Low vitamin B6 levels and the risk of recurrent venous thromboembolism

Gregor Hron, Rossana Lombardi, Sabine Eichinger, Anna Lecchi, Paul A. Kyrle, Marco Cattaneo

From Unità di Ematologia e Trombosi, Ospedale San Paolo, Dipartimento di Medicina, Chirurgia e Odontoiatria, Università di Milano, Milano, Italy (MC); Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria (GH, SE, PAK); Dipartimento di Medicina e Specialità Mediche, IRCCS Fondazione Ospedale Maggiore, Mangiagalli e Regina Elena, Università di Milano, Italy (RL, AL).

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Correspondence:
Marco Cattaneo, MD, Unità di Ematologia e Trombosi,Ospedale San Paolo, Università di Milano, Via di Rudinì 8, 20142 Milano, Italy.
E-mail: marco.cattaneo@unimi.it

ABSTRACT

Low plasma vitamin B6, measured as pyridoxal-5'-phosphate (PLP), is associated with an increased risk of first venous thromboembolism (VTE). In a prospective cohort of 757 patients with first VTE we investigated the association of PLP levels with risk of recurrent VTE. After 4 years, the likelihood of VTE recurrence was 22.5% (95% CI 13.6-31.5%) among patients with PLP ≤23.3 nmol/L and 14.4% (11.5-17.4%) among those with PLP >23.3 nmol/L (p=0.01). Patients with PLP ≤23.3 nmol/L had 1.8-fold higher recurrence risk (1.01-3.14) than patients with PLP >23.3 nmol/L (adjusted for confounders including homocysteine). Therefore, low vitamin B6 is a risk factor of recurrent VTE.

Key words: deep vein thrombosis, risk factors, pulmonary embolism, homocysteine, vitamin B6.

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The association between severe hyperhomocysteinemia and recurrent venous thromboembolism (VTE) is well established, and treatment of these patients with homocysteine-lowering vitamins decreases the incidence of recurrent events.[1] Mild hyperhomocysteinemia is related to an increased risk of first and recurrent VTE,[2,3] but evidence that it is causally related to VTE is lacking. The debate about whether it may be a marker of other disorders that are associated with VTE, or even a consequence of VTE still continues.[4] In a multicenter trial, no effect of vitamin supplementation on the risk of recurrent VTE was seen in patients with hyperhomocysteinemia. However, a risk reduction was seen in patients with normal homocysteine levels which approached statistical significance.[5] These data suggested a homocysteine-independent effect of B vitamins on the risk of thrombosis. Homocysteine metabolism involves several vitamins, including vitamin B6, vitamin B12 and folic acid. Accumulation of homocysteine due to reduced levels of these vitamins has been thought to be the main pathophysiologic link between levels of these vitamins and venous disease. However, each of these vitamins exhibits pleomorphic biologic functions, and indications for a homocysteine-independent role of these vitamins in venous disease have been found. Low folate concentration in erythrocytes gives a seven-fold increased risk after adjustment for homocysteine and other confounders.[6] Low vitamin B12 levels were independently associated with an almost four-fold increased risk of VTE in older men.[7] In a case-control study, mild hyperhomocysteinemia, low folate and low vitamin B12 levels were independently associated with an increased risk of VTE.[8] In a case-control study, we found no significant increase in risk of first VTE in patients with low vitamin B12 or folate levels.[9] However, levels of pyridoxal-5´-phosphate (PLP), the coenzyme form of vitamin B6, < 21.7 nmol/L gave a two-fold higher thrombotic risk. This was independent of folate, vitamin B12 and homocysteine levels. We hypothesized that low vitamin B6 levels may give an increased risk of recurrent VTE. In a prospective cohort of 757 patients with first,

unprovoked VTE, we assessed the relationship between vitamin B6 levels and the risk of VTE recurrence.

Design and Methods

To enter this prospective, cohort study, patients over 18 years of age had to be treated with anticoagulants for at least three months after a first unprovoked VTE (diagnosed by venography, colour duplex sonography, ventilation-perfusion scanning, or spiral computed tomography).[10] The ethics committee of the Medical University of Vienna approved the study. Patients with lupus anticoagulant, antithrombin-, protein C- or protein S-deficiency, homozygous or combined congenital clotting defects, cancer, and patients requiring long-term antithrombotic therapy were excluded. Patients were referred by general practitioners, internists, other clinics, or presented themselves independently. They were consecutively evaluated according to inclusion and exclusion criteria and entered the study at the time of withdrawal of anticoagulation. They were then observed at regular intervals. Routine ultrasonography or ventilation/perfusion lung scanning on follow-up was not performed. Patients were given written information about symptoms of VTE and instructed to report if any of these symptoms occurred.

The study endpoint was recurrent symptomatic deep vein thrombosis (DVT) confirmed by venography, duplex sonography (in case of DVT in the contralateral leg), and/or pulmonary embolism (EP), confirmed by perfusion/ventilation lung scan, and/or spiral-computed tomography.[9]

Blood sampling and laboratory analysis

Blood samples were taken after overnight fasting in 129 mM sodium citrate 3-12 weeks after the withdrawal of anticoagulation. Plasma was stored at -80°C. Antithrombin, protein C, protein S, factor VIII activity, lupus anticoagulant, factor V Leiden and prothrombin G20210A were determined by routine methods. Plasma PLP was measured by the tyrosine decarboxylase method,[11] and plasma total homocysteine (tHcy) by high performance liquid chromatography (Waters, USA), using a commercially available assay (Immundiagnostik, Germany).

Statistical analysis

Time to recurrence (possibly uncensored) was analyzed using survival time methods. Probability of recurrence was estimated according to Kaplan-Meier. To test for homogeneity between strata, we applied the log-rank. Categorical data were checked for homogeneity using contingency table analyses (χ^2 test). The Mann-Whitney test was used for linear data. A Cox proportional-hazard model was used to analyze the association between the risk of recurrence and PLP levels. Adjustments were made for age, sex, duration of anticoagulation, hyperhomocysteinemia (divided at the 75th percentile of thrombosis patients), high factor VIII (divided at the 90th percentile), and presence of factor V Leiden or prothrombin G20210A mutation. All data are given as mean±SD unless otherwise indicated. SPSS 12.0.1 was used for statistical analysis.

Table 1. Relative risks of rrecurrent venous thromboembolism according to various parameters.

Parameter	*Unadjusted Relative Risk (95% CI)*	*Adjusted Relative Risk (95% CI)**
Age (per 10 year increase)	1.2 (1.1-1.3)	1.1 (1.0-1.2)
Male sex (vs. female)	3.0 (2.1-4.4)	3.1 (2.2-4.6)
Duration of anticoagulation (per 3 mo. increase)	1.02 (1.00-1.05)	1.02 (1.00-1.05)
Factor VIII ≥ 230 IU/dl (vs. < 230 IU/dl)	2.7 (1.7-4.2)	2.6 (1.6-4.3)
Factor V Leiden (vs. absence of mutation)	1.2 (0.8-1.7)	1.3 (0.9-1.8)
Factor II G20210A (vs. absence of mutation)	1.4 (0.8-2.4)	1.3 (0.9-1.9)
Hyperhomocysteinemia	1.3 (0.9-1.9)	1.3 (0.9-1.9)

** including all listed parameters*

Results and Discussion

A total of 757 patients were followed for a median of 46 months (range 1 week-155 months). However, 235 patients left the study because of cancer (16), antithrombotics for reasons other than thrombosis (146), pregnancy (34), death (total 18 of which 2 of recurrent VTE), or lost to follow-up (21). All patients were followed until they left the study or died. Data were then censored.

VTE recurred in 130 (17%) patients (78 DVT, 52 PE±DVT). High factor VIII and male sex were major determinants of recurrent VTE (Table 1).

In a Cox proportional hazard model, relative risk (RR) of recurrence was 0.99 (95% CI 0.98-1.03) for each 10 nmol/L increase of PLP. We next investigated strength and linearity of an association between PLP levels and risk of recurrence by calculating relative risks for various PLP levels. The relation between PLP levels and risk of recurrence was nonlinear, and the strongest association was found at a cut-off of 23.3 nmol/L, which corresponds to the 15th percentile (Table 2). This level favorably compares with the results of our previous case-control study which found an association between PLP <21.7 nmol/L and an increased risk of first venous thrombosis.[8] Compared with patients with PLP ≥68.1 nmol/L, recurrence risk was almost twice as high among patients with PLP ≤23.3 nmol/L, and increase was independent of potential confounders including hyperhomocysteinemia (RR 1.72, 95% CI 1.00-3.00). Levels of vitamin B12 and folate were not measured and, therefore, the

Table 2. Relative risk of recurrent VTE according to vitamin B6 levels.

PLP (nmol/L)	Percentile	N. of patients	N. of recurrences	Unadjusted Relative Risk (95% CI)	Adjusted Relative Risk (95% CI)*
≥ 68.1	≥ 75th	189	27	1	1
≥ 44.6 - 68.1	51st - 75th	190	33	1.15 (0.69-1.92)	1.13 (0.68-1.89)
≥ 30.1 - 44.5	26th - 50th	190	35	1.72 (0.99-3.00)	1.22 (0.74-2.02)
>23.3 - 30.0	15th - 25th	75	9	0.80 (0.38-1.71)	0.56 (0.25-1.28)
≤ 23.3	< 15th	113	26	1.79 (1.05-3.08)	1.72 (1.00-3.00)

**Adjusted for age, sex, factor V Leiden, factor II G20210A, high factor VIII, duration of anticoagulation, and hyperhomocysteinemia*

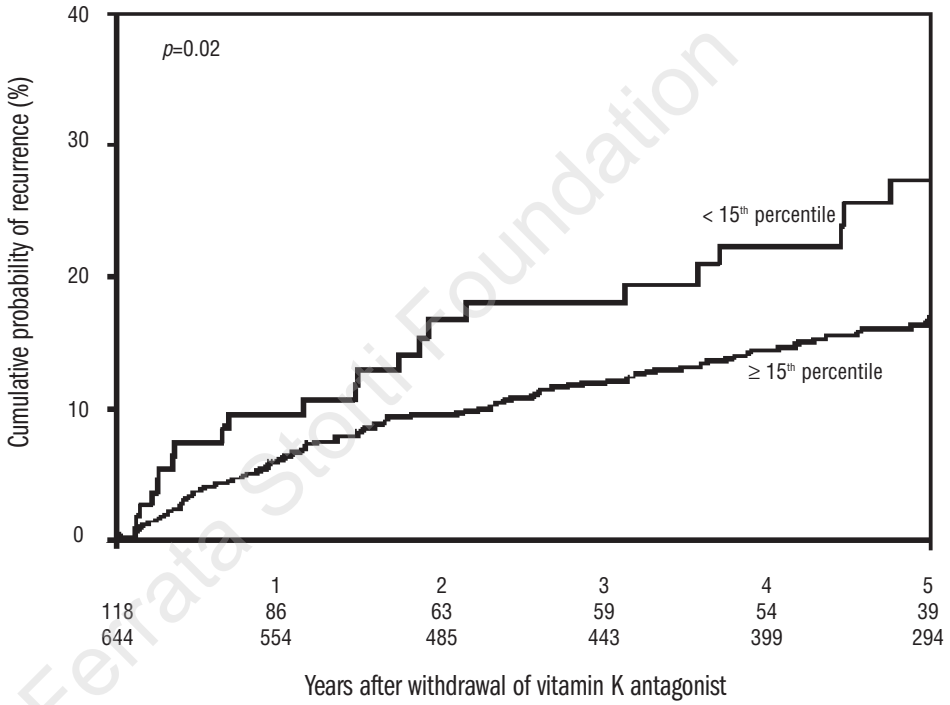

Figure 1. Kaplan-Meier estimates of the probability of recurrent venous thromboembolism in patients with low and high vitamin B6 as measured by PLP plasma levels.

effects of these vitamins on recurrence risk are not known. However, in our previous study, levels of folate, vitamin B12 and B6 were measured, and a homocysteine-independent association with the risk of first DVT was only found for low vitamin B6.[8]

Cumulative probability of recurrent VTE after four years was 22.3% (95% CI, 13.5%-31.1%) among patients with PLP ≤23.3 nmol/L and 14.4% (95% CI 11.5%-17.4%) among patients with higher levels (p=0.02, Figure 1). After adjustment for confounders including tHcy, RR of recurrence was 1.78 (95% CI 1.01-3.14) among patients with PLP ≤23.3 nmol/L compared with patients with higher levels.

Patients with low PLP levels were older (55±15 *vs.* 47±16 years, p<0.001) and had higher factor VIII (177±48 IU/dl *vs.* 163±50 IU/dl, p=0.002). The proportion of carriers of factor V Leiden was lower among patients with low PLP levels than among those with high levels (22% *vs.* 32%, p=0.03). Hyperhomocysteinemia was more frequent among patients with low PLP levels (44% vs 23%, p<0.001). The prevalence of prothrombin G20210A was identical in the two groups (8% vs. 8%, p=0.9).

Our study indicates that low vitamin B6 is associated with an increased risk of recurrent VTE. Until recently, the thrombotic risk associated with low vitamin status was entirely attributed to impaired homocysteine metabolism. But since doubts have been raised about the causal role of homocysteine in thrombotic disease,[4] other functions of B vitamins need to be considered. Vitamin B6 is a co-enzyme in the metabolism of aminoacids, carbohydrates, neurotransmitters and lipids,[12] and administration of vitamin B6 inhibits platelet function.[13] Low vitamin B6 has also been related to elevated C-reactive protein levels and other markers of inflammation,[14,15]. In fact, patients with chronic inflammatory diseases, who are at heightened risk of VTE, exhibit low vitamin B6 levels.[16] A relationship between

inflammation and venous thrombosis has not yet been firmly established, but investigating the biology of vitamin B6 in VTE might further clarify this pathogenic link.

Studies on homocysteine and vitamins are potentially subject to methodological shortcomings and biological variations in these parameters. In our study, care was taken to ensure the immediate processing of fasting plasma. However, over the 10-year course of the study, laboratory methods, particularly those for tHcy determination, varied slightly. Our study participants were predominantly of central European origin, and interpretation of the results must take into consideration the ethnic characteristics of the study population and a diet without vitamin-fortification.

Risk of recurrent VTE is reduced by anticoagulants. The duration of anticoagulant treatment is tailored to the individual patient by balancing risks of recurrence and bleeding. In patients with low vitamin B6, vitamin supplementation would be a safe alternative. Evidence exists that vitamin B6 supplementation could protect in thrombosis patients with severe hyperhomocysteinemia.[1,4] In addition, data from the VITRO study showed a 45% lower recurrence risk in patients who had low vitamin B6 levels and were on vitamins, compared with patients treated with placebo.[17] These observations tend to contradict three intervention trials in patients after myocardial infarction or ischemic stroke. The risk of recurrence in these patients was not reduced by vitamins.[18-20] However, venous and arterial thrombotic disease have different pathogenic mechanisms and sensitivity to antithrombotic therapies,. Therefore, additional information from interventional trials in venous thrombosis patients is needed.

Authors' Contributions

GH: analysis of data, drafting the manuscript, final approval of the version to be submitted; RL: acquisition of data, revising the manuscript, final approval of the version to be submitted; SE: conception and design, revising the manuscript, final approval of the version to be submitted; AL: acquisition of data, revising the manuscript, final approval of the version to be submitted; PAK: conception and design, revising the manuscript, final approval of the version to be submitted; MC: conception and design, revising the manuscript, final approval of the version to be submitted.

Conflict of Interest

The authors reported no potential conflicts of interest.

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