Platelet reactivity and endothelial function in children of patients with early acute myocardial infarction

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Aims
To assess whether platelet reactivity is increased in offspring of patients with early acute myocardial infarction (AMI) and its possible relation with endothelial dysfunction.

Methods and results
We studied 23 healthy children (15 ± 3 years, 13 males) of patients with early AMI (≤ 50 years old; Group 1) and 21 healthy children of healthy subjects without any history of cardiovascular disease (14 ± 3 years, 10 males; Group 2). Platelet reactivity was assessed by flow cytometry as the increase in monocyte-platelet aggregates (MPA) and CD41 and PAC-1 platelet expression in response to exercise stress test (EST), adenosine diphosphate (ADP) stimulation ($10^{-7} M$), or both. Endothelial function was assessed by measuring brachial artery dilation during post-ischaemic forearm hyperaemia [flow-mediated dilation (FMD)]. Both EST and ADP induced a higher percentage increase in platelet receptor expression in Group 1, compared with Group 2, with the most significant difference being shown for the response to the combined stimuli (e.g. MPA, 23.1 ± 12 vs. 5.63 ± 8%, $P = 0.001$; platelet PAC-1, 57.7 ± 47 vs. 13.2 ± 7%, $P = 0.001$). Compared with Group 2, Group 1 children showed lower FMD (10.7 ± 3.1 vs. 8.0 ± 2.9%, respectively; $P = 0.007$). However, no significant association was found between FMD and platelet reactivity.

Conclusion
Our results show increased platelet reactivity in children of patients with early AMI; the finding was not significantly correlated with endothelial dysfunction, suggesting that other mechanisms are mainly involved in the enhanced platelet response to agonistic stimuli.

Keywords
Platelet aggregation • Endothelial dysfunction • Family history of acute myocardial infarction

Introduction
Family history of early coronary artery disease (CAD) is an independent risk factor for the development of coronary events during lifetime.1–6 Although environmental and behavioral factors may contribute to CAD events in these subjects, genetic factors likely play an important role.7,8 In most cases, a family history of early CAD is related to heritable forms of known cardiovascular risk factors (CVRFs) (e.g. diabetes, hypercholesterolaemia, and hypertension).9–11 In several cases, however, no apparent CVRFs are present in family members, suggesting the involvement of still unknown mechanisms in the pathogenesis of CAD.

Platelets play a pivotal role in acute coronary events12–18 and they may also contribute to atherogenesis.19 Several factors may increase platelet activation in patients, including an impairment of endothelial function.20 The normal endothelium, indeed, exerts anti-platelet effects through the release of anti-adhesive and anti-aggregant substances, including NO and PGI2.20,21 Accordingly, endothelial dysfunction, which is the earliest vascular alteration in the chain of events that eventually lead to atherosclerosis,20–23 may favour platelet activation,24 together with vasoconstriction23 and inflammation.25

Previous studies have shown that endothelial dysfunction can be detected in young adults with a familial history of early acute myocardial infarction (AMI).26,27

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In contrast, no data are currently available about platelet reactivity in these young subjects. Thus, in this study, we aimed at assessing whether offspring of patients with early AMI have abnormalities in platelet reactivity; we also assessed whether these abnormalities might be related to endothelial dysfunction.

Methods

Population
In this study, we enrolled children with a family history of early AMI. To this aim, we identified from our hospital database all patients discharged in the year 2008 with a diagnosis of first ST-segment elevation AMI (STEMI) at age ≤ 50 and who had children with age 10–20 years (Group 1). Patients were contacted by phone and invited to give their informed consent to allow one of their children to participate in the study. Only apparently healthy children, according to clinical history, physical examination, and electrocardiogram (ECG), were included. In case more children were suitable in a family, the youngest was selected for the study.

As controls, we studied a group of children of the staff of our hospital (Group 2), comparable as to age and gender to children of AMI patients, who were apparently healthy according to clinical history, physical examination, and ECG and had no history of CAD in the two previous generations.

Subjects were excluded in the case of evidence of any cardiac or systemic disease, including any acute or chronic inflammatory or allergic disease. The presence of CVRFs was assessed in each subject. Hypertension was defined as systolic and/or diastolic blood pressure ≥ 95th percentile of published reference values for children and adolescents.\(^{28}\) Overweight was defined as a body mass index (BMI) of ≥ 25 kg/m\(^2\). Hyperglycaemia was defined as blood glucose level ≥ 110 mg/dL, hypercholesterolaemia as total blood cholesterol levels ≥ 200 mg/dL, and hypertriglyceridaemia as blood triglyceride levels ≥ 170 mg/dL. Active smoking was diagnosed if the subjects had smoked any cigarette in the last 3 months. Passive smoking was defined as habitual smoking at home by at least one parent.

Written informed consent for participation of children in the study was obtained from their parents. The study was approved by our institutional review board.

Exercise stress test
All subjects underwent a treadmill Bruce exercise stress test (EST). Leads II, V2, and V5 were monitored continuously throughout the test. A 12-lead ECG was printed at the end of each stage, when clinically indicated, and at 1 min intervals in the recovery phase. Blood pressure was measured at baseline and during the last minute of each stage, unless otherwise indicated. The test was stopped in the case of physical exhaustion, symptoms, hypotension (systolic blood pressure decrease > 40 mmHg compared with previous measurements), hypertension (systolic blood pressure > 200 mmHg and/or diastolic blood pressure > 120 mmHg), or ventricular arrhythmias. ST-segment depression (≥ 1 mm) was not a cause for stopping EST.

Blood sampling
In all children, a blood sample was collected at baseline and within 5 min of peak EST. Through a clean, non-traumatic puncture, and with minimal venous occlusion, blood samples were drawn directly through a 19 G needle from a brachial vein. Blood was collected in lithium fluoride for plasma glucose estimation, lithium heparin for lipid fraction analysis, and EDTA for haemochrome evaluation. Three millilitres of blood were collected into a tube filled with a 0.129 mol/L (3.8%) tri-sodium citrate solution (Vacuette, Greiner Bio-One, Monroe, NC, USA), kept at room temperature, and assayed within 1 h for platelet reactivity assessment. Six millilitres were centrifuged at 3000 g for 20 min at 4°C, and the resulting plasma was stored at 80°C until assayed.

Platelet reactivity
Platelet reactivity was assessed by measuring monocyte-platelet aggregates (MPA) and expression of platelet receptors, glycoprotein (GP) IIb/IIIa (CD41), and its active form (PAC1), by flow cytometry. Appropriate fluorochrome-conjugated isotype-matched mAb purchased from the different manufacturers were used as control for background staining. Monocyte-platelet aggregates and platelet receptors were assessed: (i) at rest, (ii) after adenosine diphosphate (ADP) stimulation at rest, (iii) at peak EST, and (iv) after ADP stimulation at peak EST.

Monocyte-platelet aggregates
Blood (100 μL) was labelled within 10 min of collection with a saturating concentration of fluorescein isothiocyanate (FITC)-conjugated CD41 monoclonal antibody for 15 min at room temperature, in order to specifically stain platelets by using one of its membrane receptors as an antibody target. Following incubation, erythrocytes were lysed with buffered ammonium chloride, and samples were immediately analysed by FACSscan. Monocytes were selectively gated on forward scatter (FSC) and side scatter (SSC) signals and aggregates measured as the ratio between mean fluorescent intensity (MFI) of the population staining positive for phycocerythin (PE)-conjugated CD14 (monocyte marker) and MFI of the population staining positive for CD41. A minimum of 30 000 monocytes were counted for each test.

Platelet surface receptors
Blood was diluted 1:10 in phosphate-buffered saline (PBS) and labelled within 10 min of collection by incubation with specific antibodies. Blood aliquots (5 μL) were incubated for 15 min at room temperature with saturating concentrations of PE-conjugated CD41 and FITC-conjugated PAC-1 to study the basal and the active form of platelet expression of GP IIb/IIIa receptor (Becton–Dickinson, Milan, Italy), respectively. Following incubation, samples were diluted with 200 μL of PBS and immediately analysed by a flow cytometer Becton–Dickinson FACScan. An acquisition gate was first established on FSC/SSC signals. These were collected in a logarithmic mode to improve discrimination between viable platelets and unwanted events (erythrocytes, white blood cells, debris, and aggregates). The purity of the gate was always confirmed by backgating on CD41 staining. A low flow rate was used to minimize coincident events. A minimum of 10 000 platelets were counted for each test. Fluorescence data were evaluated as MFI.

Stimulation with adenosine diphosphate
Blood samples were incubated with ADP (10\(^{-7}\) M) for 15 min at room temperature and labelled as previously described for the assessment of MPA and platelet receptors. The concentrations of ADP chosen for the study were the lowest that were found to activate platelets in adults showed highly reproducible results with average per cent differences ≤ 1% and maximal percent differences ≤ 5% for any variable.
Flow-mediated dilation

Endothelial function was assessed by measuring flow-mediated dilation (FMD) of the brachial artery by the same expert operator. Subjects rested in the supine position for at least 10 min in a warm, quiet room (22–24°C) before testing. A 10 MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Siemens™ Acuson Sequoia) was used to acquire images of the right brachial artery. The probe was kept in a fixed position by a mechanical support. After baseline images of the right brachial artery were obtained for 1 min, a forearm cuff, positioned 1 cm under the antecubital fossa, was inflated to 250 mmHg. The cuff was released 5 min after the inflation to induce forearm reactive hyperaemia. Brachial artery diameter was analysed using an automated edge detection software, which identifies and tracks the walls of the artery via the intensity of the brightness of the walls vs. the lumen of the vessel. The software provides a diameter measurement every second. The basal diameter is defined as the average of measures collected during the first minute, and FMD is calculated as the maximum per cent change of the brachial artery diameter compared with the basal value.15 Maximal Doppler flow velocity was also measured at baseline and at peak of forearm hyperaemia using the lowest insonation angle (always < 60°), which did not vary during each study.

After recovery of the brachial artery diameter to basal values, a dose of 25 μg of sublingual glyceryl trinitrate was administered to the patient. Nitrate-mediated dilatation (NMD) was calculated as the maximum per cent change of the brachial artery diameter compared with the basal value.

Preliminary assessment of variability of FMD measurements in two different days in 20 normal healthy adults at our Centre showed a per cent difference in the FMD values of 3.2 ± 7%.

Blood assays

Plasma levels of vascular cell adhesion molecule-1 (VCAM-1) were assessed using an enzyme-linked immunosorbent assay kit (Human VCAM-1, Elisa kit, TEMA ricerca, Bologna, Italy). Plasma von Willebrand antigen (vWF antigen) concentrations and activity were determined by agglutination of latex particles coated with vWF-specific polyclonal antibodies (HemosIL vWF antigen and activity, Instrumentation Laboratory, Milano, Italy). Serum C-reactive protein was measured by an immunonephelometric assay (IN Latex CRP mono, Dade Behring, Germany).

Statistical analysis

Continuous variables were compared between the two groups using independent t-test or Mann–Whitney U-test, as indicated, whereas proportions were compared by Fisher’s exact test. Correlation analyses were done by Spearman’s test.

Differences between the two groups in the response to ADP, EST, and EST + ADP of flow cytometry variables were assessed by repeated-measure two-way analysis of variance (ANOVA). In the case of significant difference in group-agonist interaction, multiple within-group and between-group comparisons were performed by paired or unpaired t-tests, respectively, applying Bonferroni’s correction. Multivariable ANOVA was applied to adjust significant results for variables that showed a definite difference or a tendency to difference (P ≤ 0.1) between the two groups (i.e. BMI, smoking, cholesterol levels, and, for platelet response to EST and/or ADP, peak heart rate and systolic blood pressure).

Multivariable linear regression was performed to verify the independent association of group belonging with platelet reactivity and with FMD. As far as platelet reactivity, we constructed linear regression models in which the per cent variations to the combined stimulus EST + ADP of each platelet cytometry variable (MPA and CD41 in the MPA gate, and CD41 and PAC-1 in the platelet gate) were entered as a dependent variable, and all potential explanatory variables (age, gender, serum glucose, cholesterol, triglyceride and C-reactive protein levels, blood pressure, BMI, and both active and passive smoking) were simultaneously included in the models together with the variable ‘Group’ (Group 1 = 1; Group 2 = 0). Tests for collinearity excluded any significant relation of group variable with any other explanatory variable (variance inflation factor < 10 for all tests).

Power calculations showed that all statistical tests performed in the study had a statistical power of >80% to detect as significant at P level <0.05 all observed differences.

Data are presented as mean ± standard deviation, unless otherwise specified. All tests were two-sided. A P-value of <0.05 was considered statistically significant. The software SPSS 17.0 (SPSS Italia, Florence, Italy) was used for statistical analysis.

Results

General findings

The main clinical characteristics of the two groups of children are summarized in Table 1, whereas Table 2 shows the main clinical features of the index parents of Group 1 children.

Group 1 included 23 children (15.0 ± 3.4 years; 10 females) of patients with early AMI. The mean age of their index parents at the time of AMI was 45.9 ± 4 years (range 28–50). Group 2 included 21 healthy children (age 14.6 ± 2.6 years, 11 females). There were no significant differences between the two groups in the main

Table 1 Main clinical findings of subjects

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 23)</th>
<th>Group 2 (n = 21)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.0 ± 3.4</td>
<td>14.6 ± 2.6</td>
<td>0.69</td>
</tr>
<tr>
<td>Male/female</td>
<td>13/10</td>
<td>10/11</td>
<td>0.76</td>
</tr>
<tr>
<td>Smokers</td>
<td>3/23</td>
<td>0/21</td>
<td>0.23</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>115 ± 7</td>
<td>112 ± 9</td>
<td>0.36</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>9 (39%)</td>
<td>7 (33%)</td>
<td>0.76</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4 ± 4.0</td>
<td>19.4 ± 1.7</td>
<td>0.044</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>162.0 ± 38.0</td>
<td>147.0 ± 24.0</td>
<td>0.13</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>51.0 ± 13.0</td>
<td>50.3 ± 24.0</td>
<td>0.24</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>86.9 ± 24.1</td>
<td>99.1 ± 56.3</td>
<td>0.37</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>85.3 ± 8.5</td>
<td>83.9 ± 10.0</td>
<td>0.61</td>
</tr>
<tr>
<td>Haemoglobin A1C (%)</td>
<td>4.9 ± 0.8</td>
<td>4.9 ± 0.4</td>
<td>0.81</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9 ± 1.8</td>
<td>0.8 ± 1.5</td>
<td>0.33</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>41.5 ± 2.8</td>
<td>41.8 ± 3.1</td>
<td>0.76</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>13.7 ± 1.2</td>
<td>13.7 ± 1.2</td>
<td>0.99</td>
</tr>
<tr>
<td>Red blood cell count (10⁶/mm³)</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.6</td>
<td>0.58</td>
</tr>
<tr>
<td>Leucocyte count (10⁹/mm³)</td>
<td>7.6 ± 1.4</td>
<td>6.8 ± 1.1</td>
<td>0.34</td>
</tr>
<tr>
<td>Platelet count (10³/mm³)</td>
<td>264.0 ± 66.2</td>
<td>280.0 ± 30.7</td>
<td>0.32</td>
</tr>
</tbody>
</table>

BMI, body mass index.

*Comparisons between groups assessed by unpaired t-test (continuous variables) or Fisher’s exact test (categorical variables).
clinical and laboratory characteristics, with the exception of BMI that was slightly higher in Group 1.

Exercise stress test
In all children, the EST was stopped for fatigue. No symptoms or ECG abnormalities were observed during the test in both groups. Heart rate and systolic blood pressure at peak exercise were higher in Group 1 compared with Group 2, although differences were not significant; no other differences were observed in EST parameters between the two groups (Table 3).

Platelet reactivity
Platelet reactivity results are summarized in Table 4. There were no significant differences between the two groups in basal MPA and platelet membrane receptors. However, ADP and EST, in particular when combined, induced a higher MPA formation and membrane platelet receptor expression in Group 1 compared with Group 2. Examples of the increased MPA formation following EST + ADP stimulation in Group 1 subjects are shown in Figure 1.

Adjustment of results for BMI, smoking, cholesterol levels, and peak heart rate and blood pressure did not influence the results. Moreover, in multivariable linear regression, belonging to Group 1 was the only variable independently associated with the response to EST + ADP of all platelet variables (data not shown; $P \leq 0.003$ for all).

Peripheral vascular function
Flow-mediated dilation was significantly lower in Group 1 compared with Group 2 ($8.0 \pm 2.9$ vs. $10.7 \pm 3.1%$; $P = 0.007$), even after adjustment for BMI and smoking ($P = 0.002$). Multivariable linear regression showed that belonging to Group 1 was the only independent variable associated with FMD values ($\beta = -0.63, P = 0.001$). On the other hand, no significant difference was observed in NMD between the two groups ($13.8 \pm 4.6$ vs. $14.8 \pm 3.1%$; $P = 0.39$; Table 5).

Plasma assay
No differences were found between the two groups in plasma levels of C-reactive protein ($P = 0.63$), VCAM-1 ($P = 0.22$), and vWF antigen ($P = 0.36$) and activity ($P = 0.31$; Table 5).

Relation between endothelial dysfunction and platelet reactivity
No significant correlation could be found between FMD and platelet receptor expression, both at rest and after ADP and/or EST stimuli, in Group 1 children (Table 6).

Discussion
Our study is the first to show that children of patients with early AMI display an increased platelet reactivity in response to agonistic stimuli such as ADP and EST, in particular when combined. Our results also confirm the presence of impaired endothelial function in these children, but no correlation could be demonstrated between endothelial dysfunction and increased platelet reactivity, suggesting that they are likely largely related to independent mechanisms.

Platelet function
Although platelets constitute a key element in the mechanisms responsible for acute thrombotic events, no previous study assessed platelet function in subjects with an early history of AMI, as well as in their children, although elevated fibrinogen plasma levels were found in healthy male relatives of patients with early CAD.

At rest, we did not find any differences in platelet activation between our study subjects and controls. However, exercise and ADP induced a greater platelet reactivity in study children, with differences being greater when platelets were stimulated with ADP after exercise.
The molecular mechanisms of the increased platelet reactivity in the offspring of early AMI patients remain to be established. However, belonging to Group 1 was the only variable consistently associated with an increased platelet reactivity in response to EST + ADP, suggesting that this finding can likely be related to some genetically mediated factor.

**Mechanisms of platelet activation**

In this study, we also investigated whether early impairment of endothelial function could be a cause of increased platelet activation in children of patients with early CAD. Previous studies, indeed, reported endothelial dysfunction in these young subjects.26,27

Impaired endothelial function is the earliest abnormality in the complex process that eventually leads to atherosclerosis.20–22 Importantly, endothelial dysfunction may also be involved in the pathogenesis of acute coronary syndromes,30 which also involves increased platelet activation.24 Accordingly, clinical studies have shown an association between endothelial dysfunction and increased risk of cardiovascular events in several groups of patients.20,21

Our findings extend the previous observations of endothelial dysfunction in children of patients with early CAD,26,17 showing that FMD is reduced very early (i.e. in the second decade of life) in these young subjects. Of note, the fact that no other variable was independently associated with reduced FMD suggests that genetic, rather than behavioral or environmental, factors might again be mainly involved in the vascular abnormality.31,32

Although endothelium-mediated vasodilation was reduced, however, there were no differences between the two groups in blood levels of VCAM-1 and vWF, as well as in vWF activity. This may suggest that endothelial activity at this age only shows very early functional abnormalities, whereas abnormal levels of vWF and VCAM-1 might have suggested a more advanced grade of endothelial damage.33–35

Our results failed to demonstrate any significant relation between endothelial dysfunction and the increased platelet reactivity in young subjects with a family history of early CAD, suggesting that the mechanisms responsible for the two abnormal findings can largely be independent.

A possible contribution to the increased platelet activation during exercise in the study group might reside in an enhanced adrenergic activation, as suggested by a higher heart rate and blood pressure achieved at peak exercise (Table 2). Catecholamines, indeed, stimulate platelets through \( \alpha \)-receptor-mediated stimulation.36 However, the differences in platelet activation between the two groups persisted after correction for exercise heart rate and blood pressure; furthermore, an increased platelet reactivity was present in Group 1 also after ADP stimulation alone.

Finally, in contrast with previous studies,37 we did not find any differences in serum C-reactive protein levels, suggesting that subclinical inflammation did not have any significant role in the

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**Table 4 Platelet cytometry variables in the two groups**

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P-value(^a)</th>
<th>P-value for changes(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPA (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>30.4 ± 4.0</td>
<td>31.8 ± 1.3</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>33.8 ± 4.5</td>
<td>34.9 ± 1.9</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>EST</td>
<td>33.0 ± 4.1</td>
<td>31.8 ± 3.2</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>ADP + EST</td>
<td>37.4 ± 6.3</td>
<td>33.6 ± 2.9</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>CD41 in MPA gate (mfi)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21.1 ± 2.7</td>
<td>22.2 ± 2.4</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>23.6 ± 3.3</td>
<td>23.6 ± 2.5</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>EST</td>
<td>23.2 ± 3.4</td>
<td>22.5 ± 2.8</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>ADP + EST</td>
<td>25.3 ± 3.3</td>
<td>23.3 ± 2.6</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>CD41 in platelet gate (mfi)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>92.5 ± 19.5</td>
<td>97.4 ± 9.1</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>105.6 ± 21.9</td>
<td>105.1 ± 7.8</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>EST</td>
<td>97.8 ± 21.6</td>
<td>97.8 ± 7.2</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>ADP + EST</td>
<td>115.4 ± 24.1</td>
<td>104.5 ± 7.6</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>PAC-1 in platelet gate (mfi)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.04 ± 1.0</td>
<td>6.47 ± 1.2</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>7.47 ± 1.2</td>
<td>7.40 ± 1.3</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>EST</td>
<td>7.11 ± 1.8</td>
<td>6.80 ± 1.10</td>
<td>0.87</td>
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</tr>
<tr>
<td>ADP + EST</td>
<td>9.32 ± 2.1</td>
<td>7.30 ± 1.2</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

ADP, adenosine diphosphate; EST, exercise; mfi, mean fluorescence intensity; MPA, monocyte-platelet aggregates.

\(^a\)Comparisons between the two groups of basal measurements assessed by unpaired t-test.

\(^b\)P-values for the change of each variable in response to the agonistic stimuli compared with basal values (group-agonist interaction), assessed by repeated-measure ANOVA.
increased platelet reactivity, as well as in the reduced FMD, observed in our study subjects.

**Clinical implications**

A potential clinical implication of our study relies on the possibility that the increased platelet reactivity, as well as the early impairment of endothelial function, may contribute to the known increased risk of cardiovascular disease of the offspring of patients with early AMI. This, however, can only be established by large prospective follow-up studies, which should also investigate...
whether specific lifestyle behaviours and/or interventions may reduce the cardiovascular risk of these subjects in the adult age.

Limitations of the study

The number of subjects included in this study is relatively small, and therefore, our data should be confirmed in larger groups of subjects. Several methods have been proposed to assess platelet function. In this study, platelet reactivity was assessed by flow cytometry. Confirmation of our data with a different and more generally acknowledged method, such as optical aggregometry, might have strengthened our results. However, we explored both the response to specific agonistic stimuli of platelet receptors typically involved in the process of platelet activation and a functional consequence of platelet activation (i.e., MPA formation). Furthermore, previous studies showed a high correlation between platelet reactivity assessed by aggregometry and platelet receptor expression assessed by flow cytometry, supporting the reliability of our results.

Conclusions

In this study, we show that children of patients with early AMI present an increased platelet reactivity and a reduced FMD, with no significant relation between the two kinds of abnormalities. The possible role of these findings in the known increased risk of cardiovascular events at adult age in these subjects deserves appropriate investigation.

Conflict of interest: none declared.

References


A 71-year-old male with highly symptomatic persistent atrial fibrillation for 6 years (EHRA III) was admitted for pulmonary vein isolation after failure of antiarrhythmic drug therapy. After vascular access, a steerable decapolar catheter was placed into the coronary sinus via the left femoral vein (Livewire®, St Jude Medical, St Paul, MN, USA). A transseptal puncture was accomplished by utilizing fluoroscopic imaging and pressure monitoring with the use of a long 8 Fr sheath (Daig SL1®, BRK trans-septal needle®, St Jude Medical). Once the needle tip crossed the interatrial septum, its positioning in the left atrium was confirmed by dye injection through its lumen to visualize the left atrial cavity. During this manoeuvre, the distal tip of the transseptal needle dislocated into the left atrium (Figure 1A–C, white arrow; see Supplementary material online, Movie S1) and ended in the left superior pulmonary vein. The needle showed a loss of the distal part compared with an intact one (Figure 1D, white arrowhead). Multiple attempts of retrieval of the embolized fragment failed. Therefore, we decided to place a bare-metal stent for fixation just before the fragment in the very peripheral left superior pulmonary vein.

The patient developed no symptoms and was discharged a few days later. Although transseptal catheterization is associated with a high success and low complication rate, interventional cardiologists should be aware of this particular unusual complication with the used system for transseptal access.

**Supplementary material**

Supplementary material is available at *European Heart Journal* online.

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**Figure 1** Fluoroscopic imaging left anterior oblique 60° of dislocation of needle tip (white arrow) while dye injection after transseptal puncture (A–C) and needle tip with loss of distal part (white arrowhead) compared with intact transseptal needle (D).

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**CARDIOVASCULAR FLASHLIGHT**

**Loss of the tip of a transseptal needle in the heart**

Gerold Mönning*, Holger Reinecke, and Lars Eckardt

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A 71-year-old male with highly symptomatic persistent atrial fibrillation for 6 years (EHRA III) was admitted for pulmonary vein isolation after failure of antiarrhythmic drug therapy. After vascular access, a steerable decapolar catheter was placed into the coronary sinus via the left femoral vein (Livewire®, St Jude Medical, St Paul, MN, USA). A transseptal puncture was accomplished by utilizing fluoroscopic imaging and pressure monitoring with the use of a long 8 Fr sheath (Daig SL1®, BRK trans-septal needle®, St Jude Medical). Once the needle tip crossed the interatrial septum, its positioning in the left atrium was confirmed by dye injection through its lumen to visualize the left atrial cavity. During this manoeuvre, the distal tip of the transseptal needle dislocated into the left atrium (Figure 1A–C, white arrow; see Supplementary material online, Movie S1) and ended in the left superior pulmonary vein. The needle showed a loss of the distal part compared with an intact one (Figure 1D, white arrowhead). Multiple attempts of retrieval of the embolized fragment failed. Therefore, we decided to place a bare-metal stent for fixation just before the fragment in the very peripheral left superior pulmonary vein.

The patient developed no symptoms and was discharged a few days later. Although transseptal catheterization is associated with a high success and low complication rate, interventional cardiologists should be aware of this particular unusual complication with the used system for transseptal access.

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