EDITORIAL

Vitamin B12

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In this issue of the *Netherlands Journal of Medicine*, the authors of the paper 'Association of vitamin B12, methylmalonic acid and functional parameters'[1] recommend increased awareness of the complicated recognition of what they call symptomatic vitamin B12 deficiency. Their study is based on a large NHANES dataset that is a cross-sectional sample of the general US population aged 19 years and older from 2012-2014. The dataset contains much biochemical data, including measurements of serum vitamin B12 and methylmalonic acid levels, in addition to functional parameters of physical and mental state, well-being, and use of medical care, as collected by questionnaires. These functional parameters are the focal point in the current discussion on their eventual relation to vitamin B12 deficiency (vitB12).

The results of this study confirm earlier observations that macrocytic anaemia occurs only in a minority (4.4%) of individuals with subnormal vitB12 levels. The study does not state whether cases with the typical neurological symptoms of ataxia, paraesthesias, and impaired sense of touch had been found. The results confirm the well-known inverse relation between serum concentrations of vitB12 and methylmalonic acid (MMA); MMA is the substrate of the vitB12-dependent enzyme MMCoA-mutase.[2] In addition, the authors show that only 50-60% of the participants with a low serum vitB12 have an increased MMA and that a normal vitB12 does not exclude an increased MMA. Remarkably, the observed mental and physical parameters are not, or only weakly, associated with vitB12, but are significantly associated with MMA. The authors conclude that MMA has proven to be a more reliable predictor of poor functional performance and may assist with diagnosing vitB12 deficiency in uncertain cases where serum vitB12 concentrations are above the lower reference value, thereby assuming that the observed functional performance parameters are related to vitB12 status.

How convincing are these conclusions? In the first place, the list of investigated health and functional parameters includes more aspects than the parameters that are included in the above-mentioned discussion. In fact, they are more closely covered by only the domains 'mental health & depression', 'physical functioning', and 'cognitive functioning'. Even in those three domains, the associations with vitB12 are very weak and are significantly stronger with MMA. The median scores and ranges are however low. These observations, therefore, only weakly support the concept that these three domains indeed belong to the spectrum of symptoms of vitB12 deficiency and should be diagnosed and treated as such. What might then be an explanation for the relation to MMA?

There is no doubt that vitB12 deficiency results in congestion of MMA if MMA is not converted to succinate, and it has been shown that this leads to increased MMA concentrations in the blood. But analysing the absolute numbers rather than the percentages of increased MMA concentrations in the vitB12 categories low, intermediate, and high, one can calculate that, in addition to the 89 cases in the low vitB12 range, another 647 cases have increased MMA in the intermediate (372) and high (275) vitB12 ranges. This is approximately four times the total number of cases in the low range. The reasons for this apparent imbalance between alleged cause (vitB12 deficiency) and consequence (MMA rise) are still unknown.

One possible explanation is that serum vitB12 is not always representative for intracellular vitB12 and that MMA more reliably reflects this intracellular vitB12 status. Either the vitamin deficiency is developing but not yet in a stage of depletion at tissue level (in the category low vitB12), or the tissues are depleted but unusual amounts of vitB12 are bound to other vitB12-binding proteins, such as haptocorrin, that cannot reach the peripheral tissues. This has been reported in typical forms of cancer and chronic inflammation.[3,4] It is however, unlikely that this occurs as frequently as seen in this cross-sectional sample. The variability of serum MMA appears to be influenced by serum vitB12 in fewer than 17% of healthy volunteers,[5] but MMA concentration increases strongly with decreasing glomerular filtration rate (GFR).[6] The percentage of participants in the study with a normal vitB12 and an increased MMA is reduced from 14% to 5% when participants with an estimated GFR < 60 ml/min are

excluded. Of course, in addition to MMA, impaired renal function effects many other plasma components such as homocysteine, which is increased in vitB12-deficiency as well. Therefore, the more relevant question is whether disturbed mental or physical functioning is more closely associated with renal function than with MMA, but unfortunately the data in this paper was not analysed in a manner that can answer this question.

It therefore remains unsure whether there is indeed a functional relation between vitB12 and the functional parameters. An association is not proof of a causal relationship and both vitB12 and MMA can represent other parallel acting parameters. From this perspective, hyperhomocysteinemia has been shown to have an effect on neurotransmitter concentrations in the cerebral spinal fluid and may therefore influence brain functions.[7] Vitamin deficiencies are individually linked to a variety of clinical symptoms but these lists of symptoms overlap considerably. Most B vitamins are active as co-factors in important enzymatic reactions that occur in most tissue cells all over the body. Shortages in vitamins, either single or combinations, may lead to different expression levels in various organs. Nutrient intake might be another modifying factor. Although the body is capable of neutralising large differences in the diet, the differences may have more influence at extreme deviations. Thus, it is conceivable that a methionine-poor diet causes a higher sensitivity to a shortage of vitB12, because of the role of methylcobalamin in the re-methylation of homocysteine to methionine.

Despite the results of the above study, the many outstanding questions prevent complete confidence in the mentioned functional parameters and their relevance to vitB12 status and MMA, and the conclusion that they are an effective diagnostic substitute for vitB12. Further epidemiological studies, with even larger patient numbers and new parameters will not solve the question of whether a specific blood component can bear the role of reliable biomarker as long as it remains uncertain which symptoms are proven to be linked to vitB12 status, and are effectively treated with vitB12 suppletion. The only way to identify this is by taking the initiative to conduct a blinded intervention study with properly defined patients and controls and by applying standard treatment modalities that have established their effectiveness in proven vitB12-deficiency conditions such as pernicious anaemia. Strict standardisation of the scoring of investigated clinical symptoms before, during, and after the intervention period is an essential condition.

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