High Frequency of Aspirin Resistance in Patients with Acute Coronary Syndrome

GULTEKIN F. HOBIKOGLU, TUGRUL NORGAZ, HUSEYIN AKSU, ORHAN OZER, MEHMET ERTURK, ZEKERIYA NURKALEM and AHMET NARIN

Siyami Ersek Thoracic and Cardiovascular Surgery Center, Cardiology Department, Istanbul, Turkey

Aspirin has an important role in the secondary prevention of cardiovascular disease and lowers the rate of death, myocardial infarction (MI) and stroke by 25% (Antiplatelet Trialists’ Collaboration 1994). Accordingly, most of the patients with coronary artery disease (CAD) use aspirin. But the effect of aspirin is not standard in all patients and different studies report that 5.5 - 45% of patients are resistant to aspirin (Helgason et al. 1994; Gum et al. 2001). It has been shown...
that the patients who have acute coronary syndrome (ACS) while taking aspirin have worse outcomes (Alexander et al. 1999). Because of this, using aspirin for last 7 days is accepted as one of the high risk indicators in risk stratification of ACS. There was no enough study to evaluate effects of aspirin resistance in this patients group. This study was planned to investigate the frequency of aspirin resistance in this group of patients and to compare with that of stable CAD patients. We also aimed to evaluate the relationship between aspirin resistance and angiographic severity of CAD in patients with ACS.

MATERIALS AND METHODS

The present study included 104 patients (75 male, 60.4 ± 10.8 years) who were hospitalized because of ACS between November 2002 and October 2003 while using aspirin for at least last 7 days. The diagnosis of ACS was based on presence of at least two of three features: angina pectoris (new onset within 1 month, or onset at rest crescendo pattern), electro-cardiogram (ECG) findings (new ST segment depression or T wave changes), and release of cardiac markers including cardiac troponin T (cTnT) or creatine kinase-myocardial band (CK-MB). The patients who had elevated cTnT levels were considered as non-ST elevation myocardial infarction (NSTEMI). The control group included 100 patients (73 men, 57.6 ± 10.6 years) with diagnosis of stable CAD, which was documented by coronary angiography, history of revascularization or MI and the use of aspirin for at least last 7 days. None of them had MI or ACS for at least last 3 months.

The study was reviewed and approved by the hospital ethic committee. All participants gave informed consent before enrollment. Exclusion criteria were; known bleeding disorders, use of ticlopidin, clopidogrel, dipyridamol and non-steroid anti-inflammatory drugs, platelet count < 150,000 /μl and > 450,000 /μl and/or hemoglobin < 8 g/100 ml. Cardiovascular risk factors were identified patient interviews. The risk factors included current smoking, hypertension (HT) (on antihypertensive drugs or with known and untreated hypertension), diabetes mellitus (DM) (the use of insulin or oral hypoglycemic agent), a family history of premature coronary heart disease and hypercholesterolemia (the use of cholesterol lowering drugs, or a plasma low density lipoprotein cholesterol of > 130 mg/100 ml or total cholesterol of > 200 mg/100 ml).

Fasting blood samples were taken into 7.5% EDTA and hematological parameters (hemoglobin, mean platelet volume, platelet, red and white blood cell counts) were measured by using an autoanalyzer (Gen-S, Coulter corp., Miami, FL, USA). Serum values of urea, creatinine and glucose were also determined.

Coronary angiography

Selective coronary angiography was performed by the femoral approach using Judkin’s technique. Multiple views were obtained in all patients by using cineangiographic equipment (Philips Integris H 3000, Netherlands). Coronary angiograms were scored according to three techniques:

Vessel score. This was the number of vessels with a significant stenosis (50% or greater reduction in lumen diameter). Degree of stenosis was defined as the greatest percentage reduction of luminal diameter in any view compared with the nearest normal segment and was determined visually. Scores ranged from 0 to 3, depending on the vessels involved. Left main artery stenosis was scored as single vessel disease.

Severity score. This was the Gensini score, which had been described previously (Gensini 1975). Briefly, coronary arterial tree was divided into segments with multiplying factors according to the functional importance of any given segment and the percent of reduction in lumen diameter of each narrowing was assigned a score. The sum of scores of all segments gives the Gensini score, this score places emphasis on the severity of the disease.

Negri extent score. Any segment with evidence of atherosclerosis (intimal irregularities to severe stenosis) receives 1 point for left main, whole left anterior descending coronary artery, proximal parts of circumflex and right coronary artery and 0.5 point for the other segments. The sum of score gives indices of CAD extent (Negri et al. 1993).

The scores were determined by one of the investigators (T.N. and G.H.) without the knowledge of the aspirin resistance.

Detection of aspirin resistance. Blood samples were obtained with 18-gouge needle by venipuncture and drawn into 4.5 ml vacutainer tubes anticoagulated with 3.8% sodium citrate. Platelet function analyses were performed within the next 2 hours. Aspirin effect on platelet function was assessed with the PFA-100 (Dade Behring W., Sacramento, CA, USA),
which simulates primary homeostasis at injured blood vessel. The PFA-100 uses disposable test cartridges that have collagen coated membrane infused with either ADP or epinephrine. The analyzer aspirates whole blood at high shear rate through the capillary where it comes into contact with the membrane, where platelets adhere to the membrane surface and aggregate. A platelet plug forms with occlusion of the aperture and cessation of blood flow. The closure time (CT) reflects platelet function in the sample evaluated.

With the PFA-100, dysfunction of platelets caused by aspirin can be detected as a prolonged closure time with collagen/epinephrine cartridge but normal with collagen/adenosine diphosphate (ADP) cartridge (< 114 sec) (Kundu et al. 1996). Aspirin resistance by PFA-100 was defined as having a normal collagen/epinephrine CT (< 170 sec) despite aspirin treatment.

Statistics
Continuous variables were presented as mean ± standard deviation. Categorical variables were presented as frequencies and percentages. The unpaired t-test for continuous variables and chi-square test for categorical variables were performed to compare patients groups and ACS patients with and without aspirin resistance. The SPSS 10.0 for Windows was utilized for the entire statistical work-up. A p value of < 0.05 was considered to be statistically significant.

RESULTS
Demographic variables of ACS and stable CAD groups are summarized in Table 1. There were no statistically significant differences between ACS and stable CAD groups by means of sex, age, tobacco use, and presence of DM and HT (p > 0.05).

Aspirin resistance was present in 27 of 100 (27%) patients with stable CAD and 42 of 104 (40.3%) patients with ACS (Fig. 1). The difference was statistically significant (p = 0.04). Among the patients with ACS, patients with aspirin resistance were older (58.2 ± 10.1 vs 63.6 ± 11.1, p = 0.015) than the patients without aspirin resistance (Table 2). But there were no statistically significant differences for sex, presence of DM, HT, hyperlipidemia, family history of CAD and tobacco use (p > 0.05). During admission the blood levels of urea (29.6 ± 6.4 mg/100 ml vs 31.1 ± 7.1 mg/100 ml) and creatinine (1.2 ± 0.3 vs 1.3 ± 0.5 mg/100 ml) were not different between patients with and without aspirin resistance (p > 0.05).

ACS group includes both unstable angina patients (patients who have rest angina and ST segment depression on ECG but no cardiac enzyme elevation) and NSTEMI patients (patients who have rest angina, ST segment depression on ECG and also cardiac enzyme elevation). According to this, among patients in the ACS group, the frequency of NSTEMI was 52% (22 of 42) in patients with aspirin resistance and 35% (22 of 62) in patients without aspirin resistance. Patients with aspirin resistance tend to have more NSTEMI but this difference was not statistically significant (p = 0.08). Also the degree of myocardial damage was higher in patients with aspirin resistance according to cTnT values (1.06 ± 1.2

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>Stable CAD (n = 100)</th>
<th>ACS (n = 104)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.6 ± 10.6</td>
<td>60.4 ± 10.8</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, n (% women)</td>
<td>27 (27)</td>
<td>29 (28)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>44 (44)</td>
<td>42 (40)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>45 (45)</td>
<td>56 (54)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>25 (25)</td>
<td>36 (35)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>35 (35)</td>
<td>39 (37)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>27 (27)</td>
<td>32 (31)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.
Eighty-three (80%) patients with ACS had undergone coronary angiography in the ACS group (33 had aspirin resistance and 50 had no aspirin resistance) (Table 3). The number of vessels with disease (2.3 ± 0.8 vs 2.1 ± 0.9, \( p = 0.4 \)), the Gensini score which emphasizes severity of CAD (58.8 ± 29.6 vs 55.9 ± 40.2, \( p = 0.7 \)) and the Negri score which emphasizes the extent of CAD (5.9 ± 2.2 vs 6.1 ± 3.1, \( p = 0.8 \)), were not statistically different between patients with and without aspirin resistance (Table 3).

**DISCUSSION**

It is known that prognosis is worse in patients who develop ACS while using aspirin but the frequency of aspirin resistance and relationship to the prognosis have not been evaluated in this subgroup. We have demonstrated that the frequency of aspirin resistance was higher in patients who developed ACS when using aspirin compared to patients taking aspirin for stable
CAD. This may be the reason for recurrent vascular thrombotic events, since the central role of platelet activation and aggregation in the pathophysiology of ACS and MI has been well documented (Fitzgerald et al. 1986; Mizuno et al. 1992). Two studies have directly linked the presence of aspirin resistance and adverse clinical outcomes. Gum et al. (2003) have followed up stable CAD patients who had aspirin resistance for two years and reported a four fold increased risk of death and MI. In a subgroup analysis of Heart Outcomes Prevention Evaluation (HOPE) study (Eikelboom et al. 2002), urine metabolite of thromboxan A$_2$, 11-dehydro thromboxan B2 (DHTB$_2$) which is a marker of effectiveness of aspirin, was measured in patients who took aspirin, and the 5-year follow-up showed that the patients at the upper quartile for urine DHTB$_2$ levels had a two fold increased risk for MI and 3.5 fold increased risk for cardiovascular death when compared with patients who had DHTB$_2$ levels at the lowest quartile.

If patients who have recurrent events despite aspirin are resistant to the antiplatelet effects of aspirin, they may derive particular benefit from the addition of other potent and effective antiplatelet or antithrombin therapies. A suggestion of such a preferential effect has been reported in the large trials, such as the Platelet IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin Theraphy (PURSUIT) trial of the glycoprotein IIb/IIIa inhibitor tirofiban. In these trials, prior aspirin users had a worse outcome than patients not using aspirin prior to presentation.

It has been reported that the response of platelet reactivity to exercise is increased in patients with CAD but not in patients with normal coronary arteries (Lanza et al. 2003). However the relationship between responsiveness of platelets for aspirin and severity of CAD has not been previously evaluated. We have found no differences for angiographic severity and extent of CAD among patients with or without aspirin resistance.

In our study, the ACS patients with aspirin resistance were older. Some investigator also reported that aspirin resistance was associated with advanced age (Spranger et al. 1989; Gum et al. 2001) but others did not support this relationship (Sane et al. 2002).

Many possible mechanisms for development of aspirin resistance have been proposed. These include insufficient dosing (Helgason et al. 1994), polymorphism of glycoprotein IIIa (Undas et al. 1999; Macchi et al. 2003), production of prostaglandin H$_2$ in monocyte and endothelial cells by COX-2 pathway (Cipololone et al. 1997), increased levels of COX-2 as a result of increased platelet degradation (Weber et al. 1999), and increased sensitivity of platelets to ADP (Macchi et al. 2002).

**Limitations of the study**

The appropriate method for detecting aspirin resistance is still controversial and the absolute definition is not available. We detected aspirin resistance with PFA-100 and did not use aggregom-

### Table 3. Scores of angiographic severity and extent of CAD in patients with ACS

<table>
<thead>
<tr>
<th></th>
<th>AR (+) (n = 33)</th>
<th>AR (-) (n = 50)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel score</td>
<td>2.3 ± 0.9</td>
<td>2.1 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Gensini score</td>
<td>58.8 ± 29.6</td>
<td>55.9 ± 40.2</td>
<td>NS</td>
</tr>
<tr>
<td>Extent score</td>
<td>5.9 ± 2.2</td>
<td>6.1 ± 3.1</td>
<td>NS</td>
</tr>
</tbody>
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AR, aspirin resistance; NS, not significant.
eter. Aggregometer uses platelet rich plasma but PFA-100 utilizes whole blood and simulates primary homeostasis which is a more physiologic approach to evaluate the platelet function. We have recruited patients according to their history of aspirin usage and did not measure the blood salicylate levels.

**CONCLUSIONS**

The frequency of aspirin resistance in patients who develop ACS while using aspirin is higher in patients who use aspirin for the stable CAD. The ACS patients with aspirin resistance are older, but angiographic extent of CAD was similar when compared with ACS patients who had no aspirin resistance. Follow up studies are needed to reach a conclusion about aspirin resistance and recurrent events in patients with ACS.

**References**


