

## Summary and concluding remarks

How are platelet activity and atherothrombosis interrelated in postmenopausal women on HRT and patients with renal disease?

### *Elaboration on this question*

The question how platelet activity relates to atherothrombosis has at least two dimensions. Firstly, this question concerns the mechanism by which altered platelet activity contributes to the progression, morbidity and mortality of atherothrombosis-related diseases. The multitude of interactions with soluble and cellular elements, the heterogeneity of blood platelets and even the influence of circulating factors and bone marrow stroma on megakaryocytes contribute to a magnitude of possible interactions. Furthermore, it is conceivable that the role of altered platelet reactivity is modified by other risk factors of atherothrombosis. The second dimension is to what quantitative extent does platelet activity contribute (independently) to an increased risk of atherothrombosis. Both of these dimensions are key elements in answering of the clinically relevant question: whether, and if so, by which strategy, is extension of influencing platelet activity, beyond what is now generally believed to be the standard of care, useful in the prevention of atherothrombosis-related disease? After all, there are many possibilities, besides aspirin, to influence platelet activity<sup>1-5</sup>. This knowledge should open up the way to a rational design of clinical trials, which ultimately will provide answers to this question.

### *Ideal studies*

It is impossible to answer these questions once and for all by one or two observational cohort studies followed by one or two clinical trials. Actually, the pathogenesis of atherothrombosis is, in different patient groups, and with respect to different clinical end-points rather heterogeneous and the time course of the disease spans several decades. Therefore, selection of certain patient groups at high risk for cardiovascular disease seems a logical first step in finding a solution to this problem. In this thesis we focused on two of these categories: postmenopausal women on hormone replacement therapy and renal impairment patients. What did we do and could we, in retrospect, have done better?

The most accurate way to investigate the relationship between platelet activity and atherothrombosis risk in these categories is to perform large, long-term follow-up prospective cohort studies, excluding persons with manifest cardiovascular disease<sup>6</sup>. To increase internal validity, the cohort must be restricted to one ethnicity and one age group, or the cohort must be so large that sub-group analysis would yield reliable data. At baseline, platelet function should be measured. In consideration of the fact that platelet function can be assessed in a variety of ways all pinpointing different aspects of platelet activity, several methods should be used simultaneously. Furthermore, "all" other factors contributing to atherothrombosis – haemostatic as well as non-haemostatic – should be measured carefully. It is important to measure all these variables repeatedly. Firstly, to avoid underestimation of the true association with atherothrombotic clinical end-points (regression dilution bias – since most of

these variables have a long-term within-person variability) <sup>7</sup>. Secondly, to study compensatory interactions between all these variables over time. Outcome measures should be clinically relevant end-points, such as coronary artery disease, peripheral arterial disease or ischaemic cerebrovascular disease.

After the above mentioned studies will have revealed where and by which mechanism platelet activity contributes to an increased risk of atherothrombotic disease, the next step would be to design randomized controlled trials to investigate by which therapeutic regimen platelet activity can be changed in order to diminish the risk of the occurrence of these atherothrombotic disease. Not only obvious platelet inhibitors tested in various dosage regimens, but also other modes of platelet function altering, like diminishing oxidative stress, inhibiting P-selectin binding, or modulating intracellular signaling pathways, could be subjected to these investigations. In addition, platelet function assays could be used to tailor platelet inhibition individually in these therapeutic strategies <sup>8;9</sup>. Again, the end-points of these studies should be occurrence of clinical atherothrombotic disease, not surrogate end-points or even 'altered platelet function'.

#### *To what extent have we conducted ideal studies?*

It goes without saying that it is almost impossible to perform such studies sequentially. Nevertheless, we have tried to contribute to answering some of the above raised issues. After thoroughly reviewing the literature on platelet function assays (chapter 2A) we decided to use fluorescence cytometry in the following chapters. It would have been more prudent to incorporate more platelet function assays in our studies, all the more so because all these function tests focus on different aspects of platelet function.

The influence of estrogen or progestagens on platelet function is best evaluated in a double-blind, randomized, controlled clinical trial. We performed such a trial (chapter 3B), but the power to detect small differences and the precision of the point estimates of the effects were not large. Obviously, this is less of a problem when the platelet function assay we used would have had a clear clinical correlate (e.g. x% increase of platelet activity is paralleled by y% increase in atherothrombotic disease burden), but such a relationship is not known, particularly in the case of healthy postmenopausal women. Whether the differences we found translate into clinically relevant outcomes should be investigated further. Even so, the results of this trial could contribute to the generation of hypotheses concerning the risk of atherothrombotic disease in post-menopausal women with or without HRT.

We studied the effect of different haemodialysis membranes on platelet activity in patients with end-stage renal disease (chapter 4). In a way, it would have been better to have studied a larger population, in a two-armed (not cross-over) design, with a longer follow-up period, and with a more standardized time schedule of blood sampling (at various time points during a dialysis session). In addition, to evaluate not only the effects of the dialysis membrane, but also the effect of the blood pump, it would have been better to have blood samples taken at three points simultaneously (instead of two) <sup>10;11</sup>. Furthermore, the study group was very heterogeneous with respect to the disease leading to end stage renal failure. This is partly inevitable, but could have been overcome by increasing the size of the study population. Nevertheless, assuming that platelet function contributes to the greater incidence of atherothrombotic disease in these patients, the fact that there are

differential effects of different dialysis membranes should be taken into account when evaluating the potential clinical benefit of new dialysis membranes. Our study helps to emphasize that issue.

The cross-sectional design of our study on platelet activity in patients with mild-to-moderate renal impairment (chapter 5) precludes conclusions about a causal role of greater platelet activity contributing to the greater risk of cardiovascular disease in these patients. Therefore, this study should be regarded as a prelude to a large prospective cohort study to evaluate this issue. Nevertheless, the results of this study may help future investigators to estimate a reasonable study size, and to choose the right platelet function parameters.

Subsequently, we studied the effect of a treatment strategy aimed at reducing oxidative stress (consisting of cholesterol-lowering and homocysteine-lowering medication, and vitamin E) in this cohort (chapter 6). In this study, we failed to show a very profound general effect of this treatment strategy on blood platelet activity. Instead, we could only show a statistically significant decrease of platelet lysosomal degranulation. Whether this finding reflects a specific effect of this treatment regimen on blood platelets, or is merely the consequence of a low power to detect a difference in the other parameters of platelet function, cannot be easily analyzed. After all, the 95%-confidence intervals of the change of the other platelet function parameters do not preclude an effect of this treatment strategy. Whether or not such an influence (if any) could be pathophysiologically or even clinically relevant, cannot be concluded at this point in time, again in the absence of a precise estimate of a relationship between platelet function and atherothrombotic end-points.

### *Conclusions and implications*

This thesis allows several conclusions on platelet activity and atherothrombosis. Firstly, there is a clear need for standardization for the assessment of platelet function. Good candidates are cartridge-based and fluorescence-cytometric based assays, although the latter needs to be automated further. After standardization, this platelet function parameter should be prospectively studied to determine its pathophysiological and clinical importance. Secondly, part of the higher risk of atherothrombotic disease in post-menopausal women taking hormone replacement therapy could be due to increased platelet function. Thirdly, it is likely that there are differences between dialysis membranes with respect to their influence on blood platelets. The most biocompatible dialysis membrane (defined by its attenuated effect on the complement system) does not automatically have the least influence on blood platelets. Fourthly, mild-to-moderate renal impairment is associated with greater platelet activity. Finally, a treatment strategy aimed at reducing oxidative stress in patients with mild-to-moderate renal impairment could at least have *some* inhibitory effect on platelet activity.

From this several lines of research may develop. Assuming that there are no financial, technical or working hours constraints, what then are future possibilities? Firstly a large prospective cohort study would be necessary to further evaluate the role of platelet function assessment in predicting atherothrombotic disease burden. Secondly, newer forms of hormonal replacement therapy could be evaluated with these platelet function assays. Thirdly, dialysis membranes (existing and newly developed) could be evaluated on the basis of these platelet function assays. Finally pathophysiology-guided platelet inhibitory strategies could be developed to decrease

the atherothrombotic disease burden in high risk groups (such as patients with mild-to-moderate renal impairment) with specific platelet inhibitory regimens and, perhaps using platelet function assessment to tailor therapy individually.

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