Clinical studies on hereditary thrombophilia : a focus on resistance to activated protein C (factor V:Q506)

Chapter 8

SUMMARY AND PERSPECTIVES

The work presented in this thesis comprises a number of studies on hereditary thrombophilia, with the principal aim of investigating the clinical relevance of APC resistance (due to Arg to Gln mutation in activated factor V, factor V:Q506), the recently described genetic determinant of thrombosis. As our Thrombosis Center is specialized in the diagnosis of coagulation abnormalities predisposing to thrombosis, mainly young patients with unexplained events (i.e., free from neoplastic, autoimmune or systemic illnesses) are referred to us. This selected population represents a good model in order to study the genetic and environmental interactions, required to bring about thrombosis.

APC resistance and factor V:Q506 were first described by Dahlbäck and Bertina who found a very high prevalence of the defect (up to 7%) in the general population of Northern Europe. A few months later, it became evident that the prevalence of the mutation differed in various ethnic populations and also in various countries. Rees et al (Lancet 1995;346:1133-4), reporting the distribution of mutant factor V throughout the world, found that it was not present in Italians. Since this finding was probably conditioned by the small number of individuals tested, we investigated a cohort of 344 healthy Italians subjects (Chapter 2), and found a prevalence of heterozygous factor V:Q506 of 2.6%, thus indicating that it was also present in Southern Europeans. Chapter 2 also shows that the association of factor V:Q506 with deficiency of the naturally occurring inhibitors confers an increased thrombotic risk, but the prevalence of combined defects was lower (3%) than in Northern Europeans (19-38%).

In Chapter 3 we wanted to determine the prevalence and clinical characteristics of APC resistance in 493 consecutive patients referred to our Thrombosis Center for a first episode of thrombosis occurring at a young age. The prevalence of APC resistance was significantly higher in patients than in healthy controls (15% versus 2%). The most frequent clinical thrombotic manifestation in APC-resistant patients was deep vein thrombosis. With the aim to make a direct comparison of the thrombotic risk and the

clinical manifestation in individuals with APC resistance, antithrombin, protein C or protein S deficiency, we carried out a study on more than 700 relatives of the index patients with one of the four defects (Chapter 3). The estimated risk was 9 to 12 times higher in carriers of deficiencies of the naturally occurring inhibitors, and only 4 times higher in those with APC resistance, than in non-carriers. The age at the occurrence of thrombosis was similar in the four defects, but patients with APC resistant had more episodes of superficial vein thrombosis. Considering that APC resistance is highly frequent in the general population and taking into considerations the molecular mechanisms of the defect (activated factor V is only partially resistant to the inactivation by activated protein C), a lower thrombotic risk in comparison to deficiency of the naturally occurring inhibitors would be expected. On the other hand, it has been recently addressed that APC resistant patients have the same thrombotic risk of those with protein C deficiency, as assessed on the basis of the age at the occurrence of the first event. In addition, a state of biochemical hypercoagulability assessed by measuring the plasma levels of markers of coagulation activation, such as prothrombin fragment 1+2 and thrombin antithrombin complexes, was identified in the same proportion of patients (one-third) with APC resistance and in any deficiencies of the naturally occurring inhibitors (Chapter 4).

A high prevalence of APC resistance was also found in patients with cerebral vein thrombosis, a rare but very harmful thrombotic manifestation (Chapter 5). The presence of acquired predisposing conditions (surgery, trauma, immobilization, pregnancy, oral contraceptives) was also evaluated; both the prevalence of the mutation (20% versus 3%) and oral contraceptive intake (75% versus 42%) were significantly higher in patients than in controls. In contrast, the prevalence of APC resistance (as well as other hypercoagulable states) was not increased in patients with deep vein thrombosis of the upper extremities (Chapter 5). The most common risk factor for the so-called "primary" deep vein thrombosis of the upper extremities was strenuous muscular activity of the arms, which may precipitate thrombosis aggravating the extrinsic compression of the veins caused by pre-existing anatomic abnormalities. Hence, a laboratory screening for coagulation abnormalities should not be done in patients with thrombosis of the upper extremities.

The association between factor V:Q506 and venous thromboembolism has been clearly demonstrated by many investigators, with case-control or family studies. Surprisingly, (Chapter 5), we found that its prevalence in patients with isolated pulmonary embolism (i.e., without a concomitant deep vein thrombosis of the lower extremity) was not as high (12%) as in those with deep vein thrombosis complicated or not, by pulmonary embolism (23%), and was not significantly higher than that found in healthy controls (3%).

Whether or not factor V:Q506 is to be considered a risk factor for arterial thrombosis is still a matter of debate. Ridker et al (N Eng J Med 1995;332:912-7) demonstrated in a

large prospective study that the mutation was not a risk factor for stoke in a population of American physicians with a stroke after 45 years of age. As advancing age is a risk factor for thrombosis per se, the same conclusions may not be drawn for stroke in the young. Hence, we studied a cohort of patients who had a stroke at an age below 45 years (Chapter 6); an interesting observation was that, although the prevalence of factor V:Q506 was similar in patients and in controls (4.2% and 1.6%), factor V:Q506 was mostly present in the subgroup of patients with criptogenetic stroke, i.e., without any apparent cause (12%), suggesting a possible role in this special cases.

As regarding arterial thrombosis, it is known that elevated plasma homocysteine levels are associated with an increased risk of stroke, myocardial infarction, peripheral artery disease, and extracranial arterial thrombosis. More recently, the evidence that hyperhomocysteinemia is a risk factor also for venous thrombosis has been provided by several studies. In Chapter 7 we reported that the prevalence of hyperhomocysteinemia in 89 consecutive patients with deep vein thrombosis of the lower extremities was higher (13.5%) than in healthy controls (6.7%). Furthermore, we underlined the importance to measure homocysteine levels in the fasting state and after a methionine loading, since the combination of the two tests identified a larger number of patients with thrombosis and impaired homocysteine metabolism than either test alone.

There is the evidence that patients with a certain coagulation defect develop thrombotic symptoms of varying severity. A discrepancy in clinical expression has been observed for example in protein C deficiency, which may be clinically recessive and clinically dominant. Since this discrepancy could not be explained by different mutations underlying the defect, the most plausible explanation arose from the view that more than one abnormality may be required to cause thrombosis. It has been shown that when APC resistance is present with deficiency of protein C, protein S or antithrombin in the same individual, it causes a higher thrombotic risk than each of these abnormalities separately. So, from a concept of thrombophilia due to a single gene defect, at the present time it is accepted that in some families the co-segregation of two genetic defects can occur and then thrombophilia is a multiple gene disorder. It is likely that genetic defects particularly frequent in the general population may aggravate the risk of thrombosis, more than carrying a high risk per se. This also explains the high risk of thrombosis among non-deficient family members with respect to the general population; many of them may carry APC resistance, or hyperhomocysteinemia, or the newly identified mutant prothrombin (due to a point mutation in the 3'-untranslated region of prothrombin [20210 AG] that accounts for approximately 10% of the cases), or other genetic defects still unknown. An explosion of knowledge on thrombophilia has been verified in the last few years, but all genetic influences and their interaction are not yet understood, since even in patients from families selected on the basis of a high number of unexplained thromboses, only in about half an underlying defect will be found.