**Assessing biomarker status of vitamin B12 in the laboratory: no simple solution**

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Cobalamin (vitamin B12) deficiency causes megaloblastic anaemia and irreversible neurological disease leading to death if untreated. Early detection and treatment are therefore essential. Severe deficiency of vitamin B12 arises with pernicious anaemia, an autoimmune gastritis characterised by B12 malabsorption owing to loss of intrinsic factor.1 A more subtle depletion of vitamin B12 status can however arise from mild atrophic gastritis leading to reduced gastric acid production (hypochlorhydria), thereby diminishing B12 absorption from food because of the essential role of gastric acid in the release of B12 from food proteins.2 Food-bound B12 malabsorption commonly occurs in older adults (reported to affect up to 20%)1 and leads to sub-clinical deficiency, with metabolic evidence of deficient status but without the classical haematological or neurological signs of deficiency.3 Of note low B12 status found in older adults is rarely attributable to dietary insufficiency, and is typically the result of malabsorption related to atrophic gastritis or use of proton pump inhibitors or other gastric acid suppressant drugs.4 Emerging evidence indicates that low (though not necessarily deficient) biomarker status of B12 is associated with increased risk of various chronic diseases of ageing including cognitive dysfunction, cardiovascular disease and osteoporosis.4 Therefore, assessing B12 status and correction of low/deficient status should be public health priorities.

Vitamin B12 status is assessed using up to 4 biomarkers, both direct (total B12 and holotranscobalamin; holoTC) and functional (homocysteine and methylmalonic acid; MMA) biomarkers. Measurement of serum total vitamin B12 has been the standard clinical test for many years, with B12 deficiency generally identified as B12 concentrations ~ <148pmol/L, however, this can vary between laboratories.5 For this purpose, the microbiological assay is generally considered to have good sensitivity for diagnosis of clinical B12 deficiency but, owing to its time consuming and laborious nature, has been replaced in almost all clinical laboratories by automated competitive protein binding assays.3 The diagnostic sensitivity of some assayshas caused concern, however, with falsely elevated results reported in patients with pernicious anaemia.6

Measurement of metabolites of vitamin B12-dependent reactions provide functional indicators of B12 status. Methionine synthase uses vitamin B12 as a cofactor in the remethylation of homocysteine to methionine. The activity of this enzyme is impaired with vitamin B12 depletion leading to an elevation of total homocysteine that can be readily measured in plasma using a variety of automated analytical techniques.7 It should be noted however that plasma homocysteine is not specific to vitamin B12 as it is influenced by other nutrient (most notably folate) and non-nutrient factors including renal function, greatly limiting its use as a biomarker of B12 status. Vitamin B12 is also a cofactor for methylmalonyl CoA mutase. In vitamin B12 depletion, reduced activity of methylmalonyl CoA mutase leads to an accumulation of the by-product MMA which can be measured in plasma or urine. Measurement of MMA, unlike homocysteine, provides a specific biomarker for vitamin B12. The majority of patients with vitamin B12 deficiency will have elevated serum MMA and it has also proven to be a useful biomarker in monitoring subclinical deficiency in population-based studies.3 Limitations of serum MMA as a biomarker of B12 status include the fact that it is greatly influenced by renal dysfunction and genetic variation, along with high running costs.5

Measurement of HoloTC (or ‘active B12’) is theoretically attractive because, unlike serum total B12 (which measures concentrations of the total vitamin, 80% of which is metabolically inert), it represents the metabolically active fraction of vitamin B12 available for cellular processes. HoloTC was reported to be better correlated with tissue stores of vitamin B12 and was found to be superior to serum total B12 and MMA in diagnosing B12 tissue deficiency in an older Irish population.8 Other reports however showed that HoloTC was only marginally better than serum total B12 in diagnosing clinical deficiency (as defined using MMA rather than tissue stores of the vitamin).9,10 Also, HoloTC is found to be elevated in patients with renal insufficiency, liver disease and cancer and is influenced by genetic factors, limiting its use as a first line diagnostic tool for identifying vitamin B12 deficiency.11.

Given the limitations of individual assays, experts in the field now recommend that more than one biomarker is used to accurately diagnose vitamin B12 deficiency,2,11 with the recent emergence of approaches that identify deficient status using combinations of two or more biomarkers. The National Health and Nutrition Examination Survey (NHANES) opted to use the combination of serum total vitamin B12 and MMA to monitor B12 status in the United States population.12 Furthermore, algorithms have been developed to diagnose vitamin B12 deficiency, including the ‘Fedosov’s Wellness Score’, a combined B12 index which uses two, three or four B12 biomarkers in combination and accounts for age and folate status, with results expressed as probable deficiency, possible deficiency, and low, adequate and elevated vitamin B12 status.13 The usefulness of Fedosov’s Wellness Score has been demonstrated in a small number of studies, however, the high costs involved are likely to make this approach prohibitive for the purposes of routine clinical practice.7 More feasible is the approach adopted by a number of laboratories which have developed first and second line diagnostic procedures, whereby one biomarker (usually serum total B12 or holoTC) is measured, with additional measurements (usually MMA) performed in samples with indeterminate results.7

In summary, accurate assessment of vitamin B12 is problematic and there is no consensus as to the best biomarker for use in clinical laboratories. HoloTC shows promise as a reliable biomarker of vitamin B12 status, but the influence of confounding factors needs to be more fully elucidated. The use of a sole biomarker of vitamin B12 status should be avoided and two or more biomarkers should be used in combination. This will ensure that vitamin B12 deficiency is diagnosed and treated in patients, and vitamin B12 status optimised in older populations generally, to ensure that any adverse health consequences of deficient and low B12 status are prevented.

**References**

1. Stabler SP. Vitamin B12 deficiency. *The New England journal of medicine*. 2013; 368: 2041-2.

2. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *The American journal of clinical nutrition*. 2011; 94: 348s-58s.

3. Carmel R. Diagnosis and management of clinical and subclinical cobalamin deficiencies: why controversies persist in the age of sensitive metabolic testing. *Biochimie*. 2013; 95: 1047-55.

4. Hughes CF, Ward M, Hoey L and McNulty H. Vitamin B12 and ageing: current issues and interaction with folate. *Annals of clinical biochemistry*. 2013; 50: 315-29.

5. Green R, Allen LH, Bjorke-Monsen AL, et al. Vitamin B12 deficiency. *Nature reviews Disease primers*. 2017; 3: 17040.

6. Carmel R and Agrawal YP. Failures of cobalamin assays in pernicious anemia. *New England journal of medicine*. 2012; 367: 385-6.

7. Harrington DJ. Laboratory assessment of vitamin B12 status. *Journal of clinical pathology*. 2017; 70: 168-73.

8. Valente E, Scott JM, Ueland PM, Cunningham C, Casey M and Molloy AM. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B12 status in the elderly. *Clinical chemistry*. 2011; 57: 856-63.

9. Risch M, Meier DW, Sakem B, et al. Vitamin B12 and folate levels in healthy Swiss senior citizens: a prospective study evaluating reference intervals and decision limits. *BMC geriatrics*. 2015; 15: 82.

10. Nexo E and Hoffmann-Lucke E. Holotranscobalamin, a marker of vitamin B-12 status: analytical aspects and clinical utility. *The American journal of clinical nutrition*. 2011; 94: 359s-65s.

11. Hannibal L, Lysne V, Bjorke-Monsen AL, et al. Biomarkers and Algorithms for the Diagnosis of Vitamin B12 Deficiency. *Frontiers in molecular biosciences*. 2016; 3: 27.

12. Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of vitamin B-12 status in NHANES: a roundtable summary. *The American journal of clinical nutrition*. 2011; 94: 313s-21s.

13. Fedosov SN, Brito A, Miller JW, Green R and Allen LH. Combined indicator of vitamin B12 status: modification for missing biomarkers and folate status and recommendations for revised cut-points. *Clinical chemistry and laboratory medicine*. 2015; 53: 1215-25.