Hyperhomocysteinemia and functional cobalamin deficiency due to granulocytosis-induced alterations in the cobalamin-binding protein

Lodewijk Thomas Vlasveld
Gerard M.J. Bos
Anton A.M. Ermens
Jaap A. Bakker
Jan Lindemans

Serum cobalamin and homocysteine levels were studied in patients with chronic myelogenous leukemia (CML) and in stem cell donors treated with granulocyte-colony stimulating factor (G-CSF). Cytoreductive treatment in patients with CML resulted in a decrease of cobalamin and homocysteine levels. In stem cell donors cobalamin and homocysteine levels increased after G-CSF administration. The increase of homocysteine level was accompanied by a decrease in the serum levels of the cobalamin-binding protein transcobalamin. We hypothesize that the increased homocysteine levels in patients with CML and donors treated with G-CSF may be the result of a functional methylcobalamin deficiency due to decreased transcobalamin levels.

Key words: chronic myelogenous leukemia, granulocyte-colony stimulating factor, homocysteine, cobalamin, cobalamin-binding proteins.

Haematologica 2006; 91:394-396

From the Department of Internal Medicine, Bronovo Hospital, Bronovolaan 5, 2597 AX The Hague, The Netherlands (LTV); Department of Internal Medicine, University Hospital Maastricht, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands (GMJB); Department of Clinical Chemistry, Amphia Hospital, Langendijk 75, 4819 EV Breda, The Netherlands (AAME); Department of Clinical Chemistry, University Hospital Maastricht, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands (JAB); Department of Clinical Chemistry, Erasmus University Medical Center, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands (JL).

Correspondence:
Lodewijk Thomas Vlasveld,
Department of Internal Medicine,
Bronovo Hospital, Bronovolaan 5,
2597, AX The Hague,
The Netherlands.
E-mail: tomvlasveld@hotmail.com

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder characterized by leucocytosis with myeloid cells in all stages of development.[1,2] A metabolic hallmark of CML is a large increase in serum vitamin B12 (cobalamin) concentration.[3,4] Cobalamin occurs in a number of different analogs of which methylcobalamin and adenosylcobalamin are the metabolically active forms in humans.[5,6,7] In the circulation cobalamin is bound to transcobalamin and haptocorrin.[8] Indeed, 80-85% of circulating cobalamin is bound to haptocorrin. Haptocorrin carries mainly methylcobalamin, while transcobalamin binds the other forms of cobalamin relatively more. Cells of the myeloid lineage are responsible for the production of circulating haptocorrin.[4,5,7,9] The exact physiological role of haptocorrin is not fully established.[5,7] In patients with CML and other myeloproliferative disorders the plasma cobalamin concentration may be elevated due to increased production of haptocorrin by the proliferating myeloid cells.[4,5] Since haptocorrin preferentially binds methylcobalamin, in patients with CML the levels of this cobalamin analog are raised more than are the other forms of cobalamin.[8] Only 5-20% of cobalamin is bound to transcobalamin. The major sources of circulating transcobalamin are considered to be hepatocytes, enterocytes, endothelial cells and monocytes.[5,7,10] Binding to transcobalamin is a prerequisite for cobalamin to be able to enter the cell. Within the cell adenosylcobalamin acts as a co-factor of methylmalonyl CoA mutase, which regulates the formation of succinyl CoA from methylmalonic CoA. Methylcobalamin acts as a co-factor for methionine synthase, which regulates the conversion of homocysteine to methionine and is connected to thymidylate synthesis.[7] The methionine metabolite S-adenosylmethionine is the most important donor of methyl groups needed for protein and DNA methylation, as thymidylate is one of the four building blocks of DNA.[11,12] In order to investigate the influence of changes of the cobalamin concentration on the plasma homocysteine levels in patients with CML, various parameters were determined in CML patients before and after cytoreductive treatment. Then, in order to determine whether the observed changes of cobalamin and homocysteine levels were disease-related or determined solely by the increased number of circulating leukocytes, the effects of granulocyte colony-stimulating factor (G-CSF) on cobalamin and homocysteine levels were studied in healthy peripheral stem cell donors treated with G-CSF for stem cell mobilization. In addition, the concentration of the cobalamin-binding proteins haptocorrin and transcobalamin were studied to better understand the relationship of high cobalamin and homocysteine levels in these individuals.

Design and Methods

We investigated 13 consecutive patients with CML enrolled in the study between 1997 and 2004. Before cytoreductive treatment complete blood count and an extensive biochemical blood profile including cobalamin, folic acid and homocysteine levels were determined. When the leukocyte count had been below the upper normal limit for at

least 4 weeks these variables were reassessed. These variables were also determined in 24 healthy blood stem cell donors before and after treatment with 5 g/kg G-CSF for 5 days. In 12 donors and in five CML patients the serum cobalamin-unsaturated-transcobalamin (apoTC), serum cobalamin-unsaturated haptocorrin (apoHC) and cobalamin-saturated-transcobalamin (holoTC) levels were studied as well. ApoHC and apoTC were measured by differential absorption assay using microfine silica,[13] whereas holoTC was measured using a radio-immunoassay from Axis-Shield (Oslo, Norway). Homocysteine was measured by high performance liquid chromatography,[14] using a procedure modified by Ubbink *et al.*[15] For this purpose the samples were collected on ice. The results were compared using the Student t-test for paired or unpaired samples. A p value of < 0.05 was considered statistically significant.

Results and Discussion

Thirteen patients were treated for CML with either hydroxyurea, or imatinib in combination with cytosine arabinoside. At presentation all patients had elevated serum cobalamin levels with a mean value of 1727 pmol/L (normal 130-750). The serum folic acid level was normal (6-39 nmol/l) in all but one patient. The plasma homocysteine level was elevated in nine of the patients with a mean value of 23.5 μmol/L (normal < 15.6). As summarized in Table 1 the plasma homocysteine level and cobalamin concentration decreased significantly after cytoreductive treatment. In the healthy peripheral stem cell donors there was a statistically significant increase in the homocysteine and cobalamin levels at the end of treatment with G-CSF, while there was a moderate but significant decrease of the serum folic acid levels (Table 2). Levels of cobalamin-binding proteins were assessed in five of the CML patients before and after cytoreductive treatment. As expected the initially greatly raised levels of apoHC decreased significantly (Table 3), while the serum holoTC levels increased and the serum apoTC levels decreased, both to a degree with a borderline statistical significance (p=0.07). Having observed the rise in homocysteine and cobalamin levels in healthy stem cell donors after administration of G-CSF, we analyzed cobalamin-binding proteins in 12 of these donors. A dramatic increase in the serum apo-HC concentration was observed after treatment with G-CSF (Table 3) with a statistically significant increase in apo-TC, and a significant decrease in holo-TC concentration.

In the total group of individuals with either CML-associated or G-CSF-induced leukocytosis there was a moderate correlation (r=0.686) between the leukocyte count and the homocysteine level while the correlation between the leukocyte count and the cobalamin level was weaker (r=0.445) in a simple regression model. In the group in which the cobalamin-binding proteins were determined, the mean holoTC concentration was significantly lower during leukocytosis than at the time of normal leukocyte counts (mean holoTC: 49.5 versus 83.0 pmol/L, p=0.003). We found that elevated serum cobalamin and homocysteine levels are frequent in patients with CML and in healthy stem cell donors at the end of a 5-day course of G-CSF. Serum cobalamin and homocysteine decrease after normalization of the leukocyte count as a result of cytoreductive treatment in patients with CML. The fact that hyperhomocysteinemia occurs during leukocytosis in patients with CML as well as in healthy G-CSF-treated peripheral stem cell donors indicates that the hyperhomocysteinemia represents a general metabolic phenomenon during expansion of cells of the myeloid lineage. To our knowledge high cobalamin levels have not previously been demonstrated to be associated with elevated homocysteine levels, either in CML patients, or in healthy G-CSF-stimulated donors. Our observation that elevated levels of cobalamin coincide with elevated levels of homocysteine may indicate that a functional cobalamin deficiency exists despite high serum cobalamin levels. Based on the findings in five CML patients and in 12 G-CSF-stimulated donors we hypothesize that the increase in the

Table 1. Changes in the leukocyte count and serum homocysteine, cobalamin and folic acid levels in 13 patients with CML treated with hydroxyurea or imatinib in combination with cytosine arabinoside.

	Before treatment	*After treatment*	*p value*
Leukocytes (×10⁹/L)			
mean	167	3.8	< 10⁻³
range	62-330	0.7-7.2	
Homocysteine (μmol/L)			
mean	23.5	13	0.003*
range	7.8-45.3	9.5-16	
Cobalamin (pmol/L)			
mean	1727	507	0.008
range	773-5299	171-1035	
Folic acid (nmol/L)			
mean	10.8	10.7	ns
range	4.3-19.8	6.5-18.6	

**n: 10 paired samples; ns: not significant.*

Table 2. Changes in the leukocyte count and serum homocysteine, cobalamin and folic acid levels in 24 healthy volunteers treated with G-CSF for peripheral blood stem cell mobilization.

	Before G-CSF	*After G-CST*	*p value*
Leucocytes (×10⁹/L)			
mean	7.0	40.2	< 10⁻³*
range	3.9-10.6	18.7-60.4	
Homocysteine (μmol/L)			
mean	11.7	15.2	< 10⁻³*
range	5.7-22.4	6.7-38.8	
Cobalamin (pmol/L)			
mean	259.2	453.5	< 10⁻³#
range	132-443	218-738	
Folic acid (nmol/L)			
mean	14.3	13.2	10⁻³#
range	6.3-25.2	4.9-25.0	

**n: 23 paired samples; #n: 21 paired samples.*

Table 3. Changes in the cobalamin binding proteins in five CML patients before and after cytoreductive therapy and 12 stem cell donors treated with G-CSF.

	Before treatment	*After treatment*	*p value*
CML patients (n=5)			
apoTC (pmol/L) (normal 350-970)			
mean	1204	1050	0.07
range	848-1440	717-1356	
apoHC (pmol/L) (normal 55-370)			
mean	3599	270	0.03
range	1367-6840	114-477	
holoTC (pmol/L) (normal 35-170)			
mean	55.8	81	0.07
range	16-79	41-118	
stem cell donors apoTC (pmol/L) (n=12)			
mean	1011.3	1149.2	0.023*
range	679-1431	633-1512	
apoHC (pmol/L)			
mean	112.9	8166.8	$< 10^{-3}$*
range	76-212	5319-11448	
holoTC (pmol/L)			
mean	79.1	44.1	0.002*
range	37-160	19-85	

**n: 9 paired samples.*

plasma homocysteine level may be due to changes in the cobalamin binding proteins and reflects a methylcobalamin deficiency at the cellular level. The rise in apo-HC and the fall in holo-TC are compatible with this hypothesis since a low holo-TC level may reflect diminished cobalamin availability.[16] In previous studies, however, no evidence of cobalamin deficiency was reported in patients with untreated CML using the deoxyuridine suppression test of bone marrow cells and the urine excretion of formiminoglutamic acid and methylmalonic acid.[8,17]

There may be several explanations for the observed changes in the plasma level of the transcobalamin-bound cobalamin. Unsaturated haptocorrin is liberated from the proliferating myeloid leukocytes and becomes rapidly saturated with cobalamin. Due to the relatively long survival time in the circulation, a substantial amount of circulating haptocorrin-bound cobalamin is trapped in the circulation.[4] For this reason insufficient cobalamin may be available for binding to transcobalamin. An alternative explanation is that the relatively low holoTC levels during G-CSF-evoked leukocytosis and in CML patients could suggest heavy cobalamin consumption by the hyperproliferating cells of the myeloid lineage. In order to meet the needs of increased DNA synthesis and production of proteins, the proliferating cells must be in a state of active methylation with increased formation of methionine, and its metabolite S-adenosylmethionine from homocysteine. The increased conversion from homocysteine to methionine, catalyzed by the enzyme methionine synthase, may consume methylcobalamin leading to upregulation of the transcobalamin receptor on myeloid cells in order to maintain the intracellular methylcobalamin level.[18] As a consequence, increased binding of the transcobalamin-cobalamin complex to the proliferating myeloid cells may lead to a decrease in the circulating transcobalamin-cobalamin complex with diminished availability of cobalamin in other tissues and a subsequent increase in the serum homocysteine level. Further studies are needed to elucidate whether the presumed functional methylcobalamin deficiency is the result of insufficient binding to transcobalamin as the result of cobalamin trapping by myeloid cell-produced haptocorrin or whether it is caused by heavy methylcobalamin consumption by the hyperproliferating myeloid cells.

LThV, GMJB and JL conceived the study. The study was performed in the group of LThV and GMJB, AAME, JAB and JL performed all the pilot experiments and most of the analysis.

LThV performed the benchwork and wrote the manuscript with contributions from other authors. The authors declare that they have no potential conflicts of interest.

Manuscript received September 15, 2005. Acceptted January 2, 2006.

References

1. Clarkson B, Strife A, Wisniewski D, Lambek CL, Liu C. Chronic myelogenous leukaemia as a paradigm of early cancer and possible curative strategies. Leukemia 2003;17:1211-62.
2. Garcia-Mareno G, Daderl S, O'Brien S, Cortes J, Talpaz M, Kantarjian HM. Chronic myelogenous leukaemia: a review and update of therapeutic strategies. Cancer 2003;98:437-57.
3. Gimsing P, Overballe-Petersen C, Hippe E. Cobalamin and cobalamin-binding proteins in plasma related to the clinical condition in chronic myelogenous leukemia. Leukemia 1995; 9:1604-9.
4. Gimsing P. Cobalamin metabolism in chronic myelogenous leukemia. Dan Med Bull 1998;45:459-79.
5. Ermens AAM, Vlasveld LT, Lindemans J. Significance of elevated cobalamin (vitamin B12) levels in blood. Clin Biochem 2003;36:585-90.
6. Kapadia CR. Vitamin B12 in health and disease. Part I – inherited disorders of function, absorption, and transport. Gastroenterologist 1995;3:329-44.
7. Markle HV. Cobalamin. Crit Rev Clin Lab Sci 1996;33:247-356.
8. Gimsing P, Nexo E. The turnover of 57Co-labeled cyanocobalamin bound to cobalamin binding proteins in patients with chronic myelogenous leukemia. J Lab Clin Med 1994; 123:264-72.
9. Neale G. B12 binding proteins. Gut 1990;31:59-63.
10. Carmel R. Cobalamin deficiency. In: Carmel R & Jacobsen DW. Homocysteine in health and disease. Cambridge University Press, 2001. p. 289-305.
11. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. Sem Thromb Hemostast 2000;26: 219-25.
12. Perry DJ. Hyperhomocysteinaemia. Ball Clin Haematol 1999;12:451-77.
13. Jacob E, Herbert V. Measurement of unsaturated granulocyte-related (TC I and III) and liver-related (TC II) B12 binders by instant batch separation using a microfine precipitate of silica (QUSO G32). J Lab Clin Med 1975;86: 505-12.
14. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422:43-52.
15. Ubbink JB, Vermaak WJ, Bissbort SH. Rapid high-performance liquid chromatography assay for total homocysteine levels in human serum. J Chromatogr 1991;565:441-6.
16. Carmel R. Measuring and interpreting holo-transcobalamin (holo-transcobalamin II). Clin Chem 2002;48:407-9.
17. Gimsing P, Hippe E. Cobalamin-dependent metabolism in chronic myelogenous leukemia determined by deoxyuridine suppression test and the formiminoglutamic acid and methylmalonate excretion in the urine. Am J Hematol 1995; 49:121-30.
18. Seetharam B. Receptor-mediated endocytosis of cobalamin (vitamin B12). Ann Rev Nutrit 1999;19:173-95.