Response:

Traumatic lumbar puncture at diagnosis of childhood acute lymphoblastic leukemia

We agree with the recommendations of Kebelmann-Betzing et al that initial lumbar puncture should be performed by an experienced clinician and followed immediately by intrathecal therapy. As also stated in our article,[1] we now routinely perform this procedure with patients under short-acting general anesthesia. When the diagnosis of leukemia is uncertain because of the lack of circulating leukemic cells, we postpone lumbar puncture and intrathecal therapy until the diagnosis is established by bone marrow examination.

It is difficult to compare the frequency of traumatic lumbar puncture in the series of Kebelmann-Betzing et al with that of ours because the status of their patients was determined retrospectively on the basis of "preserved" cerebrospinal fluid; in contrast, we used fresh samples of cerebrospinal fluid. The integrity of the erythrocytes and the morphology of the leukocytes are expected to be altered in preserved samples. Since we first implemented steps to reduce this iatrogenic complication, we have substantially reduced the frequency of traumatic lumbar punctures with blast cells from 11% to 5% and that of traumatic lumbar punctures without blast cells from 10% to 7%. It should be noted that we stringently define traumatic lumbar puncture as at least 10 erythrocytes per microliter.

As shown by Total Therapy Study XIII,[2] early intensive intrathecal and systemic therapy is now more successful in treating and preventing central nervous system (CNS) leukemia, even in patients whose leukemic blast cells are iatrogenically introduced into the cerebrospinal fluid by traumatic lumbar puncture. Therefore, cranial irradiation is seldom necessary in contemporary treatment programs. But others have reported neuropsychologic deficits in children who received CNS-directed therapy consisting solely of approximately 20 intrathecal treatments of methotrexate, hydrocortisone, and cytarabine over 3 years.[3] The challenge now is to optimize intrathecal and systemic therapy to maximize efficacy and minimize toxicity. Developing appropriate measures to avoid traumatic lumbar puncture and hence the need of extra intrathecal therapy is but one step toward this goal.

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To the editor:

Transcobalamin polymorphism and homocysteine

Namour et al[1] suggest that codon 259 of the transcobalamin *(TC)* gene is a major determinant of TC polymorphism in Caucasians and that heterozygous individuals have higher plasma homocysteine. Total serum homocysteine (tHcy) is increased in patients with Alzheimer disease (AD) and may be a risk factor for cognitive decline.[2-5] Furthermore, TC saturation declines with age in AD.[6]

We therefore determined nonfasting tHcy, TC phenotype, and codon 259 polymorphism in 144 (93 female, 51 male) healthy elderly volunteers (73) and dementia patients (71) recruited to a study of tHcy and cognition (the COBALZ II project) after ethical committee approval. Phenotypes were identified by polyacrylamide gel electrophoresis (PAGE) of neuraminidase-treated radiolabeled serum samples (Figure 1) and genotypes by solid-phase minisequencing (Figure 2).[7,8] Nonfasting tHcy was assayed with the Drew Scientific DS30 Hcy Analyser (Barrow in Furness, England); Vitamin B_{12} and folate were assayed with the Bayer ACS 180 Automated Chemiluminescence System (Newbury, England).

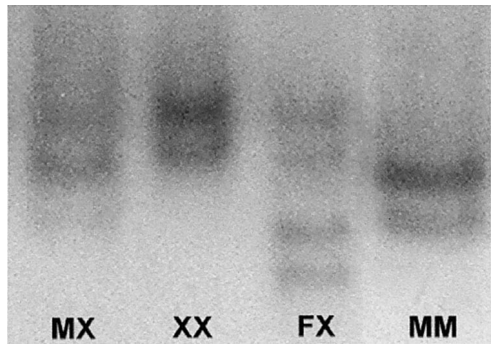

Figure 1. TC phenotypes revealed by PAGE. Serum (25 μL) saturated with 10 μL of radioactive B_{12} (^{57}Co-B_{12}; 200mCi/mg, 1 μCi/mL [7.4 GBq/mg, 37 kBq/mL]; Amersham Pharmacia Biotech "CT2" diluted 1:5 N-saline) was incubated with 25 μL of neuraminidase (0.2 IU/mL) in 25 μL of buffer (0.05 M sodium acetate, 0.1% $CaCl_2$, and 0.95% NaCl, pH 5.5) at 37°C for 30 minutes. Twenty-five μL was replaced with buffer (65% [wt/vol] sucrose) and bromophenol blue as an albumin- and front-marker. PAGE was performed in a 10% polyacrylamide gel (1.5 mm × 7.0 cm × 12.5 cm) with 3.5% concentrating top gel (1.5 cm high) and Tris-glycine electrode buffer at pH 8.3 and electrophoresed at 250 V.

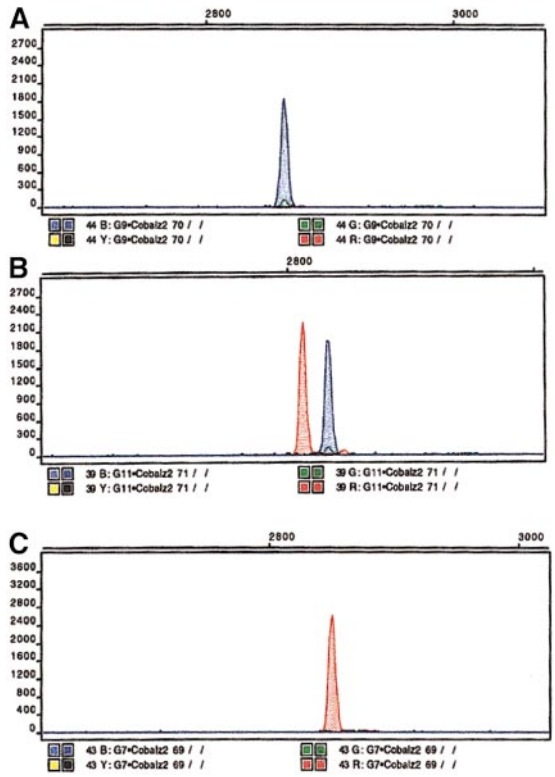

Figure 2. TC genotypes identified by solid-phase minisequencing. (A) TCII-259C homozygotes (PP); (B) TCII-259C/G heterozygotes (PR); (C) TCII-259G homozygotes (RR). Genomic DNA was amplified by PCR using the sense primer biotin-5′-GTGCGAGAGGAGATCTTGAA-3′ and the antisense primer 5′-GTAGGTCT-TGTGGTTCAGAA-3′. Biotinylated products were bound to streptavidine-coated microtiter plates (Wallac, Turku, Finland) and denatured with NaOH. Thermo Sequenase DNA polymerase (Thermo Sequenase Dye Terminator Kit, Amersham, Buckinghamshire, United Kingdom), fluorescent ddNTP, and the antisense detection primer 5′-CTGTTCCCAGTTCTGCCCCA-3′ were added to generate an allele-specific pattern. After the minisequence reaction, the plates were washed and the extended sequence primers were released by incubation with formamide. These were separated and analyzed by capillary electrophoresis and laser-induced fluorescence in an ABI 310 genetic analyzer (Perkin-Elmer, Cambridge, England).

Codon 259 polymorphism and phenotype were highly concordant (Table 1). PAGE reveals a doublet pattern in homozygotes, triplets due to overlapping bands in heterozygotes, and a quadruplet pattern in FX phenotypes (Figure 1). Alternative cleavage of the signal peptide might generate doublets rather than definitive isotypes.[9] Weaker radiolabeling of TC-X in heterozygotes resulted in misclassification of some phenotypes; it perhaps reflects differential transcription.[1] Codon 259 polymorphism and phenotypic distributions paralleled previous studies (PP = 32.6%, PR = 49.3%, and RR = 18.1%), (MM = 34%, MX = 43.7%, XX = 21.5%, and FX = 0.07%).[1,7]

Table 2. Results of a generalized linear model of known tHcy determinants together with diagnosis as an additional independent variable

	Log likelihood	χ^2	*P*
Sex	−317	0.0007	.98
Age	−317	0.05	.83
TC isotype	−317	1.41	.49
B_{12}	−318	2.79	.09
Diagnosis	−319	4.77	.09
Folate	−324	14.89	< .001
Creatinine	−346	58.19	< .0001

Thirty individuals receiving B vitamins or homocysteine-disruptive medication were excluded from further analysis. Vitamin B_{12}, folate, and tHcy did not differ among isotypes (Table 1). The apparent trend for increasing tHcy in the presence of an X variant or codon 259R allele was not significant using the Jonckheere-Terpstra test (asymptotic *P* value for genotype was .16 one-sided and .33 two-sided; *P* value for phenotypes was .08 one-sided and .17 two-sided) or using ANOVA with a Bonferroni correction. Table 2 shows the results of a generalized linear model of known tHcy determinants, together with diagnosis as an additional independent variable.

We therefore confirm that codon 259 is a major determinant of TC polymorphism in populations where M and X phenotypes predominate. The genetic basis underlying F and S phenotypes remains elusive. TC isotype is not a significant risk factor for the development of hyperhomocysteinemia in elderly Caucasians, especially when considered in relation to established risk factors such as creatinine and folate.

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Table 1. Distribution of TC phenotypes and genotypes with their respective median and interquartile ranges for serum B_{12}, folate, and tHcy

	259 Polymorphism			Serum values		
	PP (n = 47)	PR (n = 71)	RR (n = 26)	B_{12} (ng/L)	Folate (μg/L)	tHcy (μM)
Phenotype						
MM (n = 49)	42	7	0	326 (276-354)	10.7 (6.6-14.2)	9.9 (7.8-13.2)
MX (n = 63)	4	58	1	320 (261-389)	8.9 (6.9-12)	11.0 (8.7-13.3)
XX (n = 31)	1	5	25	353 (303-468)	10.4 (9.2-15.1)	11.8 (9.1-16.2)
FX (n = 1)	0	1	0	NA	NA	NA
Serum values						
B_{12} (ng/L)	317 (276-348)	324 (268-391)	416 (311-468)	NA	NA	NA
Folate (μg/L)	9.8 (6.4-14.5)	9.1 (7-12)	10.4 (9.2-18.1)	NA	NA	NA
tHcy (μM)	10.2 (8.2-13.4)	11.0 (8.5-13.2)	11.8 (9.1-16.2)	NA	NA	NA

Interquartile ranges, where applicable, are given in parentheses.

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Response:

Transcobalamin polymorphism, homocysteine, and aging

The DNA sequencing of the transcobalamin *(TC)* gene expressed in various cells identified 5 amino acid substitutions at codons 198, 219, 234, 259, and 376.[1,2] Codon 234 and 259 variability corresponded to the replacement of proline by arginine.[2] By analyzing the DNA and sera from 159 healthy Caucasians from 3 regions of northeastern France (Lorraine, Franche-Comté, and Bourgogne), we found that the codon 259 substitution was a single nucleotide polymorphism with a biallelic distribution, while no substitution was found in the other codons.[3] There is therefore no doubt that codon 259 is a major genetic determinant of the TC polymorphism.

We compared the TC genotype to the isoelectric point of TC isoforms and found a concordance between the amino acid substitution and the isoelectric point. Urea was used to avoid the formation of aggregates in our isoelectric focusing (IEF) analysis of TC. McCaddon et al confirm our findings on the TC codon 259 polymorphism's biallelic distribution. They compared it to a SDS-PAGE phenotyping of TC, which identifies 4 phenotypes, instead of the 2 isoforms observed in IEF. Apparently, no urea was used in their electrophoresis analysis. Urea avoids the formation of TC aggregates and induces denaturation of TC conformation, as shown by the comparison of IEF profiles of TC from HT-29 cells with and without urea.[3,4] In our opinion, the role of alternative cleavage of the signal peptide in generating TC isoforms needs therefore to be confirmed, as only 2 isoforms, not 4, were found in IEF with urea. The TC codon 259 polymorphism affects apo (unsaturated) TC isoforms concentration as shown by IEF analysis of heterozygous HT-29 cells and by sera apo TC assay from homozygous and heterozygous subjects.[3] This is why we thought that this polymorphism could affect the vitamin B_{12} cellular availability and, consequently, the homocysteine concentration.

Surprisingly, the relatively lower concentration of apo TC concentration observed in the Arg/Arg homozygous subjects was not related to an increase of homocysteinemia. Indeed, a significantly higher homocysteine concentration was found in heterozygous subjects than in subjects with either homozygous genotype, while the highest apo TC concentration was observed in subjects with Pro/Pro genotypes. An explanation could be that the binding of TC to the dimeric TC receptor described by Bose et al[5] is associated with a dimerization of TC and that the heterodimer of Pro/Arg isoforms has a lower affinity than Pro/Pro or Arg/Arg homodimers. This hypothesis is currently under investigation. McCaddon et al found no relation between homocysteine concentration and TC codon 259 polymorphism. They performed their study in elderly patients, but no description of the age distribution is given.

Since the publication of our paper, we performed *TC* genotyping and homocysteine analysis in a population of 76 healthy subjects from a preventive medicine center in Lorraine, including 40 elderly subjects. The data summarized in Table 1 confirm the influence of the TC codon 259 polymorphism on homocysteine concentration. But the difference in homocysteine concentration between heterozygous and homozygous subjects was not significant in our elderly subgroup. The influence of age as a determinant of homocysteine concentration may explain the results obtained by McCaddon et al in elderly subjects. Our and McCaddon et al's data suggest therefore that investigations of *TC* genotype in diseases associated with mild hyperhomocysteinemia should take into consideration the age distribution. With regard to the literature, the homocysteinemia increase in the elderly is not fully understood. One should be careful not to transpose to the elderly the role of genetic and nutritional homocysteine determinants characterized in the general population.

Table 1. Plasma homocysteine concentration according to transcobalamin codon 259 genotype in elderly and younger subjects

		Homocysteine		
	Age	Codon 259 TC homozygous	Codon 259 TC heterozygous	*P* (Mann-Whitney)
Entire group, n = 76	61 (33-80)	8.7 (5.5-16.1)	11.0 (5.9-21.6)	.0027
Elderly subgroup, n = 40	69 (60-80)	9.3 (6.2-16.1)	11.0 (5.9-21.6)	.1219
Younger subgroup, n = 36	53 (33-59)	8.4 (5.5-11.5)	10.7 (6.0-18.1)	.0063

Median and extreme values are given in μM. Ranges, where applicable, are in parentheses.

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