

### Blood cell diseases and thrombosis

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**Background and Objectives.** In recent years knowledge about thrombophilia and the mechanisms underlying the pathogenesis of thrombosis has increased greatly. Nevertheless the role of leukocytes and red cells in thrombogenesis is not well established and is probably underestimated.

**Evidence and information sources.** The contribution of leukocytes and red cells to thrombogenesis has been reviewed. Moreover, the prevalence of thrombosis as a complication of hematologic diseases has been examined. The authors are involved in the investigation and management of acute and chronic hematologic diseases as well as in investigation of thrombophilia. Pub-Med was employed as a source of information.

**State of the art.** Thrombosis is a major problem in myeloproliferative disorders such as polycythemia vera and essential thrombocythemia. A clonal involvement of megakaryocytopoiesis resulting in elevated levels of platelet-specific proteins, increased thromboxane generation, and expression of activation-dependent epitopes on the platelet surface is regarded as the main origin of thromboembolism; nevertheless, activation of leukocytes and the consequent release of elastase and alkaline phosphatase could play an important role, determining endothelial damage. Thrombosis is a relevant problem in some hemolytic anemias such as paroxysmal nocturnal hemoglobinuria and drepanocytosis. Thrombotic events in hemolytic anemias with membrane defects have been attributed, at least in part, to hypercoagulability related to the exposure of phosphatidylserine of red cell membrane activating plasma prothrombinase and supplying a procoagulant phospholipid anionic surface. A moderate but well-established risk for thrombosis occurs in acute promyelocytic leukemia and acute lymphoblastic leukemia; this risk could be increased by antileukemic drugs affecting the procoagulant activity of cells and the production of coagulation inhibitors from the liver.



**haematologica** 2001; 86:1236-1244

[http://www.haematologica.it/2001\\_12/1236.htm](http://www.haematologica.it/2001_12/1236.htm)

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**Perspectives.** Thrombotic complications during hematologic diseases other than thrombophilia due to plasma alteration could be decreased not only by anticoagulant and antiaggregating agents but also by drugs inhibiting activation of leukocytes and red cells and their interaction with platelets.

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Key words: thrombosis, chronic myeloproliferative diseases, acute leukemia, red cell membrane disorders.

A triad of factors, including vessel wall damage, reduction of blood flow and alterations of blood was proposed in 1860 by Virchow to explain the pathogenesis of thrombus formation. The basic principles of this triad have stood the test of time. Interactions between plasma and cellular components of the blood and damaged vessel wall promote the activation of the hemostatic process and ultimately thrombosis. On the other hand, thrombi are composed of fibrin and blood cells. The relative percentage of each cellular type with respect to fibrin is influenced primarily by hemodynamic factors, thus explaining why the composition of arterial thrombi differs from that of venous thrombi. An arterial thrombus develops in a condition of high blood flow and is primarily composed of platelet aggregates in a reticulum of fibrin. A venous thrombus develops in a condition of low blood flow or stasis and is primarily composed of red cells in an abundant quantity of fibrin with relatively few platelets. Some leukocytes are found early during thrombus formation as already noticed by Bizzozzero,<sup>1</sup> others are recruited by chemotactic agents released by aggregating platelets and are included in the thrombus.

Considering obvious the dominant relevance of platelets in the arterial thrombotic process and the role of the plasma hemostatic system with procoagulant and anti-coagulant factors in venous

thrombosis<sup>2</sup> and considering that the topic of this review is thrombotic complications during blood cell diseases, we will focus on the role of leukocytes and red cells in thrombogenesis, an aspect which has been underestimated.

Monocytes and neutrophils participate in the hemostatic cascade through 3 mechanisms:<sup>3</sup> 1) leukocytes express or secrete molecules with procoagulant or anticoagulant activity; 2) leukocytes cause functional alterations in vascular and perivascular cells (endothelial cells, platelets, other leukocytes) that in turn regulate blood coagulation; 3) aggregating leukocytes are trapped and obstruct the microcirculation under particular conditions.<sup>4</sup> According to some authors, the activation of blood coagulation, defined as the transformation through cleavage of amino acid chains of a soluble plasma protein (fibrinogen) into an insoluble protein (fibrin), occurs predominantly on the surface of monocytes. Monocytes can express tissue factor (TF) at different levels of intensity<sup>5,6</sup> and through TF they can bind factor VIIa, a serine protease, thus providing the first proteolytic event of the hemostatic cascade, the conversion of factor X zymogen into its active form, factor Xa.<sup>7</sup> Furthermore, monocytes express the natural inhibitor of the extrinsic pathway (tissue factor pathway inhibitor, TFPI), so that in the same cell, there is a balanced mechanism of activation of the extrinsic pathway.<sup>8</sup> Factor Xa and fibrinogen are located on the surface of monocytes and this facilitates the hemostatic process initiated by TF activity.<sup>9-10</sup>

Furthermore, monocytes participate in the activation of the hemostatic process by interacting with platelets; monocytes bind to platelets through P-selectin, thrombospondin and the integrin Mac-1, which bind the platelet glycoprotein 1b $\alpha$ .<sup>11-13</sup> Moreover plasmin-independent fibrin degradation may occur in monocytes because of the presence of elastase.<sup>14</sup>

Procoagulant activity of monocytes is stimulated by different agents including lipopolysaccharide and protein C, endotoxins,<sup>15</sup> and different cytokines (tissue necrosis factor, interleukin 1, interferon  $\gamma$ )<sup>16</sup> and is modulated by polymorphonuclear leukocytes.<sup>17</sup>

The presence of TF or other procoagulant molecules on neutrophils is doubtful<sup>18,19</sup> but abnormal promyelocytes do express both TF and cancer procoagulant.<sup>20,21</sup> Moreover the presence of TF activity derived from leukocytes has recently been demonstrated in circulating blood;<sup>19</sup> monocytes, and possibly polymorphonuclear leukocytes, are involved in the transfer of TF-positive particles to

**Table 1. Intervention of leukocytes and erythrocytes in hemostasis and thrombosis.**

*Leukocytes*

Express or secrete molecules that have procoagulant or anticoagulant activities; cause functional changes in vascular cells regulating coagulation; are found in the thrombus (mostly arterial thrombus); contain multiple adhesion molecules that could be activated during thrombosis.

*Erythrocytes*

Are the most important cells in determining viscosity; interact with platelets via ADP; are able to expose phosphatidylserine on their surface; contain multiple adhesion molecules that could be activated during thrombosis.

**Table 2. Clinical data supporting the role of leukocytes and erythrocytes in hemostasis and thrombosis.**

*Leukocytes*

Neutrophils accumulate with platelets in arterial thrombus; the leukocyte count correlates with the risk of myocardial infarction; in leukemia blood coagulation is frequently activated and thrombohemorrhagic events are related not only to platelet count but also to leukocyte type and number.

*Erythrocytes*

Erythrocytes accumulate with platelets in venous thrombus; excessive bleeding can be treated by elevation of hematocrit; overproduction of erythrocytes leads to increased frequency of thrombosis; inherited erythrocyte abnormalities can predispose to thrombosis.

platelets.<sup>22</sup> Neutrophils can also bind to platelets through P-selectin and  $\beta$ -2 integrin expressed on activated platelets;<sup>23</sup> platelets also secrete substances such as PF4 and PDGF that activate neutrophil chemotaxis. Neutrophils, in turn, may produce enzymes acting on the endothelium and causing the exposure of thrombogenic subendothelium (elastase),<sup>14</sup> or inducing platelet activation (cathepsin G).<sup>24</sup> Granulocyte colony-stimulating factor (G-CSF) induces an increase of plasma elastase antigen level, together with a parallel increase of markers of endothelial damage (thrombomodulin and von Willebrand factor) and plasma markers of blood coagulation activation.<sup>25</sup> The potential relevance of neutrophils in thrombus formation is supported by epidemiological studies showing a correlation between neutrophil count and the risk of myocardial infarction and ischemic stroke.<sup>26</sup>

In conclusion, leukocytes seem to play an important role in the physiological process of hemostasis and in the mechanism of thrombus formation. Leukocytes regulate coagulation through the production and release of molecules with procoagulant or anticoagulant activity as well as by providing spe-

cific receptors that serve as direct molecular links between inflammation and hemostasis with the intervention of the platelets (Tables 1 and 2).

The role of red cells in hemostasis is supported by clinical and laboratory data.<sup>27</sup> It is already known that the bleeding time in patients with anemia, thrombocytopenia or thrombocytopathy normalize after a single red blood cell transfusion.<sup>28,29</sup> In contrast to patients with a low hematocrit, patients with an elevated hematocrit, secondary to different etiologies, are prone to develop thrombosis which is not related to platelet count.<sup>30,31</sup> From clinical experience it is well known that patients with hereditary stomatocytosis frequently develop thrombosis.<sup>32</sup> Mice lacking the erythroid protein band-3 or with spherocytosis and  $\beta$ -spectrin deficiency show an elevated neonatal mortality due to diffuse thrombotic foci;<sup>33</sup> this thrombotic tendency can be transferred into unaffected mice through hematopoietic stem cell transplantation.<sup>34</sup> Similarly a mutant mouse with severe elliptocytosis resulting from defective conversion of spectrin dimers to tetramers develops thrombosis and infarction soon after birth.<sup>35</sup>

A simple explanation of the role of red cells in thrombosis derives from their preponderant effect on blood viscosity. The increase in blood viscosity may be related to an increased number of red cells, to the presence of red cell aggregates or to the reduction of red cell deformability. Red cells take part directly in the thrombotic event by interacting with platelets through ADP release which stimulates platelet aggregation<sup>36</sup> or through the exposure of adhesion molecule receptors on the red cell surface<sup>37</sup> or through the exposure of phosphatidylserine<sup>38</sup> (Tables 1 and 2).

#### *Clinical relevance of thrombosis in blood cell diseases*

From the above mentioned comments, it is clear that diseases causing qualitative or quantitative modifications of red cells and leukocytes besides those of platelets may induce a thrombotic diathesis. From a clinical point of view, thrombosis is a prevalent problem in myeloproliferative disorders such as essential thrombocythemia and polycythemia vera, is relevant in some hemolytic anemias such as paroxysmal nocturnal hemoglobinuria, drepanocytosis, thalassemia and is not negligible during leukemia, particularly acute promyelocytic leukemia and acute lymphoblastic leukemia (Table 3). Indeed, with current therapies, thrombotic risk + fatal events have exceeded the hemorrhagic ones in such leukemias.

**Table 3. Hematologic diseases and thrombotic risk.**

<i>High thrombotic risk</i>
Polycythemia vera
Essential thrombocythemia
Paroxysmal nocturnal hemoglobinuria
Drepanocytosis
<i>Moderate thrombotic risk</i>
Acute promyelocytic leukemia
Acute lymphoblastic leukemia
Spherocytosis
Elliptocytosis
$\beta$ -thalassemia

In this review we will focus on the latest acquisition of knowledge about thrombosis during the course of these diseases.

#### *Myeloproliferative diseases*

*Polycythemia vera (PV).* Thrombotic episodes are reported in 4.1% to 9.8% of patients with PV, with an annual incidence of 2% to 9% depending on the presence of additional risk factors; the most important risk factor is the type of therapy, the incidence of thrombosis being higher among patients treated with phlebotomy alone than in patients treated with hydroxyurea.<sup>39-41</sup> It is still unknown whether interferon is able to reduce the rate of vascular complications.<sup>42</sup> Age is another important factor; in fact the annual incidence of thrombosis is 1.8% in patients under 40 years of age while it rises to 5.1% in patients over the age of 70.<sup>41</sup> The incidence of thrombotic events increases during the 2-3 years before the diagnosis of polycythemia vera.<sup>41</sup> These epidemiological data suggest that latent myeloproliferative disorders constitute a prothrombotic status and are concordant with the frequent finding of a latent or evident myeloproliferative disorder documented in young patients with unexplained thrombosis.<sup>43-45</sup>

*Essential thrombocythemia (ET).* The prevalence of thrombosis in patients with ET reported in the literature is extremely variable, ranging from 15 to 89% with an annual incidence of 5%.<sup>46-48</sup> This variability is also related to the fact that symptoms of peripheral ischemia, such as acrocyanosis, are sometimes reported as thrombosis; it is however well established that the thrombotic risk largely exceeds the hemorrhagic risk. The thrombotic risk does not depend on the platelet count.<sup>46-48</sup> *In vivo* leukocyte activation, associated with laboratory signs of endothelium damage and coagulation sys-

tem activation, has recently been demonstrated in PV and ET patients; these data suggest that polymorphonuclear cells may be involved in the pathogenesis of the thrombophilic state.<sup>49</sup> According to some authors, thrombotic complications are considered to be rare in patients younger than 60 years of age, particularly in ET patients without previous thrombotic episodes.<sup>50</sup> The concept of ET being relatively benign is not, however, shared by other authors.<sup>51</sup> The diagnosis of ET is one of exclusion; therefore, it is possible that other diseases are included under the name of ET. It is noteworthy that only a small portion of patients (<50%) with a diagnosis of ET according to PVSG criteria have a clonal myelopoiesis as documented by X-chromosome inactivation pattern (HUMARA).<sup>52,53</sup> It is also relevant that the incidence of thrombosis is higher in female patients with clonal myelopoiesis.<sup>53,54</sup> Although the thrombotic risk does not seem to be related to the platelet count,<sup>55</sup> treatment with hydroxyurea, aimed at maintaining a platelet count below  $600 \times 10^9/L$ , reduces the incidence of thrombosis drastically.<sup>56</sup>

**Prophylaxis.** Thrombotic episodes in individuals with PV or ET are mostly of arterial type (50% to 70%); furthermore more than 80% of fatal thromboses in PV are arterial.<sup>41</sup> In the light of this, aspirin has been proposed and is widely used for thrombotic prophylaxis in patients with myeloproliferative diseases. While the efficacy of aspirin in reducing or eliminating the symptoms of peripheral ischemia due to disturbances of the microcirculation (acrocyanosis) is well recognized, the same cannot be said for prophylaxis of large vessel thrombosis. It has been demonstrated by the *Gruppo Italiano per lo Studio della Policitemia Vera* that low doses of aspirin (40 mg/day) are well tolerated and are able to inhibit PGG/H synthase in PV.<sup>57</sup> The *European Collaboration on Low-dose Aspirin in Polycythemia Vera* (ECLAP) study, which is a randomized trial designed to assess the risk/benefit ratio of low-dose aspirin in PV, is currently ongoing.<sup>58</sup>

Venous thrombosis represents 30% to 40% of thrombotic complications; frequently splanchnic and cerebral veins are involved.<sup>41</sup> In particular hepatic vein thrombosis (Budd-Chiari syndrome) is extraordinarily frequent in PV patients and about 60% of idiopathic Budd-Chiari syndrome could be ascribed to a latent or incompletely expressed myeloproliferative disorder.<sup>43,44</sup> Splanchnic venous thrombosis requires life-long anticoagulation.

**Chronic myeloid leukemia (CML).** The risk of thrombosis in CML is very low. In a recent report concerning 430 patients referred to the Ham-

smith Hospital for allogeneic bone marrow transplantation no thrombosis was seen at onset of the disease although thrombocytosis was common.<sup>59</sup> Anecdotal cases of thrombosis of abdominal veins, and also a case of Budd-Chiari syndrome,<sup>60</sup> have been reported in CML patients submitted to splenectomy;<sup>60</sup> furthermore therapy with  $\alpha$ -interferon seems to increase the incidence of thrombotic thrombocytopenic purpura.<sup>61</sup>

**Idiopathic myelofibrosis.** The true incidence of thrombosis in idiopathic myelofibrosis is not well known. There are anecdotal reports of mesenteric and hepatic vein thrombosis. However, thrombosis following splenectomy is considered an important problem, being reported in about 15% of cases. Why there are such different incidences of thrombosis in chronic diseases all affecting a pluripotent progenitor cell and resulting in trilineage hematopoietic proliferation is not well known. A clonal involvement of megakaryocytopoiesis, resulting in elevated levels of platelet-specific proteins, increased thromboxane generation, and expression of activation-dependent epitopes on the platelet surface, is regarded as the main origin of thromboembolism;<sup>62</sup> nevertheless, activation of leukocytes and the consequent release of elastase and alkaline phosphatase could play an important role, causing endothelial damage, as recently demonstrated by Falanga *et al.*<sup>49</sup> in ET and PV.

#### *Acute leukemia*

Thrombotic events have been increasingly reported during acute leukemia, both myeloid and lymphoid, and also in thrombocytopenic patients. In particular, in our experience which has been recently reported,<sup>63</sup> among 228 patients with acute leukemia (162 with ANLL, 19 of them with M3 leukemia, and 47 with ALL) observed from 1993 to 1998, the incidence of thrombotic complications (both arterial and venous) was 4.4% at diagnosis and 8.8% within 5 months from diagnosis. The thrombotic risk was higher in patients with a platelet count  $>80,000/mm^3$  at diagnosis and in patients with M3 leukemia, independently of their platelet count. Several studies, including our own, evaluated the influence of the presence of thrombophilic traits on the development of thrombosis during acute leukemia.

While in acute promyelocytic leukemia, thrombotic risk seems to have exceeded hemorrhagic risk since the introduction of all-trans retinoic acid, it has not been possible to show an increased risk of thrombosis in patients with inherited thrombophilia.<sup>64</sup> In contrast, among children with ALL this

predisposing factor does seem to play a role.<sup>65</sup> Perhaps still greater importance in determining the thrombotic risk is attributable to the chemotherapy adopted. In M3 leukemia all-trans retinoic acid caused a remarkable reduction of hemorrhagic events, through the rapid normalization of hemostatic parameters,<sup>66</sup> but several thrombotic complications, both arterial and venous, have been consistently reported in elderly patients with acute promyelocytic leukemia treated with this drug.<sup>67,68</sup>

Retinoic acid, both *in vivo* and *in vitro*, reduces procoagulant activity mediated by TF or by cancer procoagulant from differentiating promyelocytes,<sup>69,70</sup> but it increases adhesion of leukemic promyelocytes to the endothelium<sup>71</sup> and rapidly improves hemostatic parameters. The increased incidence of thrombotic events is probably due to several factors, including a preponderant resolution of the hemorrhagic syndrome and increased survival of patients with acute promyelocytic leukemia. Therapy is also crucial in the development of thrombosis in ALL: the incidence of thrombotic complications varies among different series from 2.4% to 11.5% according to the age of the patient and the treatment used, particularly of asparaginase.<sup>72-79</sup>

Asparaginase derived from *E. Coli*, and to a lesser extent asparaginase from *Erwinia Carotovora*, reduces hepatic biosynthesis of antithrombin III, protein C and protein S.<sup>75-77</sup> The incidence of thrombosis including central venous catheter thrombosis) in pediatric patients treated according to the BFM protocol was 11% (32 cases out of 289 patients).<sup>65,78</sup> The incidence of thrombosis was significantly influenced by genetic prothrombotic factors (prothrombin G20210A, factor V Leiden, antithrombin III, protein C and protein S deficiency and increase of Lp[a]).<sup>65</sup> In fact 27 thrombotic events were recorded in 58 patients harboring at least one genetic mutation, whereas only 5 events occurred in 231 patients with no genetic mutations. It is noteworthy that cerebral venous thrombosis was diagnosed in the majority of cases (n = 15), in five cases associated with a central line placed in the internal jugular vein; thrombosis of a superior cava vein was documented in 13, occlusion of femoral and pelvic veins in 2, and superficial venous occlusion in a further 2 patients.<sup>65</sup> It is clear from this experience that children with ALL should be screened for inherited thrombophilia before treatment. The same group found that the incidence of thrombosis was reduced in children treated with a different protocol in which the asparaginase was postponed.<sup>79</sup>

### *Hemolytic anemias*

Among the hemolytic anemias, the highest incidence of thrombosis occurs in those with paroxysmal nocturnal hemoglobinuria (PNH): 40% of patients develop one or more thrombotic episodes during the course of the disease, with a cumulative incidence of 28% at 4 years.<sup>80,81</sup> The manifestation is often characterized by Budd-Chiari syndrome.<sup>80,81</sup> The thrombotic risk in PNH seems to be due to complement-mediated hemolysis followed by inhibition of fibrinolysis and platelet activation.<sup>82</sup> The latter mechanism has been highlighted by a recent paper showing an increase of membrane-derived procoagulant microparticles (phosphatidylserine) stemming from the platelets of PNH patients.<sup>83</sup>

In sickle cell disease (SCD) organ damage is caused by vaso-occlusive crises related to red cell sickling because of polymerization of deoxygenated HbS. Some sickled cells adhere to endothelial cells, leading to vaso-occlusion; small vessels are more frequently involved. Vaso-occlusive crises are very painful, and can cause acute chest syndrome, leg ulcers, stroke, and priapism.<sup>84</sup> Nevertheless tissue ischemia, particularly of the brain (8% of children may develop a stroke under the age of 14 years), can be worsened by secondary thrombosis.<sup>85</sup> Thrombosis can be favored by high levels of erythropoietin,<sup>86</sup> but does not seem to be enhanced by genetic mutations associated with thrombophilia, such as factor V Leiden and prothrombin G20210A.<sup>87</sup>

Subjects with SCD, even those asymptomatic, demonstrate significantly increased platelet activation, elevated plasma levels of PF4 and  $\beta$ TG, and increased plasma concentrations of F1+2, thrombin-antithrombin complexes (TAT), plasmin-antiplasmin complexes (PAP) and D-dimer.<sup>85,88,89</sup> During painful episodes in these patients, platelet activation increases and erythrocytes express procoagulant activities. SCD painful episodes are also associated with elevated plasma levels of F1+2, TAT, PAP and D-dimer. The frequency of painful episodes correlates with enhanced platelet procoagulant activity and elevated plasma fibrinolytic activity measured during periods without pain.<sup>89</sup>

Thus, asymptomatic subjects with SCD exhibit ongoing platelet activation, thrombin generation, and fibrinolysis that all increase during episodes of pain. These changes are predictive of the frequency of pain and of the interval to the next pain episode, thereby implicating thrombogenic activity in the development of the painful episodes.<sup>89</sup> However convincing evidence of the benefit of anti-throm-

botic and antiplatelet therapy is lacking, and anticoagulation is not currently indicated to prevent vasocclusive episodes.<sup>84</sup> Interestingly, it has recently been reported that treatment with dietary polyunsaturated fatty acids for one year reduces the frequency of painful episodes.<sup>90</sup> Treatment with dietary n-3 fatty acids concurrently decreased plasma levels of F1+2, D-dimer, and PAP, implying that the reduction in painful events was related to n-3 fatty acid-dependent inhibition of thrombosis.<sup>90</sup>

Spherocytosis and elliptocytosis can also be complicated by thrombosis; these events are frequently encountered after splenectomy.<sup>32,91</sup> Portal and mesenteric veins are more frequently involved.

Thromboembolic phenomena have been described in patients with thalassemia intermedia and major, although there are relatively few epidemiological data on the overall frequency of these complications.

A high prevalence of thromboembolic events has been found in patients with thalassemia intermedia carrying factor V Leiden<sup>92</sup> and in those who have been splenectomized.<sup>93</sup> These patients had high plasma levels of markers of coagulation and fibrinolysis activation. Furthermore, thalassemic red cell and erythroid precursors from splenectomized patients with thalassemia intermedia showed an enhanced capacity to generate thrombin.<sup>92</sup> The most frequent site of thrombosis was the portal vein followed by deep veins of the legs.<sup>92</sup>

Thrombotic events in hemolytic anemias caused by membrane defects have been attributed, at least in part, to hypercoagulability related to the exposure of phosphatidylserine of red cell membrane activating plasma prothrombinase and supplying a procoagulant phospholipid anionic surface.<sup>83,94</sup> However, erythroid phosphatidyl exposure was reduced in young SCD patients with high F hemoglobin level and mild coagulation activation<sup>95</sup> and was not predictive of thrombotic risk in mice with hemolytic anemia.<sup>96</sup>

### Conclusions

Thrombosis is a prevalent problem in myeloproliferative disorders such as essential thrombocythemia and polycythemia vera, is relevant in some hemolytic anemias such as paroxysmal nocturnal hemoglobinuria, drepanocytosis, and thalassemia and is not negligible during leukemia particularly acute promyelocytic leukemia and acute lymphoblastic leukemia. The causes of thrombophilia are not completely elucidated and are probably different in the various conditions considered here: nevertheless alterations in interac-

tions between leukocytes, red cells and platelets could play a major role. Contribution of neoplastic cells,<sup>97</sup> autoimmune mechanisms,<sup>98</sup> or cytokines<sup>99</sup> to thrombophilia might be not negligible in some of the examined conditions. Inherited plasma prothrombotic defects<sup>100</sup> play a role in lymphoblastic leukemia. Thrombotic episodes could be reduced not only by antiaggregating agents, but also by drugs inhibiting activation of leukocytes and red cells and their interactions with platelets.

### Manuscript processing

*This manuscript was peer-reviewed by two external referees and by Prof. Vicente Vicente, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. Vicente and the Editors. Manuscript received June 25, 2001; accepted September 28, 2001.*

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