REVIEW

Management of chronic immune thrombocytopenic purpura: targeting insufficient megakaryopoiesis as a novel therapeutic principle

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Abstract: Traditionally, anti-platelet autoantibodies accelerating platelet clearance from the peripheral circulation have been recognized as the primary pathopysiological mechanism in chronic immune thrombocytopenia (ITP). Recently, increasing evidence supports the co-existence of insufficient megakaryopoiesis. Inadequate low thrombopoietin (TPO) levels are associated with insufficient proliferation and differentiation of megakaryocytes, decreased proplatelet formation, and subsequent platelet release. Recently two novel activators of TPO receptors have been made available: romiplostim and eltrombopag. In several phase III studies, both agents demonstrated increase of platelet counts in about 80% of chronic ITP patients within 2 to 3 weeks. These agents substantially broaden the therapeutic options for patients with chronic ITP although long-term results are still pending. This review will provide an update on the current conception of underlying mechanisms in ITP and novel, pathophysiologically based treatment options.

Keywords: immune thrombocytopenia, romiplostim, eltrombopag, megakaryopoiesis

Immune thrombocytopenic purpura (ITP) is an autoimmune hematologic disorder characterized by low platelet count and bleeding. Recently, an international working group has suggested new definitions of the clinical phases of ITP. Chronic ITP regularly affects primarily adults with persistence of thrombocytopenia for >12 months.[1] Persistent ITP was suggested for patients with ITP up to 12 months after diagnosis, as spontaneous remissions still occur. In children, ITP is usually an acute disease with spontaneous remissions within a few days or weeks, virtually never lasting longer than half a year.

ITP can be separated, pathophysiologically, into a primary and a secondary form. In primary ITP no other disorder can be identified. Secondary forms of ITP are associated with various diseases such as infections (eg, hepatitis C, HIV or *Helicobacter pylori*), autoimmune disorders (eg, lupus erythematosus or antiphospholipid syndrome) and last but not least, malignancies (eg, chronic lymphatic leukemia or lymphomas). This review will focus on the recently recognized role of impaired megakaryopoiesis in patients with chronic ITP.

Symptoms and laboratory findings

The characteristic bleeding sites in ITP are petechiae and mucosal bleeding in the oral cavity, the gastrointestinal tract or the urinary tract. Most of these bleeding episodes are mild; in patients with persistently low platelet counts substantial mortality is associated

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with bleeding episodes and infections.[2] Patients presenting with thrombocytopenia due to ITP demonstrate typically isolated thrombocytopenia and an otherwise unremarkable peripheral smear as well as normal plasmatic coagulation. Other causes of thrombocytopenia have to be excluded. Taking a thorough history is essential for ruling out drug-induced thrombocytopenia (eg, heparins, quinine, trimethoprim-sulfamethoxazole, ranitidine). A family history might give clues to rare types of inherited thrombocytopenia such as Bernard-Soulier syndrome associated with giant platelets. Last but not least, pseudo thrombocytopenia must be excluded. A thorough work up is essential to detect underlying disorders associated with secondary immune thrombocytopenia.[3] In patients with platelet counts >50,000/μL, diagnosis of ITP is usually made incidentally. Patients with platelet counts <50,000/μL may develop bleeding complications either spontaneously or after minor trauma. Patients with platelet counts of <10,000 to 20,000/μL, especially elderly, are at high risk for severe bleeding complications including major bleeding to internal sites and spontaneous intracerebral hemorrhage.[4] A bone marrow examination is usually not required as number of megacaryocytes can actually vary substantially and do not necessarily correlate with extent of thrombocytopenia. However, we recommend performing a bone marrow biopsy for treatment of refractory disease and in patients over 60 years owing to an increased incidence of myelodysplastic syndrome. Testing for antiplatelet antibodies is usually not recommended. Antiplatelet autoantibodies are not pathognomonic for ITP and can be associated with other disorders such as viral hepatitis, B-cell lymphomas or myelodysplasia. Detection of platelet-bound antibodies does have an estimated positive predictive value of ~80% t; a negative test result cannot rule out the diagnosis of ITP.[5,6] The predictive values for assays to test free plasma antibodies are even lower. Up to 50% of ITP patients do not have detectable antiplatelet autoantibodies.[7] Therefore the American Society of Hematology and a recently published international consensus report do not recommend antiplatelet autoantibody testing in patients with ITP.[8,9]

Autoantibody-dependent platelet destruction and impaired megakaryopoiesis

Physiologically, in response to a blood vessel injury, platelets adhere to the subendothelial matrix via von Willebrand factor, subsequently become activated and secrete tissue factor and other procoagulatory molecules. This first step

of hemostasis essentially depends on platelet count and functionality. Platelet counts of <30,000/μL are associated with a substantially elevated bleeding risk. Autoantibodies against platelets antigens are detectable in ITP patients. However sensitivity (approx. 50%) and specificity (approx. 70%) are low. Furthermore antiplatelet antibodies have been detected in non-ITP patients.[10,11] IgG autoantibody coated platelets have a reduced life span.[12] Macrophages and phagocytes in spleen and liver-expressing Fc-receptors are the effector cells of accelerated platelet clearance. The corresponding platelet antigens are primarily fibrinogen-receptor (glycoprotein IIb/IIIa) and von Willebrand-receptor (glycoprotein Ib/V/IX).[13] Characteristically, these molecules are expressed at a high density on the platelet surface (up to 100,000 per platelet). However, antigens with a lower expression, such as glycoprotein-complexes Ia/IIa for collagen-receptor (~1000/platelet) can also induce autoantibody formation.[14] Moreover, phagocytosis of platelets by antigen-presenting cells yields neoantigens, resulting in sufficient antibody formation to cause thrombocytopenia. This mechanism may be a likely explanation for the observation, that in some patients no autoantibodies against common platelet – receptors are detectable.[15] The autoantibody-producing cells are a limited number of B-cell derived clones with somatic mutations and antigen-driven affinity.[7] These clones, however, require costimulatory signals from activated T-helper cells. Accordingly, increased numbers of HLA-DR+ T cells and decreased numbers of T regulatory cells are often observed in these patients.[16,17]

Autoantibody-mediated accelerated peripheral platelet destruction is not the only principle in the pathophysiology of chronic ITP. Ineffective thrombopoiesis has been recognized as another important underlying mechanism. Obviously, the very same autoantibodies directed against platelet glycoprotein Ib/V/IX and IIb/IIIa are also capable of inhibiting megakaryocyte growth. It was demonstrated in the 1980s that sera of patients with ITP actually inhibit megakaryocyte growth in culture, supporting the concept of suboptimal platelet production as a contributing factor to thrombocytopenia.[18,19] Furthermore, thrombopoietin (TPO) levels in patients with ITP were only modestly increased compared to other thrombocytopenic disorders like amegakaryocytic thrombocytopenia or aplastic anemia with TPO levels up to 30-times higher, and thus are inadequately low.[20–22] Physiologically, a negative feedback loop regulates megakaryocyte proliferation and formation of platelets. TPO is primarily produced in the liver and binds to membrane

receptors on platelets with a high affinity to be internalized thereafter.[23,24] At high platelet counts, most of the circulating TPO is taken up by the platelets and little is left to bind to TPO receptors on megakaryocytes. If platelets are low, TPO serum levels are increased, just as observed in patients with amegakaryocytic thrombocytopenia. Surprisingly, considering the often extremely low platelet counts in ITP patients, TPO levels are only minimally and thus inadequately elevated.[19,25] Similar to ITP in other disorders with platelet destruction such as thrombotic-thrombocytopenic purpura or hemolytic-uremic syndrome, TPO levels in these patients are also only moderately increased.[26,27] Considering these principles, stimulation of megakaryopoiesis has been recognized to be a potentially effective treatment strategy to increase platelet counts in patients with chronic ITP.

Treatment of ITP

For treatment of chronic ITP, 3 different strategies based on pathopysiological mechanisms can be used: (1) increasing platelet production by stimulating thrombopoiesis; (2) increasing platelet survival by reducing phagocytosis by macrophagocytes via splenectomy, immunoglobulins or vinca loaded platelets; and (3) decreasing autoantibody production by immunosuppression.

Stimulating thrombopoiesis and platelet formation: steroids, romiplostim and eltrombopag

Patients without bleeding symptoms and platelet counts of >50,000/μL do not have an increased risk of spontaneous bleeding and should generally only be observed closely. In patients with platelet counts between 20,000 and 50,000/μL treatment decisions should be made individually considering age, comorbidity and concurrent medication. Even with these platelet counts, we usually do not recommend treatment for patients under 60 years of age in the absence of oral anticoagulation, recent surgery, uncontrolled hypertension, peptic ulcer disease or other disorders associated with an elevated bleeding risk.[9] For patients with a bleeding history or <20,000 platelets/μL a therapeutic intervention is usually warranted. Steroids are thought to increase platelet counts through their immunosuppressive action; however, recent data suggest that they may also work by increasing platelet production.[28,29] Steroids are still considered to be the first line of therapy. A common prednisone regimen (1 mg/kg daily for 3 weeks) has response rates of approximately 60%.[30,31] Alternatively, a pulsed dexamethasone regimen (40 mg for

4 days per month) results in a more rapid response (within 2 to 4 days) but is associated with more side effects such as hyperglycemia, insomnia and edema.[32]

Unfortunately, despite high initial response rates, long-term remissions with glucocorticoids range only from 10% to 30% for patients with chronic ITP.[33,34] Considering the findings of suboptimal thrombopoietin levels, stimulating megakaryopoiesis via the TPO receptor seems a reasonable treatment approach. Several TPO receptor agonists have been developed, 2 of which have already shown efficacy in phase III trials and are licensed: romiplostim and eltrombopag.

Romiplostim is composed of 2 identical peptide sequences targeting the TPO receptor. The peptides are linked via polyglycine and covalently bound with 2 Fc-fragments to prolong their half-life in the circulation. Binding to TPO receptor with a high affinity induces megakaryocyte differentiation via phosphorylation of JAK2 and STAT5. In clinical trials, romiplostim induced a dose-dependent increase in platelet counts, peaking 12 to 16 days after onset of treatment. In a phase I trial 6 out of 8 healthy volunteers receiving 2 to 10 μg/kg subcutaneously doubled their baseline platelet counts.[35] The highest applied dose in this pilot study resulted in a nearly 6-fold rise in platelet counts after a single dose of romiplostim. Platelet counts were back close to the baseline by day 28 after drug application. Subcutaneous use did not differ from intravenous administration. The most commonly observed adverse reactions were moderate headache and a sore throat. A randomized pilot study in patients with ITP led to a dose-dependent increase of the platelet count to >50,000/μL in 12 out of 16 patients without significant adverse events.

Encouraged by these promising results, 2 placebo-controlled randomized trials were initiated.[36] 42 splenectomized and 41 non-splenectomized patients with ITP with <30,000 platelets/μL received romiplostim sc once a week for a total of 24 weeks. Doses of study drug were adjusted to maintain platelet counts between 50,000 and 200,000/μL. As controls, 21 ITP patients after splenectomy and 21 ITP patients without splenectomy were randomized to a placebo group. A durable response was achieved in 16 of 42 splenectomized patients given romiplostim versus none of 21 given placebo ($P = 0.0013$), and in 25 of 41 non-splenectomized patients given romiplostim versus 1 of 21 given placebo ($P < 0.0001$). Moreover, in the treatment group, an overall response rate including transient response was achieved in 88% of non-splenectomized (36/41) and 79% of splenectomized patients (33/42), respectively,

compared with 14% of non-splenectomized (3/21) and 0% splenectomized patients (0/21), respectively, given placebo ($P < 0.0001$). In the romiplostim group, platelet counts of 50,000/μL were achieved by 25% of patients after 1 week and by 50% within 2 to 3 weeks. Forty-nine percent of romiplostim-treated patients and 2.4% of placebo-treated patients achieved a durable platelet response, defined as a platelet count 50,000/μL for >6 of the last 8 weeks of treatment. Interestingly, baseline TPO levels and response did not correlate. One patient experienced worsening of increased bone marrow reticulin after 7 weeks of treatment but returned to baseline 14 weeks after cessation of the drug. Thrombotic events were observed in 2 patients on romiplostim (popliteal artery thrombosis, cerebrovascular accident) and 1 on placebo (fatal pulmonary embolism). None of the patients developed antibodies against romiplostim or TPO. This study indicates that romiplostim seems to be safe and effective for patients with chronic ITP.

Another TPO-targeting agent is eltrombopag, a small non-peptide molecule that stimulates proliferation and differentiation of megacaryocytes via JAK2/STAT-signaling pathway.[37] This drug interacts with a transmembrane part of the TPO receptor. Eltrombopag is taken orally once daily. As its absorption can be significantly affected by food, it needs to be taken 2 hours before or after meals. Pharmacodynamics and pharmacokinetics are comparable to those observed with romiplostim, ie, after a median of 8 days eltrombopaq induces a dose-dependent increase of platelet counts in healthy volunteers with a peak after 16 days.[38]

A placebo-controlled trial evaluated eltrombopag in 118 adults with chronic ITP and platelet counts <30,000 /μL at 3 dose levels (30, 50, and 75 mg, respectively).[39] The primary end-point, a platelet count of >50,000/μL, was achieved in 28%, 70%, and 81% of patients, respectively, and in only 11 percent of patients in placebo group. By day 15, >80% of patients on 50 or 75 mg eltrombopag had an increased platelet count. The incidence and severity of adverse events were similar in the placebo and treatment group and consisted primarily of headache (placebo 21%, eltrombopag 15%). These results were confirmed in another phase III, placebo-controlled trial, including adult patients with chronic ITP and platelet counts of <30,000/μL.[40] Seventy-six patients received initially 50 mg eltrombopag and subsequently 75 mg if platelet count did not reach 50,000/μL (ie, the primary endpoint) within 3 weeks. After seven weeks 43 of 73 patients in the eltrombopag group (59%) and 6 of 37 patients in the placebo group (16%)

had a response ($P < 0.0001$). There was no correlation of response rate to eltrombopag and concomitant use of ITP drugs, splenectomy or number of previous ITP treatments. Of 34 patients in the eltrombopag group who subsequently received a higher dose of eltrombopag, 10 (29%) successfully achieved >50,000 platelets/μL. Platelet counts generally returned to baseline values within 2 weeks after the end of treatment. Bleeding events were significantly reduced (39% vs 60%) in the eltrombopag group, whereas the frequency of adverse drug reactions was similar in both groups (each 3%). Thus, efficacy and safety of eltrombopag seems to be comparable with that of romiplostim.

Despite these promising results, there are still considerable concerns about TPO receptor agonists. First, cessation of drug results in a rapid decline of platelet counts back to baseline, and thus a continuous application is required. Second, time to response is 1 to 4 weeks, and therefore in case of emergency, treatment with TPO agonist alone is inadequate. Third, long-term effects of these drugs are still unknown. The significance of reticulin accumulation in bone marrow is unclear. It is possible that thrombopoietic agents inherently bear the risk for reticulin deposition. Another concern is that long-term intake of TPO agonists could contribute to carcinogenesis, as the neoplastic cells in many hematopoietic malignancies express TPO receptors.[41] Likewise, in 6 of 44 patients with myelodysplasia treated with romiplostim, a transient increase of peripheral blasts was seen. After cessation of TPO agonist this effect was reversible in all but 1 patient, who progressed to secondary acute myeloid leukemia.[42]

Increasing platelet survival: splenectomy, immunoglobulins and vinca loaded platelets

The spleen is regarded as the main site for removal of autoantibody-coated platelets from the circulation. Treatment options include either splenectomy or suppression of macrophages by immunoglobulins, or vinca loaded platelets. Splenectomy is the only surgical treatment option for ITP. Macrophages within the spleen are the effecter cells mediating rapid platelet clearance from the circulation. Accordingly, splenectomy is effective in approximately 85% of patients and durable responses necessitating no further treatment are seen in about two thirds of patients with ITP.[43] Risk factors associated with an inferior outcome after splenectomy are refractory disease, advanced age, dominant hepatic platelet sequestration and secondary forms

of ITP.[44–47] Laparoscopic procedures are, at least, as safe as open surgery.[48] Recommending splenectomy remains an individual decision that requires consideration of patient risk factors as described above as well as anesthesia-related risks. In general, every patient should be vaccinated against *Haemophilus* type B, *Pneumococcus* and *Meningococcus* as prophylaxis against overwhelming bacterial sepsis.[49] To minimize surgical procedure-related complications, a preoperative rise of platelet count is often mandatory. Intravenous immune globulins (IVIG) blocking Fc-gamma receptors on macrophages remain the mainstay of therapy in this setting, resulting (in most patients) in a reliable increase of platelet counts within a few days, lasting sometimes for weeks to even months.[50] In case of an acute major bleeding complication, IVIG can be used in combination with platelet transfusions or even activated factor VII. Another approach is the transfusion of vinca loaded platelets (VLP). *Ex vivo* complexing of vinca alkaloids to platelets is a hypothetically attractive means to target the chemotherapy to effecter phagocytes. Recently we, and others, have seen promising results in heavily pretreated ITP patients using a facilitated method of generating VLP.[51]

Reducing autoantibodies production by immunosuppression

Patients who fail to respond to the treatments described above might be candidates for monoclonal antibodies or immunosuppressive therapy. Depletion of B-lymphocytes by the anti-CD20 antibody rituximab in patients with chronic ITP results in response rates of up to 50% within 1 or 2 months.[52,53]

Immunosuppressive drugs, such as azathioprine, mycophenolate mofetil and cyclophosphamide, mainly target T-lymphocytes. Remission rates vary and time to response can be months. Furthermore, potentially severe adverse events such as neutropenia, renal failure or hepatitis limit their use.[54,55]

In conclusion, pathopysiological cofactors beyond accelerated clearance of autoantibody-coated platelets have been recognized in patients with chronic ITP. Recently, increasing evidence highlights insufficient megakaryopoiesis to be a major contributor to disease manifestation in these patients. Therefore stimulating megakaryopoiesis with TPO agonists such as romiplostim and eltrombopag are promising new treatment options. Still, incorporation of these new agents into existing treatment algorithms remains a clinical challenge, especially since long-term results are still pending.

Therefore, first-line treatment should still be corticosteroids. Patients failing those should be considered candidates for splenectomy. If contraindications against splenectomy are present, treatment with the thrombopoietin receptor agonists should be considered. Patients failing to these should be considered candidates for alternative immunosuppressive treatment.

Finally, two main questions need to be addressed for the long-term treatment with thrombopoietin receptor agonists: What is the significance of possible bone marrow fibrosis? Is there an increased incidence of myelodysplastic syndrome? Prospective studies are scheduled to answer these questions.

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Disclosures

The authors declare no conflicts of interest.

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