European Journal of Clinical Investigation (2001) **31**, 928–930

Commentary

Total homocysteine is making its way into pediatric laboratory diagnostics

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In adults, the diagnostic utility of total homocysteine (tHcy) determination is well established. Elevated tHcy is a risk factor for adverse pregnancy outcomes [1,2], impaired cognitive function [3] and occlusive arterial and venous disease [4], and the use of tHcy has been recommended in cardiovascular risk assessment [5]. Levels of tHcy are increased in folate and cobalamin deficiencies, and they serve as a useful test for the diagnosis and follow up of these deficiency states [6]. Application of tHcy in laboratory diagnostics requires knowledge on the reference values for tHcy, which vary according to age, sex, lifestyle, physiological [7,8] and genetics factors [9]. Levels of tHcy are also increased in certain diseases, particularly in those associated with renal dysfunction [4,10].

During the last 5 years, the literature on tHcy levels in healthy and diseased children has accumulated, and several research groups have published reference levels for tHcy from birth until puberty. In children aged below 12 years, the mean tHcy concentration is about 4–8 μM, which is 60% of the values detected in adults [11–13]. It increases moderately as a function of age [14]. Some [15,16] but not all [13,17–20] studies demonstrate a slightly higher tHcy level in boys than in girls, and this gender effect is enhanced during and after puberty ($>$ 15 years) [18,21].

Several lifestyle factors [15] and diseases [22], in particular renal dysfunction [23–26], affect tHcy in children, as previously reported in adults, but folate and cobalamin status are the most important tHcy determinants. Of particular importance is the observation that the association between these vitamins and tHcy is age-related. In newborns and infants, tHcy shows a strong correlation with serum cobalamin but not with serum or erythrocyte folate [27,28]. Hyperhomocysteinaemia in a significant portion of infants in this age group has been attributed to impaired cobalamin status [28]. This contention is supported by the consistent findings of high serum or urine concentrations of methylmalonic acid (MMA) in many newborns [28,29]. In older children, tHcy is determined by both folate and cobalamin status, as observed in adults [13–15,18,21].

There are only a few reports evaluating tHcy in the diagnosis and follow-up of folate or cobalamin deficiencies in children. Hyperhomocysteinaemia due to cobalamin deficiency frequently develops in breast-fed newborns or infants of vegetarian [12] or malnourished mothers, as has been encountered in developing countries [30], but MMA may afford better accuracy than tHcy to detect impaired cobalamin status in these children [12,31]. Elevated tHcy reflecting impaired folate status has been demonstrated in children given high-dose methotrexate [11,32], or anti-epileptic drugs [33,34] and in girls with anorexia nervosa [35].

In this issue of *European Journal of Clinical Investigation* Vilaseca *et al.* [36] demonstrate hyperhomocysteinaemia or low serum folate in more than 40% of 69 human immunodeficiency virus (HIV)-infected children. tHcy was strongly correlated to folate but not to serum cobalamin, which was normal in the affected children. Thus, HIV infection in these children is associated with impaired folate status, whereas cobalamin deficiency, which is occasionally detected in adult patients, had not developed. Notably, folate deficiency (as determined by tHcy and serum folate) was not related to the clinical status of the patients, but was more frequent in patients treated with protease inhibitor [36].

The article of Vilaseca *et al.* is important since it demonstrates the application of tHcy measurement to identify folate-deficient subjects in a considerable portion of HIV-infected children. Supplementing these patients with folic acid may be a safe and inexpensive strategy to improve their clinical status. Folic acid is expected to reduce tHcy, particularly in subjects with elevated levels [37], and the metabolic response can be monitored as tHcy reduction. This reduction in tHcy may by itself be advantageous, since hyperhomocysteinaemia in children

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Received 14 August 2001; accepted 16 August 2001

has recently been identified as a risk factor for occlusive vascular disease [38–41], including stroke, which is a frequent complication of acquired immune deficiency syndrome in children [36].

In conclusion, high plasma tHcy is a responsive marker of impaired folate or cobalamin function in tissues [6]. Recent reports have demonstrated that its measurement, in conjunction with the cobalamin-marker MMA or with vitamin concentrations, is an efficient strategy for the diagnosis of these deficiency states in children. Metabolite determination may be particularly useful for the diagnosis of subtle deficiency states which lack the typical clinical signs of anaemia and megaloblastosis [6]. Deficiencies of folate or cobalamin among infants are probably more common than hitherto recognized [28,42], and B-vitamin deficiencies are the main indications of metabolite determination in the paediatric setting.

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