Homocysteine what does it mean and have we been led astray?

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firmed, yet, pro-brain natriuretic peptide (BNP) analysis was performed in only 9% of the patients.

The wealth of information provided by the implementation of the Sentinel Network and highlighted by Devroey and Van Casteren should stimulate serious considerations for establishing similar systems in countries that lack the appropriate venues capable of tracking the course of HF in a represented sample of the outpatient population.

Disclosures

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EDITORIAL

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Homocysteine – what does it mean and have we been led astray?

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The hypothesis that elevated levels of homocysteine in the blood are causatively associated with vascular disease can be traced back to the observation by Gibson et al. of a high incidence of cardiovascular events in patients with homocystinuria (1). Since then, an enormous amount of time, energy and money has been invested in exploring this hypothesis, and many thousands of patients have been included in clinical trials of homocysteine lowering therapies. Elevated homocysteine is strongly associated with the development of cardiovascular events in prospective epidemiological studies and there are plausible biological mechanisms by which homocysteine might be toxic to the vascular system. However, the results of clinical trials have been mainly negative. In the January issue of the journal, Wang et al. report a meta analysis of trials of homocysteine lowering therapy in patients with pre-existing cardiovascular, cerebrovascular or renal disease (2). They conclude that there is no evidence that homocysteine lowering therapy (mainly folic acid) will reduce the incidence of further vascular events or all-cause mortality. What, then, is the meaning of homocysteine and have we been misled as to its importance?

1 Very high levels of homocysteine may be harmful, whereas minor elevations are not. The homocysteine hypothesis, as noted above, originated from observations made in patients with homocystinuria, most commonly caused by the deficiency of the enzyme cystathione beta synthase. Such patients have markedly elevated levels of homocysteine, typically over 100 μmol/l, and experience atherothrombotic events in early life (3). At these grossly elevated levels, homocysteine promotes blood clotting (4), which is likely to be a major contributor to the vascular complications observed in homocystinuria. It is unclear whether such mechanisms are relevant in individuals

Elevations in homocysteine may induce a hypomethylation state with profound effects on cellular metabolism

with mildly elevated homocysteine (15–30 μmol/l), in whom atherosclerosis rather than thrombosis is the main proposed vascular pathology. If this is the case, homocysteine lowering might be of benefit in individuals with very high levels, but not in patients with mild to moderate elevation. Many studies reporting mechanisms by which mildly elevated homocysteine might be harmful to the vascular system were flawed, as a result of using free homocysteine rather than homocysteine in the form of protein mixed disulphides (5–7). In addition, some studies failed to include non-specific thiol controls. Mechanisms identified or proposed based on flawed *in vitro* studies may not be of clinical relevance.

2 Homocysteine may be a risk marker rather than a risk factor. A risk marker is associated with risk, but does not itself mediate risk. Therefore, measures to reduce a risk marker will generally not result in benefit, unless they coincidently also improve a biological relevant pathway. The homocysteine hypothesis was built on epidemiological studies, which clearly and consistently showed that homocysteine was elevated in patients with vascular disease compared with healthy controls (8–11), and that in prospective studies mildly elevated homocysteine levels were associated with an increased risk of cardiovascular events (12–14). However, this merely demonstrates association with risk and not causality. In recent years, similar associations have been seen with use of hormone replacement therapy and cardiovascular disease (15), and antioxidant intake and cardiovascular disease (16). In both cases, plausible biological mechanisms were identified, which lent strength to the observed associations and resulted in numerous clinical trials, but the trials found no beneficial effects, and in some cases showed evidence of harm (17,18). Epidemiological observations can, therefore, be misleading. This might be attributable to residual confounding, which is not adequately allowed for. For instance, individuals with low levels of homocysteine are likely to have high intakes of folate, which typically may reflect a high intake of leafy green vegetables. Individuals who eat a lot of green vegetables are likely to have a generally healthy lifestyle, with an increased prevalence of many health behaviours and characteristics, and avoidance of unhealthy lifestyle behaviour (19). While correction can be made for this in statistical analysis, some residual confounding is likely to remain.

Even mild renal impairment will elevate homocysteine significantly

Perhaps more importantly, renal impairment is an important cause of elevated homocysteine levels (20,21), and it has been suggested that even mild renal impairment will elevate homocysteine significantly. Renal function is now recognised as an important driver of cardiovascular risk (22–24), and in most epidemiological studies it is unclear if any attempt has been made to include a sensitive marker of renal function as a co-variate in analysis. Homocysteine may, therefore, be a surrogate marker of renal function and lowering homocysteine would not in these circumstances be of benefit.

3 Interventions used in clinical trials may have started too late and not lasted long enough. Atherosclerosis is a condition which originates in childhood and develops progressively throughout adult life, presenting its clinical manifestations in middle age or later. If homocysteine does play a role in the development of atherosclerosis, then the pathological effects will reflect 50 years or more of tissue exposure to moderately elevated homocysteine levels. In these circumstances, it may well be unrealistic to expect that 5 years or less of homocysteine lowering in individuals with well established disease will reverse pathological damage, which has been built up over many years. While this is an argument which is easy to make, it gives rise to an hypothesis which will be very difficult to test, and which would require a fifty year or longer randomised trial starting in early adulthood. Similar arguments have been made in relation to antioxidant intake and cardiovascular disease (25).

4 The wrong interventions may have been used in clinical trials. The metabolism of homocysteine is complex, and includes both methylation and transsulphuration (26). Numerous enzymes are involved in the homocysteine pathways, and these are dependent on a range of vitamins including folate, B12, B6 and riboflavin among others. Important metabolic intermediates are linked to homocysteine metabolism, including glutathione and asymmetric dimethylarginine (27,28). Elevations in homocysteine may, therefore, have a range of consequences, including the induction of a hypomethylation state with profound effects on cellular metabolism (29). Alternatively, hyperhomocysteinaemia may lead to increased oxidative stress by a variety of mechanisms, altering cellular redox balance (30). Homocysteine can also directly interact with proteins to modify key thiol groups and alter function (31). Interventions to lower homocysteine tend to rely on folate supplementation, with variable use of the other vitamins, which in general have a less potent homocysteine lowering effect (32). The effect that these interventions might have on the various pathways described above is not clear, and targeted interventions to address, for instance, hypomethylation might be effective whereas a blunt homocysteine lowering intervention might not.

5 Interventions used to lower homocysteine might have additional harmful effects, which outweigh any benefit resulting from homocysteine lowering. As discussed above, supplementation with folic acid has been the mainstay of trials aiming to lower homocysteine. Particular concern has been expressed by some about the potential of folate to increase methylation while lowering homocysteine. Many genes contain CpG islands in their promoter regions, and their expression is altered by changes in methylation status (33). This could have profound effects on cellular function, including alterations in cell division (34) and expression of a range of pro-atherogenic molecules (35). Therefore, folate in particular could lower homocysteine while exerting other effects that negate any benefit which might otherwise result.

What, therefore, should we conclude with regard to homocysteine measurement and homocysteine lowering therapies? In younger people with recurrent thrombotic events, it remains prudent to measure homocysteine in plasma as recommended in current guidelines (36). If homocysteine is markedly elevated in these circumstances, then measures to reduce it, including supplementation with folate and other relevant B-group vitamins, should be pursued, largely based on experience gained in homocystinuria. The evidence that homocysteine is a risk marker for cardiovascular disease is very strong, but there is little evidence that it adds value to current risk prediction equations (37). Therefore, there seems no basis on which to recommend measurement of homocysteine as a cardiovascular risk factor. Based on current evidence, use of homocysteine lowering therapies as a secondary prevention measure in individuals with renal failure or cardiovascular disease cannot be recommended. With regard to primary prevention of vascular disease, current evidence is also not supportive, although some large trials are still ongoing.

If we have been led astray in relation to homocysteine, it is because we were persuaded by epidemiology and plausible mechanisms. There are now at least three clear examples – hormone replacement therapy, antioxidants and homocysteine – where clinical trials have failed to confirm insights obtained from epidemiological studies. There are other areas where some are currently promoting widespread use of supplements based on similar types of evidence, such as vitamin D deficiency. Properly controlled clinical trials remain essential to confirm insights derived from epidemiological studies.

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None.

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