Hyperhomocysteinemia and venous thrombosis : studies into risk and therapy

Willems, H.P.J.

Chapter 9

General discussion

General discussion

In the studies described in this thesis we address several aspects of hyperhomocysteinemia in relation to venous thrombosis.
After the relationship of elevated homocysteine concentrations and venous thrombosis was established by our research group and others in earlier studies a main issue is whether lowering of homocysteine concentration might prevent recurrence of venous thrombosis (homocysteine can be lowered by the treatment with B vitamins, including vitamin B11 (Folic acid), Vitamin B12 and Vitamin B6).
This question is of interest for two reasons. The first reason is to gather more insight into the pathophysiology of homocysteine metabolism and the possible causal relationship of homocysteine and venous thrombosis. Because laboratory- and animal studies have not yet found a definite mechanism of action, it is of interest to further elucidate the relationship with data from epidemiological, intervention studies. In a randomized trial like ours where homocysteine-lowering therapy by B-vitamins was used to study if lowering homocysteine values does reduce the incidence of venous thrombosis, such an effect would strongly support the causal relationship. The second reason is obvious: reducing the incidence of venous thrombosis would benefit the patients. The outline of the study named **VITRO** (**VI**tamins and **Th**ro**O**mbosis) is described in chapter 2.
Before we could start the VITRO trial we had to find a suitable collection medium for blood samples for homocysteine determination. The determination of homocysteine in EDTA tubes is known to pose problems to epidemiological field studies since homocysteine values rise if the samples are not placed onto ice immediately. Also in clinical practice the assay may lead to false results as a consequence of blood handling errors. We showed that tubes containing acidic citrate are a suitable collection medium for determination of homocysteine concentrations (chapter 3). This observation made it possible for us to use this tube for the intervention study described in chapter 2 and 8, where it was necessary to screen a large group of persons (>4000) with venous thrombosis, under circumstances where immediate processing of samples was not possible. Furthermore we showed that the concentrations measured in the acidic citrate tubes highly correlate to the concentrations measured in EDTA blood. However, reference values need to be established in each laboratory since basic concentrations differ in EDTA blood in comparison to acidic citrate blood (Chapter 4). By performing these studies we became aware of the lack of standardization procedures in homocysteine determination. This implies that homocysteine concentrations or cut-off points from studies should be evaluated and compared with caution. Comparison with

concentrations measured in other studies or even in individual patients is therefore often not possible.

The study described in chapter 5 deals with the effect of oral anticoagulants on homocysteine concentrations. We found no relevant effect of oral anticoagulants on homocysteine concentrations. Therefore for the interpretation of research studies and in individual patients with thrombosis where patients were on anticoagulant at the time of homocysteine measurement this treatment does not have to be taken into account.

When hyperhomocysteinemia is a cause of venous thrombosis, one expects to find an association between homocysteine and components of the clotting system. For this reason we studied the endogenous thrombin potential (ETP) to seek for an association between the ETP and homocysteine values. The ETP is a method to measure the potential to generate thrombin, which is a crucial component of the clotting cascade. In patients with inherited risk factors for venous thrombosis it has been demonstrated that the ETP might be elevated. Therefore we reasoned that when high plasma homocysteine values induce thrombosis this phenomenon might be reflected by elevated ETP values. However, we did not see any association between the ETP and homocysteine levels (Chapter 5).

Venous thrombosis is common in the elderly. The incidence rises from 25 / 100,000 / year at the age of 25 to 500 / 100,000 / year over the age of 80. Since plasma homocysteine levels increase exponentially with age, homocysteine might play an important role in the development of venous thrombosis in this age group. Two previous studies have reported on the risk of hyperhomocysteinemia and the development of venous thrombosis and the relation with age, with conflicting results. We therefore performed a case-control study among elderly patients to evaluate whether elevated homocysteine levels are a risk factor for venous thrombosis in this age group, as described in chapter 7. In this study we found that the homocysteine concentration in plasma is a risk factor for venous thrombosis in elderly individuals, as it is among younger people. Furthermore, we found a graded increase in the risk with increasing homocysteine concentrations. The risk estimate was similar to those reported in studies in younger patient groups. This may imply that the absolute effect of hyperhomocysteinemia is greater among the elderly, because the incidence of thrombosis is much higher.

Finally the results of the VITRO trial are described in chapter 8.

We observed a small effect (15% reduction of venous thrombosis) which did not reach statistical significance. However, the sample size of our study was not large enough to rule out a modest beneficial effect of homocysteine lowering therapy at this level.

Since our study was underpowered for a small effect it cannot answer the question whether homocysteine is of pathophysiological significance in

developing venous thrombosis. In the past years several studies have been published which suggested an association between thrombosis and the MTHFR C677T mutation. Results are however still conflicting since not all studies demonstrate this association. This mutation is associated with higher homocysteine values, especially in those with lower vitamin B11 and B12 levels. Since this mutation is a genetic factor it cannot be influenced by the disease itself or surrounding factors. Though the association between MTHFR C666T and venous thrombosis is at most weak it suggests a causal relation[1-3].
The second reason – but in fact the most relevant one for patients – for designing our study was for clinical purposes. When homocysteine is a cause of thrombosis homocysteine treatment by vitamins was expected to reduce the incidence of recurrent venous thrombosis.
This trial was the first randomized trial that evaluated the effect of vitamin supplementation on venous thrombosis. There have been randomized studies which evaluated vitamin supplementation in arterial thrombosis. One study evaluated the effect of folic acid as prevention for re-stenosis after coronary angioplasty[4,5]. The authors found a clearly beneficial effect of the therapy on the need of revascularization and on composite vascular endpoints (death, non-fatal myocardial infarction and revascularization). A second study, however, found no effect of folic acid on composite endpoints in patients with stable angina pectoris[6]. A third randomized trial compared high versus low dose multivitamin suppletion in the secondary prevention of ischemic stroke, coronary heart disease and death[7]. In this study, no effect of therapy was found on the clinical end-points.
In our study we found no effect, at least not at a statistical significant level. A negative result should always be evaluated in the light of the power of a study. In our case the number of events (recurrent venous thrombosis) was lower than expected, especially in the control arm with high homocysteine levels. This reduced the power.
In our trial we found a non-significant 16% reduction of recurrent events. Therefore we cannot exclude a modest effect, or the absence of any effect. Theoretically a reduction in risk of 25% is to be expected according to estimations by Wald *et al.*[1]. A trial sufficiently powered to demonstrate such an effect would need approximately 4000 patient years in both treatment arms. We do not expect that such a study will be performed. If homocysteine lowering would indeed reduce recurrence by about 15%, the question would be whether this is a clinically relevant reduction that would lead to incorporation of this treatment. The recurrence risk after a first event of venous thrombosis in our study was about 7% per year. A reduction with 15% would imply that about 100 patients would need to receive vitamins to prevent one event of thrombosis per year. In chapter 8 we conclude our research project with the statement that although multivitamin supplementation seems to be safe and is not expensive,

this number needed to treat indicates that vitamin supplementation is not a clinically relevant option in the secondary prevention of venous thrombosis, even in those with hyperhomocysteinemia.

References

1. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002;325:1202.
2. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 2002;288:2023-31.
3. Davey SG, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1-22.
4. Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberli FR et al. Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N Engl J Med 2001;345:1593-600.
5. Schnyder G, Roffi M, Flammer Y, Pin R, Hess OM. Effect of homocysteine-lowering therapy with folic acid, vitamin B(12), and vitamin B(6) on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. JAMA 2002;288:973-9.
6. Liem A, Reynierse-Buitenwerf GH, Zwinderman AH, Jukema JW, van Veldhuisen DJ. Secondary prevention with folic acid: effects on clinical outcomes. J Am Coll Cardiol 2003;41:2105-13.
7. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH, Stampfer M. 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.JAMA. 2004;291:565-75.