Quantitative target metabolomics using LC-MS/MS improves the diagnosis of vitamin B12 deficiency & saves cost and time

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Introduction

Vitamin B12 is a water-soluble vitamin with a key role in the vital functioning of the brain and nervous system, as well as in the formation of blood. Furthermore, it is also involved in cell metabolism, energy production, fatty acid synthesis, DNA synthesis and regulation. Vitamin B12 deficiency is common in the general population, particularly in elderly people and vegetarians [1]. It causes hematologic and/or neuropsychiatric diseases and increases the risk of age-associated disorders like dementia, depression and bone fractures. The vitamin B12 deficiency-related neurological sequela are mostly non-specific, but can be predicted if it is treated rapidly. Diagnosis of vitamin B12 deficiency at an early stage is crucial for preventing the development of irreversible clinical complications. Total serum B12 fails to detect the deficiency in the majority of cases with intracellular B12 deficiency [2]. Low dietary intake of vitamin B12 explain the deficiency only in vegetarians, but not in elderly people where only 5% of them are known to show low serum vitamin B12 [3], while up to 20% show biochemical evidence of deficiency [4, 5]. The metabolic marker methylmalonic acid (MMA), which increases in plasma and urine in the case of vitamin B12 deficiency, fulfills both the sensitivity and specificity criteria [6]. By analysing plasma MMA levels, it is possible to distinguish persons according to their dietary habits (vegetarians, omnivores etc.) to diagnose vitamin B12 deficiency in Methylamin users, a diabetic drug, and to optimise the success of vitamin B12 supplementation. However, until now, MMA levels have mainly been measured with GCMS methods, which can be expensive and are often not available in modern laboratories.

The aim of the study was to compare the performance of combining MMA, with active B12 (holotranscobalamin, holoTC) or the traditional assay of total serum B12. MMA was measured by GC-MS in a 2-step-diagnostic algorithm after total or active B12 in 1034 samples referred to our laboratory for B12 status testing. In addition, a reagent kit for the specific determination of MMA in plasma/serum and urine using LC-MS/MS (Chromsystems GmbH) was compared with the GCMS method.

Results

Serum levels of MMA were higher in vegetarians, and in particular in those who avoid all kinds of animal products (vegans), especially, when they did not receive any kind of B12 supplements (Fig. 2a). MMA levels were > 271 nmol/L (the upper limit of the reference range) in approximately 65% of vegetarians and 85% of vegans who were not taking additional supplements. In lowering MMA after B12 treatment in this group can provide the final evidence for B12 deficiency. holoTC decreased) or normal (both MMA and holoTC are normal). In a subgroup of the population (~15%) holoTC was normal and MMA was elevated, suggesting contradicted finding. We obtained a better results when combining MMA and holoTC levels (Fig. 3). In this case, samples can be classified to either deficient (MMA elevated, holoTC decreased), depleted (only holoTC decreased) or normal (both MMA and holoTC are normal). In a subgroup of the population (~15%) holoTC was normal and MMA was elevated, suggesting contradictory finding. Lowering MMA after B12 treatment in this group can provide the final evidence for B12 deficiency. Plasma MMA concentrations measured using the commercial kit (LCMS/MS) and the GC-MS method revealed strong correlation (linear regression curves y = 0.941x + 41.681 (r² = 0.994), indicating good agreement between both methods. The LCMS/MS method has 1.5 fold lower costs and requires 20-fold less time from sample preparation to reporting results.

Conclusion

Vitamin B12 deficiency in screening studies can be detected using modern metaboalites adopted to LC-MS/MS instrumentation. Using this approach by routine laboratories improves early diagnosis and treatment monitoring, hence reducing irreversible complications. Early diagnoses and treatment are cost effective compared to treating diseases caused by the deficiency. The LCMS/MS method is characterised by fast sample preparation, minimal matrix effects, high precision, high robustness and cost-effectiveness.

References