Whole Blood Impedance Platelet Aggregometry and Ischemic Heart Disease
The Caerphilly Collaborative Heart Disease Study

Peter C. Elwood, Andrew D. Beswick, Dan S. Sharp, John W.G. Yarnell, Stephen Rogers, and Serge Renaud

The Caerphilly Collaborative Heart Disease Study is based on a large cohort of men who were ages 49 to 64 years at the time of the study. We report the results for platelet aggregation measured in whole blood from a subsample of 308 men. The index of sensitivity used was the minimum concentration of adenosine diphosphate that produced a defined degree of impedance change in the Chronolog 560 aggregometer. There was a marked association between aggregation and prevalent ischemic heart disease (IHD). The odds ratios and 95% confidence intervals (CI) for prevalent IHD in men with the most sensitive platelets compared with those with the least sensitive platelets were 3.6 (95% CI: 1.1 to 12.2) for angina; 7.3 (95% CI: 2.0 to 24.3) for previous myocardial infarction (MI); and 2.7 (95% CI: 1.0 to 7.6) for electrocardiogram evidence of ischemia. The confidence limits for these odds ratios are large because of the small sample size, but the estimates of odds ratio are relatively large compared to similar relationships between the traditional risk factors of serum cholesterol, blood pressure, smoking, and prevalent IHD (1.5 to 2.5). A number of factors that might confound the relationships between platelets and IHD were examined, but the associations remained statistically significant when these were taken into account. (Arteriosclerosis 10:1032–1036, November/December 1990)

Evidence is accumulating that platelets play a key role in ischemic heart disease (IHD). Case–control studies demonstrate positive relationships between increased platelet aggregation and IHD but of course suffer from the inferential dilemma of whether differences in platelet aggregation preceded the IHD event or were caused by it. Platelet aggregability is usually assessed by turbidimetric methods with the use of platelet-rich plasma (PRP) prepared by centrifugation and diluted to a standard platelet concentration. These preparatory procedures are likely to modify the subsequent behavior of the platelets, and in addition to this, red blood cells and other components of whole blood that may affect platelet behavior are removed and are not involved in the test. A more realistic procedure for public health screening would seem to be the testing of platelets in whole blood. In this article, we report the association between aggregation measured in whole blood by an impedance method and IHD in a population sample of older men.

Methods

In the Caerphilly Heart Disease Study, a major emphasis is put on tests of hemostasis, including the measurement of platelet aggregation to adenosine diphosphate (ADP) in PRP. Toward the end of the second phase of examinations of the men in this cohort, resources became available for the inclusion of a test of platelet aggregation in whole blood.

In all, ADP-induced aggregation in whole blood was measured in 308 men ages 49 to 66 years. Fasting venous blood samples were collected with minimal stasis into plastic syringes containing 0.13 M sodium citrate adjusted to pH 7.4 with citric acid (nine parts blood to one part citrate solution), and samples were kept sealed at room temperature for at least one-half hour before testing. Most of the tests were completed within 1.5 hours after venesection and all, within 2.5 hours.

A Chronolog 560 whole-blood platelet aggregometer (Chronolog Corporation, Haverton, PA) was used. This records the changes in the impedance of blood as platelet aggregates adhere to an electrode. The minimum concentration of ADP (Sigma, Poole, UK) that produced a change of 1.5 Ω in impedance within 2.5 minutes was found by serial testing with a range of doses of ADP in increments of 0.25 μmol/l, from 0.5 to 2.5 μmol/l, 1:1, blood/saline. Tests were done at 37°C. The presence or absence of an impedance response at any given dose of ADP could be identified within 2.5 minutes. Therefore, this time was chosen as a balance between a sufficient period to elicit an impedance response and the logistical demands of completing multiple tests on a large number of subjects. The criterion of a 1.5 Ω response was chosen by noting a bimodal distribution of the impedance response at 2.5 minutes by use of an ADP dose of 1.0 μmol/l in the first 50 men. This was the cut point of impedance change that best separated the two modal groups.
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Of 13 subjects who had whole-blood platelet aggregation impedance measurements made on two separate occasions up to 2 weeks apart, 11 had identical ADP threshold doses, and two subjects had threshold doses differing by only one dose. Platelet impedance response tracings in whole blood were stable between 30 minutes and 5 hours after venipuncture. This was demonstrated by identical titration curves over an extensive range of ADP doses. However, stored blood must not be exposed to the atmosphere because pH changes leading to variation in platelet "aggregation" responses may occur.4

This multiple testing procedure identified a "threshold" concentration and in what follows we subdivide by this measure. Men whose platelets responded to 0.75 μmol/l of ADP or less are described as having the "most sensitive" platelets. Those who responded only at 1.5 μmol/l ADP or greater are referred to as those with "least sensitive" platelets. The remaining subjects are described as having platelets of "medium sensitivity."

The extent of primary platelet aggregation in PRP (platelet count adjusted to 300x10⁹/l) induced by a single dose of ADP (0.725 μmol/l) was also measured for each subject with the turbidimetric method as originally described by Born5 and O'Brien6 and modified by Renaud et al.7 Platelet aggregation in PRP was measured as the percentage of optical density difference between PRP and platelet-poor plasma. These PRP measurements were part of a much larger study of the entire Caerphilly cohort of over 2000 men and are the topic of another publication.8

Men were grouped by three prevalent outcomes of IHD based on World Health Organization criteria: angina, past myocardial infarction, and electrocardiogram evidence of IHD. We have defined these elsewhere, and we showed that the prevalence rates for the various manifestations of IHD are similar to those reported for other large population studies.9

The relationships between the IHD and ordered categories of the platelet sensitivity in whole blood were assessed using multiple logistic regression models. The odds of the disease in men with high platelet sensitivity relative to men with low sensitivity were used as measures of association. Before these analyses were done, however, the effects of medications and of smoking were examined.

Results

Among the 308 men evaluated by whole-blood platelet aggregation, 52 were taking medications known to affect platelet function (aspirin, etc.). Table 1 confirms an association between platelet sensitivity and medication usage. Among men with platelets of low sensitivity, there was the highest proportion on such medications. Unfortunately, there were too few men on medication to conduct a separate analysis on this subgroup. These 52 men were therefore omitted from all further analyses.

Table 2 shows that platelet reactivity appears to be similar in smokers, exsmokers, and nonsmokers. However, among men who smoke, there is evidence demonstrating a gradient of an increasing proportion of men having high sensitivity with smoking more proximate to venipuncture (30%, 10%, 3%), consistent with other evidence of an effect of proximate smoking on aggregation in PRP.10

Further analyses were based on men who were not taking medication affecting platelet aggregation and for whom we have complete data on all the covariates mentioned later. This reduced the number of men to 244. Of these, 24 had evidence of angina, 19 had had a myocardial infarction, and 34 had electrocardiographic evidence of myocardial ischemia. These categories are not mutually exclusive, and 53 men (22%) showed some manifestation of IHD. Table 3 shows that within the groups of men with each manifestation of IHD, a much higher proportion had highly sensitive platelets than did those without evidence of IHD.

Another way of summarizing the data in Table 3 is in terms of the odds ratios of IHD within various subgroups defined by platelet sensitivity to ADP. This was done by using multiple logistic regression models and dummy coding the three categories of platelet sensitivity. The odds ratio of men with the most sensitive platelets to men with the least sensitive are:

- For angina: 3.6 (95% CI: 1.1 to 12.2)
- For myocardial infarction: 7.3 (95% CI: 2.0 to 26.3)
- For ECG ischemia: 2.7 (95% CI: 1.0 to 7.6)
- For any IHD: 3.5 (95% CI: 1.5 to 8.6)

These analyses take into account both smoking status and, among smokers, the time since the last cigarette. This was done by using dummy-coded variables reflecting the categories described in Table 2 and by including these variables as covariates in the logistic regression models.

A wide variety of possible hematological determinants and correlates of aggregation were examined, and a series of multiple regression models were examined to test the effect of all possible combinations of the covariates. Strong and consistent relationships were noted for several possible determinants, namely, primary ADP aggregation of PRP, platelet count, and red blood cell count. Marginal relationships were also shown with fibrinogen and with plasma viscosity (Table 4). Other than for primary aggregation to ADP in PRP, the inclusion of none of these made any appreciable difference to the relationships between whole-blood platelet aggregation and IHD.

In the present data set, all the relationships with possible determinants and correlates (Table 4) are biased low.11 This is because, in the estimation of the
Table 2. Relationships between Smoking Status and Platelet Sensitivity in Whole Blood and (in Smokers Only) between Platelet Sensitivity and Smoking Immediately before Venesection

<table>
<thead>
<tr>
<th>Sensitivity to ADP</th>
<th>Smoking status</th>
<th>Minutes since last cigarette</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsmoker</td>
<td>Exsmoker</td>
</tr>
<tr>
<td>Low</td>
<td>29 (57%)</td>
<td>35 (42%)</td>
</tr>
<tr>
<td>Medium</td>
<td>16 (43%)</td>
<td>34 (41%)</td>
</tr>
<tr>
<td>High</td>
<td>6 (19%)</td>
<td>14 (17%)</td>
</tr>
<tr>
<td>Totals</td>
<td>51 (100%)</td>
<td>83 (100%)</td>
</tr>
</tbody>
</table>

*2 = 3.81; df = 4

p = 0.43

Table 3. Numbers of Men with Various Manifestations of Ischemic Heart Disease Who Had Three Levels of Sensitivity of Platelets in Whole Blood to Adenosine Diphosphate

<table>
<thead>
<tr>
<th>Sensitivity to ADP</th>
<th>Angina</th>
<th>Myocardial infarction</th>
<th>EKG ischemia</th>
<th>Any ischemic heart disease</th>
<th>All men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>9 (7%)</td>
<td>6 (5%)</td>
<td>16 (13%)</td>
<td>23 (19%)</td>
<td>124 (100%)</td>
</tr>
<tr>
<td>Medium</td>
<td>9 (10%)</td>
<td>6 (7%)</td>
<td>10 (11%)</td>
<td>17 (19%)</td>
<td>88 (100%)</td>
</tr>
<tr>
<td>High</td>
<td>6 (19%)</td>
<td>7 (22%)</td>
<td>8 (25%)</td>
<td>13 (41%)</td>
<td>32 (100%)</td>
</tr>
</tbody>
</table>

χ² for trend 3.32 7.62 1.59 4.62

p = 0.07 p = 0.006 p = 0.21 p = 0.03

ADP = adenosine diphosphate, EKG = electrocardiogram.

Table 4. Correlations Between Whole Blood Sensitivity to Adenosine Diphosphate and Other Factors Relevant to Thrombosis

<table>
<thead>
<tr>
<th>Aggregation in whole blood</th>
<th>Correlation coefficients</th>
<th>p level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet-rich plasma aggregation</td>
<td>0.217</td>
<td>0.002</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.269</td>
<td>0.001</td>
</tr>
<tr>
<td>Red blood cell count</td>
<td>–0.138</td>
<td>0.049</td>
</tr>
<tr>
<td>Plasma fibrinogen</td>
<td>0.154</td>
<td>0.027</td>
</tr>
<tr>
<td>Plasma viscosity</td>
<td>0.134</td>
<td>0.056</td>
</tr>
</tbody>
</table>

Correlations were calculated so that increasing sensitivity to adenosine diphosphate in whole blood reflects increasing degrees of impedance change associated with adherence of platelet aggregates onto the electrode. Because of truncation of the distribution, 37 men with low sensitivities were excluded. Those with high sensitivities were allotted an arbitrary threshold concentration of 0.5 μM/mL.

sensitivity of platelets to ADP in whole blood, we used a relatively small number of dilutions of ADP. This inevitably led to considerable censoring of the distribution of sensitivities at both ends of the distribution, but especially at low sensitivities.

The interrelations of whole blood aggregation, aggregation in PRP, and IHD are of particular interest. The two platelet aggregation tests are correlated (r = 0.22), and the odds ratio for a history of myocardial infarction in the group with high whole-blood platelet sensitivity compared to those with low sensitivities decreased from 7.3 to 5.6 when PRP aggregation was included in the regression but remained statistically significant (p < 0.01). Inclusion of PRP aggregation in the statistical model did not appear to demonstrate any association with myocardial infarction that was not already taken into account by whole-blood platelet aggregation. This might suggest that the two measures of aggregation reflect similar biological mechanisms.

Discussion

There is growing evidence of the involvement of platelets in IHD and, in particular, in myocardial infarction. Thus, most subjects who have evidence of myocardial infarction at autopsy have an occlusive platelet thrombus in a coronary artery. Almost all patients with IHD who die suddenly have either an occlusive thrombus or a fissured arterial plaque and associated thrombosis. Angiography shortly after infarction has demonstrated coronary artery occlusion in a high proportion of patients, and studies of men shortly after infarction have shown enhanced platelet activity. Platelets may also play a key role in sudden death, either by the obstruction of microcoronary vessels with aggregates or by vasoconstriction induced by thromboxane A₂ released by platelets. The protective effect of aspirin against myocardial infarction provides further evidence supportive of a major role of platelets in infarction.
The present study is part of a larger prospective study of platelets and incident IHD, but it is of particular interest because it appears to yield the first evidence of an association between platelet aggregation in whole blood and IHD. Although the numbers were small, the magnitude of associations was large, particularly in subjects with a past history of myocardial infarction. Furthermore, the associations were robust, in that they remained significant after the effects of a range of confounding factors were allowed for, and they appeared to be largely independent of other hemostatic factors, fibrinogen and viscosity, which are strongly predictive of myocardial infarction.\(^{20}\) In fact, the two factors that were found to be of greatest relevance to platelet aggregation in whole blood (Table 4) were both measures of some aspect of platelets (PRP aggregation and platelet count).

Our data are, of course, subject to the limitation of all case–control studies in that they are based on prevalent disease. Incident IHD data will become available in due course, but already there is evidence that suggests that changes in platelets and megakaryocytes precede infarction.\(^{21}\) These relate to the morphological characteristics of platelets and megakaryocytes and especially to the relationships between ploidy number, size, density, and aggregation activity.

The inverse relationship between red blood cell count and platelet sensitivity in whole blood (Table 4) is perhaps unexpected.\(^{22,23}\) This finding may be due to an artifact related to the varying effects of citrate concentration on calcium concentration brought about by variation in hematocrit between subjects. However, there are other suggestions that the presence of red blood cells does act to inhibit platelet aggregation,\(^ {24}\) possibly by the action of ATP-ase and other nucleotidase activity within red blood cells.\(^ {25}\) The nature of this relationship is more fully examined and discussed in another publication.\(^ {25}\)

As to the relative merits of platelet tests based upon whole blood and tests on PRP, this must be assessed in the context of the testing of whole blood, without tests on PRP, this must be assessed on the basis of context. The testing of whole blood, without adjustment of platelet count and without the removal of other blood constituents, is closer to in vivo conditions than are tests involving the separation of platelets. Thus, the best evaluation of the value of tests on whole blood is in the context of public health utility as a predictor of IHD. Our current studies will yield such information as incident data become available.

The value of tests in PRP in which other cellular components are removed and adjustments are made either for platelet count or refractive indices related to surface area\(^ {27}\) should be judged more in the context of assessment of mechanisms of action and individual clinical characterization. Thus, we would suggest that tests of whole blood would better lend themselves to public health screening activities, while tests of PRP platelet aggregation may be better clinical and research tools to discriminate between various mechanisms impacting on aggregation.

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