Published in final edited form as:

Clin Chem Lab Med. 2013 March 1; 51(3): 671–675. doi:10.1515/cclm-2012-0637.

Trends in clinical laboratory homocysteine testing from 1997 to 2010: the impact of evidence on clinical practice at a single institution

Corinne M. Klykov and

Department of Internal Medicine, The University of Iowa Carver College of Medicine, Iowa City, IA, USA

Steven R. Lentz*

Department of Internal Medicine, The University of Iowa Carver College of Medicine, 200 Hawkins Drive, C32 GH, Iowa City, IA 52242, Iowa, USA

Abstract

Background—During the past decade, several clinical trials investigating the potential benefits of homocysteine-lowering therapy for the secondary prevention of vascular events were completed and published. The objective of the study was to determine trends in clinical laboratory testing for homocysteine at a single institution over the time period from 1997 to 2010.

Methods—A single-center, retrospective analysis was performed at a large, academic, tertiary care medical center in the USA. The database was searched for clinical laboratory measurements of plasma or serum homocysteine between January 1, 1997 and December 31, 2010. Individual medical records were reviewed for a subset of 221 unique patients in the 10-year period from 2001 to 2010 who had homocysteine values $\geq 20 \mu\text{mol/L}$.

Results—From 1997 to 2003, there was a 16-fold increase in the annual number of homocysteine assays performed. Testing for homocysteine declined in 2004 and 2006, coinciding with the publication of intervention trials that failed to support a clinical benefit of homocysteine-lowering therapy for the secondary prevention of vascular events. Subgroup analysis suggested that homocysteine testing for the indication of suspected nutritional deficiency or hypercoagulability remained steady despite a decline in testing for the indication of cardiac risk assessment.

Conclusions—The frequency of clinical laboratory testing for plasma or serum homocysteine changed bimodally over the time period from 1997 to 2010. These observations suggest that clinical practice was impacted by emerging evidence from association studies and intervention trials investigating homocysteine as a potentially treatable cardiac risk factor.

Keywords

cardiac risk factor; homocysteine; hypercoagulability; vitamin B12

*Corresponding author: **Steven R. Lentz**, MD, PhD, Department of Internal Medicine, The University of Iowa Carver College of Medicine, 200 Hawkins Drive, C32 GH, Iowa City, IA 52242, Iowa, USA, Phone: +1 319 356 4048, Fax: +1 319 353 8383, steven-lentz@uiowa.edu.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Introduction

Homocysteine is a naturally occurring amino acid that is found in micromolar concentrations in blood plasma. Measurement of total homocysteine [1] in plasma or serum is often performed in the clinical laboratory to aid in the diagnosis of vitamin B12 deficiency or an inherited disorder of folate or vitamin B12 metabolism [2]. Clinical laboratory methods to accurately measure plasma or serum homocysteine became increasingly available in the 1980s and 1990s. During the same time period, several retrospective case control and observational studies demonstrated an association between elevated homocysteine levels and increased risk for coronary heart disease and stroke [3, 4]. Elevated homocysteine was also found to be associated with an increased risk for deep vein thrombosis [5, 6]. Results from these retrospective studies were confirmed by several large prospective cohort studies that evaluated risk for vascular events in subjects with elevated plasma homocysteine [7, 8]. By the year 2000, there was growing awareness among clinicians that homocysteine may be a clinically relevant, independent risk factor for cardiovascular and thrombotic disease.

Beginning in the late 1990s and continuing into the early 2000s, several large, prospective, randomized clinical trials were initiated to define the potential benefits of homocysteine-lowering therapy for the secondary prevention of adverse vascular outcomes. These trials assessed the effects of supplementation with folic acid and other B vitamins on plasma homocysteine concentration and subsequent vascular outcomes in patients with a history of kidney disease or a prior vascular event, such as myocardial infarction, ischemic stroke, or venous thromboembolism. All of the intervention trials showed a significant reduction in plasma homocysteine concentration in subjects receiving supplemental B vitamins. However, none of the trials demonstrated a significant overall clinical benefit of homocysteine-lowering therapy for the secondary prevention of vascular events [9–11].

The result of the first major homocysteine-lowering intervention study, the VISP (Vitamin Intervention for Stroke Prevention) trial, was published in 2004. This trial failed to demonstrate that lowering homocysteine through B vitamin supplementation decreased the incidence of recurrent ischemic stroke [12]. The results of two additional intervention trials, the Heart Outcomes Prevention Evaluation 2 (HOPE-2) trial [13] and the Norwegian Vitamin Trial (NORVIT) [14] were published in 2006. In HOPE-2, supplementation with B vitamins lowered homocysteine but did not decrease the primary endpoint of combined risk of death from cardiovascular disease, myocardial infarction or stroke [13]. Similarly, NORVIT failed to demonstrate a benefit of homocysteine-lowering therapy for the prevention of recurrent myocardial infarction, stroke, or sudden death attributed to coronary artery disease [14].

To assess the impact of the homocysteine-lowering intervention trials on clinical and laboratory practice, we performed a retrospective analysis to determine trends in clinical laboratory testing for plasma or serum homocysteine over the time period from 1997 to 2010 at a single academic tertiary care medical center in the USA.

Materials and methods

This study was conducted at the University of Iowa Hospitals and Clinics, a 760-bed teaching hospital and tertiary care medical center in Iowa City, Iowa. A Structured Query Language algorithm was used to search the patient information database for all occurrences in which plasma or serum total homocysteine [1] concentration was ordered between January 1, 1997 and December 31, 2010. For each occurrence, the associated date,

laboratory value, patient identifier, ordering provider, laboratory identifier, and laboratory result were retrieved. Individual medical record reviews were performed on the subset of unique patients in the 10-year period between January 1, 2001 and December 31, 2010 who had homocysteine levels $\geq 20 \mu\text{mol/L}$ ($n = 221$). Information retrieved from the medical record included the ordering provider's specialty, the clinical indication for the measurement, and the final diagnosis, if available. The study protocol was reviewed and approved by the University of Iowa Institutional Review Board.

Results

From 1997 to 2010, 5748 clinical laboratory assays for plasma or serum homocysteine were performed at the University of Iowa Hospitals and Clinics. From 1997 to 2003 there was a marked and steady increase in homocysteine assays performed, from 46 in 1997 to 748 in 2003 (Figure 1). Clinical orders for homocysteine assays declined to 482 in 2004, rebounded to 607 in 2005, and then fell to a steady state level of approximately 400–500 orders per year thereafter. The declines in homocysteine assay orders in 2004 and 2006 corresponded with the publication of the VISP study in 2004 [12] and the HOPE-2 and NORVIT trials in 2006 [13, 14] (Figure 1).

During the 10-year period between January 1, 2001 and December 31, 2010, 5374 homocysteine assays were performed on plasma or serum samples from 3966 unique patients. There were 1407 (35%) patients who had homocysteine values $\geq 10 \mu\text{mol/L}$ and 221 (5.6%) had values $\geq 20 \mu\text{mol/L}$ (Figure 2). Thirty-four patients had severe hyperhomocysteinemia, with a serum or plasma homocysteine level $\geq 50 \mu\text{mol/L}$. Of the patients with severe hyperhomocysteinemia, 24 had a documented nutritional deficiency (including deficiencies of B12, vitamin B6, and/or folate), six had chronic kidney disease, four had alcoholism, three had inborn errors of vitamin B12 metabolism (cobalamin C deficiency, cobalamin G deficiency, and homozygous MTHFR deficiency) and two had no identifiable etiology for hyperhomocysteinemia. Four patients had both chronic kidney disease and a nutritional deficiency and one patient had both alcoholism and a nutritional deficiency.

For the subset of 221 unique patients in this 10-year period who were found to have homocysteine levels of $20 \mu\text{mol/L}$ or greater, the medical records were reviewed to determine the ordering provider's medical specialty and the clinical indication for requesting the homocysteine measurement. For most patients, the clinical indication for measuring homocysteine fell into one of three general categories: 1) assessment of cardiac risk; 2) hypercoagulability evaluation; or 3) suspected vitamin B12 deficiency (Table 1). Patients in the latter category often presented with either a hematological abnormality (including anemia with or without megaloblastic change, thrombocytopenia, leukopenia, and pancytopenia) or a neurological abnormality (such as altered mental status, cognitive impairment, peripheral neuropathies, ataxia, diplopia, nystagmus, optic nerve atrophy, weakness, or dizziness). Others were considered nutritionally “high risk” because of a known history of vitamin B12 deficiency or decreased nutritional intake, cachexia, alcoholism, or other conditions associated with malabsorption. Homocysteine laboratory testing was ordered for the indication of cardiac risk assessment most often by cardiologists, lipid specialists, or nephrologists. In contrast, homocysteine testing was requested for the indication of hypercoagulability evaluation or suspected nutritional deficiency most often by primary care providers (i.e., family medicine physicians, internists, pediatricians, or gynecologists), hematologists, neurologists or psychiatrists (Table 1).

The number of patients identified to have homocysteine levels of $20 \mu\text{mol/L}$ or greater after testing for the indication of cardiac risk assessment peaked in 2003, mirroring the overall

trend in homocysteine testing over the 10-year period, and then declined precipitously (Figure 3A). In distinction, the number of patients identified to have homocysteine levels of 20 $\mu\text{mol/L}$ or greater after testing for other indications remained steady from 2001 until 2008 and then increased in 2009 and 2010 (Figure 3B).

Discussion

The major finding from this single-institution retrospective study is that the frequency of clinical laboratory testing for plasma or serum homocysteine changed bimodally over the time period from 1997 to 2001. The marked 16-fold increase in homocysteine testing from 1997 to 2003 likely reflects budding awareness by clinicians of homocysteine as a risk factor for cardiovascular disease due to the publication of several retrospective and prospective association studies in the late 1990s [7, 8]. The subsequent decline in homocysteine testing corresponded with the publication of the negative results of the VISP study in 2004 [12] and the HOPE-2 and NORVIT trials in 2006 [13, 14]. These observations suggest that clinical practice at this institution was impacted by emerging evidence from association studies and intervention trials investigating homocysteine as a potentially treatable cardiac risk factor.

Limitations of this study include its retrospective design, the relatively small sample size, and the fact that the data reflect clinical practice at a single US institution. Due to these limitations, it is not possible to generalize the findings to other institutions or practice settings, especially outside of USA. Nevertheless, the patterns of utilization of clinical laboratory homocysteine testing observed in this study illustrate how emerging evidence may have impacted clinical practice at one institution and highlight potential areas of practice improvement for the future.

In patients ultimately found to have a homocysteine level $\geq 20 \mu\text{mol/L}$, we found that the medical indication for ordering a laboratory test for homocysteine varied between different medical specialists (Table 1). Cardiologists, lipid specialists and nephrologists accounted for almost all of the requests for homocysteine testing for the indication of cardiac risk stratification; moreover, these specialists ordered laboratory testing for homocysteine almost exclusively for this indication. In contrast, primary care providers requested homocysteine testing frequently for the indication of suspected vitamin deficiency and occasionally as part of a hypercoagulability evaluation, but almost never for the indication of cardiac risk stratification (Table 1). This may reflect practice patterns at a US academic tertiary care center, where many patients with cardiac disease are cared for by cardiologists rather than primary care providers. Alternatively, primary care providers may have been slower than cardiologists to adopt the practice of utilizing homocysteine testing for cardiac risk assessment when homocysteine became recognized as an independent risk factor in the 1990s. Interestingly, the 10-year pattern of homocysteine testing for the indication of cardiac risk assessment in this patient subset (Figure 3A) mirrored the overall trends for the entire patient population (Figure 1).

Among hematologists, the indications for ordering homocysteine were split between hypercoagulability evaluation and evaluation of hematologic abnormalities potentially attributable to B12 deficiency. Neurologists and psychiatrists ordered homocysteine testing mostly for evaluation of neurologic symptoms that were potentially attributable to B12 deficiency (such as ataxia, cognitive impairment, altered mental status, neuropathies, dizziness, diplopia, or nystagmus) and occasionally for hypercoagulability evaluation in the context of ischemic stroke. Other specialists who requested homocysteine testing include gastroenterologists, who tended to order testing for the indication of suspected nutritional deficiency in the setting of gastric bypass, chronic diarrhea, or proton-pump inhibitor use,

and ophthalmologists, who ordered testing for the indication of hypercoagulability evaluation in the context of central retinal vein occlusion.

The pattern of homocysteine testing for evaluation of suspected nutritional deficiency has remained steady, or perhaps increased, after the publication of the negative results of the homocysteine-lowering intervention trials (Figures 1 and 3B). The use of homocysteine testing, along with testing for methylmalonic acid and holotranscobalamin II, may continue to increase in the future as clinicians become more aware of populations at risk for vitamin B12 deficiency, including (often elderly) patients with malabsorption, alcoholism, inflammatory bowel disease, or conditions requiring long-term gastric acid-suppression therapy [15, 16].

Acknowledgments

The authors would like to acknowledge John Hellman for developing the algorithm used to retrieve patient data from the clinical information database.

References

1. Mudd SH, Finkelstein JD, Refsum H, Ueland PM, Malinow MR, Lentz SR, et al. Homocysteine and its disulfide derivatives: a suggested consensus terminology. *Arterioscler Thromb Vasc Biol.* 2000; 20:1704–6. [PubMed: 10894806]
2. Carmel, R.; Jacobsen, D. Homocysteine in health and disease. Cambridge University Press; Cambridge: 2009.
3. Clarke R, Daly L, Robinson K, Naughton E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med.* 1991; 324:1149–55. [PubMed: 2011158]
4. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. *J Am Med Assoc.* 1995; 274:1049–57.
5. den Heijer M, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med.* 1996; 334:759–62. [PubMed: 8592549]
6. Cattaneo M, Martinelli I, Mannucci PM. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med.* 1996; 335:974–5. [PubMed: 8786784]
7. Homocysteine Studies C. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *J Am Med Assoc.* 2002; 288:2015–22.
8. den Heijer M, Rosendaal FR, Blom HJ, Gerrits WB, Bos GM. Hyperhomocysteinemia and venous thrombosis: a meta-analysis. *Thromb Haemost.* 1998; 80:874–7. [PubMed: 9869152]
9. den Heijer M, Willems HP, Blom HJ, Gerrits WB, Cattaneo M, Eichinger S, et al. Homocysteine lowering by B vitamins and the secondary prevention of deep vein thrombosis and pulmonary embolism: a randomized, placebo-controlled, double-blind trial. *Blood.* 2007; 109:139–44. [PubMed: 16960155]
10. Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Inter Med.* 2010; 170:1622–31.
11. Clarke R, Halsey J, Bennett D, Lewington S. Homocysteine and vascular disease: review of published results of the homocysteine-lowering trials. *J Inher Metab Dis.* 2011; 34:83–91. [PubMed: 21069462]
12. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *J Am Med Assoc.* 2004; 291:565–75.

13. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med.* 2006; 354:1567–77. [PubMed: 16531613]
14. Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med.* 2006; 354:1578–88. [PubMed: 16531614]
15. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. *Am J Clin Nutr.* 2011; 94:348S–58S. [PubMed: 21593511]
16. Chatthanawaree W. Biomarkers of cobalamin (vitamin B12) deficiency and its application. *J Nutr Health Aging.* 2011; 15:227–31. [PubMed: 21369672]

Figure 1. Annual number of number of clinical laboratory measurements of plasma or serum homocysteine performed at the University of Iowa Hospitals and Clinics from 1997 to 2010. The arrows indicate the years of publication of the VISP, HOPE-2, and NORVIT trials.

Figure 2.
The number of unique patients with plasma or serum homocysteine values $\geq 10 \mu\text{mol/L}$ during the time period from 2001 to 2010.

Figure 3.

The number of unique patients found to have plasma or serum homocysteine values ≥ 20 $\mu\text{mol/L}$ after testing was performed for the indication of cardiac risk assessment (A) or an indication other than cardiac risk assessment (B) during each calendar year between 2001 and 2010.

Table 1

Medical specialty of the ordering physician and clinical indication for measuring homocysteine in patients with homocysteine 20 µmol/L (n=221^a).

	n	Cardiac risk assessment	Hypercoagulability evaluation	Suspected nutritional deficiency	Other/unable to determine
Primary care provider	65	1	20	40	4
Cardiologist or lipid specialist	49	46	3	0	0
Hematologist	52	0	22	30	0
Neurologist or psychiatrist	25	0	7	18	4
Nephrologist	21	19	2	0	0
Other specialty	23	0	10	13	5
Total	235	66	64	101	13

^aFourteen patients had two clinical indications for measuring homocysteine; these patients are included in both categories.