

CHAPTER 2A

Which platelet function parameters predict cardiovascular disease?

A qualitative review of prospective cohort studies

Which platelet function parameters predict cardiovascular disease? A qualitative review of prospective cohort studies

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Abstract

Background

Several tests exist to study platelet function in humans. This review summarizes prospective cohort studies on the association of concentration of platelet activity markers and aggregometry- and fluorescence-cytometry-measured platelet activity with cardiovascular disease incidence at follow-up.

Methods

With a sensitive, PUBMED-based search strategy we looked for prospective cohort studies on the association of beta-thromboglobulin (BTG), platelet factor 4 (PF4), thromboxane A₂ metabolites and P-selectin concentrations, optical-densitometric platelet aggregation tests, cartridge-based aggregation tests and platelet fluorescence cytometry with coronary artery disease, ischaemic cerebrovascular disease and peripheral artery disease. We extracted data from these studies in a prespecified manner, and systematically judged the quality of the reports.

Results

We found 67 reports on prospective cohorts including over 23000 persons with a median follow up of 1 day to 13.5 years. The definition of greater platelet activity differed greatly between studies. The association of plasma or urinary concentrations of BTG, PF4 and thromboxane A₂ metabolites or P-selectin with cardiovascular disease was studied in 14 studies. In 10 of these studies the association was not statistically significant. The association of optical-densitometric platelet aggregation tests and cartridge-based aggregation tests with cardiovascular disease was in almost all studies (41 of 47) statistically significant. The association of fluorescence-cytometry-measured platelet activity with cardiovascular disease was least studied (10 studies) and was statistically significant in eight.

Conclusion

Greater platelet activity measured by optical-densitometric platelet aggregation tests, cartridge-based aggregation tests and platelet fluorescence cytometry is associated with a greater incidence of cardiovascular events. Concentrations of platelet activity markers are not useful in this regard. The magnitude of this association is not well known, because - among others - very varying definitions of greater platelet activity are used.

Introduction

Cardiovascular diseases (e.g. coronary artery disease, stroke or peripheral arterial disease) are the leading causes of morbidity and mortality in the industrialized world. Traditionally, the pathogenesis of these diseases is divided into an early, inflammatory phase, called atherosclerosis, and a later, acute phase, mediated by plaque rupture and subsequent thrombosis on the thus exposed subendothelial surface. Already 35 years ago, the term atherothrombosis was used, emphasising the intertwining nature of these inflammatory and thrombotic processes¹. Atherosclerosis is primarily a disease affecting the intimal layer of elastic arteries, accompanied by dysfunction of endothelial cells, inflammatory cells and vascular smooth muscle cells. Thrombosis occurs when plaque distortion leads to plaque rupture; in this phase, blood platelets and the plasmatic coagulation system play a crucial role. However, it is increasingly thought that blood platelets also contribute to the earlier (atherosclerotic) phase, since platelets have, apart from their indisputable role in haemostasis and thrombosis, proinflammatory, growth regulatory and endothelial activating properties. Thus, in all phases of atherothrombotic lesion progression blood platelets may play a specific role (table 1²).

Several tests exist to study platelet function in humans³. Many of these function tests have been used in retrospective cohort, case-control, and cross-sectional studies in humans to investigate the role of platelet activation in atherothrombosis⁴. However, the gold standard to evaluate which of these tests are associated with cardiovascular disease are prospective cohort studies measuring platelet activity at baseline and cardiovascular disease incidence at follow up⁴. We here summarize these studies. We included both cohorts of healthy subjects and of patients at (high) risk of (recurrent) cardiovascular complications.

Methods

Platelet function tests

Three aspects of platelet function can be tested: (i) their release of several substances; (ii) their ability to adhere or aggregate; and (iii) their capacity to change the antigenic repertoire of their cell membranes. Methods testing platelet function can also be divided into three categories, e.g.: (i) plasma or urinary measurement of platelet specific products; (ii) platelet aggregation; or (iii) fluorescence cytometry. In this article we have focused on studies on beta-thromboglobulin (BTG), platelet factor 4 (PF4), thromboxane A₂ metabolites and soluble P-selectin concentrations, optical-densitometric platelet aggregation tests, cartridge-based aggregation tests and fluorescence cytometry with any type of platelet antibody (table 2).

End points

In this systematic review we focused on clinical endpoints: acute coronary syndromes (e.g. myocardial infarction, angina pectoris, stent occlusion), peripheral arterial disease and ischaemic cerebrovascular disease (i.e., transient ischaemic attack or ischaemic stroke).

Cohorts

We included any type of prospectively followed cohort that had an estimate of platelet function at baseline and followed individuals for the occurrence of clinical endpoints as defined above.

Search strategy

We used electronic databases to identify relevant reports. After reviewing the Cochrane database on systematic reviews, our primary database was MEDLINE (January 1966 to October 2008). On specifying our search strategy it became clear that studies on platelet activity and clinically relevant outcomes have been differently labeled in various ways. Therefore, we eventually used three wide, sensitive sets of predefined search terms: (1) platelet function tests; (2) clinically relevant outcomes; (3) type of study design. Table 3 shows the final versions of these three search strategies. We did not restrict ourselves to any language. Furthermore, we tried to identify additional studies by searching the reference lists of relevant studies and reading editorials, reviews and letters on this topic. In addition, we used the option 'related articles' in MEDLINE and forward citation options of relevant articles to further increase the sensitivity of our search strategy.

Study selection and data extraction

To be included, studies had to meet the following inclusion criteria: (1) the study contained a description of one of the aforementioned methods to measure platelet activity; (2) the study contained a baseline description of the population studied; (3) the study reported data on occurrence of acute coronary syndromes, peripheral arterial disease and ischaemic cerebrovascular disease; (4) the study report was a full-text article. We used a prespecified data collection sheet to extract information regarding study population, design of the study, year of publication, sample size, duration of follow up, method(s) of assessment of platelet activity and type of cardiovascular event reported on.

In order to evaluate the quality of the different study reports we developed a scoring system adapted from the STROBE (STrengthening the Reporting of OBServational studies in Epidemiology) initiative checklist (table 4)⁵. We applied this scoring system to quantify the quality of each of the selected study reports..

Results

Article set (Tables 5, 6 and 7)

The initial search strategy revealed 837 articles. Of these, 69 were reports on prospectively followed cohorts concerning selected platelet function parameters. Because of the language we were not able to extract data from two articles, one written in the Serbian¹¹, and one in the Czech language⁵⁶, the latter cohort also reported on in another article⁵⁵. After studying the reference lists of the selected articles and with use of forward citation, we retrieved another 10 articles. Therefore, we studied 68 reports in total. Five of these articles described more or less the same results of two cohorts^{42-44;55;56}. Furthermore, two articles reported different results on one cohort^{31;32}. For this reason we mentioned the latter two articles separately in table 6. Together, all cohorts contained data on over 23000 persons with a median duration of follow-up of 1 day to 13.5 years.

38 of these 68 articles contained information on a cohort followed for up to 1 year, the remaining articles reported on cohorts followed more than one year (maximum median follow up time, 13.5 year). Most of these articles (61) contained information on the clinical end-point acute coronary syndromes, while a minority (also) contained data on peripheral arterial disease (5) or ischaemic cerebrovascular disease (14). Very few articles (8) reported on cohorts consisting of persons without manifest cardiovascular disease at baseline, while half of the articles (32) reported on cohorts of patients who underwent some form of percutaneous transluminal coronary angioplasty (PTCA). As expected, since it is one of the oldest techniques to study platelet function, the majority of articles (33 of 68) was dedicated to classical aggregometry, in frequency followed by articles (18 of 68) describing results of more recently developed cartridge-based platelet function tests.

There was a wide variety in the way the authors presented their results. Firstly, some handled platelet function as a dichotomous variable (active or not-active, the cut-off level being arbitrarily chosen by the authors), others divided outcome parameters into tertiles, quartiles or quintiles, and again others considered platelet activity as a continuous variable (e.g. percentage CD62P-positive platelets). Secondly, some authors provided only unadjusted estimates of cardiovascular risk in the presence or absence of platelet activation, while others provided adjusted estimates of this risk. Adjustments were made a variety of variables, including age, sex, other haemostatic variables, lipid levels, medication use and type of intervention. However, these adjustments varied widely between the different reports.

The quality score of all these reports on the cohort studies as assessed by our criteria ranged from 4.5 to 15 points (out of a maximum of 15 points). The majority (35 of 68) of the reports had a score of equal to or more than 10 points. A score of less than 10 points was mostly due to lacking of a precise description of the participant selection, a precise definition of clinical endpoints, a clear description of efforts to address potential sources of bias and a precise description of the 'flow' of participants during each stage of the study (table 4, items 2, 3, 4 and 7). In nearly all (55) studies, a point estimate of an effect was given together with a parameter of statistical significance. Half of the studies reported a p-value, half a 95-percent confidence interval, the latter being more easily interpretable than the former.

Release tests (Table 5)

Studies on the plasma or urinary concentrations of platelet release products (n=12) did not show an unequivocal relationship between 'increased platelet activity' and future occurrence of cardiovascular events. The point estimates ranged from 1 to about 5 times increased risk in the release test defined increased platelet activation patient groups. The majority of studies, however, did not show a statistically significant positive relationship (9 of 12).

Aggregation tests (Table 6)

Light transmission aggregometry (n=33)

Studies on the relationship between platelet activity as measured by classical aggregometry virtually all (27 of 33) pointed to the same conclusion: platelet activity was associated with future coronary events. The point estimates ranged from 1 to about 35 times increased risk in aggregometry-defined increased platelet activation

patient groups, depending on the definition of 'increased platelet activity'. The increased risk was found not only in the short term in people at high risk for a (recurrent) event shortly before or after an invasive procedure (eg, PTCA for myocardial infarction) but also in a cohort of healthy men followed for a average period of 13.5 years⁴⁶. This relationship was hardly investigated with respect to peripheral arterial disease and ischaemic cerebrovascular disease: in only two reports was peripheral arterial disease mentioned, and there was only one mentioning data on stroke separately.

Cartridge-based 'aggregometry'

We found cartridge-based tests to have only been evaluated in patients at high risk, foremost in the context of an acute coronary syndrome with or without PTCA (17 of 18). Duration of follow-up was between 1 day and 2.5 years. The point estimates ranged from 1 to about 12 times increased risk in the cartridge based test defined increased platelet activation patient groups. Virtually all studies with a follow-up period of less than one year (at least 9 out of 12) indicated a worse prognosis, which was statistically significant, in patients with increased platelet activity with or without single, dual or even triple antiplatelet therapy (cyclooxygenase inhibitors, purine receptor antagonists and (or) fibrinogen receptor antagonists). The studies evaluating the risk of cardiovascular events after one year all pointed to the same direction (a higher risk with a greater level of platelet activation): 4 of 6 studies showed a statistically significant relationship. These 4 studies showed a two- to fourfold increased risk in the increased platelet activation patient groups.

Fluorescence cytometry (Table 7)

Prospective data on the association between cardiovascular disease and some form of fluorescence cytometry-measured platelet activity virtually all (10 of 11) pointed to a greater risk in patients with higher levels of platelet activation. The point estimates ranged from 1.5 to about 90 times increased risk in the release-test-defined increased platelet activation patient groups. Again, not all point estimates of increased risk in these studies reached statistical significance (9 of 10 reached statistical significance). There were no data available on cohorts consisting of participants without manifest cardiovascular disease, nor on the outcome parameter peripheral arterial disease. Indeed, fluorescence cytometry appeared the least studied form of platelet function with respect to cardiovascular end points.

Additional analysis – restriction to high-quality reports

Our conclusions did not substantially change when we restricted our analysis to reports with a quality score between 10 and 15 points (34 out of 68 articles, data not shown). All high-quality studies on optical aggregometry and cartridge-based tests so defined pointed to the conclusion that platelet activation was statistically significantly associated with increased cardiovascular risk. In fact, the articles reporting a not statistically significant relationship all fell in the lesser-quality studies. The same held true for fluorescence cytometry-based studies, although there were few of these studies (score of 10 or more: 5 out of 11).

Discussion

This study has five main findings. Firstly, conventional platelet aggregometry is the most thoroughly studied platelet function test with respect to clinical relevant cardiovascular outcomes. Secondly, release-reaction-based platelet function assays are not reliably associated with cardiovascular outcomes. Thirdly, the relatively new cartridge-based platelet function assays and fluorescence cytometry have not been evaluated in cohorts of healthy volunteers. However, when tested in patients at high risk for developing recurrent clinically relevant cardiovascular disease, these tests are associated with clinical outcomes both in cohorts with short as well as with long-term follow-up. Fourthly, not much research has been done on platelet function as a risk determinant of clinically relevant cardiovascular outcomes in cohorts of healthy participants. This even applies to the most often studied clinical outcome, i.e. coronary artery disease. Finally, there is a wide variety in the way greater platelet activity is defined, clinical end-points are defined, and the baseline cardiovascular disease risk of the cohort participants.

The strength of our study lies in the systematic nature of the retrieving and reviewing process. We prespecified inclusion criteria and used a sensitive search strategy, and thus believe to have retrieved all relevant studies. When we specified our search strategy, it became clear that studies on platelet activity and clinically relevant outcomes have been labeled in various ways. This, of course, hampers complete retrieval of all relevant articles. Our strategy to reduce this bias was to use forward and backward citation and to carefully study the reference lists of all PUBMED-retrieved reports. We can, however, not completely rule out that we missed some relevant articles.

Next, we tried to quantify the quality of the reports of the selected studies with the help of the STROBE initiative checklist⁵. We by no means tried to qualify the studies themselves, but only to qualify the reports on these studies⁷⁴. Furthermore, we did not use this quality instrument – which has not been prospectively tested – to accept or reject studies, it only helped us in systematically judging the manuscripts.

Although, in general, we found that greater platelet activity was associated with adverse outcomes, the Caerphilly cohort deserves special attention^{48;51;75-80}. Not only is this the most extensively studied cohort of healthy people with regard to platelet aggregometry, it is also the study with one of the longest follow-up periods. In this cohort consisting of 2176 adults, there was, at 5 years of follow-up, no definite relationship between increased platelet susceptibility for agonists of aggregation and future acute coronary syndromes. Moreover, in 2000 persons followed for 10 years with platelets in the least susceptible quintile (i.e. with the least platelet activation), the incidence of stroke was in fact highest. In the quintiles 2 to 5 there was an increased incidence in stroke paralleling an greater platelet activity at baseline. The finding in the lowest quintile is perhaps contradictory to findings of others, and the authors explain this unexpected u-shaped result by allostasis⁸⁰. Allostasis, in this context, represents a chronic compensatory physiological platelet response to repetitive injury by dysfunctioning intima, in patients with advanced atherothrombotic disease. In this view there is an overrepresentation of patients with advanced intimal disease in the quintile with the lowest platelet activity. For that matter, in a cohort of patients suffering from peripheral arterial disease a similar relationship was found¹⁴. Whether these findings are contradictory to the results of other studies, is difficult to

judge: most studies regard platelet activity dichotomously, precluding the possibility of finding a U- or J-shaped association between platelet activity and cardiovascular disease.

The heterogeneity of the summarized studies was large from several points of view. Firstly, there was a large variety in baseline cardiovascular disease risk of the cohort participants. This was paralleled by a large variety in the use of platelet-inhibiting drugs. Indeed, several studies were designed to test whether so-called platelet inhibitor resistance occurs and has an implication for the prognosis of patients. However, this does not affect the direction of the association between platelet function and cardiovascular disease, it at the most diminishes the relationship between platelet activity and cardiovascular disease. On the contrary, even in the presence of platelet-inhibiting drugs, greater platelet activity was associated with cardiovascular outcome in most of these studies⁸¹⁻⁸³. Secondly, the end-points and their definitions differed between studies. Thirdly, the way in which platelet activity was defined differed greatly between studies (even when the same type of platelet function test was used). Notwithstanding the variability of the studies, we found that greater platelet activity, particularly measured by aggregometry or fluorescence cytometry, was associated with increased risk of future cardiovascular events. However, as a result of the variability described above we were not able to precisely quantify this increased risk.

The way in which the authors handled potential confounders also differed greatly between studies. In some studies, only crude estimates of an effect were given, in others adjustments were done for usual cardiovascular risk factors, medication use, and/or other variables reflecting haemostasis or endothelial function. In studies where correction for (some of these) risk factors was performed, the adjusted point estimates of the relationship between platelet activity and cardiovascular outcomes did not differ greatly from the nonadjusted point estimated. Indeed, to test the hypothesis that activation of blood platelets plays a role in the causative chain leading to atherosclerosis, correcting for these variables is not always appropriate. This is e.g. the case in the following putative chain of events: hypercholesterolaemia leads to platelet activation⁸⁴; activated platelets activate endothelial cells⁸⁵ and stimulate monocyte adhesion⁸⁶; phagocytosis of lipoproteins by monocytes eventually lead to lipid loaded foam cells in the vessel wall: the beginning of a fatty streak. In this model, increased blood platelet activity may not remain a risk factor independent of e.g. hypercholesterolaemia. Nevertheless, its role in the pathogenetic sequence in this model is evident.

In conclusion, although it is beyond reasonable doubt that platelet activation plays a central role in the pathogenesis of cardiovascular disease⁸⁷, the strength of this relationship is currently not very precisely known. By virtue of their relatively easy applicability, cartridge-based platelet function assays are good candidates to be of help in future large prospective cohort studies. Fluorescence cytometry is the other technique that is promising in this regard, for several reasons. Firstly, to date all published studies have been able to find a relationship between fluorescence-cytometry-measured platelet activation and clinically relevant cardiovascular disease. Secondly, this technique could be more fully automated than is now the case, making it more suitable for high-throughput cohort studies. Finally, this type of platelet function assay is able to simultaneously reveal several different modes of platelet activation pathways.

Research aimed at improving platelet activity measurement could free the way to large prospective studies to further quantify the extent to which type of platelet activity is causal in (the early phase of) atherothrombosis. This may further elucidate the role of platelets in this disease. With regard to the issue of residual platelet activity despite the use of platelet inhibitors, it needs to be studied whether platelet activity measurement-guided intensification of platelet inhibition (e.g. by combining different classes of platelet inhibitors (ref) or doubling the dosage frequency of single platelet inhibitors) improves clinically relevant patient outcomes. Finally, standardization of the definition of 'greater platelet activity' is needed⁴, to increase the comparability of different studies and to make these tests useful in a clinical setting.

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Table 1. Platelet involvement in atherothrombosis

<i>Early lesion</i>	activated platelets activate endothelial cells activated platelets adhere to activated endothelial cells endothelium induction of lipid and lipoprotein peroxidation propagation of an inflammatory and procoagulant response in endothelium increase of expression of adhesion molecules facilitation of adhesion of monocytes and platelets interaction of blood platelets with tissue macrophages contributes to foam cell formation circulating monocyte-platelet conjugates facilitate monocyte homing to the arterial wall
<i>Lesion progression and clinical events</i>	endothelial activation, erosion or non-occlusive plaque rupture triggers platelet adhesion, activation and aggregation and the formation of mural thrombi platelet incorporation into the plaque leads to rapid expansion of the mural lesion (intermittent claudication, stable angina pectoris) release of growth factors induces smooth muscle cell proliferation, migration and differentiation enhancement of adhesion and infiltration of leukocytes
<i>Thrombosis and clinical events</i>	occluding platelet-rich thrombi are formed upon rupture of a vulnerable plaque (myocardial infarction) embolization of platelet aggregates (ischaemic gangrene, ischaemic stroke) arterial spasms can be triggered by vasoconstrictors released from activated platelets (unstable angina pectoris) activated platelets contribute to a prothrombotic state

Adapted from reference 2.

Table 2. Platelet function tests

<i>test type</i>	<i>specimen</i>	<i>detects</i>
measurement of released products		
platelet factor 4	plasma	marker for the degranulation of alpha granules
Beta thromboglobulin	urine, plasma	marker for the degranulation of alpha granules
sCD62P	plasma	marker for the degranulation of alpha granules
thromboxane metabolites	urine, plasma	measurement of end products of thromboxane A2 degradation
platelet aggregation		
light transmission aggregometry	platelet rich plasma	low shear platelet aggregation in response to classic agonists (e.g. ADP, AA, collagen)
VerifyNow	whole blood	fully automated platelet aggregometer, mostly used to measure antiplatelet therapy
PFA100	whole blood	fully automated, high shear platelet aggregation and adhesion during formation of a platelet plug
fluorescence cytometry		
e.g. by different fluorescent-conjugated monoclonal antibodies	platelet rich plasma, whole blood	measurement of platelet glycoproteins and activation markers among others by fluorescent monoclonal antibodies, e.g. alpha granule degranulation (CD62P) lysosomal degranulation (CD63) flip-flop of membrane phospholipids (Annexin V) increased expression of fibrinogen, collagen and VWF receptor (CD42b) platelet aggregates platelet-monocyte aggregates activation of fibrinogen receptor (PAC-1)

sCD62P: P-selectin, ADP: adenosine diphosphate, AA: arachidonic acid, PFA: platelet function analyser

Table 3. Search strategy

Study type	"Epidemiologic Studies"[Mesh] OR "Sensitivity and Specificity"[Mesh] OR "Predictive Value of Tests"[Mesh]
Clinical endpoint	"Brain Ischemia"[Mesh] OR "Stroke"[Mesh] OR "Intracranial Arteriosclerosis"[Mesh] OR "Myocardial Ischemia"[Mesh] OR "Intermittent Claudication"[Mesh] OR ("intima media" AND (ultrasonography OR thickness)) OR "Intimal Medial Thickness"
Platelet function test	"Platelet Function Tests"[Mesh] OR "Blood Platelets"[Mesh] OR "Platelet Activation"[Mesh] OR "P-Selectin"[Mesh] OR "beta-Thromboglobulin"[Mesh] OR "Platelet Factor 4"[Mesh] OR "Flow Cytometry"[Mesh] OR "thromboxanes"[mesh] OR CD41[tw] OR CD42b[tw] OR CD62p[tw] OR CD63[tw] OR PAC-1[tw]

AND, OR: Boolean operators

Mesh: Medical subheadings

CD41: fibrinogen receptor, CD42b: Von Willebrand factor receptor, CD62p: P-selectin

CD63: glycoprotein 53, PAC-1: activated fibrinogen receptor

Table 4 Criteria for the quality assessment of the selected cohort studies.

<i>Item</i>	<i>Mark</i>	<i>Criteria</i>
Methods		
1 Setting	1	The setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection are described
2 Participants	1	The eligibility criteria, and the sources and methods of selection of participants are given. Methods of follow-up are described
3 Variables	3	(a) All exposures or predictors are clearly defined. (b) All potential confounders, and effect modifiers are clearly defined. (c) All outcomes are clearly defined. Diagnostic criteria, if applicable, are given.
4 Bias	1	Efforts to address potential sources of bias are described
5 Quantitative variables	1	The handling of quantitative variables in the analyses is explained
6 Statistical methods	4	(a) All statistical methods, including those used to control for confounding are described (b) Any methods used to examine subgroups and interactions are described (c) There is a description of how missing data were addressed (d) There is a description of how loss to follow-up was addressed
Results		
7 Participants	1	(a) Numbers of individuals at each stage of study are reported (b) Reasons for non-participation at each stage are given
8 Descriptive data	1	(a) Characteristics of study participants and information on exposures and potential confounders are given (b) Number of participants with missing data for each variable of interest is indicated (c) Follow-up time (e.g. average and total amount) is summarised
9 Outcome data	1	Numbers of outcome events or summary measures over time are reported
10 Main results	1	(a) Unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval) are given (b) When continuous variables were categorized category boundaries were reported

Adapted from the checklist accompanying the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) initiative (reference 5)

Despite the close relationship between a p-value and a 95% confidence interval, only reporting of the latter was sufficient to score 1 point in item 10

Table 5 see supplementary sheet

Table 6 see supplementary sheet

Table 7 see supplementary sheet

