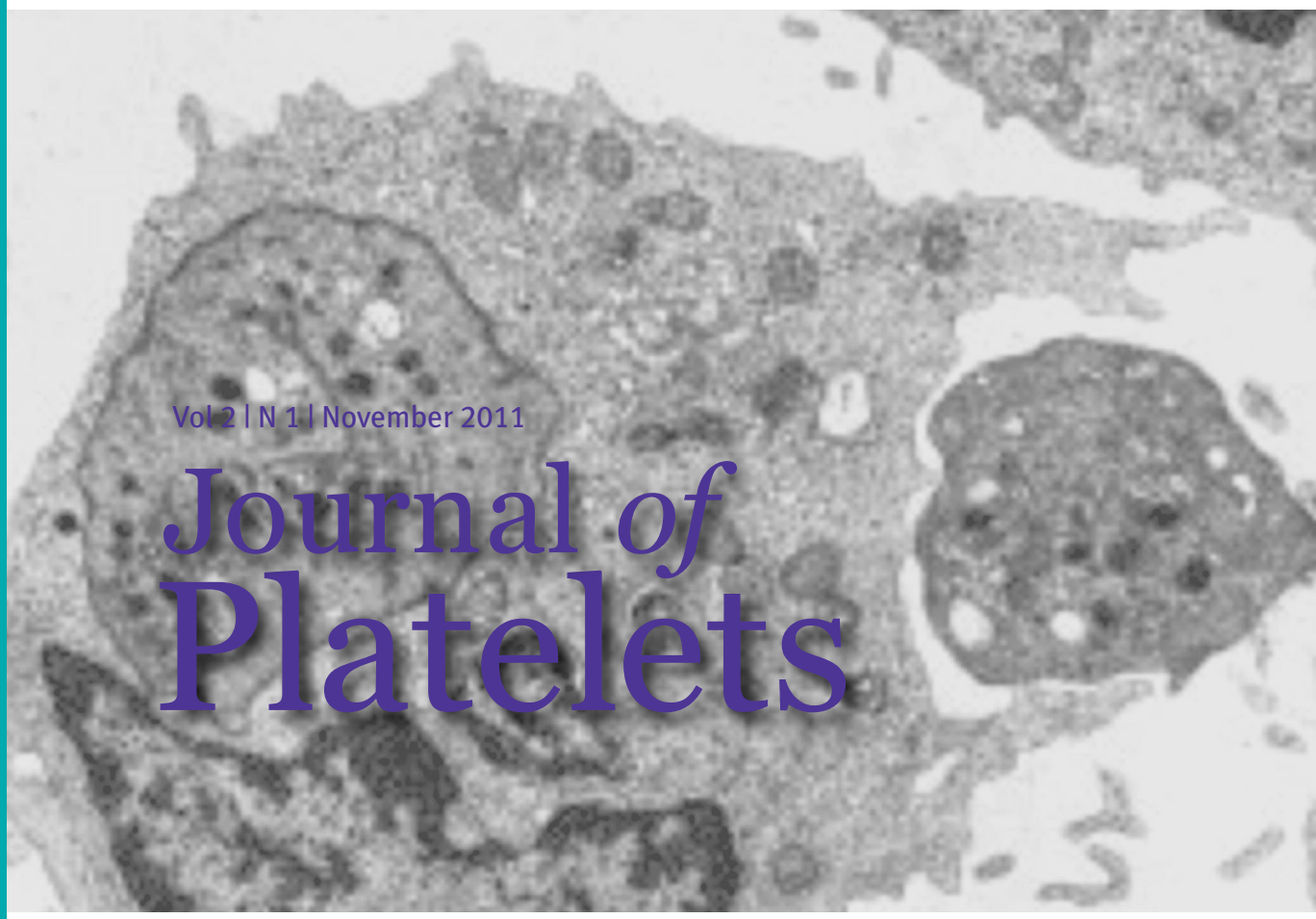

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Welcome to this issue of the Journal of Platelets, which opens the second volume. It is the time to think about the results obtained against the goals set for the first volume. When the first issue appeared the Editorial Board stated "*we look forward to providing a regular program of in depth reviews, analysis of current literature and topical issues, opinion, reports*". The first volume was conceived according to this format and we believe that the scientific goal "*to offer a forum for discussion, evaluation and dissemination on the field of platelets and thrombocytopenia*" has been achieved. The medical community has welcomed this new journal and thus has encouraged us to continue.

With the goal of furthering our understanding of platelet disorders, this volume will focus on several primary and secondary alterations in platelet production. This is particularly relevant within the context of the multidisciplinary environment, where the challenge of managing alterations in platelet production are associated with the presence of comorbid conditions such as HCV/HIV coinfection, hematologic malignancies and liver disease.

Once more it is clear how platelet alteration can herald different syndromes or diseases. *Gabriele Gugliotta and Michele Baccarani* describe the different causes of reactive thrombocytosis. Their in-depth review of the physiopathology of platelet production, facilitates the reader's understanding into why thrombocytosis can herald several inflammatory and neoplastic conditions. An approach towards a rational way to differentiate primary from reactive thrombocytoses is described and a guideline of practical interest with first and second levels of exams is provided.

Myelodysplastic syndromes are an emerging medical problem. Thrombocytopenia is frequently present alone or associated to other cytopenias. Until recently only support therapies, including platelet transfusions, were available. The introduction of hypomethylating agents has been shown to improve the quality of life and to delay the leukemic transformation in a subset of patients. The treatment, however, even when effective can transiently worsen the thrombocytopenia. *Gianluca Lunghi and Maria Gaidano* describe the clinical course of myelodysplastic syndromes and discuss the possible relevance of thrombopoietic agents in improving the tolerance to specific therapy and the quality of life.

In patients with HCV/HIV coinfection the control of HCV proliferation with interferon and ribavirin is often difficult due to the onset of thrombocytopenia. *Evangelista Sagnelli and Caterina Sagnelli* analyze the different causes of this challenge and underline that the toxicity of concomitant antiretroviral therapy is the main reason of myelotoxicity. The authors discuss how thrombopoietic agents have the potential to counteract drug toxicity thus allowing continuation of combined antiviral treatment to the patients.

Allogeneic stem cell transplantation can cure several hematologic malignancies. Persistent thrombocytopenia has been linked to a poor prognosis. *Francesco Zaja et al.* confirm these findings in a retrospective analysis of 71 patients treated at their institution and find a correlation between the degree of thrombocytopenia and the development of c-GVHD. These data provide further support to an active management of post-transplant thrombocytopenia to improve the prognosis of allogeneic stem cell-transplantion.

The role of HP infection in the pathogenesis of ITP and the relevance of its eradication for the control of thrombocytopenia is a matter of debate. *Dino Veneri et al.* present their experience and suggest that early HP eradication might help disease control mainly in moderate thrombocytopenia. A thorough discussion of the potential immunological mechanism responsible is provided.

Luca Nassi et al. demonstrate how it is difficult to differentially diagnose thrombocytopenia in patients who frequently travel abroad, particularly to tropical countries. Through the presentation of a case report, they underscore the several infections that need to be considered before a diagnosis can be made.

Edoardo G. Giannini and Francesco Rodeghiero provide a summary report of several interesting papers presented at recent and relevant international Congress meetings, AASLD (29 October–2 November 2010) and ASH (4-7 December 2010).

During AASLD, several presentations were devoted to the pathogenesis of thrombocytopenia in patients with chronic liver disease. Data were also presented on the pharmacokinetics of eltrombopag and an analysis was made of possible predictors of thrombotic events during treatment with these agents in patients with liver cirrhosis. Thrombopoietic factors can increase platelets numbers at the risk of increased thrombotic risk and it was suggested that careful monitoring of platelet numbers, to be alert to, and to avoid, excessive platelet production, which thus might help to avoid this complication.

At the ASH meeting, several studies were dedicated to ITP in order to define and optimize treatment to decrease platelet destruction and to accelerate its production. Several papers discussed the optimal use of thrombopoietic stimulators. Some attention was devoted to improving treatment with rituximab in patients with chronic refractory or relapsing ITP, since long term results are still disappointing.

Professor Massimo Aglietta

A review of the clinical and biological features of reactive thrombocytosis

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Abstract

Thrombocytosis is a relatively common hematological abnormality, with an incidence of about 1% in the general population. Currently, thrombocytosis is defined as a platelet count exceeding 450 x 10^9/L in adults, and is mainly classified as familial/hereditary, clonal, or reactive/secondary. This review focuses on reactive thrombocytosis, and examines the differential diagnosis of this form versus the other main categories. The identification of thrombocytosis needs attentive consideration by the physician, as this condition can be associated with important diseases (such as infections, malignancies or autoimmune disorders) requiring medical intervention. Most cases of thrombocytosis occur in the setting of a systemic disorder, with about 10–20% of isolated cases reflecting a hematological disorder. Certain cytotoxic drugs and classes of antibiotics can also be associated with the development of thrombocytosis. The persistence of thrombocytosis with no identified cause requires further clinical-biological evaluation to exclude a clonal disease, which increases the risk of thrombo-hemorrhagic complications, and may need specific anti-platelet treatments.

Introduction

Platelets, which were first described by Giulio Bizzozzero in the 19th century as small anucleate circulating cell fragments and dismissed merely as particles of "blood dust", have today acquired great clinical-biological relevance and are the object of stirring research. Platelets, also named thrombocytes, are formed by segmentation of megakaryocyte (MK) cytoplasm and, together with blood vessels and coagulation factors, are specifically involved in hemostasis, contributing mechanically and releasing a wide range of pro-thrombotic and vasoactive mediators. In addition, platelets are involved in immunological processes and host defence mechanisms. Platelet disorders include alterations of structure and function, and/or the number of circulating elements, i.e. their decrease (thrombocytopenia) or increase (thrombocytosis).

The incidental discovery of thrombocytosis in apparently healthy subjects has become more frequent after the advent of automated platelet counters and represents an important diagnostic challenge.[1] Identification of the clinical relevance of thrombo-hemorrhagic complications, potential existence of severe underlying diseases and availability of numerous different therapeutic approaches require that each case of thrombocytosis be properly investigated.

In this review, we will focus on the clinical-biological aspects of reactive or secondary thrombocytosis (RT) and examine the differential diagnosis with familial and clonal thrombocytosis (CT).

Thrombocytosis

Normal platelet range in adults and children

Normal platelet count, putting aside inter-laboratory differences, ranges between 150 x 10^9/L and 450 x 10^9/L in adults. In childhood, platelet count follows an age-dependent pattern; in an analysis of more than 47,000 neonates in the USA, advancing post-natal age had a significant effect on platelet count. Two peaks, with normal values (based on the 95th percentile) of up to 750 x 10^9/L, were observed at the 2nd to 3rd week and the 6th to 7th week.[2] Thereafter, the upper limit of platelet count progressively decreased (600 x 10^9/L between the 2nd and 6th month; 550 x 10^9/L between the 6th month and 2nd year; and 500 x 10^9/L

Table 1. Incidence of reactive thrombocytosis (RT)

Reference	Subjects	N	Platelet cut-off level for thrombocytosis	Incidence of thrombocytosis (%)	RT (% of all cases)
Ruggeri *et al.*[3]	Healthy	10,000	400 x 10^9/L	0.99	96
Aydogan *et al.*[4]	Hospital	124,000	500 x 10^9/L	1.6	96.7
Santhosh-Kumar *et al.*[5]	Hospital	777	500 x 10^9/L	NA	77
Griesshammer *et al.*[6]	Hospital	732	500 x 10^9/L	NA	88
Syed *et al.*[7]	Hospital	1068	600 x 10^9/L	NA	92
		55	1000 x 10^9/L	NA	72
Buss *et al.*[8]	Hospital	280	1000 x 10^9/L	NA	82

NA, not applicable (all patients with thrombocytosis).

between the 2nd and 6th year), being similar to adults after 6 years.

Incidence

The cut-off platelet count used to define thrombocytosis varies between 400 x 10^9/L and 600 x 10^9/L among different studies; however, extreme thrombocytosis is generally referred to as a platelet count over 1000 x 10^9/L (Table 1). Ruggeri *et al.* obtained platelet counts in 10,000 apparently healthy adults in an Italian city and found that 99 (0.99%) had a platelet count over 400 x 10^9/L. Of those individuals, only eight subjects still had an elevated count a few weeks later, and four were subsequently found to have essential thrombocythemia (ET).[3]

In Turkey, in a series of more than 124,000 patients admitted to hospital over a period of 5 years, thrombocytosis (defined as a platelet count >500 x 10^9/L) was found in 2000 patients (1.6%); the most common cause (96.7%) was RT.[4]

Other incidence data for RT from over 2000 patients with different platelet count cut-offs are shown in Table 1. In summary, in 777 patients with thrombocytosis (platelet count >500 x 10^9/L), admitted to a University Hospital and studied prospectively for etiology, RT accounted for 78% of all cases.[5,6] In a series of 732 medical and surgical patients with platelet counts of 500 x 10^9/L or higher, 643 (88%) had RT.[6] In a cohort of 1068 patients retrospectively reviewed in a medical centre in Pakistan, presenting with a platelet count >600 x 10^9/L, 91.8% had RT and 8.2% had CT; in the same cohort, 55 patients had extreme thrombocytosis (platelet count >1000 x 10^9/L), which was secondary in 72.7% of cases.[7] In another series of 280 consecutive hospitalised patients (platelet count >1000 x 10^9/L), 231 (82%) had RT, 11 (4%) had thrombocytosis of an uncertain cause and only 38 (14%) had a myeloproliferative disorder.[8]

The incidence, etiology and management of thrombocytosis in childhood are not the main focus of this paper and have been extensively reviewed elsewhere.[9]

Thrombocytopoiesis and platelet kinetics

Thrombocytopoiesis, i.e. platelet formation, occurs in the bone marrow and, in a small proportion of individuals (7–15%), in the spleen and lungs. With a platelet lifespan of about 10 days, a blood volume of around 5 L and one third of platelets pooled in the spleen, an adult human produces about 1 x 10^{11} platelets each day to maintain a normal platelet count; this production can greatly increase under some conditions.[10] Hematopoietic stem cells (HSCs) give rise to mature MKs through a complex process (megakaryocytopoiesis) driven by different regulatory signals via cytokines and interactions with stromal cells and the extracellular matrix. Thrombopoietin (TPO), stem-cell factor (SCF), granulocyte-monocyte colony-stimulating factor (GM-CSF), interleukin (IL)-3, IL-6, IL-9, IL-11 and erythropoietin (EPO) stimulate in vivo and in vitro MK development. Other cytokines such as transforming growth factor-beta and TNFα (tumour necrosis factor α), as well as some interferons are capable of stimulating or inhibiting thrombocytopoiesis depending on the presence of other signals. Negative regulators of thrombocytopoiesis are IL-4 and platelet factor-4 (PF4). Therefore, megakaryocytopoiesis and thrombocytopoiesis are under redundant and combinatory control in which multiple cytokines, although with different potencies and at different levels, provide, at least in vitro, a better stimulus than individual growth factors.

The main regulators of thrombocytopoiesis are TPO and its receptor MPL (CD110); MPL is expressed on HSCs, MKs and platelets, at different levels, and determines the activation of the Janus kinase-signal transducer and activator of transcription (JAK/STAT) signal transduction pathway (JAK2, STAT1, STAT3 and STAT5). The TPO gene, cloned in 1994, is localised on chromosome 3. Numerous studies have demonstrated an inverse

relationship between the levels of circulating TPO and platelet mass (interaction between TPO and its receptor, MPL); during normal homeostasis, platelet count is stable, and circulating TPO is at its basal concentration due to a constitutive production in the liver and kidneys and a constant binding and degradation by MPL-expressing cells (clearance of TPO). In cases of thrombopoietic stress, increased TPO production may be supported by the spleen and bone marrow. Additional control mechanisms for TPO production, such as regulation of alternative splicing of TPO mRNA, have been described.[11] Administration of TPO results in an increase in MK number in the bone marrow and spleen, an increase in MK size and DNA content, an increase in specific antigenic markers and a 3–10-fold increase in circulating platelet number. However, other growth factors or microenvironment signals are required for full polyploidisation and maturation of MKs.

IL-3 is another potent cytokine capable of stimulating thrombocytopoiesis. However, IL-3 is produced only by antigen-activated T lymphocytes, suggesting that its role in maintaining basal platelet production is minimal. GM-CSF has less potency than IL-3 (1%) in stimulating MK progenitors. SCF, IL-1, IL-6 and IL-11, which are not MK lineage-specific, are able to increase the activity of other growth factors in thrombocytopoiesis. IL-11 increases MK number, DNA content and peripheral platelet count in mice and has been used in humans for the treatment of chemotherapy-induced thrombocytopenia. IL-6 is another cytokine that stimulates MK maturation, with a role partially additive to that of IL-3; its actions may not be direct, but occur indirectly by the activation of stromal cells and enhancement of the hepatic production of TPO. Stromal-derived factor-1/CXCL12 has numerous influences on megakaryocytopoiesis, and it may be responsible for thrombocytopoiesis not related to TPO.[11,12]

Under the influence of these extracellular signals, HSCs, through the development of burst-forming unit MKs, colony-forming unit MKs and megakaryoblasts, give rise to mature MKs. In the megakaryoblast, repeated cycles of DNA replication are not followed by cell division (endomitosis), resulting in functional gene amplification (polyploidisation) that allows for an increase in protein synthesis. This process is highly regulated by different intracellular molecules, including a reduction in mitosis-promoting factor, decreased expression of cyclin B and down-regulation of the Aurora-kinase AIM-1. After the completion of DNA synthesis, the MK cytoplasm expands and fills with platelet-specific proteins, organelles and membrane systems, resulting in the formation of multiple long segments of cytoplasm, called proplatelets, which will repeatedly branch and give rise to platelets. Proplatelet formation is dependent on microtubules; the bending and branching of proplatelet shafts are mediated by the actin-myosin interaction. In vitro, maturation of proplatelets ends in a rapid retraction that separates a variable portion of the proplatelets from the residual cell body. In vivo, the finished platelet may be shed into the blood as a mature disk or released in a "preplatelet" form (with a long cytoplasmatic projection residual of the attachment to the cell body).

Many transcription factors have been shown to be involved in MK maturation and platelet formation. Nuclear factor (NF)-E2 is a protein that regulates the transcription of genes like beta-1-tubulin (TUBB1), thromboxane synthase and proteins that regulate inside-out signalling through $alpha_{IIb}$-$beta_3$ integrin. MKs of NF-E2 knock-out mice undergo normal endomitosis and proliferate in response to TPO; however, they fail to generate proplatelets in vitro. Targeted disruption of TUBB1 results in thrombocytopenia due to the failure to form proplatelets. GATA-1 and its cofactor FOG (friend of GATA-1) are necessary for the generation of MKs from a common bipotential progenitor; genetic elimination of FOG in mice results in specific ablation of the MK lineage, while elimination of GATA-1 arrests the maturation of MKs.

The number of circulating platelets is the result of the balance between platelet production in the bone marrow and their distribution and destruction in peripheral blood (circulating pool) and the spleen (storage pool). Thrombocytosis can result from an increased platelet production or by alterations in splenic storage, but not by a prolonged survival of platelets. The normal adult spleen contains 20 to 40 mL of blood and does not function as a reservoir for blood or erythrocytes. However, granulocytes and up to one third of the platelet mass are normally stored in the red pulp of the spleen and are released when cytokines affecting their adhesiveness are released.[12]

Classification

Currently, thrombocytosis is defined as a platelet count exceeding 450 x 10^9/L in adults.[1] Thrombocytosis can be subdivided into three main categories: hereditary/familiar thrombocytosis; CT (myeloproliferative neoplasm [MPN]); and RT.

In recent years, an improved understanding of the molecular mechanisms of thrombopoiesis has shown that some genetic alterations may be common to familiar and CT, suggesting that, in some cases, the difference in thrombocytosis may depend only on the inherited or acquired nature of the alteration. To underline this concept, these two categories of thrombocytosis may be referred to also as primitive thrombocytosis. On the other hand, RT does not reflect a defect in thrombocytopoiesis itself, but is related to a variety of underlying conditions (Table 2; Table 3).

Diagnosis

Thrombocytosis is a relatively common hematological abnormality, and hematologists are often consulted to exclude myeloproliferative disorders as a cause of high platelet counts. However, most cases of thrombocytosis occur in the setting of a systemic disorder, with about 10–20% of isolated thrombocytosis cases reflecting a hematological disorder.

Table 2. Classification of thrombocytosis

Primitive thrombocytosis	
Familiar thrombocytosis	
Clonal thrombocytosis	Essential thrombocythemia, polycythemia vera, chronic myeloid leukemia, primary myelofibrosis, myelodisplastic syndromes
Secondary/reactive thrombocytosis	
Physiopathological conditions	Exercise, hypoxia, ovulation, pregnancy, purpura
Acute inflammations	Infections (bacterial and viral), acute vasculitic-allergic states
Chronic inflammations	Autoimmune diseases (rheumatoid arthritis, enteropathies, Wegener granulomatosis, polyarteritis nodosa, temporal arteritis) chronic pneumonitis, tuberculosis, sarcoidosis
Tissue necrosis	Post-partum, post-surgery, bone fractures, organ infarction
Neoplasia	Lung, stomach, pancreas, ovary, uterus, breast, kidney, lymphoma
Bone marrow rebound	Post-trauma and acute bleeding, post-chemotherapy, post-acute thrombocytopenia, post-hemolytic crisis, post-vitamin B_{12} or folate correction
Drugs	Vincristine, vinblastine, ciclosporin, cytarabine, trans-retinoic acid, adrenergic agonists, corticosteroids, amoxicillin, ceftazidime, tazobactam, ciprofloxacin, miconazole, heparin, oral contraceptives
Asplenic/hyposplenic states	Congenital asplenia, hyposplenism, splenectomy
Sideropenic anemia	

Table 3. Most frequent causes of reactive thrombocytosis

Cause	Incidence (%)
Infections	22–50
Tissue damage	11–42
Rebound thrombocytosis	10–19
Chronic inflammation	10–13
Malignancy	6–13

Primitive thrombocytosis and RT can be distinguished by clinical and laboratory features; however, only few of these features, which are not always present, establish a definitive diagnosis (Table 4; Table 5). In some cases, it is easy to link an elevated platelet count to an underlying cause; an accurate anamnesis, the presence of specific symptoms or alterations in common laboratory findings may guide the clinician in the diagnosis of RT, which can then be simply confirmed by a subsequent normal platelet count when the underlying cause is no longer present. Among routine laboratory tests, acute phase reactants (C-reactive protein, erythrocyte sedimentation rate and fibrinogen) should be evaluated if an inflammatory/infective state is suspected; the presence of autoimmune disorders should be investigated (antinuclear antibodies and rheumatoid factor), especially in young women; in case of concomitant microcytic hypochromic anemia, an iron assay becomes mandatory.

Regarding basic hematological parameters, the absolute platelet number, number of reticulated platelets, mean platelet volume and platelet distribution width are not reliable to correctly differentiate CT from RT. A blood smear is necessary to evaluate morphological abnormalities of platelets or other blood cells. In selected cases, a chest X-ray and echocardiogram of the abdomen may be helpful to exclude possible occult malignancies of the lung, gastro enteric tract, urinary tract and reproductive organs, which may be associated with thrombocytosis, or a splenomegaly, which is more easily linked to MPN.

Only in cases with no clinical, laboratory and instrumental alterations should the possibility of MPN be deeply investigated with bone marrow aspirate, bone marrow histology, and cytogenetic and molecular biology. In the last years, great advances have been made by the discovery of specific genetic alterations in some cases of CT. These advances have contributed to the formulation of new WHO criteria for the diagnosis of MPN (2008).

Serum levels of TPO are not useful (in

Table 4. Clinical and laboratory evaluation of patients with thrombocytosis

Clinical evaluation	
History	A careful clinical history (including infections, drugs and familial history for autoimmune diseases) provides the best discrimination between RT and CT
Physical examination	Spleen
First level tests	
Blood counts	Hb, WBC, PLT, PDW, MPV
Acute phase reactant	ESR, CRP, fibrinogen
Iron assay	Iron, TBIC, ferritin
Immunological tests	ANA, ENA, rheumatoid factor
Second level tests (if no underlying cause of thrombocytosis has emerged)	
Peripheral blood molecular biology	BCR-ABL, JAK2 mutations, MPL mutations (myeloproliferative neoplasm)
Bone marrow cytology, cytogenetics and histology	Only in case of highly suspected CT (myeloproliferative neoplasm and myelodisplastic syndrome)
Platelet aggregation study	Not yet validated

ANA, anti-nuclear antibodies; CRP, C-reactive protein level; CT, clonal thrombocytosis; ENA, extractable nuclear antigens; ESR, erythrocyte sedimentation rate; Hb, hemoglobin concentration; JAK 2, Janus kinase 2; MPV, mean platelet volume; PDW, platelet distribution width; PLT, platelet count; RT, reactive thrombocytosis; TBIC, total iron binding capacity; WBC, white blood cell count.

Table 5. Typical clinical and laboratory features in differential diagnosis of clonal and reactive thrombocytosis

Features	CT	RT
Persistent platelet count increase	Yes	No
Known cause of RT	May be found	Yes
Thrombosis or hemorrhage	May be found	No
Splenomegaly	May be found	No
Bone marrow morphology	Abnormal	Normal
Spontaneous erythroid colony formation	May be found	No
Cytogenetics	May be abnormal	Normal
Molecular biology	May be abnormal	Normal
Acute phase reactants	Normal	Increased
Peripheral blood smear	May be abnormal	Normal
Platelet function	May be abnormal	Normal

CT, clonal thrombocytosis; RT, reactive thrombocytosis.

contrast to serum levels of EPO in polycythemia vera [PV]) for distinguishing RT and ET. It was demonstrated that peripheral blood and bone marrow levels of thrombopoietic and inflammatory cytokines (TPO, IL-6, soluble-IL-6 receptor, IL-8 and SCF) are elevated in patients with RT or CT compared to healthy controls; however, no significant differences among RT and CT patients were found. In addition, IL-11 and IL-3 were undetectable in most patients with thrombocytosis.[13]

The morphology of the bone marrow in cases of RT shows an increased number of normal MKs, with no alterations in other hemopoietic series; dysplastic, giant, clustered MKs are not observed in RT. Cytogenetic assessment of marrow cell metaphases is useful to exclude the presence of the Philadelphia chromosome, as, in some cases of chronic myelogenous leukemia, thrombocytosis may occur before white blood cell alterations or other signs appear. A clonal myelodisplastic syndrome due to the deletion of multiple genes (band q31;q32) of the long arm of chromosome 5 (the 5q- syndrome) almost always presents with thrombocytosis. Molecular biology has acquired an important role in the diagnosis of thrombocytosis, identifying specific alterations in genes involved in the regulation of thrombopoiesis. The most common is the mutation of the Janus-activated kinase 2 gene (either JAK2 V617F or JAK2 exon 12 mutations), which leads to an increased signalling of the EPO-receptor and TPO receptor (MPL), and to a large extent also of the GM-CSF receptor. However, JAK2 mutations that are detectable in more than 95% of patients with PV are also present in about 50–60% of patients with ET. Thus, while JAK2 mutation positivity is able to exclude a reactive form of thrombocytosis, a negative test cannot exclude MPN. Mutations of MPL, described in familiar cases of thrombocytosis, have been recently found to occur in 3–5% of patients with ET and 5–8% of patients with primary myelofibrosis. However, it is clear that about 40% of ET cases have not yet

been associated with a specific alteration or marker and therefore are at higher risk of being incorrectly diagnosed and differentiated from RT. This has an important consequence, as, in contrast to RT patients, ET patients are at risk of thrombo-hemorrhagic events and may require treatment. For this reason, experimental models for improving the differential diagnosis of thrombocytosis are necessary. Through a gene expression profile study of normal controls, ET patients and RT patients, a 4-biomarker gene subset able to predict ET in more than 85% of JAK2 wild-type mutations was discovered; in addition, using a panel of 11 genes, almost all patients with RT were correctly identified.[14] Analysis of larger patient cohorts is required to determine whether the various RT subtypes can be further sub-classified.

Different diagnostic approaches, based on platelet aggregation characteristics, have been investigated. It is well known that platelets in RT are normal, while abnormal platelet aggregation is frequent in chronic myeloproliferative disorders, with impaired epinephrine-induced platelet aggregation being one of the most consistently found defects. It was recently reported that the combined use of light transmission aggregometry with epinephrine and ADP and the PFA-100 (Platelet Function Analyzer; Dade Behring, Marburg, Germany) may be used in the differentiation of ET from RT; PFA-100 is a system in which platelet adhesion and aggregation following a vascular injury is simulated in vitro. Activation of platelets is determined by a membrane treated with collagen and epinephrine or collagen and ADP, and the time taken to stop the flow across the membrane (closure time) is recorded. Epinephrine-mediated aggregation (with both methods) was abnormal in ET patients and normal in RT patients; on the contrary, ADP-mediated aggregation was almost normal in both groups.[15] Therefore, these methods, once validated and available on a larger scale, could be useful, in selected cases, to differentiate ET and RT.

Specific conditions associated with RT

Infections and inflammatory diseases

Thrombocytosis is frequently secondary to an infection or to an inflammatory-autoimmune disorder (see Table 3). Platelets are active players in anti-microbial host defence, in the induction of inflammation and tissue repair. For example, platelets can bind and internalise pathogens and release proteins able to kill some bacteria and fungi in the bloodstream and strictly cooperate in leukocyte adhesion and transmigrations through the blood vessels. Thus, it is not surprising that up to 50% of cases of thrombocytosis (even extreme thrombocytosis) are secondary to an infection. Infections can be of bacterial, mycotic or viral origin (human immunodeficiency virus [HIV], respiratory syncytial virus [RSV], and cytomegalovirus). The significance of thrombocytosis after infection is not fully clarified, as it may be caused by both disease aggressiveness and a higher capacity for host defence. In a study of 500 patients hospitalised for community-acquired pneumonia, a high platelet count, but not leukocytosis, resulted in a significantly increased risk of mortality.[16] In a study of HIV-infected children, thrombocytosis was recorded in 24/400 patients (6%) and correlated with severe disease.[17] Thrombocytosis may play a role in the etiological diagnosis of an infectious process; for example, in a retrospective study of 345 patients with viral respiratory tract infections, thrombocytosis occurred in 29 patients, and in 24 cases (83%) it was due to RSV infection.[18]

Thrombocytosis in infective conditions is the consequence of several cytokines released during the inflammatory response; it has been demonstrated that TPO acts like an acute-phase protein, leading to an increased platelet count. IL-6 also plays a large role, as IL-6 can stimulate megakaryocytopoiesis directly and through liver production of TPO. Generally, in such cases, high levels of C-reactive protein are present. In the first week after infection, platelet count is normal, but TPO levels gradually increase and then drop when, during the second and third week, there is a peak in platelet count. IL-6 level has been shown to rise and fall more rapidly in the first week than TPO.[19] An increase in hepatic TPO production has been documented in rats injected with bacterial lipopolysaccharide, leading to subsequent thrombocytosis.[20]

Several inflammatory-autoimmune diseases (see Table 2) may be associated with thrombocytosis. While in normal conditions, TPO level is inversely related to the number of platelets and MKs, in inflammatory diseases with thrombocytosis, TPO levels are generally high. In a study of patients with inflammatory bowel disease, platelet and leukocyte counts were elevated in both active and inactive disease; in addition, in patients with active disease, elevated levels of TPO and IL-6 were observed, suggesting an additional mechanism underlying thrombocytosis in patients with inactive disease.[21] In a study of patients with rheumatoid arthritis with thrombocytosis, TPO levels were significantly elevated in cases of mild thrombocytosis, while levels were decreased in cases of markedly high platelet count, suggesting TPO regulation by the increased platelet mass via receptor-mediated uptake and metabolism.[22]

Trauma and surgery

In retrospective studies, one quarter of trauma patients in the intensive care unit (ICU) developed thrombocytosis. ICU-acquired infections, episodes of acute bleeding, need for splenectomy, prescription of epinephrine/norepinephrine and episodes of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) are conditions reported to predispose patients to RT. During ALI/ARDS, platelet sequestration in the lung has been described, and levels of IL-6 measured within the alveolar airspace have been shown to be high.

Thrombocytosis in such conditions is probably caused by stimulation of thrombocytopoiesis through an increment in circulating TPO and IL-6 levels (patients with the most severe injuries have the highest plasma levels), together with the absence of peripheral platelet consumption. In general, in trauma patients, thrombocytosis is associated with better survival than that predicted by the severity of illness score, notwithstanding a higher incidence of complications; moreover, changes in platelet count over time are related to outcome, with a lower rise generally associated with worse outcome. In patients who survive after trauma, the platelet count displays a bimodal response with an initial decrease below baseline values, followed by an increase above the normal range after 1 week.[23]

In a retrospective analysis of 3286 patients admitted to the ICU over a 7-year period, thrombocytosis developed in 18.7% of patients; most of the cases developed after 7 days (542 patients). Patients with thrombocytosis had higher rates of complications and thromboembolic events; however, overall mortality was significantly lower in this group of patients (3.8% vs. 18.1%, $p < 0.0001$).[24] In a large review of 6985 patients with trauma, extreme thrombocytosis (>1000 x 10^9/L) developed in 95 patients (1.4%); 41 patients (43%) were treated with aspirin and 54 were not (57%), with no difference in mortality, complications and ICU length-of-stay found between treated and untreated patients.[25]

In patients who underwent major surgery, it was observed that, as a consequence of tissue injury, the rise in IL-6 levels, but not IL-11 and TNFα levels, preceded the rise in TPO plasma levels that finally determined thrombocytosis.[26]

Malignancies

Thrombocytosis is a frequent (up to 57%) finding in cancer patients.[27] The underlying mechanisms probably involve factors released by tumour cells, bone marrow endothelial cells and MKs (acting through an autocrine loop). TPO production has been demonstrated in cases of ovarian carcinomas, hepatocellular carcinoma (HCC), and hepatoblastoma; IL-1 is overproduced in hepatoblastoma, whereas IL-6 is overproduced in mesothelioma, renal cell cancer and ovarian cancer.[28] It is well documented that proteins of the hemostatic system influence different steps of metastasis, angiogenesis and proteolytic events leading to tissue infiltration, but platelets also may play an important role in cancer progression. Activated platelets serve as procoagulant surfaces for amplifying the coagulation cascade; platelets that adhere to the endothelium facilitate adhesion and migration to the extravascular space of mononucleated cells, including cancer cells. Platelets induce angiogenesis in vivo through different mechanisms; platelets contribute directly to basement membrane and extracellular matrix proteolysis by releasing proteinases. Thus, the demonstrated multiple activities of platelets during tumour development and metastatic dissemination create the possibility of introducing antiplatelet agents into anticancer therapy, including antibodies against glycoprotein IIb-IIIa, direct thrombin inhibitors and protease-activated receptor-1-targeted therapy, as well as cycloxygenase and lypoxygenase inhibitors.[29] In an experimental model using a human ovarian cancer cell line (SKOV3), the inhibition of platelet activation with prostaglandin E1 attenuated the invasive capacity of these cells.[30]

Thrombocytosis has been investigated in numerous studies in different carcinoma types; however, no univocal conclusions have been reached.

- **Lung cancer**. TPO serum levels in lung cancer patients with thrombocytosis were higher than the levels found in ET patients, suggesting that TPO levels in lung cancer patients may be directly related to the activity of the neoplasm.[31] In two large series of more than 1000 patients each, thrombocytosis had an incidence of 17–32%; however, opposite conclusions regarding the impact of thrombocytosis on survival were found.[32,33]
- **Renal cell carcinoma (RCC).** Production of pro-inflammatory cytokines (in particular IL-6, but also IL-1 and IL-8) by renal cancer cells with a secondary increase in serum levels of TPO and GM-CSF has been demonstrated in patients with RCC.[34] Thrombocytosis is a frequent finding in RCC patients, and in the largest studies available, it was associated with worse survival.
- **Ovarian/uterine carcinoma.** Preoperative thrombocytosis was associated with a decrease in surgical cytoreductivity and worse survival in patients with epithelial ovarian carcinoma. Different mechanisms have been implicated; IL-6 has been demonstrated to be released in vitro by ovarian cancer cells, high levels of IL-6 have been found in ascites of patients with such a tumour, and these high IL-6 levels have been shown to correlate significantly with platelet count. Androgen modulation of thrombocytosis may play a role in this effect.[35,36] Thrombocytosis was described in up to 38% patients with ovarian cancer and in many studies was predictive of worse survival. In patients with carcinoma of the cervix, thrombocytosis was more common in advanced stages, in which it has been described to have a negative prognostic value.
- **Breast cancer.** In 4300 patients with early-stage breast cancer, pre-treatment thrombocytosis was observed only in 161 patients (3.7%); however, it was identified as a predictive factor for overall survival and breast cancer-related survival.[37]
- **HCC.** A low incidence of thrombocytosis has been reported in patients with HCC (31/1154, 2.7%); in these patients, thrombocytosis was a consequence of TPO overproduction by the tumour and was associated with a higher incidence of portal vein thrombosis, larger tumour volume and shorter survival.[38] Thrombocytosis has also been reported to be an adverse prognostic factor for survival in pancreatic, stomach, oesophageal, head and neck cancers.

Splenectomy, asplenia and hyposplenism

Post-splenectomy RT has an incidence of about 75%, regardless of the surgical indication. Generally, platelet count rises quickly, with a peak value between 7 and 12 days after surgery. In the next 2–3 months, platelet count normalises in the majority of patients; however, in some cases, platelet count may require more time to normalise or even persist indefinitely. The platelet count can increase even over 1000 x 10^9/L. RT is generally believed not to be associated with an increased incidence of thromboembolic complications. However, venous thrombosis was reported to occur in approximately 5% of patients after splenectomy and was generally associated with a platelet count above 600 x 10^9/L. Most cases were in portal, mesenteric and splenic veins. Less commonly, patients can experience arterial thrombosis (such as stroke or myocardial infarction).[39]

In very rare cases, thrombocytosis can be a consequence of congenital asplenia. This condition can be isolated or be associated with severe heart disease. Patients without cardiological problems are at higher risk of death by severe infections, especially at a younger age; however, a few such cases have been diagnosed in adulthood.[12]

Hyposplenism is a condition in which the spleen is not able to completely perform its immunological and hemocatheretic functions; it is characterised by typical changes in the peripheral blood, including thrombocytosis, lymphocytosis, monocytosis and the presence of Howell-Jolly bodies. Hyposplenism can be related to various conditions such as sickle cell disease, many immunological and autoimmune diseases (systemic lupus erithematosus, rheumatoid arthritis, systemic vasculitis, sarcoidosis, ulcerative colitis, celiac disease, mastocytosis, amyloidosis, chronic graft-versus-host disease or combined immunodeficiency) and different therapies (radiation therapy, corticosteroids or intravenous immunoglobulins). Hyposplenism in sickle cell disease is a consequence of multiple infarctions of the spleen; after radiation therapy, hyposplenism is related to ablation of B and T-cell function; in the other conditions, it is generally due to a reduced phagocytic function.

Iron deficiency

Abnormal platelet count has been reported in several studies in adults with iron deficiency anemia (IDA). In a series of 615 patients, thrombocytosis was detected in 13.3% of patients, while thrombocytopenia was recorded in 2.1% of cases.[40] Usually, thrombocytosis is mild to moderate, but could even be extreme (7% in another series).[41] The mechanisms underlying RT in IDA patients are still mainly unknown and controversial, possibly involving EPO, not TPO.

Drugs

Different cytotoxic drugs can be associated with thrombocytosis. One of the best-known of such drugs is vincristine, which acts through direct stimulation of megakaryocytopoiesis, and which in the past has been used in the treatment of idiopathic thrombocytopenic purpura. All-trans retinoic acid (ATRA) treatment has been described to induce transient thrombocytosis in up to 23% of patients with acute promyelocytic leukemia, generally after 4 weeks of treatment. In this setting, IL-6 may play an important pathogenetic role; in addition, it was demonstrated that ATRA is able to induce TPO transcription in bone marrow stromal cells.[42] Corticosteroids may induce thrombocytosis through inhibition of platelet uptake and increased destruction in the reticulo-endotelial system.

Different classes of antibiotics (amoxicillin-clavulanate; tazobactam; ciprofloxacin; ceftazidime) have been associated with the development of an increased platelet count; however, it is rarely possible to differentiate between infection-related and therapy-related thrombocytosis.

It is well known that heparin treatment can be complicated by thrombocytopenia (heparin-induced thrombocytopenia), especially when unfractioned heparin is used, generally as a consequence of developing antibodies against PF4; however, thrombocytosis, probably through inhibition of PF4 and subsequent stimulation of megakaryocytopoiesis, also has been associated, although rarely, with heparin treatment.[43]

Miscellanea (exercise and bone marrow recovery)

Thrombocytosis can occur after recovery from a transient thrombocytopenic state such as myelosuppression from chemotherapy or after treatment of thrombocytopenia in a severe megaloblastic state or in idiopathic thrombocytopenic purpura. It can also be an acute response to exercise, a phenomenon probably mediated by catecholamines that trigger release of platelets from the spleen.

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Conclusions

The platelet count cut-off of 450 x 10^9/L seems adequate to define thrombocytosis, including that observed in clonal myeloproliferative diseases (WHO 2008). The incidence of thrombocytosis in the general population is around 1%, with the rate of reactive forms ranging between 78% and 96% due to different platelet count cut-offs used in different studies. Identifying thrombocytosis requires attentive consideration by the physician, as the condition can be associated with important diseases (infections, malignancies or autoimmune disorders) requiring medical intervention. Moreover, the persistence of thrombocytosis with no identified cause needs further clinical-biological evaluation in order to exclude a clonal disease, which increases the risk of thrombo-hemorrhagic complications, and may require specific anti-platelet treatments. Luckily, the molecular tests now available to identify JAK2 and c-MPL mutations have improved the diagnostic approach, distinguishing RT from Ph-negative chronic MPNs and allowing for the diagnosis of ET, PV and primary myelofibrosis.

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Myelodysplastic syndromes presenting as isolated thrombocytopenia

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Abstract

Clinically relevant thrombocytopenia is a common problem in patients with myelodysplastic syndromes (MDS). Thrombocytopenia is present in approximately 40–65% of MDS patients and is an independent adverse prognostic, associated with higher incidence of fatal hemorrhagic complications and increased risk of AML transformation. This review focuses on the current and future therapeutic options for patients with MDS presenting with thrombocytopenia. Platelet transfusions are the only current treatment option and 6–30% of patients with MDS become dependent on platelet transfusion. Hypomethylating agents can ameliorate platelet count and increase patient survival, but these drugs are predominantly indicated in high-risk MDS and cause thrombocytopenia as a transitory adverse effect in more than half of patients. New preclinical and clinical studies suggest that thrombopoietin receptor (TPO-R) agonists can effectively increase platelet count and reduce bleeding events in patients with MDS, without causing significant adverse effects. To confirm the efficacy and safety of TPO-R agonists in the context of MDS and other myeloid malignances, further research is required.

General concepts

Myelodysplastic syndromes (MDS) include a heterogeneous group of acquired clonal stem cell disorders identified by ineffective hematopoiesis, morphologic and functional abnormalities of hemopoietic cells, and an increased risk of transformation into acute myeloid leukemia (AML). Patients with MDS usually present with uni-, bi- or tri-lineage cytopenia despite a hypercellular bone marrow.

Thrombocytopenia in MDS occurs as a consequence of ineffective hematopoiesis. Marrow platelet production appears to be ineffective as a result of altered proliferation and maturation of megakaryocytes and their precursors.

Morphological studies of bone marrow from patients with MDS show dysplastic features such as micromegakaryocytes, hypogranulation, mononuclear megakaryocytes and dissociation between nuclear and cytoplasmatic maturation.[1] Cytogenetic and molecular studies have revealed that the majority of megakaryocytes belong to the MDS clone.[2] Beside thrombocytopenia, platelet dysfunction can also increase the risk of hemorrhagic complications in MDS.[3,4]

Prevalence of thrombocytopenia in MDS

The frequency of thrombocytopenia in MDS ranges between 40% and 65%. In particular, at the time of diagnosis, 30% of patients have thrombocytopenia, with less than 10% experiencing serious bleeding.[3] Among a large series of 2410 patients with MDS, 18% of patients had severe thrombocytopenia (defined as a platelet count of $< 20 \times 10^9$/L); 3–53% of patients had hemorrhagic complications and 14–24% died because of hemorrhagic complications.[5]

Isolated thrombocytopenia is the presenting manifestation in 5–10% of patients with MDS and may be mistaken for immune thrombocytopenia (ITP).[6] The incidence and the degree of thrombocytopenia correlate with the severity of the disease. Kantarjian et al. reported that 66% of patients with MDS develop thrombocytopenia during the course of the disease, and the frequency correlates with advanced stage of MDS (according to the WHO classification) and high risk profile (according to IPSS). Even if low platelet count and hemorrhagic complications are more common in patients with high-risk MDS who progress to AML, severe thrombocytopenia at presentation is documented in 12% of patients with low or intermediate-1 MDS.[3]

Prognostic value of thrombocytopenia in MDS

Patients with MDS are currently classified according to the 2008 WHO classification system, based on the degree of cytopenia and blast cell count. This new classification recognizes "refractory cytopenia with unilineage dysplasia" (RCUD) as a separate linico-pathological entity, and subdivides this subtype into refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT). All these three sub-categories belong to low-risk MDS, but the RT group have a worse prognosis compared to RA and RT based on median survival data, and these differences may be related to the higher rate of death in the RT group due to bleeding complications.[7]

The presence of thrombocytopenia at diagnosis is an independent adverse prognostic factor and correlates with a shorter survival in patients with MDS.[8,9] This observation is also taken into account by the IPSS and by the MD Anderson risk score.[10,11] In particular, thrombocytopenia in MDS is associated not only with higher incidence of fatal hemorrhagic complications, but also with an increased risk of AML transformation.[12] Despite the higher incidence of thrombocytopenia at diagnosis in patients belonging to high risk categories, the frequency of fatal bleeding is also distributed among all IPSS risk groups, probably due to platelet dysfunction in MDS. Moreover, bleeding complications can further aggravate coexisting anemia, another adverse prognostic factor for survival in patients with MDS.

Therapeutic options

In the clinical management of MDS, the presence of thrombocytopenia is an important factor in treatment decisions. Amelioration of thrombocytopenia decreases the incidence of thrombocytopenia-related complications/mortality and reduces the necessity for treatment (such as platelet transfusions, chemotherapy and other therapeutic options).

In patients with MDS, WHO classification, IPSS score, age, performance status and donor availability are factors that have to be considered in treatment decision.[7,11] Estimated incidence of MDS is approximately 3.5– 12.6 per 100,000 per year, which increases with age: importantly, fewer than 10% of patients with MDS are younger than 50 years, while the incidence of MDS increases to 20–50 per 100,000 in patients older than 60 years.[5] Therefore, only a small fraction of patients with MDS can be considered candidates for curative treatment (e.g. stem cell transplantation); consequently the management of the majority of MDS patients remains a challenge for clinicians.

The use of hematopoietic growth factors, such as erythropoietin and/or granulocyte colony-stimulating factor, has been successfully implemented into clinical practice for the treatment of anemia and neutropenia in MDS. However, treatment of thrombocytopenia remains a significant problem, with 14–30% of patients deaths attributed to bleeding events.[3]

Platelet transfusions are the only current treatment option for thrombocytopenia related to MDS, with approximately 6–33% of patients with MDS becoming platelet transfusion-dependent.[13]

Platelet transfusions are associated with adverse events such as febrile or allergic transfusion reactions, transmission of viral or bacterial infections, transfusion-related acute lung injury, and refractoriness to platelet transfusions due to alloimmunization.[14] This latter event is common in chronically transfused MDS patients.

Recently, three new drugs have been introduced in the MDS treatment: the hypomethylating compounds azacitidine (AZA) and decitabine, and the immunomodulatory drug lenalidomide. These treatments have changed the course of the disease, improving quality of life, transfusion requirement and survival of patients with MDS. Moreover, the new thrombopoietin receptor (TPO-R) agonists are currently under investigation in particular in MDS presenting with thrombocytopenia.

Hypomethylating drugs

Treatment with AZA is approved by the FDA and the EMA for the management of intermediate-2 and high risk MDS patients, as well as for AML patients with less than 30% of bone marrow blasts (refractory anemia with excess of blasts in transformation, according to the previous French-American-British classification).[15-16] In the randomized Cancer and Leukemia Group B (CALGB) 9221 trial, which involved 191 patients with MDS, AZA 75 mg/m^2/die subcutaneously injected in 7-day cycles, repeated every 4 weeks (n = 99), was compared with best supportive care (BSC; n = 92).[17] The study demonstrated the superiority of AZA over BSC in terms of quality of life, delayed time to AML-transformation and reduction in transfusion needs. Moreover, a major platelet response was recorded in 21% of patients in the AZA group compared with 5% in the BSC group, while the median duration of platelet-transfusion independence was 6.3 months in the AZA group compared with 2.4 months in the BSC group. Complete remission (CR) and overall response (CR, partial remission and hematological improvement) according to International Working Group 2000 criteria occurred in 10% and 47% of AZA-treated patients, respectively.[17,18] A second randomized trial (AZA-001) demonstrated comparable results.[19]

Decitabine, a cytosine nucleoside analogue, is approved by the FDA for treatment of MDS of all French-American-British subtypes and intermediate-1, intermediate-2 and high-risk IPSS groups. In a randomized phase III trial comparing decitabine versus BSC, decitabine showed a significant advantage in terms of improved quality of life and reduced transfusion needs, but no survival benefit.[20] Moreover, myelosuppression appeared to be worse at least in the early phases of treatment with decitabine than with AZA: grade 3–4 thrombocytopenia and grade 3–4 neutropenia was present

in 58 and 61% of AZA-treated patients, and in 85 and 87% of decitabine-treated patients, respectively.[19,20]

Immunomodulatory drugs

Lenalidomide is an immunomodulatory drug approved by the FDA and the EMA for treating low or intermediate-1 risk MDS patients with transfusion-dependent anemia and interstitial deletion of the long arm of chromosome 5.[21-23] This drug induces an erythroid response in 83% of patients with del(5q), compared with 57% of those with normal karyotype and 12% of those with other cytogenetic abnormalities. Currently, lenalidomide has no clear indication in MDS with thrombocytopenia.[24]

Immunosuppressive therapy

Immunosuppressive therapy (IST) with antithymocyte globulin and cyclosporine (CsA) can be indicated in younger patient with lower-risk MDS and HLA-DR15 positivity.[25] Initial reports of IST use documented achievement of transfusion independence in approximately 30% of patients,[26-28] although these results were not repeated in other studies.[29] Moreover, the serious toxicity of this regimen and its long-term immunosuppressive effect has led to reconsider the application of IST in MDS.[25]

Thrombopoietin receptor agonists

The second generation of TPO-R agonists, romiplostim and eltrombopag, share no sequence homology with endogenous thrombopoietin, preventing the production of neutralizing antibodies. These TPO-R agonists are indicated as second-line treatment for patients with ITP, and are currently under investigation in MDS patients with thrombocytopenia. Romiplostim, a TPO mimetic, is a Fc-peptide fusion peptibody protein that activates intracellular transcriptional pathways. In patients with ITP, treatment with romiplostim leads to increased platelet production via the TPO receptor and is associated with few adverse effects.[30] The safety and efficacy of weekly administration of romiplostim in thrombocytopenic patients with low or intermediate-1 risk MDS was explored in phase I/II studies. Kantarjian et al. treated 44 patients with lower-risk MDS and platelet count ≤50 x 10^9/L with romiplostim 300–1500 µg subcutaneously weekly for three courses. Approximately half of the patients (46%) achieved a durable platelet response and the incidence of bleeding events and the requirement of platelet transfusions were less common in patients with platelet response.[31] The pharmacodynamics and pharmacokinetics of three different dosing schedules of romiplostim (two subcutaneous and one intravenous) were subsequently tested in 24 MDS patients. No patient developed neutralizing antibodies or marrow fibrosis; 57% of patients achieved a complete platelet response, 8% a major platelet response and 61% did not require transfusion within 8 weeks.[32] In all these studies, romiplostim appeared to be well tolerated and serious adverse events were recorded only in 11–18% of patients, almost all of which were treated with the 1500 µg dose or the intravenous formulation. A global, multicenter, randomized, double-blind, placebo-controlled phase II, study in low or intermediate-1 risk MDS is currently ongoing (clinical trial identifier NCT00614523). Additionally, in two randomized phase II studies, romiplostim was shown to reduce clinically significant thrombocytopenic events and platelet transfusions in low or intermediate risk MDS receiving hypomethylating agents (azacitidine and decitabine).[33,34] Similar results are emerging in patients treated with lenalidomide in combination with romiplostim.[35] Preliminary results were reported at the ASH 2010 meeting from the open-label extension study evaluating the safety of long-term romiplostim treatment in patients with thrombocytopenia and MDS enrolled in previous romiplostim studies. Until now, 40 patients have been enrolled and 85% had a platelet response, with a significant reduction of bleeding events and platelet transfusion requirement after starting romiplostim treatment.[36]

Eltrombopag is a novel nonpeptide TPO-R agonist which was shown to increase platelet counts and reduce hemorrhagic complications in patients with chronic ITP or hepatitis C.[37,38] As yet, eltrombopag has not been examined in the clinical context of myeloid malignancies. In a preclinical study, the effect of eltrombopag on proliferation, apoptosis, differentiation, colony formation, and malignant self-renewal of bone marrow nuclear cell of patients with MDS, AML and healthy individuals was examined. Eltrombopag increased megakaryocytic differentiation and formation of normal megakaryocyte colonies in patients with MDS and AML without evidence of proliferation or expansion of the malignant clone.[39] These encouraging results were confirmed in a second *ex-vivo* study, which supports the progression of eltrombopag to clinical study in the context of AML and MDS.[40]

Conclusions

Thrombocytopenia is a significant clinical problem in MDS patients, not only because low platelet count is associated with increased risk of hemorrhagic complications and lower quality of life, but because MDS-related thrombocytopenia reflects an unfavourable biology of the underlying disease and an increased risk of AML progression. To date, platelet transfusions are the only therapeutic option in MDS-related thrombocytopenia.

However, in approximately one-fifth of transfusion-dependent patients, incremental platelet responses are inadequate as a result of alloimmunization. The hypomethylating agents, in particular AZA, can ameliorate platelet count and increase patient survival, but these drugs are predominantly indicated in high-risk MDS and are associated with thrombocytopenia (a transitory adverse effect) in more than 50% of treated patients. Preclinical and preliminary clinical studies have confirmed that TPO-R agonists can have efficacy in

increasing platelet count and reducing bleeding events in MDS patients, without significant side effects. TPO-R stimulators lead to thrombopoietic progenitor cell expansion, differentiation and platelet production. As the TPO receptor is predominantly expressed on the surface of cells of the myeloid lineage, there is a theoretical concern that TPO-R agonists may stimulate the progression of existing hematopoietic malignancies. In MDS patients treated with romiplostim, there were reported cases of progression to AML. In addition, cases of transient blast cell increase were observed in the romiplostim studies in MDS, but the increases in blast cell count were reversible upon discontinuation of the drug. Progression to AML is an expected clinical outcome of MDS and the relationship to TPO-R agonist treatment is unclear.[31]

Eltrombopag has not been tested in patients with MDS, but preclinical data suggest that it may increase platelet count without stimulating the myeloid neoplastic clone.[39,40] In conclusion, the TPO-R agonists might be a suitable option for the treatment of MSD presenting with isolated thrombocytopenia – ongoing trials and future combination studies will define the precise therapeutic role of these novel treatments in MDS.

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Thrombocytopenia in HIV/HCV coinfection, a factor limiting the access to treatment with pegylated interferon and ribavirin

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Abstract

Purpose: This article discusses the efficacy of peginterferon α-2a and ribavirin for the treatment of hepatitis C virus (HCV) infection in patients with HCV/HIV coinfection, the effects of thrombocytopenia on treatment of patients with HCV/HIV coinfection, and the role of eltrombopag in patients with HCV/HIV coinfection.

Summary: Treatment of HCV with peginterferon α-2a and ribavirin is considerably less effective in HCV/HIV coinfection than in HCV monoinfection, with an approximately 50% reduction in sustained virological response rate. A possible reason for this is poor adherence to antiviral treatment, due to an increased occurrence of adverse events in coinfected patients. Thrombocytopenia is a significant obstacle to successful antiviral treatment, and may occur both as a result of chronic hepatitis, and as an adverse event associated with interferon treatment. The results of a trial assessing the use of eltrombopag in patients with thrombocytopenia and chronic HCV infection were promising, with an increased proportion of patients receiving the TPO receptor agonist completing a 12-week course of peginterferon α-2a and ribavirin. However, the ELEVATE study, designed to assess the efficacy of eltrombopag in patients with thrombocytopenia due to chronic liver disease, was terminated due to an increased occurrence of portal vein thrombosis in the active treatment group.

Conclusions: Thrombocytopenia is a significant obstacle to the successful treatment of HCV/HIV coinfection. However, use of eltrombopag may represent a useful strategy to improve access to antiviral treatment in this patient group, and should be investigated further.

Introduction

Coinfection with hepatitis C virus (HCV) and HIV is associated with significant increases in morbidity and mortality rates, since more HIV/HCV coinfected patients progress to cirrhosis, liver failure and hepatocellular carcinoma and develop recurrent hepatitis C after liver transplantation than patients with HCV monoinfection.[1,2] These differences, however, are lower when HIV/HCV coinfected patients have been treated with HAART (highly active anti-retroviral therapy),[3,4] particularly if successful control of HIV replication and improvement in CD4 cell count have been obtained.[5-7]

Treatment of HCV/HIV coinfection

The combined pegylated interferon (Peg-IFN) plus ribavirin (RBV) shows a 10-15% lower rate of sustained viral response (SVR) in HIV/HCV coinfection, compared with HCV monoinfection, and is poorly tolerated; drug interactions and increased toxicity when combined with antiretroviral drugs may reduce the percentage of HIV/HCV coinfected patients that complete the required treatment.[8] Consequently, whenever possible, it is desirable that treatment for HCV infection precedes HAART administration.

The rate of SVR with Peg-IFN plus RBV in clinical studies in HCV/HIV coinfected patients ranges from 40% to 44%,[9,10] whereas for patients with HCV monoinfection, the SVR rate is about 80% for patients with genotype 2 or 3 and about 45% for those with genotype 1.[11,12] In the APRICOT Study, 868 HIV/HCV coinfected patients were randomly assigned to receive Peg-IFN plus RBV, Peg-IFN monotherapy, or IFN plus RBV.[10] The results were quite promising, with a significantly greater proportion of Peg-IFN plus RBV recipients

achieving SVR than recipients of Peg-IFN monotherapy or IFN plus RBV (40% vs. 20% and 12%, respectively; $p<0.001$ for both comparisons). In addition, of Peg-IFN plus RBV recipients infected with HCV genotype 1, or with HCV genotype 2 or 3, 29% and 62%, respectively, achieved SVR. SVR rates were significantly lower in the Peg-IFN monotherapy and IFN plus RBV treatment arms. In terms of safety and tolerability, 25% of patients in the Peg-IFN plus RBV arm discontinued treatment, compared with 31% and 39% of patients in the Peg-IFN monotherapy and IFN plus RBV treatment arms, respectively.[10] The rates of treatment-related serious adverse reactions in the three treatment arms were 8%, 10% and 5%, respectively, with neutropenia and thrombocytopenia being more frequent in patients receiving Peg-IFN. The proportion of patients with mitochondrial toxicity was low in all treatment arms. The lower frequency of SVR response to Peg-IFN plus RBV in HIV-infected subjects, compared with HCV monoinfected individuals may have several explanations, but poor adherence to treatment appears to play a relevant role.

Thrombocytopenia in HIV/HCV coinfection

In both HCV monoinfection and HIV/HCV coinfection, patients with a platelet count below 75 x 10^9/L have been excluded from registrational studies, and consequently they remain untreated in clinical practice. In addition, thrombocytopenia may be clinically relevant during antiviral treatment due to the myelosuppressive activity of IFN, with a consequent discontinuation of treatment in some cases or reduction in Peg-IFN dose in others. Thus, thrombocytopenia represents a major obstacle to successful treatment in a subset of patients. This was demonstrated in a recent study, where the achievement of SVR in patients with compensated HCV-related cirrhosis was associated with reduced development of esophageal varices, allowing endoscopic surveillance to be safely delayed or avoided.[13]

Currently, the percentage of HCV monoinfected and HIV/HCV coinfected patients requiring Peg-IFN treatment for chronic HCV infection who remain untreated because of thrombocytopenia is unknown, as is the proportion of patients who discontinue Peg-IFN treatment due to development of thrombocytopenia. Moreover, it is unknown whether thrombocytopenia in HIV/HCV coinfected patients with advanced liver disease is correlated with HIV infection or with HCV liver disease.

Thrombocytopenia is observed in patients with chronic HCV infection of varying degrees of severity, but is associated with the more advanced stages of disease both in HCV monoinfected and in HIV/HCV coinfected patients. Causes of this association include reduced production of thrombopoietin (TPO), the endogenous thrombopoietic growth factor,[14] increased sequestration of platelets due to hypersplenism,[15] and the myelosuppressive effects of HCV on the bone marrow.[16-19]

A platelet count below 150 x 10^9/L has been observed in 5.5% of non-cirrhotic patients with chronic hepatitis C, and in 64% of patients with cirrhosis.[20] In patients with chronic hepatitis, thrombocytopenia is not associated with major bleeding, whereas in patients with advanced cirrhosis the decrease of platelet count parallels the worsening in liver function and portal hypertension.[14] In patients with liver cirrhosis there is an increased risk of spontaneous bleeding and bleeding during major or minor invasive procedures.[21,22] Platelet transfusions are frequently used for the prevention of bleeding before an invasive procedure, but they expose patients to the risk of reactions, alloimmunization, and infections.[23]

Eltrombopag in HCV-related thrombocytopenia

TPO receptor agonists are able to stimulate thrombopoiesis, and have been evaluated for the treatment of thrombocytopenia in various pathologic conditions.[24,25]

Published studies report satisfactory tolerability profiles for eltrombopag in patients with idiopathic thrombocytopenic purpura,[26,27] and in patients with HCV infection-associated thrombocytopenia.[28,29] The administration of eltrombopag resulted in a dose-related increase in platelet count in healthy subjects,[28] and in patients with chronic idiopathic thrombocytopenic purpura, with a favourable safety profile.[26,27,29] Similar results were observed in thrombocytopenic patients with chronic HCV infection, who, under eltrombopag administration, showed a safe increase in platelet count, enabling patients to undergo treatment with Peg-IFN plus RBV.[30] Eltrombopag, given at three different dosages (30 mg/day, 50 mg/day, and 75 mg/day) to 74 patients with HCV infection and thrombocytopenia, assured a platelet count above 100 x 10^9/L after 4 weeks of treatment in 75%, 79%, and 95% of the patients, respectively, with a significant increase from baseline in all groups. A 12-week course of Peg-IFN plus RBV was completed by 36%, 53%, and 65% of patients receiving a daily dose of eltrombopag of 30 mg, 50 mg and 75 mg, respectively, compared with 6% in the placebo group.[30] Platelet count decreased during antiviral treatment, but remained above 50 x 10^9/L in all treatment groups. The most common side effects during the initial 4 weeks' treatment with eltrombopag were headache, dry mouth, abdominal pain, nausea and itching; these effects were of insufficient severity to require discontinuation of the drug and occurred with similar frequency in the placebo group. Throughout the study, eltrombopag was discontinued in five patients, but it was not reported whether the adverse events leading to discontinuation were related to the investigational drug or to Peg-IFN or RBV. These results are tempered by the ELEVATE study,[31] a multinational, randomized, double-blind, placebo-controlled study, performed to evaluate the safety and efficacy of eltrombopag in reducing the need for platelet transfusion in thrombocytopenic patients with chronic liver disease before invasive procedures. The

study was terminated because of an imbalance in the occurrence of portal venous thrombosis, which occurred in six patients (4%) in the eltrombopag group and one (1%) in the placebo group. Five of the six patients treated with eltrombopag showed portal venous thrombosis at platelet counts above 200 x 10^9/L. Patients included in the study presented with hepatic impairment of various etiologies and received a daily oral dose of 75 mg of eltrombopag or placebo for 14 days.[31] The data from this study suggests caution for the use of eltrombopag in patients with HCV-related chronic liver disease, both in HIV/HCV coinfected and in HCV monoinfected patients; most probably, the safety and efficacy of lower eltrombopag doses should be investigated.

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Conclusions

Thrombocytopenia is a frequent problem for patients in the more advanced stages of HCV-related liver disease who may benefit from antiviral treatment. No treatment has been approved for thrombocytopenia in patients with HCV infection. Preliminary findings have demonstrated that eltrombopag may improve access to anti-HCV treatment for such patients, suggesting that this drug deserves attention and further investigation.

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Post-transplant thrombocytopenia appears to be a strong negative prognostic factor for survival in patients undergoing allogeneic stem cell transplant

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Abstract

Background: Previous studies have highlighted the development of post-transplant thrombocytopenia as a poor prognostic factor for the outcome of patients undergoing allogeneic stem cell transplant (SCT).

Purpose: To evaluate the impact of post-SCT thrombocytopenia on patient survival.

Methods: Patients aged ≥18 years who had undergone SCT and who had survived for ≥3 months were included in the analysis. Thrombocytopenia was defined as a platelet count of ≤100 x 10^9/L ≥3 months after SCT.

Results: In total, 71 patients (median age 52 years; range 18–69 years) were included in this analysis and were followed for a median follow-up time of 21 months (range 3–44 months) after SCT. Twenty-seven patients (38%) developed thrombocytopenia with a median platelet count of 29 x 10^9/L (range 7–86 x 10^9/L). Platelet count was ≤50 x 10^9/L in 19 patients among whom 9 had ≤20 x 10^9/L. Patients with and without thrombocytopenia had similar baseline clinical characteristics, transplant type, and status at transplant. Thrombocytopenia was associated with chronic graft-versus-host disease (cGVHD) in 13 of 24 patients (54%), disease relapse in 7 patients (26%; 5 acute leukaemia, 2 lymphoma), cytomegalovirus reactivation in 4 patients, graft failure in 2 patients, and microangiopathy in 1 patient; thrombocytopenia was classified as idiopathic in 3 patients. The incidence of mortality was significantly higher in patients with post-SCT thrombocytopenia compared with patients without thrombocytopenia (70% vs. 14%; $p<0.0001$). The cause of mortality in patients with thrombocytopenia included: primary disease in 11 patients (58%; acute leukaemia in 5 patients, lymphoma in 3 patients, 1 patient each with myelodysplasia, chronic myeloid leukaemia, and multiple myeloma), cGVHD in 4 patients (21%), infections in 4 patients (21%), 1 patient each with cerebral bleeding, cardiac stroke, and multi-organ failure. In multivariate analysis only thrombocytopenia was significantly associated with overall survival (HR 9.77; 95% CI, 3.63–26.33; $p<0.0001$). Thirty-two patients developed cGVHD and 13 had concomitant thrombocytopenia. The incidence of mortality in this group was significantly higher in patients with post-SCT thrombocytopenia compared with patients without thrombocytopenia (61.5% vs. 10.5%; $p=0.005$).

Conclusion: In this retrospective analysis the occurrence of post-SCT thrombocytopenia appears to be a strong negative predictor of survival. cGVHD seems to be the most frequent pathologic condition associated with thrombocytopenia. The management of post-SCT thrombocytopenia may influence patient outcome.

Introduction

Late-onset thrombocytopenia is observed in around 20–40% of patients undergoing stem cell transplantation (SCT).[1,2] With the exception of cases in which thrombocytopenia reflects a recurrence of the underlying malignancy, several other factors may cause this complication including graft loss, infectious conditions (particularly cytomegalovirus [CMV] or other herpes virus-related infection or reactivation), secondary microangiopathy observed following utilization of calcineurin

inhibitors and, in particular, the onset of chronic graft-versus-host disease (cGVHD). Previous studies show that late-onset thrombocytopenia is a strong negative prognostic indicator of survival in transplant recipients and in particular in those cases where thrombocytopenia is related to GVHD.[1-12]

Late-onset thrombocytopenia following SCT and GVHD appears to be strongly related. Thrombocytopenia is correlated with a higher incidence of advanced acute and cGVHD, and represents one of the major prognostic factors influencing post-transplant mortality.[1,2,12]

On these grounds we retrospectively evaluated the incidence and prognostic significance of post-SCT thrombocytopenia in a single center transplant population. The results of this analysis are reported here.

Patients and methods

This is a retrospective analysis that evaluated the incidence of late-onset thrombocytopenia post-SCT as well as its clinical and prognostic relevance. The primary endpoint of this study was to evaluate the impact of thrombocytopenia (from any cause) on survival. Secondary endpoints were to look for potential relationships between thrombocytopenia, cGVHD development, and other clinical and transplant conditions. For this analysis we evaluated consecutive patients who underwent SCT from January 2007 to December 2008 at the Department of Hematology and SCT of the Udine University Hospital, Italy.

Patients (aged ≥18 years) who had undergone SCT using a related or unrelated matched donor and who had survived for ≥3 months after SCT were included in the analysis. Patients were analyzed for the development of late-onset thrombocytopenia, defined as a platelet count of ≤100 x 10^9/L ≥3 months following SCT. Evaluation of blood cell count was performed at least once per month. Patients were stratified according to the type of transplant (related vs. unrelated), stem cell source (peripheral blood vs. bone marrow), conditioning regimen (conventional [CON] *vs.* reduced intensity [RIC]), primary disease, and status at transplant. Thrombocytopenia was distinguished as *persistent* or *transient* in the presence of a ≥ or <30 day duration of platelet count reduction, respectively. Clinical conditions related or associated with the development of thrombocytopenia (i.e. cGVHD, infectious complications, relapse, microangiopathy, or other) were evaluated.

Table 1. Summary of patients' clinical and transplant baseline characteristics

Patients, N	71
Median age, years (range)	52 (18–69)
Type of transplant, N (%)	
Matched sibling	25 (35)
Matched unrelated	47 (65)
Myeloablative	28 (38)
Reduced intensity	43 (62)
Stem cell source, N (%)	
Bone marrow	14 (20)
Peripheral blood	57 (80)
CD34-positive reinfused stem cells, median number x 10^6/kg (range)	
All patients	5.6 (0.8–12.7)
From bone marrow	2.55 (0.8–4.6)
From peripheral blood	6.4 (1.6–12.7)
Primary disease, N (%)	
Chronic myeloid leukemia	2 (3)
Acute myeloid leukemia	30 (42)
Acute lymphoblastic leukemia	4 (6)
Myelodysplasia	1 (1)
Lymphoma/chronic lymphocytic leukemia	25 (35)
Multiple myeloma	7 (10)
Primary myelofibrosis	2 (3)
Paroxysmal nocturnal hemoglobinuria	1 (1)
Status at transplant, N (%)	
Complete remission	34 (48)
Partial remission	14 (20)
No response/progression	20 (28)
No previous therapies	3 (4)

Results

Baseline characteristics

In total, 71 patients were selected for analysis (baseline characteristics are summarized in Table 1). Briefly, median age was 52 years, 62% of patients received RIC, stem cells were sourced from a matched unrelated donor for 65% of patients, 80% of cases used stem cells from peripheral blood, and the median number of CD34-positive reinfused stem cells was 5.6 x 10^6/kg. Forty-two percent of patients had acute leukaemia, 35% had lymphoma or chronic lymphocytic leukemia (CLL), and 48% and 20% of patients, respectively, were in complete or partial remission at transplant.

Incidence and type of thrombocytopenia

The median follow-up after SCT was 21 months (range 3–44 months). Twenty-seven of 71 patients (38%) developed post-SCT thrombocytopenia after a median time of 3 months from transplant (range 1–13 months); with a median number of platelets of 29 x 10^9/L (range 7–86 x 10^9/L). Platelet count was ≤50 x 10^9/L in 19 patients among whom 9 had ≤20 x 10^9/L. Thrombocytopenia was transient in 5 and persistent in 22 patients; in this last group the median duration of thrombocytopenia was 3 months. Patients with and without thrombocytopenia were similar in baseline clinical characteristics, type of transplant and status at transplant (Table 2).

Medical conditions associated with post-SCT thrombocytopenia

Thrombocytopenia was associated with cGVHD in 13 out of 24 patients (54%) (3 patients with thrombocytopenia were not evaluable for cGVHD), disease relapse in 7 patients (26%; 5 acute leukaemia and 2 lymphomas), CMV infection in 4 patients (15%), graft failure in 2 patients, and microangiopathy in 1 patient; 3 patients had idiopathic thrombocytopenia. Thrombocytopenia was complicated with major intestinal bleeding and cerebral bleeding in 4 and 2 patients, respectively.

Thrombocytopenia and survival

The incidence of mortality was significantly higher in patients with post-SCT thrombocytopenia compared with patients without thrombocytopenia (19 out of 27 [70%] vs. 6 out of 44 [14%] patients; $p<0.0001$). The cause of mortality in patients with thrombocytopenia included: primary disease in 11 patients (58%; acute leukemia in 5 patients, lymphoma in 3 patients, 1 patient each myelodysplasia, chronic myeloid leukemia, and multiple myeloma), cGVHD in 4 patients (21%), infections in 2 patients (10.5%), 1 patient each with cerebral bleeding, cardiac

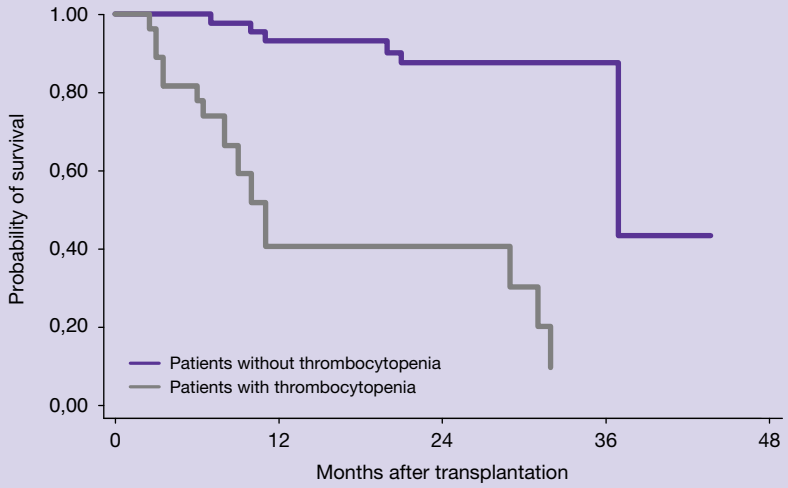
Table 2. Clinical and transplant characteristics in patients with and without post-SCT thrombocytopenia

	Patients with thrombocytopenia (N=27)	Patients without thrombocytopenia (N=44)	*p*-value
Median age, years (range)	52 (24–67)	53.5 (17–69)	0.606
Gender: male/female	15/12	24/20	0.934
Type of transplant			
Sibling/unrelated	8/19	17/27	0.440
Myeloablative/RIC	11/16	16/28	0.712
Stem cell source			
BM/PB	7/20	7/37	0.303
CD34-positive reinfused stem cells, median number x 10^6/kg (± SD)	5.29 (2.61)	6.26 (2.66)	0.137
Primary disease			
AML/ALL/MDS	14	20	0.406
NHL/HL/CLL	10	22	
CML/myelofibrosis/PNH	3	2	
Status at transplant			
Complete remission	12	22	0.057
Partial remission	2	12	
No response/progression	11	9	
No previous therapies	2	1	
cGVHD*			
Yes	13	19	0.386
No	11	25	

* Not evaluable in 3 patients with thrombocytopenia
ALL: acute lymphoid leukaemia; AML: acute myeloid leukaemia; BM: bone marrow; CLL: chronic lymphocytic leukaemia; CML: chronic myeloid leukaemia; cGVHD: chronic graft-versus-host disease; HL: Hodgkin lymphoma; MDS: myelodysplastic syndrome; NHL: non-Hodgkin lymphoma; PB, peripheral blood; PNH, paroxysmal nocturnal hemoglobinuria; RIC, reduced-intensity conditioning; SD, standard deviation

stroke and multi organ failure. Patient survival after 12, 24, and 33 months was 93%, 87%, and 87% in patients without thrombocytopenia vs. 41%, 41%, and 7% in patients with thrombocytopenia, respectively ($p<0.0001$) (Figure 1). In univariate analysis using Cox proportional hazard model, overall survival (OS) showed a significant association with status at transplant (no response/ progression vs. complete remission hazard ratio [HR] 2.74; 95% confidence interval [CI], 1.11–6.79; $p=0.03$) and thrombocytopenia (HR 9.77; 95% CI, 3.63–26.33; $p<0.0001$). In multivariate analysis only thrombocytopenia was significantly associated with OS (HR 9.77; 95% CI, 3.63–26.33; $p<0.0001$).

Figure 1. Kaplan-Meier overall survival curves in patients with and without post-SCT thrombocytopenia.



Thrombocytopenia and cGVHD

Of the 68 patients for whom cGVHD was evaluable, 32 (47%) developed cGVHD and 13 had concomitant thrombocytopenia (see Table 2). The incidence of mortality in this group was significantly higher in patients with post-SCT thrombocytopenia compared with patients without thrombocytopenia (8 out of 13 [61.5%] vs. 2 out of 19 [10.5%] patients; $p=0.005$).

Discussion

This retrospective analysis confirms the incidence and prognostic significance of post-SCT thrombocytopenia. In our survey 38% of patients developed thrombocytopenia, which was related with a higher risk of mortality. cGVHD was the most frequent condition associated with thrombocytopenia and the mortality rate in patients with cGVHD and thrombocytopenia reached 61.5%. Thrombocytopenia itself, regardless of the cause, and the combination of cGVHD and thrombocytopenia appears to be strong negative prognostic factors for survival in patients undergoing allogeneic SCT.

It is notable that thrombocytopenia was secondary to recurrence of acute leukaemia in only 5 of 27 patients (18.5%); therefore, in the majority of patients the poor prognostic significance of thrombocytopenia seems not to be strictly related to or a consequence of the activity of the primary disease, but appears to be epiphenomenon of a pro-inflammatory or pro-immunologic detrimental condition. This status may in part help understand the frequent occurrence of severe infectious complications which (rather then bleeding) likely represent the main cause of mortality in patients with post-SCT persistent thrombocytopenia.[1,3]

The pathogenic process of cGVHD-related thrombocytopenia is complex and only partially understood. Biological and clinical evidence support an autoimmune-like thrombocytopenia with increased platelet destruction;[4] this is also supported by the response to some therapeutic strategies used to treat classical immune thrombocytopenia including steroids, high dose intravenous immunoglobulin, splenectomy, and rituximab.[13-17]

However a mechanism consistent with impaired platelet production has also been suggested. Glycocalicin index, plasma thrombopoietin (TPO), and circulating B cells producing anti-GPIIb-IIIa antibodies were measured and compared in 23 SCT recipients who had prolonged and isolated thrombocytopenia with no apparent cause, SCT recipients without thrombocytopenia, and in patients with idiopathic thrombocytopenic purpura or aplastic anemia.[18] Despite the frequent occurrence of an antiplatelet antibody response, patients with post-SCT thrombocytopenia showed a glycocalicin index and TPO status similar to that seen in patients with aplastic anemia; TPO levels were normal in approximately 30% of patients.[18] Recently, Bao *et al.* showed an improved regulatory T-cell (T-regs) activity in patients with chronic primary immune thrombocytopenia (ITP) treated with thrombolytic agents, suggesting a possible role of platelet count in improving T-reg function and restoring immune tolerance.[19] Based on this and on the similarity to ITP, the stimulation of thrombopoiesis with the thrombolytic agents could be beneficial in some patients with persistent post-SCT thrombocytopenia, both on platelet count and on cGVHD manifestations.

In conclusion, post-SCT thrombocytopenia appears to be a strong negative predictor of survival. cGVHD seems to be the most frequent pathologic condition associated with thrombocytopenia. The management of post-SCT thrombocytopenia may influence patient outcome.

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Helicobacter pylori eradication in the treatment of primary immune thrombocytopenia: clinical relevance and potential underlying mechanisms

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Abstract

Background and Objectives: The detection of *Helicobacter pylori* infection and infection eradication are indicated for the diagnosis and the treatment of primary immune thrombocytopenia, also known as immune thrombocytopenic purpura (ITP). However, this approach is not consistently effective, and appears to work better in patients with moderate thrombocytopenia than in patients with severe thrombocytopenia (platelet count $<30 \times 10^9$/L). The aim of the present study was to establish whether the duration of thrombocytopenia and *H. pylori* infection affect the outcomes of ITP treatment based on *H. pylori* eradication.

Materials and Methods: Forty consecutive adult patients with ITP and a platelet count $<30 \times 10^9$/L with a follow-up of at least 1 year were included in this study, which investigated whether the combination of *H. pylori* eradication and immunosuppressive treatment was associated with a benefit for *H. pylori*-positive patients, compared with *H. pylori*-negative patients. Complete remission was defined as a platelet count $\geq 100 \times 10^9$/L 1 year after the first observation, with no need for additional treatments.

Results: *H. pylori* infection was assessed in 22/40 (55%) patients. Of these, 12/22 (54.5%) patients were positive and 10/22 (45.5%) were negative. After adjusting for ITP-relapse, the mean duration of thrombocytopenia in *H. pylori*-positive patients was longer than in *H. pylori*-negative patients (4.3±2.1 vs. 2.8±1.9 months, respectively; p=0.04). First-line immunosuppressive treatment achieved complete remission of ITP in 11 patients (50%). Complete remission was achieved in six *H. pylori*-positive patients (50%) and five *H. pylori*-negative patients (50%). Complete remission was sustained for 1 year in three patients with infection eradication (50%) and in four *H. pylori*-negative patients (80%). In *H. pylori*-positive patients, the inverse correlation between thrombocytopenia duration before eradication and response was statistically significant (p=0.01).

Conclusions: Early eradication therapy in ITP in the presence of moderate thrombocytopenia appears to be more effective than in severe long-term ITP. The reduction of the bacterial load and the inhibition of the initial platelet destruction may limit the production of cross-reacting antibodies. This, in turn, blocks the autoimmune mechanism responsible for disease perpetuation. In severe and long-term ITP, the occurrence of somatic mutations may lead to the production of antibodies specific for platelet antigens and independent from bacterial antigens, making the therapeutic approach based on *H. pylori* eradication ineffective in these patients.

Introduction

Steroids are the first-line treatment for primary immune thrombocytopenia, also known as immune thrombocytopenic purpura (ITP). In patients with the recurrent form of ITP, splenectomy, thrombopoietin (TPO) receptor agonists, rituximab and other immunosuppressive therapies are indicated. Over the past few years, eradication of *Helicobacter pylori* infection has emerged as a treatment option for *H. pylori*-positive patients with ITP.

In 1998, a case report was published describing a Japanese patient with chronic ITP who had a significant improvement in platelet count after receiving a proton pump inhibitor for the treatment of a concomitant peptic ulcer.[1] In the same year, Gasbarrini *et al.* reported a significant increase in the platelet count in ITP patients (8/11) who had successfully undergone a *H. pylori* eradication protocol. The improvement in platelet count in most patients (6/8) was associated with the disappearance of antiplatelets antibodies.[2] In the following

years, the eradication of *H. pylori* with antibiotics combined with a proton pump inhibitor was variably associated with a substantial and sustained improvement of platelet count in ITP patients. Complete and partial response rates of about 50% were reported in several studies and a meta-analysis, conducted mainly in the Italian and Japanese population,[3-6] while lower response rates were described by French, Spanish, and North American authors.[7-9] Despite these discrepancies, the Maastricht III Consensus Conference recently established ITP as one of the extraintestinal conditions for which the eradication of *H. pylori* is indicated.[10] According to the guidelines issued in 2010 by the International Consensus Report on the investigation and management of primary immune thrombocytopenia, *H. pylori* testing is among the recommendations for the basic evaluation of patients with suspected ITP.[11]

So far, no clinical characteristics and no specific predictive factors of platelet count response to *H. pylori* eradication are known. A correlation has been suggested between chronic ITP with severe thrombocytopenia (platelet count $<30 \times 10^9$/L) and a poor response to *H. pylori* eradication.[7-14] This potential correlation, however, has never been investigated systematically. To clarify this issue, in the present study we evaluated whether thrombocytopenia severity and duration before *H. pylori* eradication had an impact on the outcomes of ITP treatment.

Patients and methods

Forty-six consecutive patients with ITP presenting to our Department between 2001 and the end of 2008 with platelet counts $<30 \times 10^9$/L and a follow-up period of at least 1 year were evaluated. The diagnosis of ITP was defined by the exclusion of other possible causes of thrombocytopenia such as EDTA-induced pseudothrombocytopenia, hepatitis C virus or HIV infection, drug induced thrombocytopenia, autoimmune disease or lymphoproliferative disorders. Patients who were older than 60 years underwent bone aspiration and chromosomal mapping to exclude the presence of myeloproliferative disorders. Of the 46 patients screened, two pregnant women with a follow-up shorter than 1 year and four patients lost to follow-up were excluded. Twenty two patients who had their *H. pylori* infection status assessed were included in our analysis.

For each patient we recorded whether *H. pylori* testing had been performed and the mean duration of thrombocytopenia before the first visit in our centre.

All patients received immunosuppressive treatment with steroids, with or without immunoglobulin. *H. pylori* eradication therapy was given within 4 months of the first observation. The eradication protocol consisted of amoxicillin 1 g twice daily and clarithromycin 500 mg twice daily, both for 7 days, in combination with omeprazole 20 mg twice daily for 14 days. Eradication efficacy was evaluated at least 12 weeks after the end of the treatment using the same tests performed for diagnosis. Changes in platelet count between *H. pylori*-positive patients with eradicated infection and *H. pylori* negative patients were compared, and response was defined as a platelet count $\geq 100 \times 10^9$/L one year after the first observation, with no need for additional treatment.

Statistical analysis

Comparisons of the mean values of two groups were performed using the *t*-test and the Mann-Whitney test. Variance analysis and comparisons of the mean values of more than two groups were conducted by means of the Kruskal-Wallis test and the analysis of the frequency was performed with the Chi-square test. A p value ≤ 0.05 was defined as statistically significant.

Results

Median patient age was 52.2 years (range 15–87) and median platelet count at the first observation was 9×10^9/L (range 1–24 $\times 10^9$/L). In 34 of the 40 patients in our analysis, ITP had developed recently, while six of the 40 patients were experiencing a relapse of ITP previously diagnosed and treated at other centres. Of the six relapsing patients, four had been previously treated with steroids and immunoglobulin, and two had undergone splenectomy. Of the 22 patients who had undergone *H. pylori* testing (10 males and 12 females; 20 Caucasian and 2 South American), 12 patients were *H. pylori*-positive and 10 were negative. The method used to detect *H. pylori* infection was a urea breath test in 17 patients (77.3%), stool antigen testing in three patients (13.7%), and histological analysis of gastric biopsies obtained by esophago-gastro-duodenoscopy in two patients (9%). Mean platelet count at the first observation was similar in *H. pylori*-positive and *H. pylori*-negative patients ($9.6 \pm 4.0 \times 10^9$/L vs. $12.0 \pm 9.6 \times 10^9$/L, p=0.74). Notably, three patients with relapsing ITP were *H. pylori*-positive at the first observation.

A total of 11 patients (50%) achieved complete remission following first-line immunosuppressive treatment. Complete remission was achieved in six *H. pylori*-positive patients (50%) and five *H. pylori*-negative patients (50%). Complete remission was sustained for up to 1 year in three patients with eradicated *H. pylori* infection and in four *H. pylori*-negative patients. One *H. pylori*-positive patient who failed to respond to the immunosuppressive therapy was resistant to the eradication treatment. Low-dose steroid treatment was maintained in two *H. pylori*-positive patients (17%) and in one *H. pylori*-negative patient (10%). The findings are summarized in Table 1.

Demographic and clinical characteristics (age, gender, and median platelet count at diagnosis) were similar among *H. pylori*-positive and *H. pylori*-negative patients, and no statistically significant differences in response were detected. In *H. pylori* positive-patients, we observed a statistically significant (p=0.01) inverted correlation between thrombo-

Table 1. Treatment response 1 year after the end of therapy

	No response/ relapse; n (%)	Complete remission; n (%)	Mean platelet count at diagnosis (10^9/L)	Mean duration of ITP before treatment (months)
H. pylori-positive (n=12)	9 (75)	3 (25)	9.6±4.0	52.7±116.1
H. pylori-negative (n=10)	6 (60)	4 (40)	12.0±9.6	2.8±1.9

cytopenia duration before eradication and response at 1 year, which was confirmed by a multivariate analysis comparing sex, age, mean platelet count at diagnosis, mean platelet count at eradication treatment, and duration of thrombocytopenia before *H. pylori* eradication. Mean duration of thrombocytopenia in *H. pylori*-positive patients was considerably longer than in non-infected patients (52.7±116.1 months vs. 2.8±1.9 months, respectively), as all patients with relapsing thrombocytopenia at the first observation were from the group of infected patients. However, even after excluding the patients with relapsed ITP from the analysis, the mean duration of thrombocytopenia was longer in *H. pylori*-positive patients compared with *H. pylori*-negative patients (4.3±2.1 months vs. 2.8±1.9 months, respectively; p=0.04).

Discussion

ITP pathogenesis is complex and only partially understood. One of the mechanisms thought to be involved is the accelerated destruction of platelets in the reticuloendothelial system (RES) mediated by Fcγ-receptors expressed on monocytes and macrophages (FcγRIIA and FcγRIIIA receptors with activator function and FcγRIIB receptors with inhibitory function).[15] These receptors bind platelets that have been opsonized by autoantibodies reacting against platelet antigens, particularly against glycoprotein IIb/IIIa.[16] The origins of the antigenic epitopes that initiate the autoimmune reaction are unknown, as are as the mechanisms that sustain this reaction in the long-term. In the case of *H. pylori* infection, the sequence homology between bacterial and platelet antigens is thought to be one of the mechanisms leading to the production of autoantibodies. *H. pylori* antigens potentially involved in this process are the CagA protein[17,18] and, in patients with the required genetic background, the gene for blood group antigen-binding adhesin (BabA) expressed in some bacterial strains.[19] Furthermore, the bacterial liposaccharide (LPS) component of the outer membrane of Gram negative bacteria, including *H. pylori*, is recognized by a specific platelet receptor belonging to the family of Toll-like receptors. The binding of bacteria to this receptor induces platelet aggregation and the monocyte-platelet interaction: bacterial LPS and anti-platelet IgG autoantibodies act in synergy to promote FcγR-mediated phagocytosis by monocytes.[20] The monocytes of ITP patients with *H. pylori* infection show an activated phenotype with increased phagocytic activity and a lower expression of the inhibitory FcγRIIB receptor compared with the monocytes of non-infected ITP patients.[21] In *H. pylori*-infected patients, expression of the FcγRIIB receptor increases to the stable levels observed in non-infected patients after eradication therapy.[22,23] Some bacterial strains have also been shown to bind the Von Willebrand factor and to interact with platelet glycoprotein Ib (GPIb), leading to platelet aggregation, platelet clearance and antigen presentation.[24]

Based on all these observations, a possible mechanism for understanding the correlation between chronic *H. pylori* infection and ITP would be the production by B lymphocytes of antibodies cross-reacting with platelets. As previously mentioned, RES macrophages activated by *H. pylori* phagocytate opsonized platelets in a Fcγ-receptor mediated process. This process is enhanced by bacterial LPS and by the binding of *H. pylori* to the Von Willebrand factor. Upon platelet antigen presentation to T-helper lymphocytes, the autoimmune process is enhanced and perpetuated. Somatic mutations in the antibody repertoire could lead to a second immunoglobulin "generation" capable of recognizing bacterial antigens bound to platelets or cross-reacting with platelet antigens, thereby enhancing platelet destruction. According to the mechanism suggested here, in chronic ITP, further somatic mutations could lead to the production of a third "generation" of antibodies, no longer specific for bacterial antigens but reactive against platelets. In this later phase, infection eradication would fail completely as a disease treatment. Therefore, *H. pylori* eradication affects the platelet count by blocking RES-mediated platelet clearance.

This is achieved by modifying the expression of Fcγ-receptors. Such a mechanism would explain the rapid increase in the platelet count occasionally observed after the end of the eradication treatment, while a sustained increase in the platelet count would be achieved by preventing macrophage antigen presentation.

Our results fail to demonstrate a benefit of *H. pylori* eradication in patients with severe thrombocytopenia: in line with the data reported in the literature, the rate of complete remission after one year was 27% in these patients.[9] However, our study confirms that eradication therapy administered early in the course of ITP, when thrombocytopenia is still moderate, is more effective. At this stage of the disease, the reduction of the bacterial load and the inhibition of platelet destruction by cross-reacting antibodies could prevent the production of autoantibodies that are nonspecific against bacterial antigens and reactive against platelets. Therefore, besides well recognized factors such as genetics and the virulence of the bacterial strain,[25] early *H. pylori* testing followed by a timely eradication treatment are also crucial for the impact of *H. pylori* eradication on the outcomes of ITP treatment.

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Thrombocytopenia in patients travelling abroad: a case report and review of the literature

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Abstract

Immune thrombocytopenia (or immune thrombocytopenic purpura, ITP) is a disease characterised by a low platelet (PLT) count due to the presence of anti-PLT antibodies, resulting in increased destruction of PLTs. Many conditions, including use of medications, autoimmune diseases and infections, may lead to secondary ITP and therefore should be investigated in patients with newly diagnosed isolated thrombocytopenia. Nowadays, intercontinental travels are far more frequent than in the past, and infectious agents once rarely seen at our latitudes are becoming progressively more evident. Infectious diseases, however, represent an important cause of ITP not only in the traveller, but also in the general population. Viruses such as human immunodeficiency virus and hepatitis C virus are frequently associated with thrombocytopenia. Also, in the intercontinental traveller with newly diagnosed thrombocytopenia returning from geographic areas at risk, conditions such as dengue fever and malaria should always be suspected. The case report presented here documents the complexity of the differential diagnosis of thrombocytopenia in the intercontinental traveller.

Introduction

Immune thrombocytopenia (or immune thrombocytopenic purpura, ITP) is an autoimmune disease characterised by a low platelet (PLT) count due to anti-PLT antibodies that result in immuno-mediated destruction of PLTs in the liver, spleen and other organs of the reticulo-endothelial system. ITP is clinically characterised by an increased risk of spontaneous or secondary hemorrhages.[1] Currently, the diagnosis of ITP in daily practice is based on the exclusion of possible causes of secondary ITP.[2] Autoimmune diseases, medications and infections are the vast and complex fields that should be investigated in order to correctly evaluate the nature of newly diagnosed isolated thrombocytopenia.

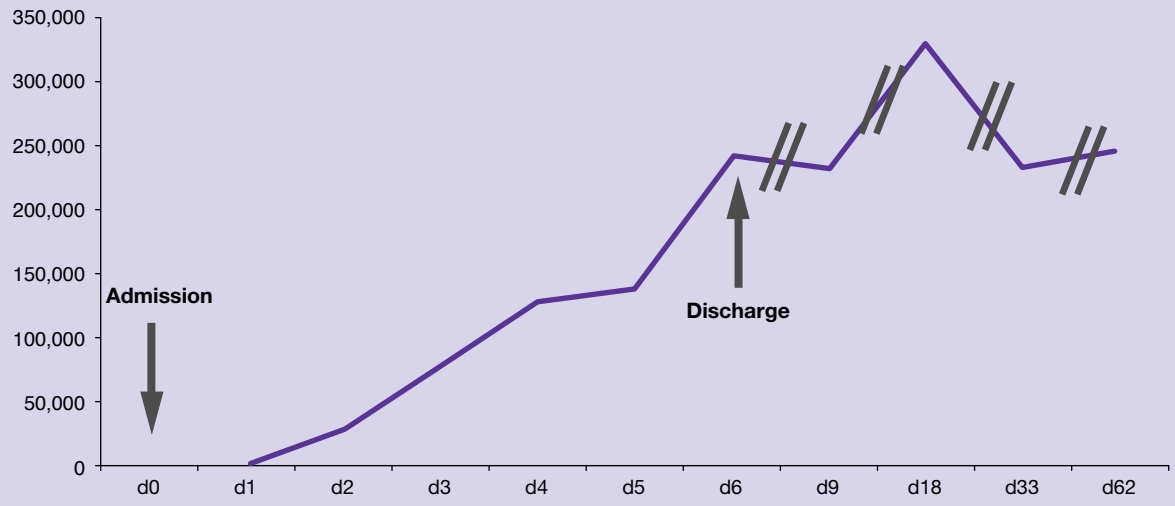
Herein, we report the case of a patient recently admitted to our hospital for severe thrombocytopenia that developed after travelling to a tropical country.

Patient and methods

A 39-year-old male patient, a Cambodian refugee living in Italy since 1980, presented at the emergency department with fever and bleeding of the gums. He had spent the previous two months in Cambodia, and approximately a week before re-entering Italy he had developed a fever (maximum body temperature 39 °C). The patient had been seen at a local hospital in Phnom Penh, where blood analysis showed a PLT count of 36,000 x 10^9/L, and where he received an unidentified intravenous (iv) treatment and was started on oral amoxicillin 1 g twice daily (bid) and paracetamol 500 mg three times daily, without any improvement. As soon as the patient came back to Italy, he was admitted to our emergency department, where blood analysis showed a white blood cell count of 5000 x 10^9/L, absolute neutrophil count of 1700 x 10^9/L, lymphocyte count of 2500 x 10^9/L, hemoglobin concentration of 16.5 g/dL and PLT count of 2000 x 10^9/L. Thrombocytopenia was confirmed following blood analysis with citrate as anticoagulant. Renal function tests were normal, with aspartate aminotransferase and alanine aminotransferase levels of 557 IU/L and 336 IU/L, respectively, lactate dehydrogenase of 1419 IU/L, and creatine kinase of 664 IU/L; C-reactive protein was 1.58 ng/L. An increased activated partial thromboplastin time was observed (60.2 s). The patient was admitted to our hematology unit.

Past medical history and familiar history were negative, except for a previous history of peptic ulcer treated with proton-

Figure 1. Increase in platelet count following discharge.



pump inhibitors. The patient denied suffering from diarrhoea, pain, weight loss, itching or cough. He denied regular drug intake and, in particular, ruled out traditional Cambodian medications. During his travels, he did not take a chemoprophylaxis against Malaria *spp*. At physical examination, we observed bleeding gums, diffuse petechiae on the lower limbs and a palpable liver (lower border 1 cm under the costal arch). The physical examination was otherwise negative for other hemorrhages, gum hypertrophy, lymphadenopathy, splenomegaly and neurological signs; body temperature was 37.1 °C after paracetamol, and other vital signs were normal. Chest X-ray and abdomen ultrasound did not show significant lesions.

A peripheral blood smear showed no signs of white cell dysplasia, normal red cell morphology and the absence of schistocytes. Bone marrow aspiration was performed; morphological assessment showed the presence of numerous megakaryocytes and no evidence of lineage dysplasia, with 2-3% of plasma cells identified as polyclonal cells. Blood parasite evaluation was negative, as well as serology tests for human immunodeficiency virus (HIV), hepatitis A virus, hepatitis C virus (HCV), cytomegalovirus and Epstein-Barr virus. Hepatitis B virus surface antigen was negative, hepatitis B surface antibody titre was 1000 IU/mL, and hepatitis B core antibody and hepatitis B early antibody were positive. Assessments for dengue virus, *Leptospira* and *Leishmania* were also negative, as well as direct and indirect Coombs tests. At admission, we started an antibiotic treatment with levofloxacin 500 mg iv bid, resulting in a response in terms of fever, and performed PLT transfusion, without any rise in PLT count. After excluding secondary causes, we considered the diagnosis of ITP as the most likely for our patient; we started methylprednisolone 1 mg/kg/day, and administered iv immunoglobulins 1 g/kg for two consecutive days, because of the high risk of major bleeding. After the start of this treatment, a prompt rise in PLT count was observed (Figure 1), paralleled by a progressive reduction in mucosal bleeding and a constant improvement of liver function tests and coagulation assays.

The patient was discharged five days after admission, with a PLT count of 243,000 x 10^9/L; he maintained a normal PLT count throughout the tapering of the steroid-based immunosuppressive therapy, and has since suspended immunosuppressive treatments.

Discussion

The differential diagnosis of primary ITP is still dependent upon the exclusion of clinical conditions such as infections, autoimmune diseases and drugs. This necessity has been recently confirmed by an International Working Group in a consensus conference held in Vicenza, Italy (the Vicenza Consensus Conference), which standardised the terminology related to ITP diagnosis, phase of disease, clinical response, and treatment.[3]

However, the distinction between primary and secondary ITP is important not only for clarity, but also because of the different origin and pathogenesis of these conditions,[4] as well as the distinct treatment, which includes targeting of the underlying medical condition for secondary ITP[5] or the withdrawal of drugs in drug-induced ITP.[6]

As clearly stated in the updated international guidelines for ITP,[2] patient history and physical examination are a relevant part of the diagnostic workout for ITP, and infectious diseases should be investigated in all patients, not only in the traveller. Infectious diseases may lead to thrombocytopenia with various pathogenetic mechanisms, such as immunological PLT destruction, inappropriate

PLT activation and consumption, and impaired megakaryopoiesis.[7]

Thrombocytopenia may be present in approximately 10-50% of HIV-positive patients, and it may also be the first symptom of infection. The pathogenesis of HIV-related thrombocytopenia is multifactorial and includes accelerated destruction of PLTs due to immune complexes, direct infection of megakaryocytes and the presence of antibodies cross-reacting with the PLT membrane.[8] In vitro studies have shown that HIV virions may be internalised by PLTs, causing the activation and expression of P-selectin by PLTs, facilitating their clearance by macrophages.[7]

HCV is another virus that frequently causes a reduced PLT count, even in the absence of liver disease, and HCV positivity may be found in up to 30% of patients with ITP.[8] As is the case for HIV, the pathogenesis of HCV-related thrombocytopenia is due to various mechanisms. These include: *i)* direct infection of PLTs and megakaryocytes by HCV; *ii)* an 'innocent bystander' mechanism involving the binding of anti-HCV antibodies to complexes formed by the surface of PLTs and HCV;[9] and *iii)* non-immune mechanisms in patients with liver disease, including sequestration of PLTs in the enlarged spleen secondary to portal hypertension (hyper-splenism), and inadequate production of thrombopoietin (TPO).[8] Treatment of HCV infection could lead to an increase in PLT count. Recently, eltrombopag, was shown to increase PLT count in most patients with HCV-related cirrhosis, permitting the start of interferon-based therapies in patients otherwise not capable of receiving this treatment.[10]

Other viral and parasitic infections should also be considered in travellers. Malaria, caused in humans by four types of *Plasmodium* spp., can be associated with thrombocytopenia if caused by *P. falciparum* or *P. vivax*.[11] Even if other symptoms are much more probable in patients with malaria, thin and thick blood smears and other diagnostic tests should be performed if thrombocytopenia is diagnosed in subjects who recently travelled in malaria transmission areas.

Another possible cause of thrombocytopenia is dengue fever, a mosquito-borne viral disease associated with important clinical manifestations. A decrease in PLT count is frequent in dengue fever, and almost constant in patients with dengue hemorrhagic fever. The pathogenetic mechanisms of thrombocytopenia in dengue virus infection are not completely clear, but an immune-mediated clearance due to anti-virus antibodies cross-reacting with normal PLTs in the presence of virus-specific antibodies has been proposed,[12] while direct infection of PLTs and megakaryocytes has not been observed.[13]

In conclusion, the diagnosis of ITP still remains difficult, and the many possible causes of secondary ITP should be evaluated and excluded. Infectious diseases should always be considered, and travel history may play an important role in patients with a recently diagnosed decrease in PLT count.

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Highlights from the 61st Annual Meeting of the American Association for the Study of Liver Disease

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Abstract

Purpose of review: This article reviews selected studies presented at the 61st Annual Meeting of the American Association for the Study of Liver Disease.

Recent findings: Studies presented at the annual meeting covered: the effects of chronic liver disease on the mobilization of CD34+ cells; the contribution of platelet activation and aggregation in the liver to thrombocytopenia in cirrhotic patients; the association between inositol triphosphate pyrophosphatase gene variants and interferon-induced thrombocytopenia in patients with chronic HCV infection; the population pharmacokinetics of eltrombopag; and an exploratory analysis of predictors of thrombotic events following treatment with eltrombopag in patients with chronic liver disease (the ELEVATE study).

Summary: Thrombocytopenia in patients with chronic HCV infection has a multifactorial aetiology, influenced by the severity of liver damage and the myelosuppressive effects of interferon treatment, which may be influenced by genetic variation. Eltrombopag may have some efficacy for the treatment of thrombocytopenia in patients with chronic HCV infection, though the pharmacokinetics are affected by race and severity of liver impairment. Orally administered TPO receptor agonists have also been associated with thrombotic events, which were found to be significantly more likely in patients with a platelet count >200 x 10^9/L.

Introduction

Thrombocytopenia in patients with chronic HCV infection was the subject of several presentations at the recent annual meeting of the American Association for the Study of Liver Disease, held in Boston, Massachusetts from the 29th of October to the 2nd of November 2010. This article will summarize the most important abstracts with findings relevant to the etiology and treatment of thrombocytopenia in patients with chronic HCV infection.

Evaluation of the influence of chronic liver disease on mobilization of CD34+ cells before and after liver transplantation

Hematopoietic stem cells expressing the CD34 antigen are usually present at low levels in peripheral blood.[1] However, CD34+ cells are mobilised from the bone marrow into peripheral blood after myeloablative chemotherapy, or the administration of granulocyte colony-stimulating factor or thrombopoietin (TPO).[2,3] TPO is produced by the liver at a constant rate,[4] and chronic liver diseases are associated with a decrease in serum TPO levels that may contribute to the thrombocytopenia frequently observed in these patients.[5] Furthermore, TPO production is restored after successful liver transplantation, followed by an increase in thrombopoiesis.[6]

Yamamoto *et al.* evaluated the association of CD34+ cell mobilization in 16 healthy subjects and 88 patients with various stages of HCV-associated chronic liver diseases (chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma).[7] In a further group of 12 patients they also analyzed platelet count and number of circulating CD34+ cells both before and after living donor liver transplantation performed for liver cirrhosis with or without hepatocellular carcinoma.[7] CD34+ cell counts were evaluated using flow cytometry, and platelet counts and serum TPO levels were also assessed. The main result of the study was that the number of

circulating CD34+ cells was significantly lower in patients with chronic liver disease, compared with healthy controls (3.78±3.93 cells/μL), and decreased with progression of liver disease: chronic hepatitis patients (1.49±1.41 cells/μL), liver cirrhosis without hepatocellular carcinoma (0.66±0.83 cells/μL), and liver cirrhosis with hepatocellular carcinoma (0.58±0.19 cells/μL). The longitudinal evaluation of patients who underwent living-donor liver transplant showed a 4-fold increase in platelet counts (from 68 x 10^9/L to 234 x 10^9/L) and a 5-fold increase in number of circulating CD34+ cells (from 0.78 cells/μL to 3.52 cells/μL) after liver transplant. Concomitantly, serum TPO levels increased.

These results indicate that not only thrombopoiesis but also the mobilization of CD34+ cells from bone marrow into peripheral blood depends on liver conditions. This finding, besides adding to our knowledge on thrombopoiesis, might also shed new light on the possible contribution of megakaryocytopoiesis to liver regeneration.

Contribution of platelet activation and aggregation within the liver to thrombocytopenia in cirrhotic patients

Thrombocytopenia is the most common hematological abnormality observed in patients with liver cirrhosis.[8] In these patients, this condition is mainly explained by splenic pooling of platelets due to portal hypertension and by decreased production of TPO in diseased livers.[9] In some patients, other mechanisms such as the presence of anti-platelet antibodies and myelosuppression due to infection with hepatitis viruses or alcohol abuse are thought to play a role in the pathogenesis of thrombocytopenia.[10] Also, it has previously been reported that activated platelet aggregation in sinusoids can cause secondary parenchymal damage in cirrhotic livers,[11] and this may also reduce the number of circulating platelets. A similar phenomenon has been reported during liver transplant with increased activation of platelets following liver reperfusion.[12]

In an autopsy-based analysis, Ikura *et al.* evaluated the possible role of platelet aggregation in cirrhotic livers in the development of thrombocytopenia.[13] They studied 55 consecutive autopsies of patients with chronic liver disease (50 cirrhotics and 43 with viral hepatitis) from the last decade. Data regarding circulating platelet counts and the spleen weight were retrieved from hospital charts and autopsy protocols, respectively. Megakaryocytes were counted in randomly chosen 10 high-power fields in each bone marrow sample. In 16 subjects, frozen liver samples were subjected to immunofluorescent staining with specific antibodies against platelet glycoprotein IIb/IIIa and P-selectin. Double-positive areas were assessed by means of computer-aided morphometry (%) and were interpreted as a signal of activated platelet aggregates. Six autopsies of non-liver disease subjects without hemostatic disorders were examined as controls. In patients with chronic liver disease both megakaryocyte count ($r=0.37$; $p=0.007$) and spleen weight ($r=-0.40$; $p=0.004$) were independently correlated with circulating platelet count. The area of activated platelet aggregation was significantly greater in cirrhotic livers than in control livers (0.33±0.13% vs. 0.21±0.04%; $p=0.044$), and was significantly correlated with circulating platelet counts ($r=-0.36$; $p=0.033$). Although these results should be evaluated with caution due to the particular study setting, they are nevertheless in keeping with the results obtained in explanted livers, and point toward a further mechanism that may contribute to thrombocytopenia in cirrhotic patients via activated platelet aggregation in livers. Furthermore, they also confirm that platelets may play an active role in progressive parenchymal extinction as a mechanism of disease progression in patients with chronic liver disease.[14]

Identification of an association between ITPA gene variants and interferon-induced thrombocytopenia in a genome-wide association study of chronic hepatitis C patients

The occurrence of thrombocytopenia during antiviral therapy with pegylated interferon for the treatment of chronic HCV infection may be dose-limiting in up to 19% of patients,[15] and a reduction in the dosage of pegylated interferon is associated with a decrease in sustained viral response.[16] The IDEAL study was the largest trial comparing pegylated interferon α-2a and α-2b with ribavirin in patients with HCV genotype 1.[17] Among the 3070 patients included in the study, 1604 patients consented to DNA testing, and Thompson *et al.* performed a genome-wide association study in these patients to identify genetic determinants of interferon-induced thrombocytopenia.[18] The primary aim of the study was to assess genetic determinants of change in platelet count after 4 weeks of treatment in patients who had received at least 80% of the scheduled pegylated interferon dose. Week 4 was chosen to minimise confounding by dose modification, and because the greatest interferon-induced myelosuppressive effect is usually seen within this time frame.[19] The genome-wide association study revealed that six single nucleotide polymorphisms (SNPs) on chromosome 20 were significantly and positively associated with platelet reduction at week 4 (top SNP rs965469; overall $p=10^{-10}$). Of note, these tag SNPs have previously been shown to be in high linkage-disequilibrium with two functional variants in the inositol triphosphate pyrophosphatase (ITPA) gene (rs1127354 and rs7270101) that cause ITPase deficiency and protect against ribavirin-induced hemolytic anemia. These two SNPs showed a strong independent association with platelet reduction (rs1127354, $p=10^{-12}$; rs7270101, $p=10^{-7}$; combined variable=10^{-20}). As hemoglobin

decrease was strongly and inversely correlated with platelet reduction at week 4 ($p=10^{-18}$), the authors tested whether the association between platelet reduction and ITPA variants indirectly reflected an association between anemia and relative reactive thrombocytosis that might have reduced the effect of interferon on platelet counts. Inclusion of hemoglobin decrease into a regression model that also included the two ITPA variants significantly reduced the strength of the association between the ITPA variants and platelet reduction from 10^{-20} to 10^{-7}. These results show that two functional variants in the ITPA gene that are strongly associated with protection from ribavirin-induced hemolytic anemia are also associated with greater thrombocytopenia during antiviral therapy. Furthermore, this association is largely explained by a relative reactive thrombocytosis in response to ribavirin-induced hemolytic anemia which attenuates interferon-related thrombocytopenia.

Determinants of eltrombopag exposure in patients with chronic liver disease and exploratory analyses of predictors of thrombotic events in the ELEVATE study

In patients affected by chronic liver disease and with thrombocytopenia, eltrombopag have been shown to allow initiation of antiviral therapy in patients with chronic hepatitis C,[20] and to significantly decrease the need for platelet transfusions in patients undergoing invasive procedures.[21] Farrell *et al.* carried out a thorough evaluation of drug exposure in patients with thrombocytopenia and varying degrees of liver impairment who had been included in various studies assessing use of eltrombopag in order to evaluate the drug population pharmacokinetics.[22] The pharmacokinetic dataset was constructed from three studies that included 28 healthy volunteers and 79 patients with chronic liver disease for a total of 786 observations. The dose of the drug ranged from 12.5 to 75 mg as a single dose, or as repeat daily doses for up to 22 days. The results of this study showed that within the chronic liver disease population, gender, race (East Asian vs. Others) and severity of chronic liver disease were predictors of drug exposure. In particular, following receipt of the same regimen of eltrombopag, women, subjects of East Asian origin, and chronic liver disease patients with a Child-Pugh score of 5 would have 61%, 110% and 87% higher exposure, compared to healthy male subjects, respectively. Increases in severity of hepatic impairment were associated with further increases in eltrombopag exposure, while body weight, creatinine clearance and liver function enzyme levels were not found to be predictive of eltrombopag pharmacokinetics in patients with chronic liver disease. Overall, the results of this study showed that, besides ethnic and demographic characteristics, severity of chronic liver disease can have a clinically significant effect on exposure to eltrombopag, which may have important implications for dosing in different patient populations.

The ELEVATE study showed that eltrombopag is effective in reducing the need for platelet transfusion in patients with advanced liver disease and thrombocytopenia undergoing invasive procedures.[21] In particular, the study results showed that platelet transfusion was avoided in 104 (72%) of patients who received eltrombopag and 28 (19%) of patients who received placebo ($p<0.0001$). However, the pharmaceutical company sponsoring the trial decided to discontinue the study to allow a complete data analysis after an Independent Data Monitoring Committee had suggested temporarily suspending the trial due to an imbalance in the occurrence of thrombotic events in the active arm of the study. Giannini *et al.* performed an exploratory analysis to assess if other factors in the study population were predictive of the occurrence of thrombotic events.[23] In particular, a univariate descriptive analysis was used to assess any association between thrombotic events and concurrent medical conditions (reported in ≥2 subjects), and a multivariate analysis was performed on demographic and important baseline characteristics. Data mining based on random Forest classifications were performed to identify or establish if there were any predictors of high platelet counts. The results of this study showed that no demographic or baseline characteristics (age, gender, race, weight, baseline platelet count, Child-Pugh score and Model for End-stage Liver Disease score) significantly and independently predicted the occurrence of thrombotic events. Furthermore, there were no significant associations between patients' medical conditions (splenomegaly, HCV infection, oesophageal varices, portal hypertension, diabetes, hepatocellular carcinoma and non-hepatic malignancies) and thrombotic events. The only parameter significantly associated with thrombotic events was maximum post-baseline platelet count ($p=0.0045$). A receiver operating characteristic curve was used to identify possible platelet count cut-points, and showed that patients with platelet counts ≤200 x 10^9/L were less likely than patients with platelet counts above this threshold to experience a thrombotic event with a high negative predictive value (>98%). This study identified maximum post-baseline platelet count as the only parameter significantly associated with the occurrence of thrombotic events in the ELEVATE study. In this regard, the identification of a platelet threshold with a very high negative predictive value could lead to risk minimisation strategies.

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Pathophysiology and treatment of immune thrombocytopenia: Highlights from the 52nd ASH annual meeting

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Abstract

Immune thrombocytopenia (or immune thrombocytopenic purpura, ITP) is a commonly occurring autoimmune disorder characterized by persistent thrombocytopenia (platelet count <150 x 10^9/L). Here we report the results of several studies presented at the recent American Society of Hematology (ASH) meeting that examined the pathophysiology and treatment of ITP. In 4–6-year extension studies, the thrombopoietin (TPO) receptor agonists, eltrombopag and romiplostim, achieved a platelet count of ≥50 x 10^9/L in 87% and 94.5% of participants, respectively, while the novel TPO receptor agonist E5501 was associated with an overall platelet response rate of 75.5%. These agents offer an effective and relatively safe approach to the management of chronic ITP, replacing more toxic treatments, such as prolonged use of corticosteroids or immunosuppression, and delaying splenectomy. Despite the uncertainty over the long-term safety of rituximab, new studies on this treatment were also presented. High-dose dexamethasone was not shown to be more effective than prednisolone in adult ITP patients. New approaches based on primary versus secondary ITP, and "patient-specific" treatments, including high-throughput screening to clone auto-antibodies and search for implicated epitopes and external antigens, may offer new perspectives for the treatment of ITP.

Introduction

At the last meeting of the American Society of Hematology, held in Orlando, Florida, on 4–7 December 2010, the pathophysiology and treatment of immune thrombocytopenia (or immune thrombocytopenia purpura, ITP) and related issues were the subject of several oral and poster presentations.

ITP and related issues were also addressed in three educational lectures. Greinacher and Selleng discussed the difficulty of diagnosing specific thrombocytopenic disorders (including ITP) in the intensive care unit patient.[1]

A general overview of ITP and its treatment, mainly based on the recent International Consensus Report,[2] was presented by Cuker and Cines,[3] whereas ITP in pregnancy was discussed by McCrae.[4] In an evidence-based mini review, Cuker and Cines also briefly analysed the use of indium-labelled autologous platelet scanning to predict the response to splenectomy in patients with chronic ITP,[5] concluding that although the technique is promising in expert centres, it cannot be recommended for routine use in predicting response to splenectomy in centres without extensive experience with this modality.

Pathophysiology

ITP is caused by the loss of the self-tolerance to some platelet membrane epitopes, mainly residing on the glycoprotein (GP) IIb/IIIa complex, which is present on megakaryocytes and circulating platelets. This results in the production of autoantibodies, which in turn cause impaired platelet production and increased removal of opsonised platelets by the reticuloendothelial system.

The pathogenetic role of B lymphocytes in ITP is well established. In this regard, Olsson *et al.*[6] showed that chronic ITP is associated with an increased number of CD20+ B-cells in the red pulp of the spleen, which provides a microenvironment where close interaction between platelets, cytotoxic T-cells and B-cells can take place. Direct T-cell cytotoxicity has also been established as an additional immune-mediated mechanism. On the contrary, the role of the complement system in ITP remains poorly defined; however, in a pilot study assessing the blood samples of 250 patients with chronic ITP, a significant number of sera containing autoantibodies were capable of activating the classical complement pathway, even if absent

according to the monoclonal antibody immobilisation of platelet antigens assay. This finding indicates that current techniques for autoantibody detection may be insufficient. Despite the finding that only 50% of sera could fix complement in a GP IIb/IIIa-dependent manner and also induce platelet lysis in vitro, it appears that complement fixation may contribute to ITP by directly damaging platelets and/or by enhancing platelet clearance via complement receptor-mediated phagocytosis.[7]

Some evidence is emerging that the phenotype of ITP can be modulated by inherited factors. For example, it is known that platelet production and function are dependent on the presence of the hematopoietic-specific β-tubulin isotype Hβ1 (Class VI), whose expression is restricted to platelets and megakaryocytes, constituting 90% of total platelet β-tubulin. Among the eight human β-tubulin isotypes, Hβ1 is the only isotype for which frequent non-synonymous single nucleotide polymorphisms (SNPs) have been described. Little is known about the role of these SNPs in platelet production and function, but it was reported that Arg307His substitution is over-represented in patients with ITP and can be used to identify patients with more severe disease.[8] Interleukin-18 (IL-18) plays an important role in Th1 and Th2 immune response, and some haplotypes of IL-18 polymorphisms, like –607A/C and –137G/C, may also be linked to more severe ITP.[9]

B and T lymphocytes participate in immune responses through the production of antibodies, antigen presentation to T-cells, and cytokine secretion. In humans, CD19+CD24hiCD38hi B-cells, originally identified as immature transitional B-cells, were recently shown to possess regulatory capacity mediated in part by IL-10. These cells, now called Breg cells, may be functionally compromised in some patients with ITP, as indicated by reduced IL-10 production and indirectly by Breg depletion studies showing an inability to dampen effector T-cell responses. Given the important role of Bregs in controlling CD4+T responses, these data implicate defective Bregs as an additional mechanism of the increased T-cell responses in patients with ITP. The impaired Breg activity may also explain the variability in response to treatment with anti-CD20 in patients with ITP. It is possible that B-cell depletion therapy in patients who have defective Breg activity will result in the removal primarily of the pathogenic B-cells, and therefore these patients will have a good response to rituximab treatment. However, in patients with intact Bregs, the same treatment will deplete both pathogenic and regulatory B-cells, causing a less effective response.[10]

Foxp3+ CD4+CD25+regulatory T-cells (Tregs) are also crucial for the maintenance of immunological self-tolerance. Indeed, defective Treg compartments have been described in several autoimmune diseases, including ITP. ITP patients on treatment with thrombopoietic receptor (TPO-R) agonists, which are known to improve platelet count, have increased Treg suppressive activity. In a cohort of 31 ITP patients who were tested at least at two different time points either before, during or after their treatment with TPO-R agonists, a positive correlation between platelet count and circulating Treg frequency (Foxp3+CD25hi, r=0.388, p=0.005), as well as in vitro suppressive activity (r= –0.556, p <0.0001), was found, suggesting that patients with elevated platelet counts have increased Treg suppressive activity. Interestingly, the patients' monocyte count was inversely correlated with platelet count, indicating that monocytes may have an inhibitory effect exclusively on the expansion of pre-existing Tregs.[11] CTLA4 engagement may also be required for these interactions.[12]

Fatigue is reported in many autoimmune disorders, and this symptom is quite relevant for some patients with ITP, but its pathogenesis remains obscure and requires full elucidation. Interestingly, a survey in adult patients with ITP conducted among 585 (31%) of 1871 members of the UK ITP Support Association and 69 (74%) of 93 patients in the Oklahoma ITP Registry showed that patients with ITP frequently report energy changes that appear to be greater when their platelet count is lower. In this analysis, the authors documented that fatigue is associated with bleeding problems and that predictors of fatigue are different among patients with and without bleeding problems. Whereas fatigue is associated with orthostatic symptoms in patients with and without bleeding problems, it is associated with active ITP only in patients with bleeding problems; however, fatigue is associated with other medical conditions and sleepiness symptoms only in patients without bleeding problems. The association of orthostatic and sleepiness symptoms with fatigue in patients with ITP suggests that the fatigue symptoms may be related to autoimmune autonomic abnormalities.[13]

TPO-R agonists

Eltrombopag

The safety and efficacy of eltrombopag treatment were evaluated in an ongoing open-label, extension study (EXTEND) in ITP patients who completed a previous eltrombopag study.[14] More than 50% of patients had a platelet count less than 50 x 10^9/L at baseline; 38% of patients were splenectomised; 33% were receiving concomitant ITP medication at baseline, and 53% had received ≥3 previous ITP therapies. A total of 249, 210, 138 and 24 patients had been taking eltrombopag for ≥26, 52, 104 and 156 weeks, respectively, with a median duration of exposure of 100 weeks at the time of data analysis. Of the 299 patients enrolled, 8% (23) completed the study, 41% (122) withdrew, and 52% (154) continue to participate in the study. Overall, 87% (261/299) of patients achieved a platelet count ≥50 x 10^9/L on treatment; 37 of these had a baseline platelet count ≥50 x 10^9/L. Median platelet counts increased to ≥50 x 10^9/L by week 2 and remained consistently ≥50 x 10^9/L through week 164. The incidence of any bleeding symptoms (WHO grades 1–4) declined

from 56% at baseline to 16% and 20% at weeks 52 and 104, respectively. Clinically significant bleeding (WHO grades 2–4) was reduced from 16% (47/299) at baseline to 3% (2/77) and 7% (3/41) at weeks 52 and 104, respectively.

Twenty-one thromboembolic events (TEE) were reported in 16 patients (5%); the incidence rate was 3.17 cases/100 patient years (95% CI 1.81, 5.15). The most common TEEs were deep vein thrombosis (DVT) [8] and myocardial infarction (MI) [4]. No association was observed between TEEs and elevated platelet count, as only 3/16 patients experienced the TEE closest to their maximum platelet count achieved during the study. In a more detailed analysis including all cases exposed to eltrombopag,[15] the majority of patients (55%, 11) had platelet counts below the normal range at the time of the TEE (<150 x 10^9/L); 4/20 patients experienced the TEE closest to their maximum platelet count achieved during the study, whereas the majority (80%, 16/20) experienced the TEE at a lower platelet count than their maximum platelet count during treatment with eltrombopag.

Hepatobiliary laboratory abnormalities were reported in 29 patients (10%). All were reversible; the majority while on therapy. Of the 299 patients enrolled, 6 (2%) were withdrawn due to a hepatobiliary adverse event. After examining bone marrow biopsies from >150 patients treated with eltrombopag for >1 year, no clinically relevant increase in reticulin fibre deposition was observed. It is noteworthy that, based on results from the RAISE placebo-controlled study, eltrombopag increased platelet counts and reduced bleeding symptoms versus placebo regardless of splenectomy status, although median dose was slightly higher in splenectomised patients.[16]

In a phase II, open-label, dose-escalation trial, eltrombopag was tested in 12 adults with inherited ITP deriving from MYH9 mutations and bleeding symptoms. Eltrombopag at a dose of 50–75 mg per day increased platelet count and reduced bleeding tendency in most patients with MYH9-related disease.[17]

Romiplostim

Kuter *et al*. reported final cumulative data from adult patients with ITP who, after completing a romiplostim study, subsequently received romiplostim in an open-label extension study from August 2004 to January 2010 for as long as 277 weeks.[18] Romiplostim was administered subcutaneously once a week, with dose adjustments to maintain platelet counts in the target range of 50–200 x 10^9/L. Patients who achieved a stable dose for 3 consecutive weeks were eligible to receive self or caregiver administration of romiplostim at home, returning to the clinic every 4 weeks for evaluation. A total of 292 adult patients, mostly female (63%), received romiplostim. The median time since ITP diagnosis was 4.9 years (range, 0.6–46.4 years) and 32.5% had undergone a splenectomy. Patients received romiplostim for a median of 78 weeks (range, 1–277 weeks) at a median dose of 4 µg/kg. After week 12, romiplostim doses remained relatively constant, with 78% of patients administered a dose within 2 µg/kg of their most frequent dose at least 90% of the time. Home administration was started by 82% of patients; 28/239 patients (12%) discontinued home administration and resumed weekly study-site injection. Almost all patients (94.5%) achieved a platelet count ≥50 x 10^9/L during the study. More than 50% had platelet counts ≥50 x 10^9/L on ≥90% of all visits. After the first week, median platelet counts remained within the target range of 50–200 x 10^9/L. Of the patients who received concurrent ITP medication at baseline, 81% (30/37) were able to discontinue or reduce their dose by >25%. Patients were divided into four cohorts, depending on protocol changes; cohorts differed with regard to baseline platelet count, duration of disease and splenectomy status. Median weekly platelet counts during the study were similar for each of the cohorts and for the overall population. Bone marrow biopsies were performed at the investigators' discretion on a small proportion of patients; bone marrow reticulin was present or increased in 11 patients. In general, patients with reticulin had a longer duration of disease, were splenectomised and received higher doses of romiplostim. Two patients developed neutralising antibodies to romiplostim that did not cross-react with thrombopoietin; these were absent on retesting after drug withdrawal. The subjects that developed a neutralising antibody response against romiplostim were associated with a trend for lower platelet counts.[19] Sixteen patients died; two deaths were considered by the investigator as possibly related to treatment (unstable angina, MI). Thrombotic events were reported in 8.6% of cases.

A recent consensus report[2] recommends delaying splenectomy for 6–12 months, in keeping with the new definition of chronic ITP.[20] In accordance with these premises, patients who had been diagnosed with ITP for ≤1 year and who participated in a recent study of romiplostim versus standard of care (SOC) were enrolled in a study that evaluated 29 patients receiving SOC and 56 receiving weekly injections of romiplostim. This randomised, open-label study was conducted in non-splenectomised adult patients with ITP within 1 year of diagnosis. The co-primary study endpoints were the incidence of treatment failure and the incidence of splenectomy. The findings of the study indicate that romiplostim may reduce the incidence of treatment failure and splenectomy in patients who have been diagnosed with ITP for 1 year or less.[21]

Assessing all patients enrolled in the extension study mentioned above demonstrated that non-splenectomised ITP patients receiving romiplostim experience greater improvements in quality of life (QoL) than patients receiving SOC. However, the clinical significance of the QoL improvements in romiplostim-treated patients relative to those receiving SOC remains uncertain.[22]

In another study, the authors analysed mortality rates in ITP patients who participated in randomised, controlled romiplostim clinical studies. In total, 354

patients (238 romiplostim, 41 placebo, 75 SOC) were included in the analysis. Of these, 238 were enrolled into the extension study (187 romiplostim, 33 placebo, 18 SOC). At the time of enrolment into the controlled studies, the median age was 55 years and 22% of patients were splenectomised; the median time since diagnosis was 3.5 years; patients had received a median of three prior ITP therapies, and 24% were receiving baseline concurrent ITP treatment. Patients were followed for up to 87 weeks in the controlled studies. Overall, 0.8% (2/238) of patients in the romiplostim arm died and 6.9% (8/116) of patients in the placebo/SOC arm died. In this large retrospective analysis, the mortality rate was significantly lower in patients receiving romiplostim than in those receiving placebo or other ITP therapies; however, the mechanisms associated with the increased survival in the romiplostim arm remain unclear and will require further study.[23]

Finally, in a pilot study in paediatric patients, the first with TPO-R agonists, 18 patients aged less than 16 years with refractory chronic ITP for more than a year who failed to maintain response on more than two therapeutic modalities received romiplostim. The drug was well tolerated without serious adverse events and was effective in most of the patients, with minimal bleeding. Lower doses compared to previous published data were used in this patient population.[24]

E5501

E5501 (previously AKR501) is a novel, orally-active, once-a-day TPO-R agonist. Data from a phase II, multicenter, randomised, double-blind, placebo-controlled, dose-ranging, parallel group (placebo, 2.5, 5, 10 or 20 mg), 28-day study showed that 58% of subjects responded to ≥5 mg of E5501 by day 7. Subjects treated with E5501 20 mg achieved a 93% response rate on day 7. None of the five placebo-treated subjects responded at any time during the study.[25] A total of 53 of the 57 subjects who completed the 28-day treatment regimen were subsequently treated with E5501 in a 6-month extension study. Subjects classified as responders in the phase II study continued to receive their original E5501 blinded dose when they entered the extension study. Subjects who were nonresponders in the phase II study initially received open-label E5501 10 mg once-daily. E5501 dose was titrated upwards in an open-label fashion in 10 mg increments every 14 days depending on subject response (to a maximum of 40 mg once-daily for nonresponders and blinded dose plus 20 mg once-daily for responders). The durable platelet response rate was 53% for all subjects, 72% for subjects who were responders in the phase II study and 36% for subjects who had been nonresponders. The overall platelet response rate was 75.5%, 88% for responders and almost two thirds (64%) for nonresponders. TEEs were reported in 4 of 64 subjects (6%). One had a grade 3 DVT, one had a grade 3 stroke, and one had a transient ischaemic attack and MI (day 20) and a grade 4 retinal artery occlusion. All three of these subjects had multiple risk factors for thrombosis. The fourth subject had grade 1 superficial thrombophlebitis.[26]

Rituximab and high-dose dexamethasone

Despite the fact that the long-term safety of rituximab remains uncertain and that only approximately one in five adults will have at least a 5-year relapse-free response to rituximab treatment, which is disappointingly low,[27] new studies on this treatment were presented at the meeting. A Dutch HOVON multicenter, randomised, open-label phase II trial analysed three rituximab dosing schemes in chronic ITP in three groups of 35 patients each (375 mg/m^2/week x 4; 750 mg/m^2/week x 2; 375 mg/m^2/week x 4 or x 2). Interim analysis of the overall response data within 71 days revealed no superior dosing scheme of CD20.[28] In another study, in 10 patients with refractory ITP who were followed-up for more than 2 years and who had all previously responded to their initial 4 weekly standard infusions of rituximab (375 mg/m^2) before relapsing, rituximab maintenance therapy with one standard dose every four months for up to six doses was safe and effective.[29] Combining rituximab with one to three cycles of high-dose dexamethasone (dex) may possibly improve these limited results, but with three cycles, some toxicity becomes apparent.[30] High-dose dex (40 mg/day for 4 days) was compared to conventional-dose prednisolone (1 mg/kg/day for 4 weeks) in adults treated front-line for ITP in a randomised, prospective, multicenter, phase III trial involving 151 patients. One or two courses of dex were not more effective than prednisolone in adults with ITP. However, since 20% of patients had a sustained response with a single course of dex, the authors suggested that initial treatment with dex could be useful for identifying patients who may not require prolonged steroid treatment.[31]

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Conclusions

ITP pathogenesis and management continue to be an active field of investigation. Clarification of the inciting factors leading to the loss of self-tolerance against platelet membrane GPs and the mechanisms of ITP may pave the way towards a novel, possibly curative approach to this disease. Meanwhile, on the basis of 4 to 6-year extension studies in several patients, TPO-R agonists may offer an effective and relatively safe approach for the management of chronic ITP, replacing more toxic treatments like prolonged use of corticosteroids or immunosuppression and delaying splenectomy. Among the possible treatment-related adverse effects of TPO-R agonists, thrombosis seems the most intriguing, requiring further study and necessitating the exclusion of subjects with risk factors for thrombosis from this treatment.
New approaches based on primary versus secondary ITP and "patient-specific" treatments, including high-throughput screening to clone autoantibodies and search for implicated epitopes and external antigens, as well as other innovative approaches, may offer new perspectives for the cure of these intriguing disorders.[5]

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