

CHAPTER 1

Introduction to and outline of the thesis

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Human blood platelets are anuclear cell fragments capable of detecting and sealing minor injuries of the inner endothelial layer of blood vessels. They stem from megakaryocytes which are mainly, but not exclusively, located in bone marrow stroma. The lifespan of platelets (about 7 days) and their number (about 10^{11} /l blood) makes it necessary that there is a huge daily production. Although the haemostatic systems of the body are composed of many elements, of which some may seem even redundant, a complete absence of blood platelets makes an individual extremely vulnerable after vessel wall: a haemorrhagic diathesis. We have learned much on the biology of blood platelets since Bizzozero described this classical function of blood platelets in 1882, about four decades after what is classically regarded as the first description of blood platelets (1;2). In the early ages platelet research was focussed on understanding the role of blood platelets in the physiology of haemostasis and on the understanding of the pathogenesis of bleeding disorders. Later, it became clear that the main epidemic of the industrialized world – cardiovascular disease – in part might be due to enhanced platelet function, and that the final clinical manifestation of arteriosclerosis was caused by the formation of an occlusive platelet rich thrombus on an injured inner layer of an artery. The term atherothrombosis emphasizes the relevance of thrombotic processes in this disease spectrum. The success of platelet inhibition in decreasing the risk of recurrent cardiovascular events has underscored the importance of platelet activity in these thrombotic (or embolic) events (3).

More recently properties of blood platelets other than participation in the haemostasis system came into sight: inflammatory, endothelial stimulating, differentiation-initiating and even bactericidal functions of blood platelets were discovered (4;5). In addition to these newly discovered properties of blood platelets, it became clear that blood platelet activation was more subtle than an all-or-nothing phenomenon only contributing to acute 'catastrophic' events: evidence emerged that blood platelets also could have a more subtle variation in their function, paving the way for the hypothesis that blood platelets could also play a pathogenetic role – in concert with other risk factors like hypertension, diabetes mellitus or hypercholesterolaemia – in the earlier stages of atherothrombotic diseases (6;7). Table 1 of Chapter 2 of this thesis summarizes some possible mechanisms of platelet involvement in the pathogenesis of cardiovascular disease.

In view of these considerations, it is relevant to explore whether blood platelet function is affected by 'classic' risk factors for cardiovascular disease, and whether treatment of these risk factors contributes to an alteration in the activity of circulating blood platelets.

This thesis explores some of these issues.

In **chapter 2a** we summarize the relationship between several platelet function tests on the one hand and major clinical end points in the spectrum of atherothrombotic disease on the other. The emphasis lies on prospective cohort studies testing three categories of platelet properties: (i) release of platelet specific substances; (ii) platelet aggregation; and (iii) fluorescence cytometry. One of these platelet categories – fluorescence cytometry – appears not to have been widely

studied in this regard, yet has some promising features. Since this technique is used in our studies reported on in chapters 3b-6, we provide a short description in **chapter 2b**. In the studies described in the following chapters, we measured platelet function in different clinical conditions related to atherothrombotic disease: hormone replacement therapy in postmenopausal women and renal disease.

Hormone replacement therapy

Until the end of the twentieth century it was thought that in postmenopausal women part of the increasing incidence of cardiovascular disease could be due to estrogen deficiency. Indeed, there were many case control studies supporting this view: hormone replacement therapy (HRT) seemed to reduce the risk of cardiovascular disease (8;9). Also there were many reports on the putative mechanism by which estrogen deficiency caused and estrogen replacement could diminish or even abolish this increased incidence (10;11). However, in the late twentieth and the beginning of the twenty-first century several large randomized controlled clinical trials changed the view of a positive influence HRT on cardiovascular risk (12-14). Indeed, the cardiovascular risk of women on HRT rather seemed to increase compared to women without such therapy. In **chapter 3a** we review what was known on the effects of hormone substitution (oestrogen only or the combination of oestrogen with progestagens) on several aspects of the haemostatic system. It became clear that not much was known on the effects of HRT on blood platelet activity. In view of these considerations, we conducted a double-blind, placebo controlled, randomized clinical trial to evaluate the effect of ERT and CHRT on platelet function in 60 healthy postmenopausal women, the results of which are described in **chapter 3b**.

Renal disease

Renal disease is a major risk factor for (the progression of) atherothrombosis. This applies both to end stage renal disease (ESRD) as well as to earlier stages of renal disease (defined by a decline of glomerular filtration rate) (15-18). Large randomized trials in ESRD patients have consistently shown no survival benefit from multiple treatment strategies aimed at reducing cardiovascular disease, such as lipid-lowering with statins (19), increased dialysis dose (20;21) or increased biocompatibility of dialysis membranes (22;23). In part, this may be because some interventions, while decreasing one or more risk factors, have adverse effects on other risk factors, such as platelet activation. In **chapter 4** we report data on a small cross-over study to explore the effect of two different types of dialysis membranes on platelet function in patients with ESRD.

Even mild-to-moderate renal impairment is associated with a high cardiovascular risk that increases as kidney function declines. The increased risk could be due to a high burden of traditional vascular risk factors (24). However, even patients with a primary non-atherothrombotic renal disease such as polycystic kidney disease have an elevated risk of cardiovascular disease (25). Traditional cardiovascular risk factors, although overrepresented in patients with mild impairment of renal function, can only partially explain the cardiovascular morbidity and mortality (26). Additional risk factors may therefore contribute to the increased incidence in this category of patients (table 1 (27)). One of these putative additional risk factors is increased platelet activity. **Chapter 5** is a report of a cross-sectional study in a cohort of patients with mild-to-moderate renal impairment. In this cohort we measured

platelet function and related it to the degree of renal impairment. An important putative risk factor for increased cardiovascular morbidity in patients with mild-to-moderate renal impairment is increased oxidative stress (table 1), as increased oxidative stress occurs in the early stages of renal impairment. In addition, oxidative stress may induce platelet activation in vitro (28;29). In vivo, the relation between oxidative stress and platelet activation is less clear. Therefore, we evaluated the effects of a treatment regimen aimed at reducing oxidative stress on platelet activity in a randomized, double-blind, placebo controlled clinical trial in the cohort described in chapter 5. These data are reported in **chapter 6**.

Finally, **chapter 7, 8 and 9** provide a summary and a critical appraisal of the foregoing chapters, and touch on possible future perspectives of this research field (in English, in Dutch, and in Dutch for persons not experienced in this particular subject, respectively).

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Table 1. Nontraditional risk factors for atherothrombotic disease

Inflammation

- C-reactive protein
- vascular and cellular adhesion molecules
- soluble CD40-ligand

Haemostasis

- fibrinogen
- von Willebrand factor antigen
- D-dimer
- platelet size
- platelet function
- platelet count

Oxidative stress

- oxidized LDL
 - hyperhomocysteinaemia
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Adapted from reference (27)