Effectiveness of serum Holotranscobalamin II and/or Vitamin B12 in diagnosis of vitamin B12 deficiency

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Background

Vitamin B deficiency is still a wide-spread health problem and with the world-wide ageing populations the significance of this problem is likely to increase. It takes up to 2 years until clinical symptoms of vitamin B12 deficiency become overt. As some of these symptoms like neurological disorders already might be irreversible at the time of diagnosis, efficient laboratory screening tests for vitamin B12 deficiency are necessary.

Cobalamin (total vitamin B12) can be used as a direct measure of the vitamin B12 status, however, the accuracy has been described as low, as cobalamin is measured that is bound to two different binding proteins, haptocorrin and transcobalamin. Only the fraction that is bound to transcobalamin (20 – 30%) represents the fraction with a proven biological activity. To increase the accuracy, Methylmalonic Acid (MMA) is frequently used as a surrogate marker for vitamin B12 deficiency as it is elevated, when the B12 stores are depleted. However, this marker is elevated relatively late and suffers from high testing costs. An ideal entry marker should therefore have a high accuracy to identify non-depletion (stop testing).

The aim of this study was to evaluate an automated measurement of the active fraction of vitamin B12, Holotranscobalamin, as a novel first line marker in comparison to total vitamin B12 and MMA.

Methods

From 01/2005 till 12/2009 we enrolled 996 healthy volunteers of the SAPHIR study, an Austrian prevention study. Exclusion criterias were any vascular or gastric diseases, thyroid dysfuntion, renal dysfunction (GFRmayo <60mL/min/1.73m²) and any medication or vitamin supplementation. For all 996 subjects total vitamin B12 and holotranscobalamin II (HTC) were measured to evaluate which of these parameters are prone to be used as an early laboratory marker for vitamin B12 deficiency. Reference values were 216ng/mL for total vitamin B12 and 43ng/mL for HTC; methylmalonic acid (MMA) values >0.3ng/mL served as external validation criterion for vitamin B12 depletion and the cut-off for folic acid were >3.5ng/mL. Cut-off levels were defined as 95% percentiles according to our own study population in absence of kidney dysfuntion and vitamin deficiency. Blood samples were collected, immediately centrifuged and serum semples were stored at -80°C till analyses were performed. We measured total vitamin B12 and HTC on the Abbott AxSYM Analyzer; MMA was analyzed by mass spectrometry (1).

Results

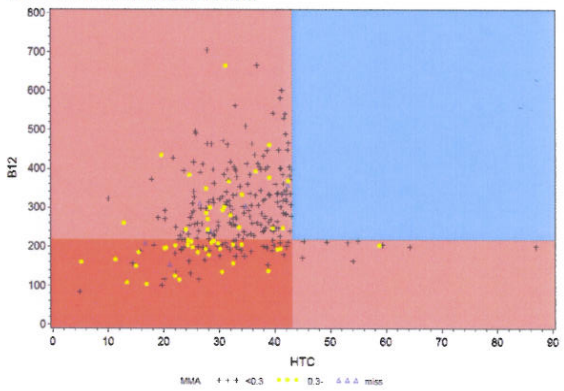
HTC and total Vitamin B12 concordance with MMA

Among all subjects 88 (8.8%) showed a vitamin B 12 deficiency defined as MMA >0.3ng/mL.

HTC < 43 pmol/L identifies nearly all subjects with MMA > 0.30 µmol/L, in contrast to total vitamin B12 (<216), which misses 41.5% of the subjects with elevated MMA.

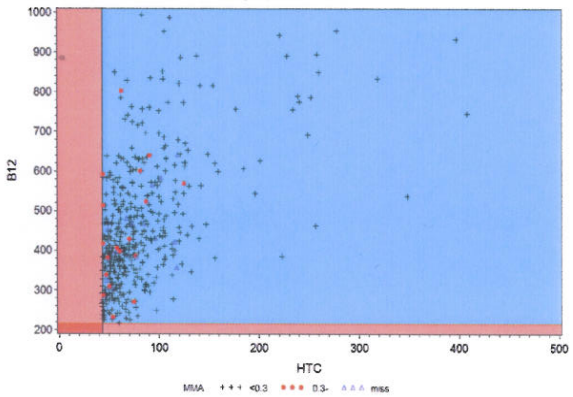
212 subjects had HTC< 43 pmol/L but no elevated MMA. Thus, if MMA is used as surrogate marker for Vitamin B12 depletion, HTC would falsely identify these patients and MMA follow up is required to confirm B12 depletion. However, it has to be noted that MMA is a relatively late metabolic marker of vitamin B12 depletion (2). So the possibility exists that HTC provides an early detection of beginning B12 depletion in some patients, where the metabolic effect of elevated MMA is not yet manifest. Valente et al. showed in a recent publication that the sensitivity and specificity of HTC exceeds that of MMA if red blood cell cobalamin < 33 pmol/L is used to define B12 depletion (3).

Figure 1. MMA distribution in depletion group(s)



Only 3.9% of subjects with a HTC > 43 pmol/L have MMA > 0.30 µmol/L. These subjects could only be identified by raising the cutoff of HTC at the cost of markedly decreased specificity (figure 2).

Figure 2. MMA distribution in non-depletion group



Results *(continued)*

ROC Analysis

In receiver operating characteristic curve (ROC) analysis, correlation of HTC and total vitamin B12 with vitamin B12 deficiency was moderate. There was a difference of 0.06 for the AUC when HTC and B12 were compared (p=0.058).

The difference of the AUC of the combination HTC / B12 to that of B12 was significant (p=0.011). However, we could not detect a significant difference between the combination and HTC only (p=0.705). This suggests, that the gain of information is not relevant if HTC together with total vitamin B12 is measured.

Figure 3. ROC curves

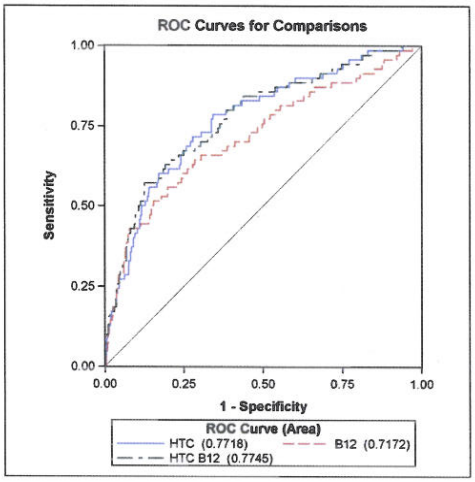


Table 2. ROC analysis

	n	AUC	95% CI
HTC	734	0.7718	0.7122 to 0.8315
B12	734	0.7172	0.6464 to 0.7880
HTC + B12	734	0.7745	0.7139 to 0.8352

Sensitivity and Specificity

Table 3. Analytical Performance

	n	cutoff	Specificity (95% CI)	Sensitivity (95% CI)	NPV (95% CI)	PPV (95% CI)
HTC	734	43ng/mL	68.1 (64.4 to 71.6)	72.9 (60.9 to 82.8)	96.0 (93.8 to 97.6)	19.4 (14.8 to 24.7)
B12	741	216ng/mL	92.4 (90.1 to 94.3)	43.7 (31.9 to 56.0)	90 (55.50 to 99.75)	37.8 (27.3 to 49.2)

Conclusions

- HTC has a high accuracy in identifying non-depletion. Therefore, it is an ideal entry marker that allows to stop further testing after identification of non-depletion.
- HTC has a significantly better positive predictive value in the depletion group than total serum vitamin B12.
- HTC is a more reliable indicator than total serum vitamin B12 in this healthy population with normal renal function and should be used to screen for vitamin B12 deficiency.
- HTC might reach an even better sensitivity and specificity than calculated from this study, if it can be established as an early marker for B12 depletion that precedes MMA elevation with high accuracy as described in a recent publication (3).

References

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2. Herbert V. Am J Clin Nutr 1994;59(suppl):1213S-22S
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