Association of Mean Platelet Volume With Risk of Stroke Among 3134 Individuals With History of Cerebrovascular Disease

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Background and Purpose—Mean platelet volume (MPV) is positively associated with measures of platelet activity and may be a useful indicator of the risk of vascular events in a variety of patient groups.

Methods—The association of MPV with the risk of stroke was assessed in the Perindopril Protection Against Recurrent Stroke Study (PROGRESS). All participants had a history of cerebrovascular disease at baseline, and analyses were adjusted for the effects of potential confounders.

Results—The study followed 3134 individuals for an average of 3.9 years (mean age, 65 years; 71% male; average MPV, 10.0 fL). Three hundred eighty-three individuals had 402 stroke events, and 160 had major coronary events. MPV was positively associated with the risk of stroke, with an 11% increased relative risk (95% CI, 3% to 19%) of stroke per femtoliter greater MPV. There was no clear association of MPV with the risk of major coronary events (9% decreased relative risk; 95% CI, −23% to 7%). Perindopril did not alter MPV.

Conclusions—MPV is an independent predictor of the risk of stroke among individuals with a history of stroke or transient ischemic attack. The measurement of MPV may add useful prognostic information for clinicians managing patients with a history of cerebrovascular disease. (Stroke. 2004;35:622-626.)

Key Words: myocardial infarction • platelets • stroke, hemorrhagic • stroke, ischemic

Platelet size, measured as mean platelet volume (MPV), is a marker of platelet function and is positively associated with indicators of platelet activity, including aggregation and release of thromboxane A₂, platelet factor 4, and β-thromboglobulin. Previous studies have documented above average levels of MPV among patients with acute stroke, myocardial infarction, chronic vascular disease, or vascular risk factors. In addition, there is evidence that an elevated MPV is associated with a poor outcome among survivors of myocardial infarction and stroke and with an increased risk of restenosis after coronary angioplasty.

The Perindopril Protection Against Recurrent Stroke Study (PROGRESS) recruited 6105 individuals with a history of cerebrovascular disease and demonstrated that a blood pressure–lowering regimen, involving an angiotensin-converting enzyme (ACE) inhibitor and diuretic, reduced the risks of stroke and major vascular events. A total of 6105 participants were enrolled within 5 years of stroke and/or transient ischemic attack from 172 collaborating centers in 10 countries (Australia, Belgium, China, France, Italy, Japan, New Zealand, Republic of Ireland, Sweden, United Kingdom). After giving written informed consent and successful completion of a 4- to 6-week run-in period on perindopril, eligible participants were randomized to either continued blood pressure lowering with a perindopril-based regimen or matching placebo. Double-blind follow-up continued for a mean of 3.9 years.

Subjects and Methods

PROGRESS Trial

A total of 6105 participants were enrolled within 5 years of stroke and/or transient ischemic attack from 172 collaborating centers in 10 countries (Australia, Belgium, China, France, Italy, Japan, New Zealand, Republic of Ireland, Sweden, United Kingdom). After giving written informed consent and successful completion of a 4- to 6-week run-in period on perindopril, eligible participants were randomized to either continued blood pressure lowering with a perindopril-based regimen or matching placebo. Double-blind follow-up continued for a mean of 3.9 years.

Participants

The MPV substudy was approved by the local research ethics committee of King’s College Hospital, London (No. 1995-0183). Individuals registered in the 5 countries (Australia, China, Japan, New Zealand, Sweden) participating in the MPV substudy were eligible for inclusion, dependent on (1) collection of a blood sample before any outcome event and (2) inclusion in the double-blind follow-up phase of the trial. For analysis of the effect of randomized study treatment on MPV, eligibility further required that (3) the baseline measurement was made on a sample collected before the start of the active run-in treatment and (4) the follow-up sample was collected at least 180 days after randomization.
Blood Collection and Measurement of MPV
Nonfasting venous blood samples were collected into EDTA tubes and transported at 4°C for analysis 24 to 48 hours after venipuncture at a central laboratory in each country. Second blood samples were sought 1 year after randomization from 10% of individuals selected at random from participating countries except China. The repeated blood samples enabled assessment of (1) the effect of perindopril on MPV and (2) errors in the baseline measurements of MPV (regression dilution bias). The hematology analyzers used were as follows: Australia (Sysmex K1000, University of Melbourne), China (Abbott Diagnostics CD1600, Fu Wai Hospital, Beijing), Japan (Sysmex SE9000, BML, Tokyo), New Zealand (Technicon H1 or H1ACTS, Greenlane Hematology, Auckland), and Sweden (Coulter StkS, Centrum for Laboratorie Medicin, Uppsala).

Quality Control
A quality control program was run by the United Kingdom National External Quality Assessment Scheme for Hematology (UK NEQAS/H). Identical pairs of blood samples were sent to each participating laboratory on 12 occasions throughout the study.

Outcomes
The primary outcome was stroke. Secondary outcomes were stroke subtype (ischemic, hemorrhagic, unknown type) and major coronary events (nonfatal myocardial infarction and death from coronary heart disease, including sudden death). All events were reviewed and validated by an adjudication committee. Only the first event of each type was included in the analyses.

Statistical Analysis
The association of MPV with the risk of an event during follow-up was done in 2 ways: (1) by estimating the absolute event rates (95% CIs) defined by fifths of baseline MPV with stratification by country and testing of the trend across the groups by \( \chi^2 \) test and (2) by calculating the percent risk of an event for each unit increase in MPV [(hazard ratio – 1) × 100] with the use of Cox proportional hazards regression to estimate hazard ratios. Analyses were adjusted for country and for random error in the measurement of baseline MPV (see below). Multivariable analyses included the following baseline characteristics: treatment allocation (active, placebo), age, sex, systolic blood pressure, smoking status, history of diabetes, atrial fibrillation, myocardial infarction, prior stroke, platelet count, red cell count, red cell volume, white cell count, and medications at baseline (including a history of aspirin or other antiplatelet therapy). Covariates were included on the basis of their known effects on the risk of vascular disease or an observed effect on the \( \beta \)-coefficient for the association of MPV with stroke. For sensitivity analyses, the association of MPV with the risk of stroke was made for subgroups defined by (1) time of blood sample collection (before/at, after registration visit) and (2) delay between blood sample collection and randomization, \( n = 550 \).

The effect of perindopril on MPV was done with the use of \( t \) tests of the differences in the mean change between treatment arms. Separate estimates of the effects were made for subgroups treated with combination drug therapy (perindopril and indapamide versus double placebo) and single drug therapy (perindopril versus placebo) with homogeneity of effects determined from \( \chi^2 \) tests.

Adjustment for error in the measurement of baseline MPV was achieved by multiplication of the \( \beta \)-coefficient obtained for MPV by the inverse of a regression dilution coefficient (0.75). The coefficient was calculated in 359 individuals with 2 MPV measurements that were separated by at least 180 days in the absence of an intervening vascular event. Accordingly, the results are reported per unit “usual” MPV rather than per unit “baseline” MPV.

Results
Participants, Baseline Characteristics, and Quality Control Program
A total of 3134 individuals were enrolled from 107 centers in 5 participating countries (Figure 1). There were moderate differences in the characteristics of participants recruited from each country (Table 1). Marked difference in baseline levels of MPV and use of antiplatelet therapy were present; however, the differences in MPV between participants who were on or off antiplatelets were small (≤0.3 fL for each country).

UK NEQAS/H received results for 66 of 120 distributed samples (55%). Results for MPV were available for every country for 1 round: New Zealand 6.3 fL, Sweden 9.3 fL, Australia 10.9 fL, Japan 11.7 fL, China 12.3 fL. The principal difference was between New Zealand, in which the autoanalyzer used an optical measurement method, and the other countries, in which measurement was based on electric impedance. Too few samples were returned for reliable assessment of the stability of the autoanalyzers over time.

Blood Collection and Analysis
MPV measurements were made at different times after enrollment: before active run-in treatment, \( n = 1694 \); before randomization, \( n = 890 \); after randomization, \( n = 550 \). The median time between collection of the blood sample and measurement of MPV was 25 hours (interquartile range, 12 to 30 hours). Forty-one percent of samples were assayed within 24 hours of collection, and 16% of samples were assayed >48 hours after collection. Information on blood collection was missing for 27 participants.

Association of MPV With Risk of Stroke
Four hundred two strokes were recorded among 383 individuals during the follow-up period: 301 ischemic strokes, 59 intracerebral hemorrhages, 42 strokes of unknown type (Table 2). Nineteen individuals experienced >1 type of stroke event, and 43 of the strokes were fatal. Stroke rates were greater among individuals with higher measurements of MPV, both overall (\( P \) for trend across fifths of MPV = 0.01) and for ischemic stroke alone (\( P = 0.01 \)) (Figure 2). There was no evidence of an association of MPV with the rates of either intracerebral hemorrhage (\( P = 0.1 \)) or stroke of unknown type (\( P = 0.5 \)). With adjustment for country of recruitment and measurement error, the strength of the overall association was...
such that each 1-fL increase in usual MPV was associated with a 12% (95% CI, 4% to 20%) increased relative risk of stroke. Inclusion of other potential confounders in the statistical model had little effect on the estimate (Table 2). This association did not differ among subgroups of participants defined by either the time of baseline measurement (P homogeneity/H0.4) or the delay between blood collection and measurement of MPV (P homogeneity/H0.8).

Association of MPV With Risk of Major Coronary Events
One hundred sixty individuals had a major coronary event: nonfatal myocardial infarction, n=93; coronary heart disease death, n=54; sudden death, n=13 (Table 2). Coronary event rates were not associated with MPV (P=0.2) (Figure 3, Table 2).

Effects of Randomized Treatment on MPV
Two hundred eighty-one participants (130 active, 151 placebo) had baseline and follow-up measurements of MPV. The

TABLE 1. Baseline Characteristics of Participants Overall and by Country of Recruitment

<table>
<thead>
<tr>
<th></th>
<th>Australia</th>
<th>China</th>
<th>Japan</th>
<th>New Zealand</th>
<th>Sweden</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>67 (11)</td>
<td>60 (8)</td>
<td>64 (9)</td>
<td>68 (9)</td>
<td>68 (8)</td>
<td>65 (9)</td>
</tr>
<tr>
<td>Male, %</td>
<td>75</td>
<td>71</td>
<td>77</td>
<td>67</td>
<td>65</td>
<td>71</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>143 (18)</td>
<td>144 (20)</td>
<td>143 (17)</td>
<td>145 (20)</td>
<td>153 (18)</td>
<td>146 (19)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83 (10)</td>
<td>87 (11)</td>
<td>84 (11)</td>
<td>82 (10)</td>
<td>87 (10)</td>
<td>85 (10)</td>
</tr>
<tr>
<td>Smoker, %</td>
<td>12</td>
<td>22</td>
<td>25</td>
<td>15</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Mean platelet volume, fL</td>
<td>11.1 (1.1)</td>
<td>10.9 (2.5)</td>
<td>11.2 (1.0)</td>
<td>7.4 (1.1)</td>
<td>8.7 (1.2)</td>
<td>10.0 (2.1)</td>
</tr>
<tr>
<td>Platelet count, ×10^11/L</td>
<td>217 (83)</td>
<td>227 (94)</td>
<td>236 (75)</td>
<td>243 (76)</td>
<td>242 (60)</td>
<td>233 (80)</td>
</tr>
<tr>
<td>White cell count, ×10^9/L</td>
<td>6.9 (2.2)</td>
<td>7.0 (4.4)</td>
<td>6.2 (1.7)</td>
<td>6.6 (4.7)</td>
<td>7.2 (2.6)</td>
<td>6.8 (3.4)</td>
</tr>
<tr>
<td>Red cell count, ×10^12/L</td>
<td>4.7 (0.9)</td>
<td>4.6 (0.8)</td>
<td>4.6 (0.6)</td>
<td>4.9 (0.5)</td>
<td>4.8 (0.5)</td>
<td>4.7 (0.7)</td>
</tr>
<tr>
<td>Mean red cell volume, fL</td>
<td>92 (6)</td>
<td>97 (5)</td>
<td>92 (5)</td>
<td>90 (5)</td>
<td>94 (5)</td>
<td>94 (6)</td>
</tr>
<tr>
<td>Prior ischemic stroke, %</td>
<td>66</td>
<td>77</td>
<td>79</td>
<td>56</td>
<td>74</td>
<td>72</td>
</tr>
<tr>
<td>Prior hemorrhagic stroke, %</td>
<td>5</td>
<td>18</td>
<td>15</td>
<td>6</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Prior stroke of unknown type, %</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>19</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Prior myocardial infarction, %</td>
<td>17</td>
<td>4</td>
<td>2</td>
<td>14</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Diabetes at baseline, %</td>
<td>12</td>
<td>10</td>
<td>19</td>
<td>8</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Antiplatelet use at baseline, %</td>
<td>80</td>
<td>59</td>
<td>65</td>
<td>81</td>
<td>74</td>
<td>70</td>
</tr>
<tr>
<td>Assigned active treatment, %</td>
<td>49</td>
<td>50</td>
<td>50</td>
<td>49</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Values are mean (SD) or frequency (%).
*Participants may have had more than one type of prior event.

TABLE 2. Percent Increases in the Risks of Stroke and Major Coronary Events for Each Femtoliter Above the Usual Mean Platelet Volume*

<table>
<thead>
<tr>
<th></th>
<th>% Increase in Risk (95% CI) per 1.0 fL Above the Usual Mean Platelet Volume, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Events†</td>
</tr>
<tr>
<td>Any stroke</td>
<td>383</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>301</td>
</tr>
<tr>
<td>Intracerebral hemorrhage</td>
<td>59</td>
</tr>
<tr>
<td>Stroke of unknown type</td>
<td>42</td>
</tr>
<tr>
<td>Major coronary event</td>
<td>160</td>
</tr>
</tbody>
</table>

*Adjusted for regression dilution bias.
†The first event alone contributes to the analysis.
‡Age, sex, systolic blood pressure, smoking status, history of diabetes, history of atrial fibrillation, history of myocardial infarction, history of prior stroke, country of recruitment, platelet count.
mean time between randomization and collection of the follow-up blood samples was 380 (SD 70) days. MPV did not differ between randomized groups: difference 0.001 fL\(^{-1}\) (95% CI, \(-0.23\) to \(+0.24\) fL\(^{-1}\)). There was no evidence of any difference in the effects on MPV of combination drug therapy (perindopril/indapamide) and single drug therapy (perindopril alone) (\(P\) homogeneity = 0.4). There was also no significant difference in the change in platelet count between treatment groups.

**Effect of Other Blood Factors on Stroke and Major Coronary Events**

When stroke was modeled as a univariate function of individual blood factors, neither platelet count (\(P=0.99\)), red cell count (\(P=0.60\)), white cell count (\(P=0.23\)), nor mean red cell volume (\(P=0.38\)) had a statistically significant association with stroke. When major coronary events were modeled as a univariate function of individual blood factors, neither platelet count (\(P=0.42\)), red cell count (\(P=0.35\)), nor mean red cell volume (\(P=0.35\)) had a statistically significant association with events. White cell count was associated (\(P=0.02\)) with major coronary events, but not when the model was adjusted for age, baseline systolic blood pressure, sex, smoking status, treatment allocation, and history of diabetes, atrial fibrillation, or myocardial infarction.

**Discussion**

This study of patients with a history of cerebrovascular disease is the first to show, in a prospective design, a positive association of MPV with the risk of stroke. The observed association was independent of other established determinants and appeared to be primarily due to an association between MPV and ischemic stroke but not hemorrhagic stroke or stroke of unknown type. The association with ischemic stroke is in accord with the known greater reactivity of larger platelets,\(^5\) the pathophysiological role of platelets in the occurrence of ischemic stroke,\(^23\) and the identified effects of antplatelet therapy on the risk of ischemic stroke.\(^24\)

In contrast to previous studies,\(^7\)\(^8\)\(^13\)\(^15\) there was no clear association of MPV with major coronary events in PROGRESS. Previous studies were done among patients with established coronary heart disease, whereas only 8% of PROGRESS participants had a history of myocardial infarction. The absence of an association of MPV with coronary events could reflect a true lack of association in this study population. A more likely explanation is that the small number of coronary events provided only limited statistical power to detect the true association of MPV with coronary disease. The CI about the estimate for the association of MPV with major coronary events in PROGRESS is wide, and positive, negative, and null associations of MPV with risk are all possible.

Several possible sources of error in the measurement of MPV may have contributed to the uncertainty surrounding the association of MPV with coronary events and attenuated the strength of the association of MPV with stroke.\(^21\) First, platelets swell in a time-dependent fashion after blood sampling in EDTA.\(^19\) The intention of conducting assays 24 to 48 hours after blood collection (when platelet swelling will have ceased\(^19\)\(^20\)\(^25\)) was intended to minimize such errors. However, a relatively large proportion was assayed outside of the proposed time window. It was not possible to adjust for the differences in the effects of platelet swelling on MPV measurements since calibration curves for time-dependent swelling were not available for each of the 5 different machines used to measure MPV. However, the sensitivity analysis of the effect of the time between sample collection and analysis (\(\leq 24\) versus \(>24\) hours) on the association between MPV and stroke showed no evidence of heterogeneity. Second, the MPV measurements were made with the use of a different machine in each of the collaborating countries over a period of approximately 2 years. The quality control program identified machine-dependent differences between the average levels of MPV in the participating countries, especially between New Zealand (where the machine used an optical mechanism, in which measured MPV appears to decrease with swelling) and other countries (where machines used an electric impedance mechanism of assay, in which measured MPV tends to increase with swelling).\(^25\) Country stratification or a country dummy variable was used in all analyses, although such adjustment is unlikely to have controlled completely for the differences between the machines.

Third, there is likely to have been error in the measurement of each participant’s usual MPV because of normal biological variation, leading to the regression dilution bias.\(^3\) The inclusion in the analyses of an adjustment for the regression dilution effect should have minimized the attenuating effects of such variation. While there were several possible sources of measurement error in this study, it seems unlikely that any would have occurred differentially between study participants who did or did not experience events. As such, the most likely effect of the measurement error that was not controlled for by the various strategies employed is underestimation of the strength of the observed associations and failure to detect other associations. The associations reported from this study are therefore likely to represent underestimates rather than overestimates of the associations of MPV with risk.

The antiplatelet effects of perindopril and other ACE inhibitors appear to be small.\(^26\) In this study there was no evidence of an effect of the randomized treatment on subsequent platelet parameters. Other studies of the ACE inhibitor
quinapril" and the angiotensin receptor blocker losartan similarly have shown little effect on MPV. The findings of this study therefore make it unlikely that perindopril had any effects on platelet production or that this could have been a mechanism by which long-term use of a perindopril-based blood pressure–lowering regimen prevented recurrent stroke in the participants of PROGRESS. Similarly, antiplatelet effects of ACE inhibitors are unlikely to have contributed importantly to the effects observed in the many other trials of ACE inhibitors that have demonstrated beneficial effects on the risks of major vascular outcomes.

In summary, this study identified MPV as an independent predictor of the risk of stroke among high-risk individuals with a history of prior cerebrovascular disease and largely excluded effects on platelet production as a blood pressure–independent mechanism by which ACE inhibitors afford vascular protection. The measurement of MPV may add useful prognostic information for clinicians managing patients with a history of cerebrovascular disease.

Acknowledgments

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References

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