

Pathogenesis of Thrombocytopenia in Cyanotic Congenital Heart Disease

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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 $<100 \times 10^9/L$. 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 system 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,⁴ 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 \pm SD 41 \pm 5).

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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

Table 1

Reticulated platelet counts in normal acyanotic controls versus thrombocytopenic patients with cyanotic congenital heart disease

	n	Platelet Count	Reticulated Platelet Count
Controls	20	246 ± 15	4,455 ± 325
Patients	26	61 ± 5	1,657 ± 443*

* p <0.0001.

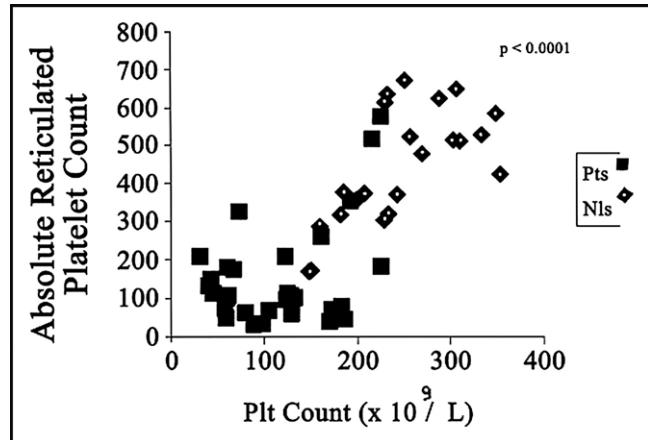


Figure 1. Absolute reticulated platelet counts in patients with CCHD (Pts) versus normal controls (Nls).

temperatures in a basal state at the same time of day in the same familiar clinic environment. In 6 menstruating women, blood samples were rescheduled. To avoid platelet activation, samples were drawn gently into an ethylenediaminetetraacetic acid (1.5g/ml) vacutainer tube (Becton Dickinson, Rutherford, New Jersey). Because electronic platelet counts are occasionally inaccurate in the presence of thrombocytopenia, electronic counts were supplemented by the flow cytometric method.⁷ Thrombocytopenia was defined as platelet counts $<100 \times 10^9/L$. Direct inspection of blood smears excluded platelet aggregation as an artifactual cause of thrombocytopenia and excluded platelet microparticles as an artifactual cause of increased platelet counts.⁸

Platelet production was assessed by flow cytometric quantification of reticulated platelets.⁹ Decreased production was based on significant reductions in absolute reticulated platelet counts ($p <0.001$) (Table 1; Figures 1 and 2). Thrombopoietin (Tpo) levels were measured with a quantitative sandwich enzyme immunoassay technique,^{10,11} and normal megakaryocyte mass was inferred from normal Tpo levels^{10,11} (Table 2 and Figure 3). Prothrombin time (PT), activated partial thromboplastin time (aPTT), d-dimers, platelet factor 4 (PF4), and β thromboglobulin (β TG)^{4,12} were determined by standard laboratory methods. Normal PT, aPTT, and d-dimers excluded disseminated intravascular coagulation. Normal or only slightly elevated PF4 and β TG indicated minimal or absent platelet activation, although Horigome et al⁸ 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.³ 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,¹³ 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.³

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 $125 \times 10^9/L$ to $332 \times 10^9/L$ (mean 155 ± 12), which includes the lower range of normal. In the 26 thrombocytopenic patients (25%), platelet counts were $<100 \times 10^9/L$ (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 $>240 \times 10^9/L$. Twelve thrombocytopenic patients were women and 14 were men aged 21 to 51 years (mean 42 ± 5). All thrombocytopenic patients had Eisenmenger syndrome² 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.

Transcutaneous systemic arterial oxygen saturations in the 105 patients were 69% to 78% (mean $74 \pm 5\%$). In the patient with patent ductus arteriosus and differential cyanosis (reversed shunt), arterial oxygen saturations were determined in a great toe. Hematocrits were 62% to 74% (mean $67 \pm 5.3\%$). Iron indexes were normal.

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, Aschoff¹⁴ proposed that megakaryocytes originated in bone marrow, migrated into the bloodstream,

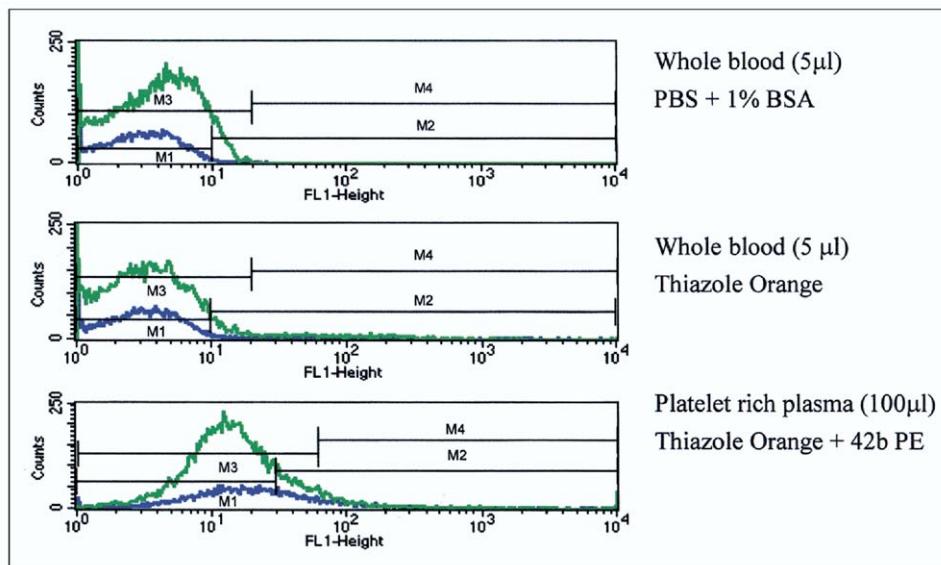


Figure 2. Fluorescence histograms of reticulated platelet content. Whole blood and platelet rich plasma are shown from healthy volunteers (upper curves, blue lines) and from patients with cyanotic congenital heart disease (lower curves, green lines).

Table 2

Levels of platelet activation markers in thrombocytopenic patients with cyanotic congenital heart disease

	BTG (n = 26)	PF4 (n = 26)	Tpo (n = 26)
Reference range	10–78	0–35	ND–196 pg/ml
Patients	68 ± 6	30 ± 4	178 ± 36

ND = near end of reference range.

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.^{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.¹⁸ Shunted megakaryocytes release platelets at systemic impact sites, but the thrombocytes so formed remain in situ without contributing to platelet counts.^{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.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.^{10,19} Three models of platelet biogenesis have been proposed: (1) platelet budding, (2) cytoplasmic

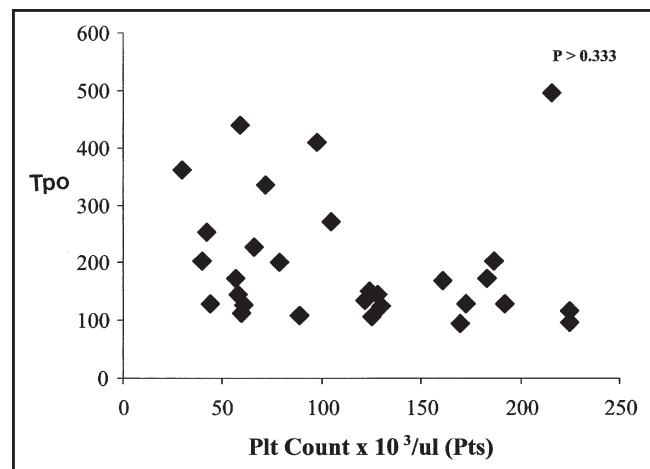


Figure 3. Tpo levels versus platelet counts in patients with CCHD. There is no significant relation between the 2 variables.

fragmentation, and (3) protoplatelet formation.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.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.¹⁰ The third proposal postulates that long, thin cytoplasmic processes emanate from megakaryocytes and contain platelet-sized beads that fragment into platelets.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 circulation²⁰ and (2) that platelet biogenesis is not

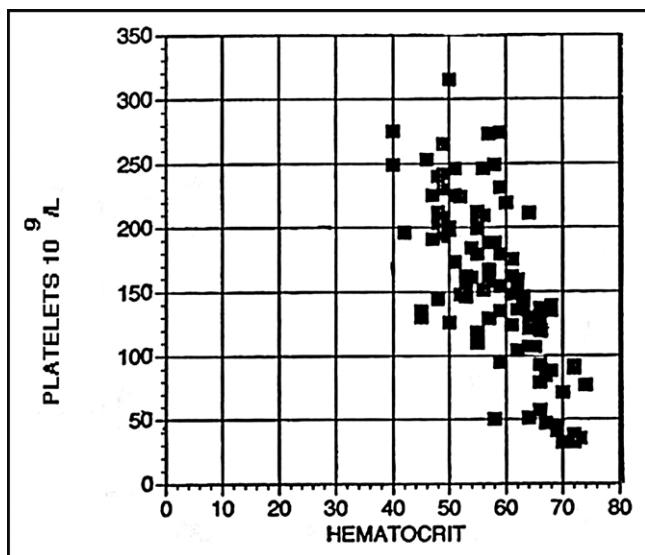


Figure 4. Platelet counts versus hematocrits in patients with CCHD.

confined to the bone marrow.^{17,19,20} In addition, cultured human megakaryocytes form functional platelets in vitro, indicating that bone marrow is not a necessary environment for platelet formation.²¹

Transmigration of whole megakaryocytes through the marrow-blood barrier via 3 to 6 μm endothelial apertures has been identified in rabbits by electron microscopy.²² A chemokine receptor is believed to govern retention of immature megakaryocytes in the marrow, while permitting mature megakaryocytes to enter the systemic venous circulation.¹⁰ An estimated 250,000 megakaryocytes reach the pulmonary vascular bed every hour,^{23–25} and platelet counts are higher in pulmonary veins than in pulmonary arteries, evidence that the pulmonary circulation is a major site of platelet formation.^{24,25} The relative percentages of platelets derived from megakaryocytes in the lungs versus the bone marrow remain to be established.^{23,24}

Megakaryocytes that lodge in the capillaries of the digits and periostium release platelet-derived growth factor and transforming growth factor- β , the cytokines and mitogens to which clubbing and hypertrophic osteoarthropathy have been attributed.¹⁶ Shunted megakaryocytes that lodge in glomerular capillaries release the same cytokines and mitogens that are responsible for the nonvascular glomerular abnormality in CCHD.²⁶

The reliability of reticulated platelet counts as a measure of the number of circulating young platelets has been debated.⁷ 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.^{10,11,27} The principal regulator of

Tpo levels is uptake by its cognate receptor on megakaryocytes and platelets.²⁷ Normal Tpo levels in the presence of low platelet counts suggest that megakaryocyte mass is also normal.²⁷ 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.^{27,28} Whether and by what means hypoxia affects Tpo production is poorly understood,¹⁰ but hypoxic erythrocytic 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,¹⁸ suggesting that chronic hypoxia does not affect Tpo production.

Normal PT, aPTT, and D-dimers excluded increased platelet destruction.²⁹ Had platelets been destroyed by increased endothelial shear stress in response to the abnormal rheology of erythrocytosis,³⁰ there should have been evidence of platelet activation, which was minimal or absent based on normal or only slightly elevated PF4 and β TG.

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 reported⁸ 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 $\geq 65\%$.^{1,3} 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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1. Maurer HM, McCue CM, Robertson JC, Haggins JC. Correction of platelet dysfunction and bleeding in cyanotic congenital heart disease by simple red cell volume reduction. *Am J Cardiol* 1975;35:831–835.
2. Niwa K, Perloff JK, Kaplan S, Child JS, Miner PD. Eisenmenger syndrome in adults. Ventricular septal defect, truncus arteriosus, and univentricular heart. *J Am Coll Cardiol* 1999;34:223–232.
3. Perloff JK, Child JS. Congenital Heart Disease in Adults. 2nd Ed. Philadelphia: WB Saunders Co, 1998;91,199,201,207.
4. Peters AM, Rozkovec A, Bell RN, Halladie-Smith KA, Goodwin JF, Lavender JP. Platelet kinetics in congenital heart disease. *Cardiovasc Res* 1982;16:391–397.
5. Territo MC, Perloff JK, Rosove MH, Moake JL, Runge A. Acquired von Willebrand factor abnormalities in adults with congenital heart disease. *Clin Appl Thrombosis/Hemostasis* 1998;4:257–261.
6. Perloff JK, Hart EM, Greaves SM, Miner PD, Child JS. Proximal pulmonary arterial and intrapulmonary radiologic features of Eisenmenger syndrome and primary pulmonary hypertension. *Am J Cardiol* 2003;92:182–187.
7. Chavda N, Mackie IJ, Porter JB. Rapid flow cytometric quantification of reticulated platelets in whole blood. *Platelets* 1996;7:189–194.
8. Horigome H, Hiramatsu Y, Shigeta O, Nagawasa T, Matsui A. Over-production of platelet microparticles in cyanotic congenital heart disease with polycythemia. *J Am Coll Cardiol* 2002;39:1072–1077.
9. Rinder HM, Munz UJ, Ault KA, Ault HA, Bonan JL, Smith BR. Reticulated platelets in the evaluation of thrombopoietic disorders. *Arch Pathol Lab Med* 1993;117:606–610.
10. Michelson AD, ed. Platelets. New York: Academic Press, 2002;21–32.
11. Kaushansky K. Thrombopoietin: the primary regulator of platelet production. *Blood* 1995;86:431.
12. Hammond WP, Wun T, Kaplan A. High concentrations of thrombopoietin activates platelets in vitro. *Clin Appl Thrombosis/Hemostasis* 1998;4:170–178.
13. Kaemmerer H, Fratz S, Braun SL, Loelling K, Eiken A, Brodherr-Heberlein S, Pietrzik K, Hess J. Erythrocyte indices, iron metabolism, and hyperhomocysteinemia in adults with cyanotic congenital heart disease. *Am J Cardiol* 2004;94:825–828.
14. Aschoff L. Ueber capillare embolie von riesenkernhaltigen zellen. *Arch Pathol Anat Physiol* 1893;134:11–14.
15. Slater DN, Trowbridge EA, Martin JF. The megakaryocyte in thrombocytopenia: a microscopic study which supports the theory that platelets are produced in the lungs. *Thromb Res* 1983;31:163–176.
16. Dickinson CJ. Etiology of clubbing and hypertrophic osteoarthopathy. *Eur J Clin Invest* 1993;23:330–338.
17. Trowbridge EA, Martin JF, Slater DN. Evidence for a theory of physical fragmentation of megakaryocytes, implying that all platelets are produced in the pulmonary circulation. *Thromb Res* 1982;28:461–475.
18. Hudson JG, Bowen AL, Navia P, Rios-Dalenz J, Pollard AJ, Williams D, Heath D. The effects of high altitude on platelet counts, thrombopoietin and erythropoietin levels in young Bolivian airmen visiting the Andes. *Int J Biometeorol* 1999;43:85–90.
19. Owaga M. Differentiation and proliferation of hematopoietic stem cells. *Blood* 1991;81:2844–2853.
20. Behnke O, Forer A. From megakaryocytes to platelets: platelet morphogenesis takes place in the blood stream. *Eur J Hematol* 1998;60:3–24.
21. Choi E, Nichol JL, Hokom MM, Hornkohl AC, Hunt P. Platelets generated in vitro from protoplatelet-displaying human megakaryocytes are functional. *Blood* 1995;85:391–401.
22. Travassoli M, Aoiki M. Migration of whole megakaryocytes through the marrow-blood barrier. *Br J Haematol* 1981;48:25–29.
23. Scheinin T, Koivuneimi A. Megakaryocytes in the pulmonary circulation. *Blood* 1963;22:82–87.
24. Levine R, Eldor P, Shoff S, Kirwin D, Tenza D, Cramer E. Circulating megakaryocytes. Delivery of large numbers of intact, mature megakaryocytes to the Lungs. *Eur J Hematol* 1993;51:233–246.
25. Pedersen N. Occurrence of megakaryocytes in various vessels and their retention in the pulmonary capillaries in man. *Scand J Haematol* 1978;21:269–275.
26. Perloff JK, Latta H, Barsotti P. Pathogenesis of the glomerular abnormality in cyanotic congenital heart disease. *Am J Cardiol* 2000;86:1198–2004.
27. Nagasawa T, Hasegawa Y, Shimizu S, Kawashima Y, Nishimura S, Susu K, Mukai H, Hori M, Komono T, Kojima H, et al. Serum thrombopoietin level is mainly regulated by megakaryocyte mass rather than platelet mass in human subjects. *Br J Haematol* 1998;101:242–244.
28. Koike Y, Yoneyama A, Shirai J. Evaluation of thrombopoiesis in thrombocytopenic disorders by simultaneous measurements of reticulated platelets of whole blood and serum thrombopoietin concentrations. *Thromb Haemost* 1998;79:1106–1110.
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.
30. Kohler A, Sun D, Kaley G. Role of shear stress and endothelial prostaglandins in flow and viscosity-induced dilatation in vivo. *Circulation Res* 1993;72:1276–1284.