with at least folic acid, riboflavin, pyridoxin, and cyanocobalamin in proper dosing.

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Response:

Homocysteine lowering and recurrent venous thrombosis: the VITRO trial

We welcome the opportunity to respond to the comments of Tsamaloukas and the comments of Marcucci and colleagues. The primary aim of our study was to investigate the effect of vitamin supplementation on the incidence of recurrent venous thrombosis. Because factor V Leiden and prothrombin G20210A are not important risk factors for recurrent events, we did not control for these genetic factors.[1] The same holds true for the *MTHFR* C677T polymorphism. which is not thought to influence recurrence risk independent of homocysteine levels. Moreover, in a randomized trial one assumes that confounding by extraneous risk factors is controlled by the randomization procedure. Nonetheless, we have measured these 3 polymorphisms[2]; their distribution is listed in Table1. Adjustment for these polymorphisms did not change the hazard ratios for treatment effect substantially.

The prevalence of hormonal therapy use at time of the first event in hyperhomocysteinemic patients was 14% in the vitamin group and 15% in the placebo group; in the normohomocysteinemic group, it was 19% in the vitamin group and 12% in the placebo group. Adjustment for hormonal use did not change the hazard ratio for treatment effect substantially. We have no data on renal function (creatinine concentration), but there is no reason to assume any bias because of the randomized nature of the study.

We agree that the homocysteine levels in Table 1 of our paper might be somewhat puzzling. The explanation for this is that the distinction between the hyperhomocysteinemic and normohomocysteinemic groups was made on the basis of homocysteine measurements in acidic citrate tubes as stated in "Patients, materials, and methods." If people met the other inclusion criteria, they were randomized to vitamin and placebo. Just before entering the study, another blood sample was taken in EDTA, which was used for the baseline value in the table. Because of variation in homocysteine levels, some patients in the hyperhomocysteinemic group had homocysteine levels lower than the cutoff level, and some patients in the normohomocysteinemic group had values higher than the cutoff level. In general, there is a clear contrast in homocysteine level between the hyper- and normohomocysteinemic groups (15.5 μM vs 9 μM). As for a trial in intermediate hyperhomocysteinemia, when we stratified patients in quartiles based on the homocysteine levels at baseline (just before they start using the study medication), there was no stronger effect in the highest quartile (with homocysteine levels between 14.7 and 108 μM).

Regarding the study medication, we intended to achieve a maximal homocysteine-lowering effect. The choice for the combination of folate (5 mg), vitamin B_{12} (0.4 mg), and vitamin B_6

Table 1. Distribution of polymorphisms in *MTHFR*, FII G20210A, and factor V Leiden in the VITRO study

	Hyperhomocysteinemic group; n = 360		Normohomocysteinemic group; n = 341	
Polymorphism	**Multivitamin; n = 177**	**Placebo; n = 183**	**Multivitamin; n = 176**	**Placebo; n = 165**
MTHFR				
cc, no. (%)	68 (40)	80 (47)	92 (52)	80 (48)
ct, no. (%)	74 (44)	70 (41)	75 (43)	65 (39)
tt, no. (%)	28 (16)	22 (13)	9 (5)	20 (12)
Total no.	170	172	176	165
FII				
gg, no. (%)	161 (96)	160 (95)	166 (96)	155 (95)
ga, no. (%)	7 (4)	9 (5)	7 (4)	9 (5)
Total no.	168	169	173	164
Factor V Leiden				
gg, no. (%)	128 (81)	139 (87)	144 (84)	135 (84)
ga, no. (%)	30 (19)	19 (12)	28 (16)	23 (14)
aa, no. (%)	0 (0)	2 (1)	0 (0)	3 (2)
Total no.	158	160	172	161

(50 mg) was based on an earlier study[3] in which this regimen showed the strongest effect on homocysteine levels; this dose is relatively high compared with several other homocysteine-lowering trials. We are aware that vitamin B_2 is a coenzyme of *MTHFR*, and that riboflavin supplementation lowers homocysteine levels in vitamin B_2–deficient patients.[4] However, no data are available on the additional effect of riboflavin next to the combination of folate, vitamin B_{12}, and vitamin B_6. Only 1 study shows a small additional effect of riboflavin next to folate alone.[5]

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To the editor:

RAG1 and *BRCA2* polymorphisms in non-Hodgkin lymphoma

A recent *Blood* paper by Hill et al[1] suggested a link between the risk of developing non-Hodgkin lymphoma (NHL) and polymorphic variations in several genes involved in DNA strand break induction and DNA repair pathways. We examined 2 of these polymorphisms where Hill et al reported statistically significant associations, in the Recombination-activating 1 (*RAG1)* gene (K820R; odds ratio [OR] = 2.7 [AA vs GG], 95% confidence interval [CI], 1.4-5.0) and the *Breast cancer 2* (*BRCA2*) gene (N372H; OR = 1.5 [AA vs CC], 95% CI, 1.0-2.1), in a United Kingdom case-control study of lymphoma.

Local ethical approval was obtained for this research. Informed consent was obtained in accordance with the Declaration of Helsinki.

A total of 741 white patients with NHL and 806 white control participants randomly selected from population registers were recruited as part of a lymphoma case-control study conducted in parts of north and southwest England.[2] Our study participants consented to give a blood sample, and represented 67% of patients with incident NHL aged 16 to 69 years in the study area and 66% of the control participants who were contacted successfully. We genotyped the participants' DNA while blinded to case-control status using TaqMan SNP genotyping assays (Applied Biosystems, Warrington, United Kingdom) and ABI PRISM 7000 software (Applied Biosystems). To assess the reliability of the Taqman assays, 20 samples were randomly selected for direct DNA sequencing: concordance with TaqMan was 100% for both polymorphisms. Among our control population, *RAG1* K820R and *BRCA2* N372H were in Hardy-Weinberg equilibrium, and the distributions of variants were consistent with those reported by Hill et al and the cancer genome anatomy project SNP500 cancer database.[3] Unmatched statistical analyses to estimate ORs and 95% CIs were conducted using unconditional logistic regression adjusting for age and sex,[4] and the likelihood ratio test was used to test for interactions.

Table 1. Number of patients and control participants, adjusted ORs, and 95% CIs for *BRCA2* N372H and *RAG1* K820R by immunophenotype

		NHL		B-cell lymphoma		T-cell lymphoma	
Genes and variants	Controls, no. (%)	Patients, no. (%)	OR (95% CI)	Patients, no. (%)	OR (95% CI)	Patients, no. (%)	OR (95% CI)
Total	806 (100)	741 (100)	NA	665 (100)	NA	59 (100)	NA
BRCA2 N372H, A > C*							
AA	387 (51.1)	375 (55.5)	1 (reference)	340 (56.4)	1 (reference)	28 (48.3)	1 (reference)
AC	307 (40.6)	253 (37.4)	0.85 (0.68-1.05)	224 (37.1)	0.83 (0.66-1.03)	22 (37.9)	1.03 (0.58-1.85)
CC	63 (8.3)	48 (7.1)	0.79 (0.53-1.18)	39 (6.5)	0.71 (0.46-1.09)	8 (13.8)	1.61 (0.70-3.75)
AC + CC	370 (48.9)	301 (44.5)	0.84 (0.68-1.03)	263 (43.6)	0.81 (0.65-1.00)	30 (51.7)	1.14 (0.67-1.96)
RAG1 K820R, A > G*							
AA	622 (81.4)	557 (81.2)	1 (reference)	496 (81.3)	1 (reference)	46 (78.0)	1 (reference)
AG	136 (17.8)	124 (18.1)	1.01 (0.77-1.33)	109 (17.9)	1.00 (0.76-1.32)	13 (22.0)	1.38 (0.72-2.65)
GG	6 (0.8)	5 (0.7)	0.94 (0.29-3.10)	5 (0.8)	1.07 (0.32-3.52)	0 (0.0)	0 (0-8.84)†
AG + GG	142 (18.6)	129 (18.8)	1.01 (0.78-1.33)	114 (18.7)	1.00 (0.76-1.32)	13 (22.0)	1.31 (0.69-2.51)

B-cell lymphoma includes International Classification of Diseases for Oncology version 3 codes 9673/3, 9679/3, 9680/3, 9684/3, 9689/3, 9690/3, 9691/3, 9695/3, 9698/3, and 9699/3; T-cell lymphoma includes 9700/3, 9701/3, 9702/3, 9705/3, 9708/3, 9709/3, 9714/3, 9716/3, 9717/3, 9718/3, 9719/3, and 9827/3.

Immunophenotype was not known for 17 patients. ORs and 95% CIs were calculated using unconditional logistic regression adjusting for age and sex.

NA indicates not applicable.

**BRCA2 N372H* and *RAG1 K820R* were in Hardy-Weinberg equilibrium among controls ($\chi^2 = 0.04$, $P = 0.85$; $\chi^2 = 0.23$, $P = 0.63$, respectively). Samples did not amplify for 65 patients and 49 control participants for *BRCA2 N372H,* and 55 patients and 42 control participants for *RAG1 K820R.*

†OR and 95% CI were calculated using exact methods.