Whole blood viscosity and erythrocyte deformability are related to endothelium-dependent vasodilation and coronary risk in the elderly

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study

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Department of Medicine, Uppsala University Hospital, Uppsala, Sweden

Abstract. It has previously been shown that a high hemoglobin value, a major determinant of whole blood viscosity (WBV), predicts cardiovascular events. One putative mechanism might be an impaired endothelial function. Erythrocyte deformability is another rheologic feature of the erythrocyte being of importance for the flow properties of the blood, especially in the capillaries. The present study evaluates the relationships between blood viscosity, erythrocyte deformability assessed as erythrocyte fluidity (EF), coronary risk and endothelial vasodilatory function in the population-based PIVUS study (1016 subjects aged 70); endothelium-dependent vasodilation (EDV) was evaluated by the invasive forearm technique with acetylcholine given in the brachial artery and the brachial artery ultrasound technique with measurement of flow-mediated dilatation (FMD). WBV, plasma viscosity (PV) and EF were measured in a random sample of 573 subjects. WBV and PV were positively and EF negatively related to Framingham risk score. EDV was inversely related to both whole blood and plasma viscosity. FMD was not related to any rheologic variable. In multiple regression analyses, WBV and EF were significantly related to EDV independently of gender, hypertension, smoking, hypercholesterolemia, obesity and diabetes. Acetylcholine-induced vasodilation in the forearm, but not FMD, was negatively related to whole blood viscosity and positively related to EF independently of traditional risk factors in elderly subjects, indicating a pathophysiological link between impaired hemorheology and coronary risk.

Keywords: Blood viscosity, endothelium, erythrocyte deformability, coronary risk, vasodilation

1. Introduction

Endothelium-dependent vasodilation (EDV) is a characteristic feature of the vasculature and an impaired EDV is seen early in the atherosclerotic process [7]. An impaired EDV has been shown to predict future cardiovascular events [8]. It has also previously been shown that a high hemoglobin value, a major determinant of whole blood viscosity (WBV), predicts cardiovascular events. One putative mechanism

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might be an impaired endothelial function. Erythrocyte deformability is another rheologic feature of the
erythrocyte being of importance for the flow properties of the blood, especially in the capillaries.

The Prospective Investigation of the Vasculature in Uppsala Seniors (the PIVUS study) was conducted
with the primary aim to evaluate the power of two different tests of endothelium-dependent vasodilation
in the peripheral circulation to predict future cardiovascular events in more than 1000 subjects aged 70
living in the community of Uppsala, Sweden. We here report one of the secondary aims of the study,
namely to evaluate the relationships between whole blood and plasma viscosity as well as erythrocyte
deformability and measurements of endothelium-dependent vasodilation in both the brachial artery and
the forearm resistance arteries.

2. Material and methods

2.1. Subjects

Eligible were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were
chosen from the register of community living and were invited in a randomized order from the start of
the study in April 2001 to the last included subject in June 2004. The subjects received an invitation by
letter within 1 months of their 70th birthday in order to standardize for age. Of the 2025 subjects invited,
1016 subjects were investigated giving a participation rate of 50.1% [4].

The study was approved by the Ethics Committee of the University of Uppsala and the participants
gave informed consent.

2.2. Baseline investigation

The participants were asked to answer a questionnaire about their medical history, smoking habits and
regular medication.

All subjects were investigated in the morning after an overnight fast. No medication or smoking was
allowed after midnight. After recordings of height, weight, abdominal and hip circumference, an arterial
cannula was inserted in the brachial artery for blood sampling and later regional infusions of vasodilators.
During the investigation, the subjects were supine in a quiet room maintained at a constant temperature.
Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated
arm to nearest mmHg after at least 30 min of rest and the average of three recordings was used. Lipid
variables and fasting blood glucose were measured by standard laboratory techniques. Characteristics of
the subjects are given in Tables 1 and 2.

As the participation rate in this cohort was only 50%, we carried out an evaluation of cardiovascu-
lar disorders and medications in 100 consecutive subjects who were invited to the study, but denied
participation. The prevalences of cardiovascular drug intake, history of myocardial infarction, coronary
revascularization, antihypertensive medication, statin use and insulin treatment were similar to those in
the investigated sample, while the prevalences of diabetes, congestive heart failure and stroke tended to
be higher among the non-participants.

2.3. The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography. A mercury in-silastic
strain-gauge was placed at the upper third of the forearm, which rested comfortably slightly above the level
Table 1
Basic characteristics, major cardiovascular risk factors and measures of endothelium-dependent vasodilation in the total sample. Means are given as ±SD or as median and 10th and 90th percentiles in parenthesis. SBP = Systolic and DBP = Diastolic blood pressure. BMI = Body mass index. EDV = endothelium-dependent vasodilation and EIDV = endothelium-independent vasodilation (invasive forearm technique). FMD = flow mediated dilatation.

<table>
<thead>
<tr>
<th></th>
<th>Total sample</th>
<th>Rheological sample</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>1016 (or as stated)</td>
<td>573</td>
</tr>
<tr>
<td>Females (%)</td>
<td>50.2</td>
<td>50.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 ± 9.1</td>
<td>169 ± 9.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 ± 14</td>
<td>77 ± 15</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>91 ± 12</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.0 ± 4.3</td>
<td>26.8 ± 4.5</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.90 ± 0.75</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>150 ± 23</td>
<td>150 ± 22</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79 ± 10</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>62 ± 8.7</td>
<td>62 ± 8.7</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.4 ± 1.0</td>
<td>5.4 ± 1.0</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.5 ± 0.88</td>
<td>3.4 ± 0.88</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.5 ± 0.42</td>
<td>1.50 ± 0.41</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.3 ± 0.60</td>
<td>1.3 ± 0.63</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.3 ± 1.6</td>
<td>5.3 ± 1.5</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>EDV (%) n = 884</td>
<td>850 (199–909)</td>
<td>532 (190–927)</td>
</tr>
<tr>
<td>EIDV (%) n = 884</td>
<td>128 (149–629)</td>
<td>367 (153–620)</td>
</tr>
<tr>
<td>FMD (%) n = 986</td>
<td>4.4 (0.0–9.7)</td>
<td>4.8 (0.0–9.7)</td>
</tr>
<tr>
<td>WBV (mPa·s)</td>
<td>4.3 ± 0.43</td>
<td></td>
</tr>
<tr>
<td>PV (mPa·s)</td>
<td>1.3 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>EF (Pa⁻¹)</td>
<td>102 ± 11</td>
<td></td>
</tr>
<tr>
<td>Hct (%)</td>
<td>43 ± 3.2</td>
<td></td>
</tr>
</tbody>
</table>

of the heart. The strain-gauge was connected to a calibrated plethysmograph (Elektromedicin, Kullavik, Sweden). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 50 mm Hg by a rapid cuff inflator. Evaluations of FBF were made by calculations of the mean of at least five consecutive recordings.

An arterial cannula was placed in the brachial artery. No more than one attempt to insert the cannula in each arm was allowed. Resting FBF was measured 30 min after cannula insertion. After evaluation of resting FBF, local intra-arterial drug-infusions were given during 5 minutes for each dose with a 20 minutes washout period between the drugs. The infused dosages were 25 and 50 μg/minute for Acetylcholine (Clin-Alpha, Switzerland) to evaluate EDV and 5 and 10 μg/minute for SNP (Nitropress, Abbot, UK) to evaluate endothelium-independent vasodilation (EIDV). The dosages of these drugs have been chosen to result in FBFs on the steep part of the dose-response curve without giving systemic effects. The drugs were given in a random order at a maximal rate of 1 ml/min.
Table 2

Self-reported history of cardiovascular (CV) disorders and regular drug intake given in percentage in the total sample and in the sample where rheology was assessed. CABG/PTCA = coronary revascularization. GTN = any nitroglycerine preparation.

<table>
<thead>
<tr>
<th>Disorder/Drug Description</th>
<th>Total investigated sample</th>
<th>Rheological sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1016</td>
<td>573</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>7.1</td>
<td>8.0</td>
</tr>
<tr>
<td>Stroke</td>
<td>3.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>8.1</td>
<td>7.5</td>
</tr>
<tr>
<td>CABG/PTCA</td>
<td>5.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8.7</td>
<td>8.9</td>
</tr>
<tr>
<td>Any regular drug</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Any CV drug</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>Any antihypertensive medication</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Diuretics</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>8.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Angiotensin II-blockers</td>
<td>8.5</td>
<td>8.7</td>
</tr>
<tr>
<td>GTN</td>
<td>3.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Digoxin</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Statins</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Other antihyperlipidemic drugs</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Oral antidiabetic drugs</td>
<td>6.1</td>
<td>6.5</td>
</tr>
<tr>
<td>Warfarin</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Aspirin/Clopidogrel</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Other antiarrhythmic drugs</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In the present study only data from the highest doses of Acetylcholine and SNP were used. EDV was defined as FBF during infusion of 50 µg/min of Acetylcholine minus resting FBF divided by resting FBF. EIDV was defined as FBF during infusion of 10 µg/min of SNP minus resting FBF divided by resting FBF. EDV and EIDV could successfully be evaluated in 87% of the participants of the study. Cannulation of the artery was not performed in the 3% of the subjects who were on regular treatment with Warfarin and in another 10% cannulation failed. We have previously shown the reproducibility (coefficient of variation, CV) for EDV and EIDV to be 8–10% [6].

2.4. The brachial artery ultrasound technique

The brachial artery was assessed by external B-mode ultrasound imaging 2–3 cm above the elbow (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA), according to the recommendations of the International Brachial Artery Task Force [1]. Depths and gains settings were optimized to identify the lumen to vessel wall interface. The subject rested in the supine position for at least 30 minutes before the first scan and remained supine during the evaluation. Blood flow increase
was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 50 mmHg above systolic blood pressure. When the cuff was rapidly deflated five minutes later, the artery was scanned continuously for 90 seconds and recorded on a super-VHS videotape for later analysis of the diameter in end-diastole. FMD was defined as the maximal brachial artery diameter recorded between 30 and 90 sec following cuff release minus diameter at rest divided by the diameter at rest. FMD was successfully evaluated in 97% of the participants. In the remaining 3%, only suboptimal images were obtained not allowing proper analysis.

We have previously shown the reproducibility (CV) to be 3% for baseline brachial artery diameter and 29% for FMD [5].

The two different techniques to evaluate endothelium-dependent vasodilation were performed in the order described above. Two technicians were employed at the laboratory, but each vasodilatory technique was performed and evaluated by one unique technician throughout the study not being aware of the results of the other techniques or any clinical data.

2.5. Hemorheological measurements

For the hemorheological evaluation, blood samples were collected in heparin-coated vacutainer tubes and tested within one hour. Hematocrit was assessed by microhematocrit centrifugation at 11000 g for 5 minutes. No correction was made for trapped plasma. The rheological variables were assessed at 37°C in a low shear rotational viscometer (LS30, Contraves AG, Zürich, Switzerland). PV was analyzed at a shear rate of 38 s⁻¹ and apparent WBV at 100 s⁻¹ (interpolated from measurements at 128.5 and 94.5 s⁻¹) at native Hct. EF, a measure of erythrocyte deformability, was, as suggested by the ISCH [2] and evaluated by Staubli et al. [9] measured by bulk viscometry at a low shear rate (1 s⁻¹ interpolated from measurements at 1.285 and 0.945 s⁻¹) as the reciprocal apparent viscosity. Before measurement the erythrocytes were separated from the plasma by centrifugation and resuspended to a Hct of 55% in isotonic phosphate-buffered saline at pH 7.4. Whole blood and plasma viscosity were for practical reasons only measured on average every second day throughout the study, resulting in 573 evaluated subjects. All hemorheological measurements were carried out by Bo Sandhagen.

2.6. Traditional risk factors

Hypertension was defined as blood pressure >139/89 mmHg or antihypertensive treatment, diabetes was defined as fasting blood glucose >6.1 mmol/l or antidiabetic treatment, obesity was defined as BMI > 30 kg/m² and hypercholesterolemia as LDL-cholesterol > 3.5 mmol/l.

2.7. Statistics

Non-normally distributed variables were log-transformed to achieve a normal distribution. Relationships between pairs of variables were evaluated by Pearson’s correlation coefficient. Multiple regression analysis was applied to relate several independent variables to a dependent variable. Two-tailed significance values were given with \( p < 0.05 \) regarded as significant. The statistical programme package StatView (SAS inc, NC, USA) was used.
3. Results

Basic characteristics, medical history and drug intake are given in Tables 1 and 2. The prevalences of hypertension, diabetes mellitus, current smoking, obesity and hypercholesterolemia were 72, 12, 11, 22 and 43%, respectively. No significant differences regarding major characteristics between the subjects in whom whole blood and plasma viscosity were assessed (n = 573) and in the other part of the sample were seen (Table 1 and 2).

3.1. Rheology vs EDV

In univariate analysis, WBV and PV were negatively and EF positively correlated to EDV \( (r = -0.16, p = 0.0004), r = -0.14, p = 0.0015 \) and \( r = 0.13, p = 0.0052 \), respectively, Fig. 1a–c). When these three measures of rheology were included in multiple regression analysis together with gender, hypertension, diabetes, smoking, obesity and hypercholesterolemia, only WBV and EF were significantly related to EDV \( (r = -0.15, p = 0.0047; r = 0.10, p = 0.032) \). The relationships between WBV and EF to EDV were still significant after adjustment for the different cardiovascular medications given in Table 2 (data not shown).

3.2. Rheology vs FMD

None of the rheological measures were significantly correlated to FMD \( (r = 0.01, r = 0.03 \) and \( r = 0.03 \), respectively, Fig. 2a–c).

3.3. Rheology vs Framingham risk score

WBV and PV were positively and EF negatively related to the Framingham risk score \( (r = 0.20, p < 0.0001 \) for WBV, \( r = 0.12, p = 0.0044 \) for PV and \( r = -0.14, p = 0.0009 \) for EF, Fig. 3 a–c). In multiple regression analysis, WBV and EF were independently of each other related to the Framingham risk score \( (p = 0.0001 \) for WBV and \( p = 0.0233 \) for EF).

4. Discussion

The present study showed that endothelium-dependent vasodilation as evaluated by the invasive forearm technique, but not FMD, was negatively related to whole blood viscosity and positively related to EF independently of other major cardiovascular risk factors and medication in a large population sample of elderly subjects.

Whole blood viscosity and EF are two main characteristics determining shear stress upon the vascular wall. Since shear stress is the main stimulus for nitric oxide release, it is not surprising that both blood viscosity and EF are related to endothelium-dependent vasodilation in the forearm resistance vessels.

It is however more surprising that these rheological variables are not related to FMD in this elderly population. There might be several reasons for this. As argued by Wendelhag et al. [10], FMD is markedly reduced by age also in healthy elderly, and with a median FMD of around 4% the power to detect relationships within such a narrow range is limited. Furthermore, Witte et al showing recently that FMD is related to cardiovascular risk only in those with a normal arterial compliance [11] and suggested that
Fig. 1. Relationships between a) whole blood viscosity (WBV), b) plasma viscosity (PV) and c) erythrocyte deformability (EF) and endothelium-dependent vasodilation evaluated with the invasive forearm technique (EDV) ln-transformed.
Fig. 2. Relationships between a) whole blood viscosity (WBV), b) plasma viscosity (PV) and c) erythrocyte deformability (EF) and flow-mediated vasodilation (FMD) In-transformed.
Fig. 3. Relationships between a) whole blood viscosity (WBV), b) plasma viscosity (PV) and c) erythrocyte deformability (EF) and the Framingham risk score.
a reduced arterial compliance limits the use of FMD. In the present age-group arterial compliance is
reduced also in the healthy part of the sample obscuring any relationship between FMD and any risk
factor. Similar results have previously been presented in a study conducted in the present PIVUS sample
[3].

Thus, to rule out the possibility that rheology characteristics are related to FMD, an investigation has
to be performed in a younger sample where FMD is less influenced by arterial compliance.

The lack of relation between FMD and WBV (Fig. 2a) might also be explained by that a higher WBV will
enhance the shear stress on the endothelium, thereby increasing the release of NO, in turn increasing the
vasodilation. This co-variation between WBV and vasodilation might neutralize the negative correlation
seen between EDV and WBV. The same explanation could be applied for PV and EF (Fig. 2b,c). A higher
PV as well as stiffer erythrocytes (lower EF) will also enhance the shear stress on the endothelium.

4.1. Limitation of the study

The present sample is limited to caucasians aged 70. So, caution should be made to draw conclusions
to other ethnic and age-groups.

The present study had a moderate participation rate. However, an analysis of non-participants showed
the present sample to be fairly representative of the total population regarding most cardiovascular
disorders and drug intake.

5. Conclusion

Acetylcholine-induced vasodilation in the forearm, but not FMD, was negatively related to whole blood
viscosity and positively related to erythrocyte fluidity independently of traditional risk factors in elderly
subjects.

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