PLATELET DYSFUNCTION–EOSINOPHILIA SYNDROME IN PARASITIZED VENEZUELAN CHILDREN

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Abstract. Platelet dysfunction was detected in six children with purpura and eosinophilia. We conducted clinical evaluations, hematologic and platelet function tests, clotting studies (bleeding time, prothrombin time, partial thromboplastin time, thrombin time, factor XIII, factor VIII, and von Willebrand factor), assays for IgG and IgM antibodies to platelets, and a search for stool parasites. Mild bleeding phenomena (echymoses, petechiae, epistaxis, and gingival) were transient. All children showed intestinal parasites and marked eosinophilia (mean count = 2,615.2 cells/μL, 95% confidence interval = 1,259.6–5,429.8). Main abnormalities included prolonged bleeding times (50%) and defective aggregation with collagen (100%) adrenaline (66%), or ADP (66%). Antibodies to platelets were not detected. Anti-parasite therapy reversed the hemorrhagic manifestations and normalized eosinophil counts and platelet alterations. No relationship could be established between excess eosinophils, intensity of bleeding, or type and degree of platelet abnormalities. Thrombocytopenic features mimicked the intrinsic defect of storage pool disease. The possible pathogenic roles of eosinophilia and parasitism are reviewed. This is the first report of this pathologic combination in Latin American children.

INTRODUCTION
Platelet dysfunction of variable clinical relevance is detectable in a wide spectrum of congenital and acquired pathologies of diverse etiology.1 Its peculiar association with hypereosinophilia has been documented almost exclusively in southeast Asia.2–4 In this setting, the eosinophilic proliferation appears intense and the thrombocytopenia is a transitory event expressed in vivo as a purpuric syndrome and in vitro as a dysfunctional state. This intriguing combination, which is unfamiliar in Latin American medicine, is reported for the first time in parasitized Venezuelan children.

PATIENTS AND METHODS
A cohort of six eutrophic otherwise healthy children less than 12 years of age were referred to our hemostasis unit due to mild dermal and mucosal bleeding episodes, mainly represented by variable-size ecchymoses (1.5–8 cm) mostly confined to the upper and lower extremities. These patients with intense eosinophilic responses (> 650 cells/μL) were referred to the parasitology department and had florid intestinal infections. They were referred over a period of 33 months from 1999 to 2001 without any association with the season of the year. Except for patient no. 3 who exhibited a three-year period of protracted recurrent symptoms and cyclical thrombocytopenia, the syndrome lasted a few weeks. No underlying illnesses or exposure to recent drug intake were elicited. Periodic follow-up studies were conducted between 15 days and 30 months post-parasite therapy. Parental permission was obtained for all procedures. The study was reviewed and approved by the Universidad Central de Venezuela and the Research Unit of the José María Vargas School of Medicine.

Hematologic studies. Venous blood was collected into tubes containing EDTA and blood cells were quantified in a Coulter Gen S System 2 analyzer (Coulter Electronics, Hialeah, FL). Platelet morphology was examined on Wright-Giemsa–stained blood smears and leukocytes were counted manually.5 Absolute eosinophils were calculated from the product of the number of leukocytes and the percentage of eosinophils in differential counts.

All coagulation procedures were done on citrated venous blood (10% [v/v] 0.129 M sodium citrate). Platelet-poor plasma (PPP) was obtained by centrifugation at 2,500 × g for 20 minutes at 4°C, and platelet-rich plasma (PRP) was obtained by centrifugation at 200 × g for 10 minutes at 22°C. Thrombin time, prothrombin time, and activated partial thromboplastin time in PPP were determined using a coagulometer (Stago Diagnostica, Asniers, France). Factor XIII was evaluated qualitatively by solubility in 5 M urea, factor VIII by the one-stage method,7 von Willebrand factor antigen by immunoelectrophoresis,8 and ristocetin cofactor activity by the MacFarlane method.9 The Simplate device (Organon Teknika, Durham, NC) was used to determine bleeding times.

Platelet aggregation was read turbidimetrically10 in PRP (Chrono-log PICA aggregometer; Chrono-Log Co, Haver- town, PA). Platelets were adjusted to a concentration of 300 × 10^9/L with autologous PPP and stimulated with 11 μM adrenaline (Pulmobronk, Caracas, Venezuela), 2 μM adenosine diphosphate (Sigma, St. Louis, MO), 2 μg/mL of collagen (Hormon-Chemie, Munich, Germany), and 1.2 or 0.6 mg/mL of ristocetin (H. Lundbeck, Paramus, NJ). Studies were conducted on patients and a healthy control 30 minutes after preparing the PRP and were completed within two hours of blood collection. Results were expressed as the percentage change in optical density and compared with previously established normal patterns. Abnormal results were verified with the same sample. In one experiment, a preincubated (37°C for 30 minutes) 1:1 mixture of normal PRP with PPP from patient 1 was tested with adrenaline and collagen. IgG and IgM antibodies to platelets11 were identified by flow cytometry (FACSort; Becton Dickinson, Franklin Lakes, NJ).

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Search for parasites in stool. All subjects, and/or their parents, were instructed to collect stool samples. A minimum of three samples from different days was requested. Coprologic methods consisted of direct observation of fresh stool, the Kato concentration technique, and a slightly modified Baermann thermotrophic test. These were complemented with fecal cultures in agar-petri dishes as an additional tool for detecting *Strongyloides stercoralis* and hookworms.

### RESULTS

The main clinical characteristics and hematologic data for the six patients (two boys and four girls, median age = 8.6 years) are shown in Table 1. The mean eosinophil count was 2,615.2 cells/µL (95% confidence interval [CI] = 1,259.6–5,429.8). All patients had with spontaneous ecchymoses; two reported mild epistaxis, one reported petechiae, and one complained of gum bleeding. Platelet morphology was adequate for size, shape, and granulation, and electronically determined platelet volumes fluctuated within normal ranges. The results of clotting assays and platelet function tests are shown in Table 2. Bleeding times were prolonged in three patients. Deficient or absent aggregability with collagen was constant; four patients did not react adequately to stimulation with adrenaline or ADP, with a common absence of second waves (Figures 1B and 2B), whereas reactivity to ristocetin remained normal. Concentrations of von Willebrand antigen and its ristocetin cofactor were not affected. The PPP derived from patient 1 failed to inhibit adrenaline- and collagen-induced platelet aggregation of PRP collected from a healthy donor. Circulating antibodies to platelets were not detected.

The salient details of the prevalent parasitism are shown in Table 1. Eosinophil counts were much higher in those harboring more than one parasite (> 2,300 cells/µL), and strikingly elevated in those co-infected with *Necator americanus* and *Ascaris lumbricoides* (> 3,000 cells/µL). In a simultaneous fashion within 10–21 days after initiating treatment with appropriate medications, purpuric lesions dissipated without further recurrences, all platelet abnormalities disappeared (Figures 1C and 2C), whereas reactivity to ristocetin remained normal. Concentrations of von Willebrand antigen and its ristocetin cofactor were not affected. The PPP derived from patient 1 failed to inhibit adrenaline- and collagen-induced platelet aggregation of PRP collected from a healthy donor. Circulating antibodies to platelets were not detected.

### DISCUSSION

Acquired platelet dysfunction coexisting with eosinophilia, which has been known since 1975, became a new pathologic

### Table 1
Clinical data, hematologic parameters, and parasitologic data of six patients*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical presentation</th>
<th>Time† (months)</th>
<th>WBCs (10³/L)</th>
<th>Eosinophils (µL)</th>
<th>PLT (10³/L)</th>
<th>MPV (fL)</th>
<th>Parasite</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>11</td>
<td>Ecchymoses</td>
<td>1</td>
<td>10.3</td>
<td>2,369</td>
<td>184</td>
<td>7.7</td>
<td><em>Ascaris lumbricoides,</em> <em>Blastocystis hominis</em></td>
<td>Piperazine, Mebendazole</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>12</td>
<td>Ecchymoses</td>
<td>3</td>
<td>8.7</td>
<td>1,479</td>
<td>299</td>
<td>6.8</td>
<td><em>Necator americanus,</em> <em>Trichuris trichiura</em></td>
<td>Tiabendazole, Metronidazole</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>12</td>
<td>Ecchymoses Epistaxis</td>
<td>36</td>
<td>8.5</td>
<td>1,615</td>
<td>86</td>
<td>7.0</td>
<td><em>Strongyloides stercoralis,</em> <em>Giardia intestinalis,</em> <em>Blastocystis hominis</em></td>
<td>Tiabendazole, Metronidazole</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>3</td>
<td>Ecchymoses</td>
<td>0.5</td>
<td>5.4</td>
<td>1,656</td>
<td>247</td>
<td>7.2</td>
<td><em>Necator americanus,</em> <em>Ascaris lumbricoides,</em> <em>Enterostrus verniculatus</em></td>
<td>Mebendazole</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>5</td>
<td>Ecchymoses Epistaxis</td>
<td>2</td>
<td>21.3</td>
<td>3,834</td>
<td>351</td>
<td>7.8</td>
<td><em>Ascaris lumbricoides,</em> <em>Trichuris trichiura</em></td>
<td>Piperazine, Tiabendazole, Mebendazole</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>9</td>
<td>Ecchymoses</td>
<td>0.75</td>
<td>21.2</td>
<td>8,904</td>
<td>188</td>
<td>7.5</td>
<td><em>Necator americanus,</em> <em>Ascaris lumbricoides,</em> <em>Trichuris trichiura</em></td>
<td>Piperazine, Tiabendazole</td>
</tr>
</tbody>
</table>

** RV = reference value.

### Table 2
Hemostasis evaluation of six patients*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bleeding time (sec)</th>
<th>Pre-ADR (%)</th>
<th>Pre-ADP (%)</th>
<th>Pre-COL (%)</th>
<th>Pre-RIS (%)</th>
<th>FvWAg/vWRCo (U/dL)</th>
<th>PAb IgG/IgM</th>
<th>Post-treatment eosinophils (µL)</th>
<th>Post-ADR (%)</th>
<th>Post-COL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180</td>
<td>5</td>
<td>70</td>
<td>5</td>
<td>81</td>
<td>70/80</td>
<td>Neg</td>
<td>584</td>
<td>69</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>720</td>
<td>5</td>
<td>50</td>
<td>50</td>
<td>75</td>
<td>100/100</td>
<td>Neg</td>
<td>810</td>
<td>69</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>480</td>
<td>68</td>
<td>65</td>
<td>60</td>
<td>75</td>
<td>120/100</td>
<td>Neg</td>
<td>456</td>
<td>83</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>240</td>
<td>31</td>
<td>56</td>
<td>37</td>
<td>75</td>
<td>140/100</td>
<td>Neg</td>
<td>345</td>
<td>79</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 600</td>
<td>25</td>
<td>62</td>
<td>31</td>
<td>85</td>
<td>80/65</td>
<td>Neg</td>
<td>563</td>
<td>68</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>75</td>
<td>81</td>
<td>0</td>
<td>81</td>
<td>100/72</td>
<td>Neg</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RV</td>
<td>&lt; 360</td>
<td>57–100</td>
<td>75–120</td>
<td>75–125</td>
<td>75–125</td>
<td>50–150</td>
<td>Neg</td>
<td>&lt; 650</td>
<td>57–100</td>
<td>75–120</td>
</tr>
</tbody>
</table>

* WBCs = white blood cells; PLT = platelet; MPV = mean platelet volume; RV = reference values.

† Time from start of symptoms.
Figure 1. Platelet aggregation with adrenaline in a control subject (A) and in case 4 before (B) and after (C) antihelminthic treatment.

Figure 2. Platelet aggregation with collagen in a control subject (A) and in case 1 before (B) and after (C) antihelminthic treatment.
entity after 1979. It predominates in native children and young adults in Singapore, Thailand, China, and India, and affects some infants visiting southeast Asia. In Latin America, it is either very rare, unreported, or undiagnosed. The six patients in our study exhibited manifestations akin to their Asian counterparts, but had milder bleeding.

In vitro platelet dysfunction was constant, highlighted by defective or absent collagen-induced platelet aggregation. As previously reported, the wide range of eosinophil responses (1,479–8,904 cells/μL) (Table 1) were not related to sites or extent of bleeding or the type or magnitude of the thrombocytopathy, but were proportional to the type and extent of parasitic loads. Unexplained thrombocytopenia may also accompany the eosinophilia–thrombocytopathy syndrome, as exemplified in patient no. 3, a boy infected with *S. stercoralis* who had a low platelet count (86 × 10^9/L) that returned to a normal level shortly after treatment with an anthelmintic. Two patients developed reinfections without recurrence of bleeding, eosinophilia, or platelet abnormalities, strengthening the belief that platelet damage is more related to eosinophil excess than to parasitic stimuli.

Although bronchial asthma and hay fever–related eosinophilias may also indicate defective platelet aggregability and prolonged bleeding times in the absence of hemorrhages, when purpura does appear parasitism is still assumed. Often leading to blind therapy, Specific antiparasitic therapy eliminated all in vivo and in vitro symptoms. In two other studies, these interventions were not given because spontaneous recoveries and a low parasitic incidence (56%) were reported. However, these conclusions remain ambiguous, since levels of eosinophils were regularly elevated (86%) and coprologic methods were not specified. These contrasting views can be reconciled because pre-existing natural immune mechanisms may kill or expel parasites. In such a scenario, the syndrome may become limited, undetectable, or even entirely prevented without medical treatment.

Various platelet disturbances could be attributed to eosinophil secretion of platelet-activating factor (PAF) or other active proteins, circulating antibodies, or specific immune complexes that are not detectable by standard flow cytometry. Platelet degranulation findings were crucial in categorizing the thrombocytopathy as a storage pool deficiency type. Such an intrinsic anomaly is supported by the experiment in which plasma from patient 1 did not inhibit normal aggregation.

Since not all individuals harboring parasites nor all those with eosinophilia are bleeders, in both conditions a surreptitious thrombocytopathy is worth considering. Future investigations may also clarify if parasitic-induced eosinophils possess adverse platelet-specific effects not encountered in those arising in allergic illnesses. The influence of genetic traits or environmental factors could also be facilitating contributors. A plasmatic deficiency of PAF acetylhydrolase, a PAF-degrading enzyme, is implicated in certain pathologic states affecting restricted ethnic groups.

In conclusion, these Venezuelan children with platelet dysfunction eosinophilia-parasite syndrome suggest that its apparent predominance in southeast Asia may represent a more widespread tropical phenomenon. We anticipate that platelet function testing in parasite-eosinophilia populations may uncover many unsuspected cases.

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