Mediterranean Macrothrombocytopenia

By Wieland E. von Behrens

Platelet count, platelet size, and circulating platelet biomass concentration estimates made with an erythrocyte-calibrated electronic sizing system on EDTA-anticoagulated blood samples gave population medians and 95% ranges for 145 asymptomatic Mediterranean and 200 healthy Northern European subjects. The Mediterraneans had lower platelet counts [161,000 (89,000–290,000)/µl compared with 219,000 (148,000–323,000)/µl] and higher arithmetic mean volumes [17.8 (10.8–29.2) cu µm compared with 12.4 (9.9–15.6) cu µm], while the individual lognormal platelet size distribution profiles were comparable [geometric standard deviations of 1.78 (1.60–1.98) against 1.70 (1.54–1.88)]; and the platelet biomass concentrations, given by count per microliter times mean volume × 10⁻⁷ and expressed as a volumetric percentage of whole blood, were almost identical [0.286% (0.216%–0.379%) against 0.272% (0.201%–0.367%)]. Mediterranean macrothrombocytopenia is, therefore, considered a benign morphologic variant that requires differentiation from thrombocytopenias in which the circulating platelet biomass concentration is decreased.

Numerous workers have documented that some apparently healthy individuals have reduced circulating platelet counts, while others show a variable increase in platelet size. In the present study the relationship between the platelet count and arithmetic mean platelet volume is examined with the Coulter technique by comparing a group of healthy Northern European subjects with a group of Mediterranean subjects, since many of the latter have both a mild reduction in their peripheral platelet count and platelets that appear large in standard blood films.

Materials and Methods

Measurements were carried out on coded blood samples from 145 healthy Mediterranean volunteers and 200 Northern European controls, all living in Adelaide, Australia. The Mediterranean subjects or their parents were born on the Italian or Balkan peninsulas or the adjacent islands, and the Northern European subjects or their parents were born in the British Isles, the Netherlands, Germany, or the Baltic states. All subjects had hematocrits which were normal for age and sex.

Venous blood of all subjects was anticoagulated with dry Na₂EDTA (2.0–2.5 mg/ml). One hundred samples were also collected into 3.8% sodium citrate solution (9:1 v/v) to be analyzed for platelet size only. The blood was mixed gently at the laboratory and allowed to sediment in flat-bottomed, open 1 x 4-cm plastic tubes. After 30–60 min, 20 µl of PRP (sampled midway between the meniscus level and the red cell layer) were diluted gently into 40 ml of essentially particle-free 0.9% (w/v) NaCl solution at 22°C. Electronic sizing and counting were completed within 10 min of dilution and in a time interval between 2 and 4 hr after blood collection.

Frequency distributions of platelet Coulter volumes were documented with a model 110 Nuclear Data multichannel pulse height analyzer coupled to a model B Coulter Counter. A standard 1967
50-μ coulter aperture tube (no. 3872) was used at an aperture current setting of 1.1 (i.e., 1 mA), and amplification switches were varied as necessary. The counter and analyzer were zeroed and initially calibrated in cubic micrometers against the modal coulter volume of fresh human red cells sized by the hematocrit technique. Subsequently calibration was monitored with inert spores or latex particles which had been sized against the erythrocytes. Background counts were negligible. Each platelet size distribution profile consisted of more than 10^5 cells, had the appearance of a lognormal distribution, and was analyzed graphically (fig. 1A) of the curve were designated A and D and yielded the geometric standard deviation (GSD) as (D/A)^(1/2). From this, the mode (Mo) was located as D/GSD. The median (Me) was computed as the product of the mode and antilog [2.3026 (log GSD)]^2, and the arithmetic mean (Ma) as the product of Me and (Me/Mo)^(1/2). A table of Mo, Me, and Ma for different values of GSD facilitated these computations.

Percentiles 2.3 and 97.7 of each EDTA platelet size distribution (fig. 1A) were computed as Me(GSD)^2 and Me(GSD)^2. The lower and upper threshold dials of the calibrated coulter counter were set to encompass the 95.45% range between these percentiles, and duplicate raw platelet counts were recorded. From these, the PRP platelet count was calculated via the truncation factor of 1/0.9545, the coincidence correction factor, and the sampling and dilution factor. The platelet count per microliter whole blood was computed via the hematocrit correction factor of method A of Bull et al. Circulating platelet biomass concentration was estimated in per cent
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as the product of the platelet count, arithmetic mean Coulter platelet volume, and the scaling factor $10^{-7}$.

The measurements of each subject population were arranged in histogram form and also plotted as cumulative percentage frequencies on lognormal probability paper, a straight line being fitted to the central points by eye. The abscissa locations corresponding to percentiles 2.3, 50, and 97.7 were designated E, Me, and F as shown in Fig. 1B, where Me is again the median of the lognormal model and the GSD can be crudely estimated as $(F/E)^{1/4}$. Since such probability paper plots indicated lognormality of the data, the Northern European and Mediterranean population samples were compared via the t test, with the sample means and sample variances calculated using logarithms of the individual measurements. The antilogarithms of these means and standard deviations are more precise estimates of the parameters $Mg$ and GSD of the lognormal model of Fig. 1. The correlation analyses were also performed on logarithms of the platelet counts and volumes of the individual subjects.

Platelets from more than 50 subjects of each group were also examined by light microscopy. A 90 $\times$ objective was immersed into a drop of PRP on a blood slide, and platelet shape was assessed both under transmission and phase-contrast illumination. Stained blood films from EDTA blood samples were also inspected. An electron-microscopic study of Mediterranean macrothrombocytes and red cells was carried out in parallel with part of the present study by the method of Stockinger et al.

RESULTS

Representative normal and macrothrombocyte Coulter distributions appear in Fig. 2. There was a large difference in the platelet counts and platelet volumes of the two subject groups ($p < 0.001$). The median platelet count of the Northern European subjects was 219,000/μl whole blood, while that of the Mediterraneans was only 161,000, and 39.5% fell below percentile 2.3 of the controls (Fig. 3, Table 1). The medians of the modal Coulter volumes were 8.14 cu μm for the Mediterranean group, and 59% of the latter fell above
Fig. 3. Platelet count and size distributions in the Mediterranean (—) and Northern European (——) subject groups. Differences between means were highly significant \((p < 0.001)\).

Table 1. Electronic Platelet Count and Volume Parameters*

<table>
<thead>
<tr>
<th>Platelet Populations</th>
<th>200 Northern Europeans</th>
<th>145 Mediterraneans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentile</td>
<td>GSD</td>
</tr>
<tr>
<td>2.3</td>
<td>50</td>
<td>97.7</td>
</tr>
<tr>
<td>Observed measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL.Counts (\times 10^{-3}) ((\mu L))</td>
<td>148</td>
<td>219</td>
</tr>
<tr>
<td>Dispersion = GSDt</td>
<td>—</td>
<td>1.54</td>
</tr>
<tr>
<td>Platelet mode = Mo   ((\mu L))</td>
<td>6.48</td>
<td>8.14</td>
</tr>
<tr>
<td>Computed measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean = Me  ((\mu L))</td>
<td>8.6</td>
<td>10.8</td>
</tr>
<tr>
<td>Arithmetic mean = Ma ((\mu L))</td>
<td>9.9</td>
<td>12.4</td>
</tr>
<tr>
<td>Biomass concentration ((%))</td>
<td>0.201</td>
<td>0.272</td>
</tr>
<tr>
<td>= count (\times \text{Ma} \times 10^{-7})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The parameters of location cited here are those of platelet populations from gravity sedimented EDTA-PRP sized at 22°C with a standard Coulter system calibrated ultimately with fresh red cells. The parameters of location for platelet populations from citrate PRP were lower by a median factor of 0.78 than those cited here, apparently because of a difference in electrical cell form factors. When the system was calibrated directly against latex spheres whose volumes were computed from their electron-microscopically estimated diameters, the parameters of location were lower by a further factor of 0.667. These parameters are expressed in cubic micrometers \((\mu m^3)\) or \(10^{-15} \text{m}^3\) or \(10^{-18} \text{m}^2\) or \(\text{fl or femtoliters}\). The parameter of dispersion (GSD) is dimensionless and not affected by sealing factors such as the cell form factors.

†The median (percentile 50) and geometric mean of a lognormal distribution coincide, and the geometric standard deviation (GSD) expresses the dispersion of such a distribution.

All the medians above differ significantly \((p < 0.05)\).
Fig. 4. Plot of median Coulter platelet volumes against subject age.
percentile 97.7 of the controls (Fig. 3, Table 1). Circulating platelet biomass concentrations of the two groups differed less; these were 0.272% for the control median and 0.286% for the Mediterranean group (Table 1).

Platelet counts and Coulter volumes showed no trend with subject age in either group (Fig. 4). However, both the counts and volumes were scattered over a wider absolute and relative range in the Mediterranean subjects ($p < 0.005$; Table 1, Figs. 3 and 4). This greater variance could be traced to an inverse correlation between platelet count and volume within each subject group ($p < 0.001$) and a striking reciprocal relationship within the pooled population (Fig. 5). When size estimates were performed on platelets from citrated PRP, the reciprocal relationship with the platelet count was preserved, but platelet volume was lower by a median factor of 0.78 (GSD = 1.06) (Table 1).

The electron micrograph of Fig. 6 shows typical platelets, red cells, and white cells from the Mediterranean subject of Fig. 2. Microscopic examination of the citrate PRP samples at 22°C showed that the platelets of the macrothrombocyte populations were larger and more globular than the predominantly discoid platelets from Northern European subjects, while the platelets of EDTA-PRP of both groups were spheroidal. When unimodal platelet populations like those of Fig. 2 were compared in stained blood films, the larger platelets within the macrothrombocyte population gave the deceptive impression of a subpopulation of giant platelets. Döhle bodies characteristic of the May-Hegglin anomaly$^6$,$^9$ were never observed in the Mediterranean blood films.

DISCUSSION

The present study shows that healthy Mediterranean and Northern European subjects have a similar peripheral platelet biomass concentration even though
they differ significantly in platelet count and Coulter platelet size. An inverse correlation was demonstrated between the latter parameters within each group and a reciprocal relationship within the pooled sample. This relationship can also be demonstrated with platelet count and size data from subjects with such rare genetic variants as the May-Hegglin anomaly, and it may be expected within every species, since the circulating platelet biomass concentration is a broad evolutionary constant analogous to the hematocrit. However, systematic errors are inherent in most platelet counting methods and in the Coulter volume parameters of Table 1. The cited platelet biomass concentration values are at best proportional to the true volumetric concentrations of platelets in whole blood, a deficiency also inherent in centrifugal thrombocrit estimates unless these are individually corrected for the trapped plasma fraction to give true thrombocytocrits.

The apparent electrical volume of any cell is actually the product of its primary Euclidean volume and an electrical form factor. The latter varies from less than 1.1 for such highly deformable cells as erythrocytes aligned within the hydrodynamic shear field of the Coulter orifice to over 1.5 for inert rigid spherical latex particles. This explains why approximately 1.5-fold higher values were recorded for Coulter volumes when the system was calibrated directly against latex particles (Footnote to Table 1) and largely explains why the apparent volume of sphered EDTA-anticoagulated platelets of this and
several other studies7,9,10,12 is approximately 1.28 times higher than that of citrate-anticoagulated platelets.

Progressive change in platelet shape probably also largely explains the observations interpreted by Bull and Zucker9 as platelet swelling. These workers documented platelet modes with a system calibrated directly against latex spheres and reported that at temperatures below 37°C the electronic size of both citrate and EDTA platelets may take about 2 hr to stabilize. Unfortunately, the precise time course of these changes varies between different subjects and patients, but over the 2-4-hr period used in the present study a temporary or final plateau has generally been reached at 22°C.7 Furthermore, randomization of blood collection and sample coding were used to exclude bias from time-dependent changes in Coulter platelet size.

Differences between the mode, median, and geometric and arithmetic mean of frequency distributions are also important in comparing reports on platelet volumes (Fig. 1). In the present study, as in other published Coulter studies involving healthy subjects and not using hydrodynamic focusing during sizing,7,9,10,12 the ratio between the above parameters of location is approximately 0.747 : 1.00 : 1.00 : 1.165. However, there are differences between subjects, as evidenced by the range of GSD values in Table 1. Differences may also result from biases introduced in the estimation of the parameters from the recorded frequencies. The estimation procedure of Fig. 1A is simple, rapid, and relatively insensitive to data truncation, while that of Fig. 1B incorporates a reasonable test for lognormality, i.e., whether a straight line fits the plot.

Data truncation is also a problem in cell counting. In earlier studies7 the upper end of macrothrombocyte populations was lost from the platelet count because the usually recommended settings for counting platelets on a model B Coulter Counter were used.10-12,17 It was also demonstrated7 that in macrothrombocytopenia larger platelets stratify rapidly within the PRP when platelet-rich plasma is harvested by centrifugation. The present electronic platelet counting method was developed to avoid these problems. Whole blood platelet counts determined by this method on subjects with marked macrothrombocytopenia agreed well with those made via the microscopic platelet counting method of Brecher et al.1 Nevertheless, the numerical blood platelet concentrations estimated on the Northern European group coincide with only some of the platelet count ranges established by other workers on basically Northern European control populations.1,2,7,10-12

The electron-microscopic study from which Fig. 6 is taken confirmed the morphologic features evident under the light microscope, namely that some platelets of macrothrombocyte populations exceed normal red cells in size and that macrothrombocytes are more globular (or have a higher thickness to diameter ratio) under conditions that maintain control platelets in a discoid state.7,18,19 Compared with control thrombocytes, the equatorial microtubular bundle of Mediterranean macrothrombocytes is arranged more haphazardly and the Golgi apparatus of some is more prominent.7,23 These features are also found in other macrothrombocytopenias.18,19

Finally, studies of platelet function and kinetics that may be influenced by size-related properties of platelets should be interpreted with caution in macro-
thrombocytopenia. Variable loss of platelets during centrifugal harvesting of PRP may produce a spurious platelet survival curve, and centrifugal loss of platelets may also exaggerate the thrombocytopenia in certain electronic platelet counting methods. Patients with incidental benign macrothrombocytopenia may, therefore, erroneously be considered to have acute or chronic ITP. On the other hand, individuals with macrothrombocyte populations are also subject to the various pathophysiologic processes affecting the thrombocyte system. Thus, as used here, the term Mediterranean macrothrombocytopenia implies a circulating platelet population of increased size, reduced count, and normal biomass in apparently healthy subjects originating from the Italian or Balkan peninsulas.

ACKNOWLEDGMENT

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REFERENCES

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