12} deficiency	Definite vitamin B_{12} deficiency
Normal renal function (n = 1651)	198	70	198	70
True positive (a)	128 (7.8)	54 (3.3)	124 (7.5)	53 (3.2)
False positive (b)	303 (18.4)	377 (22.8)	366 (22.2)	437 (26.5)
False negative (c)	70 (4.2)	16 (1.0)	74 (4.5)	17 (1.0)
True negative (d)	1150 (70.0)	1204 (72.9)	1087 (65.8)	1114 (69.3)
Sensitivity [a/(a + c)]	64.7	77.1	62.6	75.7
Specificity [d/(b + d)]	79.2	76.1	74.8	72.4
Positive predictive value [a/(a + b)]	29.7	12.5	25.3	10.8
OAPR (a/b)	1.42	1.14	1.34	1.12
Likelihood ratio ([a/(a + c)]/[b/(b + d)])	3.1	3.2	2.5	2.7
All participants[a]	390/2403	141/2403	390/2403	141/2403
True positive (a)	229 (9.5)	105 (4.4)	216 (9.0)	93 (3.9)
False positive (b)	378 (15.7)	502 (20.9)	487 (20.3)	610 (25.4)
False negative (c)	161 (6.7)	36 (20.9)	174 (7.2)	48 (2.0)
True negative (d)	1635 (68.0)	1760 (73.2)	1526 (63.5)	1652 (68.8)
Sensitivity [a/(a + c)]	58.7	74.5	55.4	66.0
Specificity [d/(b + d)]	81.2	77.8	75.8	73.0
Positive predictive value [a/(a + b)]	37.7	17.3	30.7	13.2
OAPR (a/b)	1.61	1.21	1.44	1.15
Likelihood ratio ([a/(a + c)]/[b/(b + d)])	3.1	3.4	2.3	2.4

Data are n (%) or %.

true positives for both definite and probable vitamin B_{12} deficiency. Additional analysis using holoTC and vitamin B_{12} simultaneously (using cutoff points of equal sensitivity and specificity for each test) or the ratio of holoTC/vitamin B_{12} did not decrease the excess proportion of false positives to true positives identified using either test alone (data not shown).

Discussion

This study involving 2403 randomly selected older people demonstrated a modest superior diagnostic utility of holoTC compared with conventional vitamin B_{12} testing for the detection of both definite and probable metabolic vitamin B_{12} deficiency. The superior diagnostic utility was confirmed not only in the overall population (AUC 0.85 vs 0.76; $P < 0.001$) and in those with normal renal function (AUC 0.87 vs 0.79; $P < 0.001$), but also in those with abnormal renal function (AUC 0.85 vs 0.74; $P = 0.002$). Using cutoff points of equal sensitivity and specificity at 45 pmol/L for holoTC and 200 pmol/L for vitamin B_{12}, holoTC had a better diagnostic accuracy than vitamin B_{12} (77% vs 73%) for detection of definite metabolic vitamin B_{12} deficiency in individuals with normal renal function.

The results of this study differ from a population-based study of 607 individuals in North America *(12)* that reported no difference in the diagnostic utility of holoTC compared with vitamin B_{12} to detect vitamin B_{12} deficiency. Our study, involving an older population, detected 141 individuals with definite and 390 with probable vitamin B_{12} deficiency and hence had greater statistical power to detect modest differences in the AUC for ROC plots of vitamin B_{12} deficiency than the 37 individuals detected with vitamin B_{12} deficiency in the North American study *(12)*. The results of the present study confirm the superior diagnostic utility of holoTC compared with vitamin B_{12} previously reported in highly selected populations, either people with increased MMA concentrations *(10)* or vegetarians *(17, 18)*.

The superior diagnostic accuracy of holoTC compared with vitamin B_{12} was confirmed in 2 independent populations in Oxfordshire using slightly different assay methods to measure holoTC concentrations *(13, 15)*. Moreover, irrespective of whether a Beckman or Centaur assay was used to measure vitamin B_{12} in the Banbury study, there was a modestly superior diagnostic utility for holoTC compared with vitamin B_{12} to detect metabolic vitamin B_{12} deficiency (with the exception of detection of definite metabolic vitamin B_{12} deficiency among the subset with normal renal function). Whereas the nonsignificant difference ($P = 0.07$) between the diagnostic utility of the 2 assays in the latter subset may have arisen because of random error (chance), an alternative explanation that the superior diagnostic utility of holoTC resulted from limitations of the Centaur vitamin B_{12} assay cannot be completely excluded.

It had been originally suggested that holoTC may be the earliest metabolic change when an individual enters a negative vitamin B_{12} balance, and consequently holoTC should have superior diagnostic accuracy compared with conventional vitamin B_{12} testing for detection of vitamin B_{12} deficiency *(19)*. The advent of several different methods to measure holoTC provided an opportunity to test these predictions *(13, 15)*. However, some experts have advocated caution in the interpretation of low holoTC test results and about the use of holoTC as a surrogate for a Schilling test *(11)*. Clearly, a low holoTC concentration cannot distinguish general cobalamin insufficiency (deficient intake) from malabsorption (idiopathic or drug-induced) *(11)*. Nevertheless, because holoTC is more sensitive than conventional vitamin B_{12} testing in response to low doses of oral vitamin B_{12}, use of holoTC before and after oral ingestion of vitamin B_{12} could still be developed into a test that might be suitable as an alternative to the Schilling test *(20)*.

IMPLICATIONS FOR PUBLIC HEALTH

Impaired vitamin B_{12} function and decreased vitamin B_{12} status have been associated with neurological and cognitive impairment *(4–6)*, but whether these associations are causal is uncertain. Nevertheless, detection of impaired vitamin B_{12} status and the choice of the optimum test to achieve this may be relevant to prevent and treat neurological dysfunction and prevent cognitive impairment.

In view of the high prevalence of low vitamin B_{12} status in older people *(2–4)* and the uncertainty about the proportion of such individuals who may be put at risk because of folic acid fortification, universal screening to detect vitamin B_{12} deficiency in the elderly has been advocated *(2)*. If the utility of screening is solely to detect affected individuals with metabolic vitamin B_{12} deficiency, then the present study suggests that neither test is suitable for this purpose. Clinical vigilance for vitamin B_{12} deficiency will continue to be important to minimize the risk of disability associated with vitamin B_{12} deficiency in older people, as will public health and clinical strategies to avoid an excessive intake of folic acid (>1000 μg/day folic acid where the risk of aggravation of neurological function in people with vitamin B_{12} deficiency is greatest). This study demonstrated that holoTC had a modest superior diagnostic accuracy compared with vitamin B_{12} for the detection of vitamin B_{12} deficiency, but neither test could be recommended to screen asymptomatic populations for vitamin B_{12} deficiency.

Grant/funding support: The MRC CFAS program was funded by the Medical Research Council and the Department of Health. Blood collection and analysis was supported by grants from the Medical Research Council, European Union (QLK3-CT-2002-01775), Health Foundation, London (554/1236), Food Standards Agency, Clothworkers Foundation, and the Joan Dawkins Foundation of the British Medical Association. All the analyses were carried out independently of the sources of support.

Financial disclosures: None declared. The Clinical Trial Service Unit has a policy of not accepting honoraria or other payments from the pharmaceutical industry, except for reimbursement of costs for participation in scientific meetings.

Acknowledgements: We are grateful to all the participants in the Oxford Healthy Aging Project and the Banbury B_{12} project. The MRC CFAS provided administrative support for the main interview data in the OHAP study.

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