Pathogenesis of Thrombocytopenia in Cyanotic Congenital Heart Disease

Michael C. Lill, MD, Joseph K. Perloff, MD*, and John S. Child, MD

Although a significant minority of patients with cyanotic congenital heart disease (CCHD) are thrombocytopenic, the pathogenesis and prevalence have not been established. This study was designed to address these 2 issues. We included 105 patients with CCHD (60 men and 45 women; aged 21 to 54 years). Systemic arterial oxygen saturations were 69% to 78%. Hematocrits were 62% to 74% with normal iron indexes. In 26 of 105 patients (25%), platelet counts were <100 × 10^9/L. The diagnosis was Eisenmenger syndrome in all 26 patients with thrombocytopenia. Platelet production was determined by flow cytometric reticulated platelet counts. Megakaryocyte mass was determined indirectly by thrombopoietin levels. Disseminated intravascular coagulation was based on prothrombin time, activated partial thromboplastin time, and D-dimers. Platelet activation was determined by levels of platelet factor 4 and β-thromboglobulin. Reference ranges were derived from 20 normal acyanotic controls. A reduction in absolute reticulated platelet counts implied decreased platelet production (p <0.001). Normal thrombopoietin levels implied normal megakaryocyte mass. Normal prothrombin time, activated partial thromboplastin time, and D-dimers excluded disseminated intravascular coagulation. Normal platelet factor 4 and β-thromboglobulin indicated absent or minimal platelet activation. Twenty-five percent of the patients with CCHD were thrombocytopenic because platelet production was decreased despite normal megakaryocyte mass. We hypothesized that right-to-left shunts deliver whole megakaryocytes into the systemic arterial circulation, bypassing the lungs where megakaryocytic cytoplasm is fragmented into platelets, thus reducing platelet production. In conclusion, platelet counts in CCHD appear to represent a continuum beginning with low normal counts and ending with thrombocytopenia. © 2006 Elsevier Inc. All rights reserved. (Am J Cardiol 2006;98:254–258)

A hemorrhagic tendency in cyanotic congenital heart disease (CCHD) was initially attributed to an increase in tissue vascularity, but co-existing hemostatic defects were subsequently identified and attributed to thrombocytopenia,1–4 shortened platelet survival,2 and deficient von Willebrand multimers.5 Although thrombocytopenia is well recognized in CCHD, its incidence and pathogenesis have not been established. In addressing these 2 issues, we sought to determine whether the cause of thrombocytopenia was decreased platelet production, decreased megakaryocyte production, increased platelet destruction, or increased platelet activation.

Methods

Study population: The UCLA Institutional Review Board approved the study. Subjects gave written informed consent. We consecutively recruited 105 cyanotic patients from the Adult Congenital Heart Disease Clinic (60 men and 45 women; aged 21 to 54 years, mean ± SD 41 ± 5).

Fourteen of the 105 patients had previously been included in a study of Eisenmenger syndrome,6 in which computerized tomography detected large proximal pulmonary arterial thrombi that might have served as sites of platelet consumption, but platelet counts in these 14 patients did not differ from counts in the remaining 91 study patients. No patient was taking or was known to have taken an antiplatelet or anti-inflammatory agent, and none had either Noonan or Down syndrome, in which thrombocytopenia occasionally occurs. None had abnormal liver function tests or hepatosplenomegaly.

Study protocol: Evaluation included a history, physical examination, 12-lead scalar electrocardiogram, postero-anterior/lateral chest roentgenogram, and a transthoracic echocardiogram with color flow imaging and Doppler interrogation. Eisenmenger syndrome was defined as a nonrestrictive communication at the atrial, ventricular, or great arterial level with suprasystemic pulmonary vascular resistance and a right-to-left shunt.2 Patients were not catheterized because echocardiography securely established both the anatomic and physiologic diagnoses.3 No etiology of pulmonary hypertension or pulmonary vascular disease other than Eisenmenger syndrome was identified.

Blood samples and transcutaneous pulse oximetry systemic arterial oxygen saturations were secured at room air...
...technique, and Thrombopoietin (Tpo) levels were measured with a quantitative sandwich enzyme immunoassay technique, and Thrombopoietin (Tpo) levels were measured with a quantitative sandwich enzyme immunoassay technique. Horigome et al attributed platelet microparticles to platelet factor 4 (PF4), and activated partial thromboplastin time (aPTT), D-dimers, iron indexes were determined by standard laboratory methods. Normal PT, aPTT, and D-dimers excluded disseminated intravascular coagulation. Normal or only slightly elevated PF4 and βTG indicated minimal or absent platelet activation, although Horigome et al attributed platelet microparticles to shear-induced activation. Automated electronic particle counts were used (Coulter Electronics, Hialeah, Florida) because microhematocrit centrifugation in the presence of erythrocytosis results in plasma trapping and falsely elevated hematocrits. Thrombocytopenic platelet counts were reconfirmed before inclusion in the study. Iron indexes based on mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were done routinely. Although folic acid and/or vitamin B deficiency may mask hypochromia and microcytosis and although homocystine is a serologic marker of these deficiency states, assays were not done because this information was published after our study was completed. Direct bone marrow biopsy staining was proscribed by the institutional review board. In accordance with established recommendations, no patient was phlebotomized.

Reference ranges were based on 20 controls that consisted of normal volunteers whose age and gender distributions were similar to the 26 thrombocytopenic patients with CCHD. Normal volunteers were chosen as controls because in acyanotic not operated on congenital heart disease patients, platelet activation might be affected by turbulent blood flow or increased endothelial shear stress, and in patients with acyanotic postoperative congenital heart disease patients, endothelial and cardiac valve surfaces may not be covered by normal tissue.

**Results**

Results are presented as mean ± SD. In the 79 nonthrombocytopenic patients with CCHD, platelet counts were 125 × 10^9/L to 332 × 10^9/L (mean 155 ± 12), which includes the lower range of normal. In the 26 thrombocytopenic patients (25%), platelet counts were <100 × 10^9/L (mean 68 ± 6.2), which is below the lower range of normal. In 60 acyanotic not operated on adults attending the same clinic, platelet counts were >240 × 10^9/L. Twelve thrombocytopenic patients were women and 14 were men aged 21 to 51 years (mean 42 ± 5). All thrombocytopenic patients had Eisenmenger syndrome, diagnosed echocardiographically, represented by nonrestrictive ventricular septal defect in 12, truncus arteriosus in 5, single ventricle in 3, double-outlet right ventricle in 3, inlet ventricular septal defect in 2, and nonrestrictive patent ductus arteriosus in 1.

**Discussion**

Four pathogenetic mechanisms are potentially responsible for thrombocytopenia in CCHD: (1) decreased platelet production, (2) decreased megakaryocyte production, (3) increased platelet destruction, and (4) increased platelet activation. In 1893, Aschoff proposed that megakaryocytes originated in bone marrow, migrated into the bloodstream,
and because of their massive size, lodged in the pulmonary capillary bed where platelets were produced. We hypothesized that the pathogenesis of thrombocytopenia in CCHD reflected the right-to-left shunts that necessarily deliver portions of these large formed elements from the systemic venous into the systemic arterial circulation, thus circumventing the lungs and reducing the number of platelets produced in the pulmonary bed.\textsuperscript{15–17} In accord with this hypothesis are data from high-altitude residents who have normal platelet counts despite hypoxemia and erythrocytosis because they have no right-to-left shunts to deliver megakaryocyte into the systemic circulation.\textsuperscript{18} Shunted megakaryocytes release platelets at systemic impact sites, but the thrombocytes so formed remain in situ without contributing to platelet counts.\textsuperscript{15,16}

There is a uniform consensus that platelets are derived from bone marrow megakaryocytes, which are specialized precursor cells derived from pluripotential hematopoietic progenitors whose sole function is to produce platelets and release them into the circulation.\textsuperscript{10} However, the mechanisms by which thrombocytes are formed and released from these precursor cells—platelet biosynthesis—continue to engage hematologists after more than a century of interest and investigation.\textsuperscript{10,19} Three models of platelet biogenesis have been proposed: (1) platelet budding, (2) cytoplasmic fragmentation, and (3) proplatelet formation.\textsuperscript{10} The first proposal argues that platelets are shed from blebs on the surface of megakaryocytic cytoplasm, but electron microscopy has not detected platelet organelles in these blebs.\textsuperscript{10} The second proposal contends that platelets are released by fragmentation of megakaryocyte cytoplasm along demarcating membrane system fracture lines, but platelet fields delineated by the demarcating membrane system do not exhibit structural characteristics of platelets.\textsuperscript{10} The third proposal postulates that long, thin cytoplasmic processes emanate from megakaryocytes and contain platelet-sized beads that fragment into platelets.\textsuperscript{10} Which of these 3 proposals of platelet formation is correct is not relevant to our pathogenetic hypothesis of thrombocytopenia in CCHD. What is relevant are 2 essential observations, namely: (1) that megakaryocytes normally inhabit the systemic venous circulation\textsuperscript{20} and (2) that platelet biogenesis is not
been attributed. Shunted megakaryocytes that lodge in the capillaries of the digits to which clubbing and hypertrophic osteoarthropathy have been attributed. However, the absolute number of reticulated platelets in our thrombocytopenic patients with CCHD was significantly reduced (p <0.001), which is more consistent with decreased platelet production than with dilution caused by increased plasma volume.

Tpo, a cytokine that binds a megakaryocyte-specific receptor, is constitutively produced in the liver, stimulates megakaryopoiesis, and promotes growth and maturation of megakaryocytic precursors. The principal regulator of Tpo levels is uptake by its cognate receptor on megakaryocytes and platelets. Normal Tpo levels in the presence of low platelet counts suggest that megakaryocyte mass is also normal. A relation between Tpo levels and megakaryocyte mass has been based on analysis of patients with idiopathic thrombocytopenic purpura who have decreased platelet counts without decreased megakaryocyte mass. Whether and by what means hypoxia affects Tpo production is poorly understood, but hypoxemic erythrocytotic adults acclimatized to high altitude have normal platelet counts despite oxygen saturations and elevated hematocrits in the same range as the cyanotic patients in our study, suggesting that chronic hypoxia does not affect Tpo production.

Thrombocytopenia in CCHD appears to represent the far end of a continuum that begins with low normal platelet counts and ends with thrombocytopenia. Consistent with this proposal is the inverse relation between platelet counts and the magnitude of right-to-left shunts as judged by the hematocrit and systemic arterial oxygen saturation, that is, the larger the right-to-left shunt, the lower the systemic arterial oxygen saturation, the higher the hematocrit, and the lower the platelet count (Figure 4). These relations were recently reported and were confirmed in our study.

Our observations have a potential impact on the management of patients with CCHD. Bleeding accompanied by thrombocytopenia can be treated with platelet transfusion, and the response of thrombocytopenic platelet counts to phlebotomy has therapeutic implications. Platelet counts typically increase dramatically within hours after phlebotomy, especially when hematocrits are ≥65%. The mechanism(s) responsible for the increase are unknown, but the rapidity suggests platelet release from a reservoir rather than as a response to an hematopoietic cytokine growth stimulus. Whatever the mechanism(s), the response can be used to therapeutic advantage using preoperative phlebotomy in thrombocytopenic patients with CCHD. Intraoperative blood loss cannot be relied on to induce the desired increase in platelet counts because of uncertainty that the loss will not be sufficient to stimulate the desired increase in platelets and because of uncertainty regarding the time course between intraoperative hemorrhage and the desired increase in platelet count.

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29. Emmons RV, Reid DM, Cohen RL, Meng G, Young NS, Dunbar CE, Shulman NR. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. *Blood* 1996;87:4068–4071.