Understanding the Complexity of Homocysteine Lowering With Vitamins
The Potential Role of Subgroup Analyses

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Since the publication of several randomized trials and a meta-analysis indicating that lowering homocysteine levels with B vitamins (to reduce the effects of homocysteine on the vascular endothelium) did not result in cardiovascular benefit, the use of vitamin therapy to lower homocysteine levels is widely regarded as ineffective. However, there has been renewed interest in the issue because an analysis of studies from the National Health and Nutrition Examination Survey and the Multi-Ethnic Study of Atherosclerosis showed that adding total homocysteine level to a Framingham risk score was associated with an approximately 20% net reclassification of intermediate-risk patients. A recent meta-analysis raised the question of whether population folate intake and polymorphisms of methylenetetrahydrofolate reductase may alter interpretation of these clinical trials; mendelian randomization analysis suggested that the polymorphisms might be important only among individuals with low folate status. A commentary about that article noted the hazards of the “spinal reflex” that leads to “automatic rejection of observational data when they appear to be discrepant from trials.” A similar spinal reflex, automatic rejection of subgroup analyses, is another hazard of interpretation of clinical trials.

Subgroup analyses founded in biology may have the potential to importantly inform interpretation of clinical trial results, provided sufficient caution is exercised. Thoughtfully derived subgroups, especially those formed a priori and not derived post hoc from the same data set, can stimulate further work and reinterpretation of existing data.

The question of whether B vitamin therapy to lower homocysteine levels may reduce the risk of stroke is becoming more complicated, and analyses that depended on grouping all patients together may have obscured important differences among patient (and perhaps population) subgroups. For instance, the main results of the Vitamin Intervention for Stroke Prevention (VISP) trial showed no effect of B vitamins (folic acid/pyridoxine/cyanocobalamin) on the risk of recurrent stroke, death, or myocardial infarction, whereas a subgroup analysis of the VISP trial showed benefit of B vitamins in a defined subset of the population. More recently, House et al reported that B vitamin therapy was shown to increase cardiovascular risk in patients with diabetic nephropathy. Hence, subgroup analyses have shown beneficial as well as adverse effects of B vitamin therapy.

The VISP subgroup analysis excluded patients with B12 deficiency (because they were all receiving injections of cyanocobalamin, regardless of the treatment to which they were randomized), and patients with significant renal impairment (because it was thought they would not respond to vitamin therapy). There was a significant reduction of stroke or myocardial infarction with B vitamins in the remaining participants, and this finding was more pronounced when patients were stratified by baseline serum B12 level. The difference between patients who entered the study with a serum B12 level above the median (ie, they could absorb B12 reasonably well) and received high-dose vitamins and those who entered the study with a serum B12 level below the median and received low-dose vitamins was a 34% reduction of stroke, death, or myocardial infarction (P = .02). At the time, it seemed that the findings were explained mainly by exclusion of patients receiving vitamin B12 injections, and this seemed supported by the finding in the Heart Outcomes Prevention Evaluation 2 (HOPE-2) trial (the only large trial to use an adequate dose of B12 for elderly patients) that B vitamins significantly reduced the risk of stroke. In 2011, Hsu et al reported that in the VISP trial, a subgroup of patients with the GG phenotype of transcobalamin 2, a transport protein for vitamin B12, were responsive to high-dose vitamin therapy.

Vitamin B12 may thus have a key role in stroke prevention interventions involving vitamin therapy used to lower homocysteine levels. Homocysteine increases thrombosis and is associated with a markedly increased risk of stroke in atrial fibrillation. The prevalence of atrial fibrillation increases steeply with age, as do metabolic B12 deficiency (not necessarily reflected in serum B12 levels, discussed below) and plasma total homocysteine levels. In the Framingham cohort, 1.5% of strokes at ages 50 through 59 years were attributable to atrial fibrillation, whereas by ages 80 through

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89 years the attributable risk was 23%, such that in this age group, atrial fibrillation was the only independent predictor of stroke risk. Among patients referred to a stroke prevention clinic, the prevalence of plasma total homocysteine levels greater than 14 µmol/L was 30% among patients aged 75 through 79 years and 40% among those 80 years or older. This finding is mainly attributable to metabolic B12 deficiency.

Importantly, a serum B12 level in the reference or “normal” range does not exclude metabolic B12 deficiency. Metabolic B12 deficiency is strictly defined by elevation of plasma methylmalonic acid; in folate-replete patients, elevation of plasma total homocysteine levels can substitute for methylmalonic acid levels, which are not commonly measured. With folic acid fortification of the grain supply, nearly all residents in North America are folate-replete, so vitamin B12 is now the major determinant of total homocysteine levels. Some researchers have estimated that approximately 20% of older persons have B12 deficiency. Among patients with vascular disease from one clinic database, metabolic B12 deficiency was present in 12% of those younger than 50 years, 13% of those aged 50 through 71 years, and 30% of those 71 years or older. The serum B12 level below which total homocysteine and methylmalonic acid levels become elevated is 400 pmol/L10 (well within the reference range of 160-600 pmol/L). To be confident on the basis of a serum B12 level that a patient does not have metabolic B12 deficiency, the serum level needs to be above 400 pmol/L; approximately two-thirds of patients would require further testing, such as measurement of plasma methylmalonic acid or total homocysteine levels, to exclude metabolic deficiency of B12.

The finding that B vitamin therapy was associated with increased cardiovascular risk in patients with diabetic nephropathy, and that the increased risk of events was confined to those with a low glomerular filtration rate (less than 50), sheds further light on the VISP subgroup analysis. It seems that benefit or harm from vitamin therapy used to lower homocysteine levels depends not only on adequate dosing and absorption of vitamin B12 but also on renal function.

Several biological effects may help to explain the harmful effect of B vitamins in patients with impaired renal function. High levels of folic acid not metabolized to tetrahydrofolate could increase levels of asymmetric dimethylarginine, an antagonist of nitric oxide. In addition, patients with renal failure who are given cyanocobalamin accumulate cyanide, and cyanide, excreted as thiocyanate, has a central role in the catabolism of hydrogen sulphide, an endothelium-derived relaxing factor analogous to nitric oxide. Among patients with renal failure who were given methylcobalamin rather than cyanocobalamin, levels of both plasma total homocysteine and asymmetric dimethylarginine were lowered.

Folate status, B12 status, and renal function are all crucial to interpretation of results of clinical trials of vitamin therapy used to lower homocysteine levels. The clinical implications of these issues are that metabolic B12 deficiency needs to be better detected and treated, and in patients with renal failure it would be advisable to substitute methylcobalamin for cyanocobalamin. For patients undergoing dialysis, more intensive dialysis and use of thiols such as mesna may represent other potential options.

When subgroup analyses are biologically based, thoughtfully developed, and preplanned, consideration of their results can enrich the findings from randomized trials and may lead to insights that can help explain apparently divergent results. In clinical practice it may be reasonable to consider acting on such insights, pending results from subsequent clinical trials that focus on those particular subgroups.

Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Spence reported receiving study vitamin and placebo for the DIVINE study from Pan American Laboratories and consulting fees from Medice Arzneimittel Pütter GmbH & Co KG (both are vitamin manufacturers). Dr Spence and Dr Stamper both reported serving as members of the Executive Committee of the Vitamin Intervention for Stroke Prevention (VISP) trial, funded by NIH/NINDS, and conceiving the VISP subgroup analysis.

Additional Information: Complete references are available at http://www.imaging.roberts.ca/SPARC/.

REFERENCES