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# Prevalence of aspirin resistance measured by PFA-100

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**Abstract**

*Background:* Aspirin protects from cardiovascular events. However, a number of patients who take this drug suffer events, probably due to aspirin resistance. Our objective was to determine the prevalence of aspirin resistance in patients taking this drug and to test if resistance is related to different variables.
*Methods:* Platelet function was studied in 113 patients (90 men) aged 63±9 (80 with stable ischaemic heart disease) who took aspirin (100 to 300 mg/day). By a platelet function analyzer, called PFA-100, the epinephrine closure time was studied. We also analysed the possible relationship between epinephrine closure time and the following variables: total cholesterol, LDL, HDL cholesterol, total/HDL cholesterol, triglycerides, lipoprotein(a), and C reactive protein. The possible association between aspirin resistance (epinephrine closure time <161 s) and different variables was also analyzed with the SPSS statistical package. Results are expressed in median (interquartile range).
*Results:* Aspirin resistance was found in 32% of cases. Ischaemic heart disease, smoking habit, and treatment with statins were associated with a significantly greater percent of resistance ($p$=0.049, 0.009, and 0.043, respectively). Patients with aspirin resistance had higher levels of total/HDL cholesterol: 4.46 (3.76–5.55) vs. 3.97 (3.20–4.75) ($p$=0.023); and lipoprotein(a): 57.2 (24.8–85.0) mg/dl vs. 13.1 (3.7–38.0) mg/dl ($p$=0.007).
*Conclusions:* Aspirin resistance is frequent and easily detected by PFA-100. It occurs more frequently in smokers. A mild association is found with ischaemic heart disease, some lipids, and treatment with statins. Our results support the applicability of this method to clinical practice.


*Keywords:* Aspirin; Platelet function analyzer (PFA-100); Epinephrine closure time

## 1. Introduction

Standard dose aspirin has well-known cardioprotective properties. However, despite strict adherence to a standard aspirin regimen, many patients continue to suffer cardiac ischaemic events. A recent metaanalysis [1] on this drug shows a 23% risk reduction of future cardiovascular events (nonfatal myocardial infarction, nonfatal stroke, or vascular death) after 2 years of treatment. Therefore, not every patient is protected. In addition, patients who undergo acute coronary syndrome while taking aspirin have a worse outcome than those who are not taking it [2].

There are different reasons why aspirin may not be totally effective in cardiovascular event prevention. One is the possible resistance to its antiplatelet effect. The term "aspirin resistance" has been used for years, but there is a lack of uniformity in its definition, which may be clinical or biochemical. In addition, different biochemical tests have been used for its detection. Although none of them is still universally validated, several studies [3–6] performed with different methods show a worse prognosis in patients with, than in those without, analytical aspirin resistance. In the last few years, an alternative method called PFA-100 (platelet function analyser) became available for the study of platelet function [7]. This method evaluates platelet adhesion/aggregation, and it is being ever more frequently used in clinical practice to detect haemostatic alterations. It

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is easy to handle and can identify aspirin-resistant patients [8,9]. In fact, it has been found to be more sensitive than optical platelet aggregation in the detection of aspirin resistance [9]. The aim of this cross-sectional study is to determine the prevalence of aspirin resistance with PFA-100 and to test whether resistance is related to different variables studied.

## 2. Materials and methods

*Population included*: 113 consecutive patients (90 men) who were taking aspirin (100 to 300 mg/day) for at least 1 week were included. They were being seen as outpatients in our hospital.

*Population excluded*: Patients with a platelet count $<150\times10^3/\mu l$ or $>450\times10^3/\mu l$ or a hematocrit lower than 28%. Those taking any other antiplatelet or anticoagulant or antiinflammatory drug, as well as those with any blood disease.

*Ethics*: The study complies with de Declaration of Helsinky, the locally appointed ethics committee approved the research protocol, and informed consent was obtained from the patients.

*Blood samples* for all the analyses were taken in the morning after an overnight fast.

*Platelet function tests*: They were performed with whole blood using PFA-100 (Dade Behring; [7]). It is a semi-automatic analyser of platelet function that reproduces in vivo conditions. For the analysis, citrated blood is passed through a capillary device that simulates in vivo conditions of shear stress. PFA-100 gives a punctual lecture when blood flow stops, because the capillary is occluded, due to platelet adhesion and subsequent aggregation to the exposure of platelet agonists that cover the membrane of a disposable cartridge. The final point in which blood flow stops is the closure time. Two different cartridges are used; both of them utilise a membrane which is covered with a layer of fibrillar type I collagen; in one of the cartridges, the membrane is also covered with epinephrine and in the other with adenosine diphosphate. Platelet function is altered when both epinephrine and adenosine closure times are prolonged. Aspirin only prolongs the epinephrine closure time [7]. In theory, the maximum closure time provided by PFA-100 is 300 s. In practice, any value >250 s may be considered as maximally prolonged (equivalent to non-occlusion; [7]). In our laboratory, the normal epinephrine closure time ranges between 95 and 160 s. It is prolonged from 161 s on. The calculated interassay coefficient of variation is 0.6% (0.0–1.1).

The platelet function tests were performed after at least 7 days of taking aspirin and within 24 h of the latest dose.

*Variables studied*: Apart from epinephrine closure time, the following variables were analysed: age, gender, body mass index, aspirin dose/$m^2$, presence of ischaemic heart disease, diabetes mellitus, smoking habit, total cholesterol, LDL and HDL cholesterol, total/HDL cholesterol ratio, and triglycerides. For technical problems, lipoprotein(a) was studied in only 48 cases and high-sensitivity C reactive protein in 106. Additional treatment with statins, angiotensin converting enzyme inhibitors, beta-blocking drugs, and nitrates was also analysed.

*Aspirin resistance* was defined as an epinephrine closure time <161 s.

*Statistical analysis*: By means of the Kolmoronov–Smirnov test, the distribution of the variables was checked. When it was asymmetrical, the results were expressed as median (interquartile ranges: $Q_1$–$Q_3$) instead of mean± standard deviation, and nonparametrical tests were used. The Mann Whitney's *U*-test was used to check a possible association between aspirin resistance and the quantitative variables. Differences in percentages between groups were compared with the exact test (PEPI statistical package by JH Abramson and PM Gahlinger, 1993–2000). The lineal association test was used instead of the exact test for ordinal variables. A two-tailed *p* value inferior to 0.05 was considered significant.

## 3. Results

### 3.1. Patient's characteristics

The age of the patients ranged between 36 and 84 (63±9) years. The reasons for taking aspirin were stable ischaemic heart disease in 80, hypertension in 22, previous atrial fibrillation in 6, stroke in 1, diabetes mellitus in 1, and because they wanted to in 3. Thirty-six of the 113 patients had diabetes, 14 were current smokers, and 57 exsmokers (they stopped smoking at least 1 year before the study).

### 3.2. Epinephrine closure time

In the total group, it ranged between 87 and 300 s: the median was 275 s ($Q_1$–$Q_3$=145–300). Thirty-six patients (32%) had aspirin resistance (epinephrine closure time <161 s), while 77 (68%) were aspirin-sensitive. Only two patients had an epinephrine closure time below 95 s.

### 3.3. Relationship between aspirin resistance and different variables

Table 1 shows the absence of a significant relationship between aspirin resistance and age, body mass index, or

Table 1
Relationships between aspirin resistance and quantitative variables

| | Resistance | No resistance | *p* |
|---|---|---|---|
| Age (years) | 62±8 | 63±10 | 0.224 |
| BMI (kg/$m^2$) | 28 (26–31) | 28 (25–31) | 0.744 |
| Aspirin (dose/$m^2$) | 74 (53–83) | 73 (58–91) | 0.360 |

BMI: body mass index. Numbers in brackets indicate interquartile ranges.