bjh review

Primary and secondary thrombocytosis in childhood

Christof Dame[1] and Anton Heinz Sutor[2],†

[1]Department of Neonatology, Charité – University Medicine Berlin, Campus Virchow-Klinikum, Berlin, and [2]Children's Hospital, University of Freiburg, Freiburg, Germany

Summary

This review summarizes current data on the pathomechanisms and clinical aspects of primary and secondary thrombocytosis in childhood. Primary thrombocytosis is extremely rare in childhood, mostly diagnosed at the beginning of the second decade of life. As in adults, the criteria of the Polycythemia Vera Group are appropriate to diagnose primary thrombocytosis. The pathomechansims of non-familial forms are complex and include spontaneous formation of megakaryopoietic progenitors and increased sensitivity to thrombopoietin (Tpo). Familial forms can be caused by mutations in Tpo or Tpo receptor (*c-mpl*) genes. These mutations result in overexpression of Tpo, sustained intracellular signalling or disturbed regulation of circulating Tpo. Treatment of primary thrombocytosis is not recommended if platelet counts are <1500/nl and bleeding or thrombosis did not occur in patient's history. In severe cases, decision on treatment should weigh potential risks of treatment options (hydroxyurea, anagrelide) against expected benefits for preventing thrombosis or haemorrhage. Secondary thrombocytosis is frequent in children, in particular in the first decade of life. Hepatic Tpo production is stimulated in acute response reaction to a variety of disorders. Thrombosis prophylaxis is not required, even at platelet counts >1000/nl, except for cases with additional prothrombotic risk factors.

Keywords: thrombocytosis, thrombocythaemia, thrombopoietin, megakaryopoiesis, thrombosis and c-mpl.

While primary thrombocytosis is rare in childhood, secondary thrombocytosis occurs frequently. Since the discovery of thrombopoietin (Tpo), the pathomechanisms of primary/essential thrombocytosis (ET) and secondary/reactive thrombocytosis (RT) have been better elucidated. Therefore, the current data on the pathogenesis of thrombocytosis in childhood and adolescence are summarized herein with a main focus on the biology of Tpo and its receptor (c-mpl).

Studies on the prevention of thromboembolic and haemorrhagic complications in children with thrombocytosis are also very limited. Experience and concepts to treat ET in adults can be transferred to children only to some extent, particularly regarding the adverse effects of platelet-lowering agents. The current concepts on the diagnosis and treatment of primary and secondary thrombocytosis in childhood are also summarized in this review.

Definition of thrombocytosis in childhood

Thrombocytosis in children is defined by an elevated platelet count, as in adults. The definition of normal platelet counts in the range of 150×10^9/l and 450×10^9/l is generally accepted for healthy neonates, infants, children and adolescents. However, the definition of thrombocytosis varies between platelet counts of $>400 \times 10^9$/l and $>1000 \times 10^9$/l (Sutor, 1999). To consider the characteristics and clinical implication of thrombocytosis and to compare published data, the following arbitrary classification of thrombocytosis has been chosen in current textbooks: *mild* thrombocytosis, if the platelet count is >500 and $<700 \times 10^9$/l; *moderate* thrombocytosis, if the platelet count ranges between >700 and $<900 \times 10^9$/l; *severe* thrombocytosis, if the platelet count is $>900 \times 10^9$/l; and *extreme* thrombocytosis, if the platelet count is $>1000 \times 10^9$/l (Sutor, 1999).

Classification of thrombocytosis

According to the pathogenic origin, thrombocytosis can be classified into a primary (essential) and secondary (reactive) form. Primary thrombocytosis is a myeloproliferative disease, caused by monoclonal or polyclonal abnormalities of haematopoietic cells or by abnormalities in the biology of Tpo. Secondary thrombocytosis is caused by stimulated megakaryopoiesis because of various haematological or non-haematological disorders.

Correspondence: Christof Dame, Department of Neonatology, Charité – University Medicine Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, D-13353 Berlin, Germany.
E-mail: christof.dame@charite.de

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A1	Platelet count >400 × 10^9/l and no known cause of reactive thrombocytosis
A2	Increase in and clustering of mature giant megakaryocytes with hyperploid nuclei
A3	No preceding or allied other subtype of myeloproliferative disorder or myelodysplastic syndrome
B1	Normal or elevated leucocyte alkaline phosphatase, normal erythrocyte sedimentation rate, no fever
B2	Normal or slightly increased cellularity and no or minimal reticulin fibrosis
B3	Splenomegaly on palpation or spleen length >11 cm* on diagnostic imaging procedure
B4	Spontaneous erythroid colony and/or spontaneous megakaryocyte colony formation in bone marrow progenitor assays

Table I. Diagnostic criteria for essential thrombocytosis.

A criteria, diagnostic; B criteria, confirmative (Michiels *et al*, 1999; Greist, 2002).
*A spleen length above 2 SD of normal may fulfill this criterion in children.

Diagnostic features of thrombocytosis

Counting of platelets

A gold standard for the method of blood sampling (venous or capillary blood sample) or platelet counting (automated cell counter, counting under the microscope) is still not defined. If an abnormal platelet count has been found by automated cell counting, the analysis of a blood smear is necessary to exclude morphological abnormalities of platelets or other blood cells, such as abnormal platelet granules or red cell fragmentation.

Other diagnostic criteria of thrombocytosis

In most children with thrombocytosis, clinically apparent symptoms of an active underlying systemic disease may be evident. However, in individual cases, it can be very difficult to differentiate between the clonal/essential and reactive forms of thrombocytosis. Before ascribing thrombocytosis to a clonal/essential disorder of megakaryopoiesis, which is largely a diagnosis of exclusion, extensive diagnostic tests are necessary (Schafer, 2004). As in adults, the gold standard diagnostic criteria for ET remain those proposed by the Polycythemia Vera Group, which are summarized in Table I (Michiels *et al*, 1999). Besides the morphological and genetic/cytogenetic analysis of a bone marrow specimen, many other potential diagnostic features have been studied. These include the culture of megakaryocyte colonies, clonality assays and measurement of various megakaryopoietic growth factors or cytokines. Their diagnostic value has been recently reviewed (Harrison, 2002). Currently, there is no evidence to suggest a different diagnostic value in children than in adults.

Physiology of megakaryopoiesis and platelet production

Thrombopoietin and its receptor play a major role in the pathogenesis of thrombocytosis. Tpo is the key regulator of megakaryopoiesis and platelet production. It acts on the commitment of early haematopoietic stem and progenitor cells into lineage-specific differentiation; and it stimulates the proliferation of committed megakaryocyte progenitor cells and the differentiation of megakaryoblasts to megakaryocytes (Kaushansky *et al*, 1994; de Sauvage *et al*, 1994). However, Tpo seems not to be required for megakaryocyte proplatelet formation, the final step of thrombopoiesis (Choi *et al*, 1995). The haematopoietic activity of Tpo is mediated by the binding to its specific receptor. As the Tpo receptor was characterized some years before Tpo was identified as its ligand, the Tpo receptor is still named with the acronym c-mpl for the *c*ellular homologue of a retrovirus complex inducing *m*yelo *p*roliferative *l*eukaemia. C-mpl is expressed on $CD34^+$ cells, pluripotent haematopoietic progenitor cells, megakaryocytes, platelets and endothelial cells (Debili *et al*, 1995; Cardier & Dempsey, 1998).

The current understanding of Tpo regulation is based on the relationship between Tpo gene expression levels, circulating Tpo concentrations, and the presence of c-mpl bearing cells. Tpo is primarily expressed in the liver and, to a lower extent, in the kidneys, bone marrow and other organs (Bartley *et al*, 1994; Sungaran *et al*, 1997; Wolber *et al*, 1999). Hepatic Tpo mRNA expression does not change in the presence of thrombocytopenia, implicating that Tpo production is primarily constitutive (Cohen-Solal *et al*, 1996; Fielder *et al*, 1996). This also implies that c-mpl bearing cells play a major role in regulating Tpo protein concentrations. Functional Tpo receptors remove Tpo by absorption and internalization of the cell surface complex. The concept of an 'end-cell-mediated regulation' of Tpo has been confirmed by several studies on Tpo concentrations, megakaryopoietic activity, and platelet counts in neonates, children, and adults: circulating Tpo concentrations are elevated if thrombocytopenia results from reduced megakaryopoiesis (Kuter & Rosenberg, 1995). In contrast, Tpo concentrations are normal or in the upper normal range if thrombocytopenia results from platelet destruction (Ichikawa *et al*, 1996; Cremer *et al*, 2003). This generally accepted model of Tpo regulation is illustrated in Fig 1.

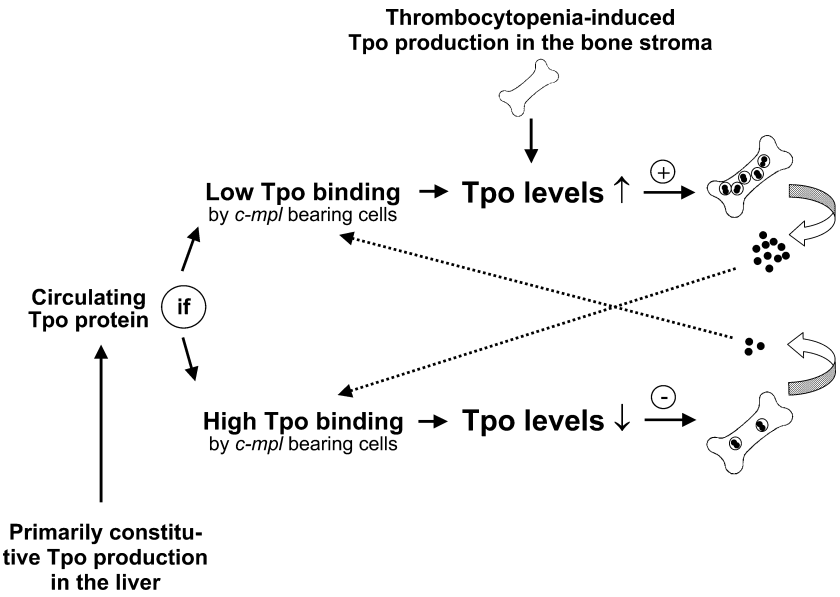

Fig 1. Model of the regulation of thrombopoietin (Tpo) concentrations. The model is based on the concept of an 'end-cell-mediated regulation' by the mass of c-mpl-(Tpo receptor) bearing cells and a mainly constitutive Tpo production in the liver and kidneys. This model implicates an auto-regulatory loop mechanism, as illustrated by dashed lines. In addition, bone marrow stroma cells contribute to Tpo production in thrombocytopenia. [Modified from Dame (2002). With permission from Taylor & Francis, Stockholm, Sweden.]

Bone marrow stromal cells are an additional site of Tpo production (Sungaran *et al*, 1997). In contrast to the liver, Tpo gene expression is up-regulated in stroma cells in response to thrombocytopenia (Fig 1). Although the exact type of stroma cell that expresses Tpo has not been identified yet, studies in primary bone marrow cultures showed that various platelet α-granular proteins are involved in this regulatory process (Sungaran *et al*, 2000).

Recent studies have indicated that the regulation of Tpo in the liver is more complex than previously thought, particularly in thrombocytosis (Dame, 2001). In contrast to the generally accepted model, Tpo concentrations do not inversely correlate with the mass of platelets in RT (Cerutti *et al*, 1999; Hsu *et al*, 1999). In fact, longitudinal Tpo measurements in infants and children with acute infection showed that the elevation of circulating Tpo concentrations precedes thrombocytosis (Ishiguro *et al*, 2002). Furthermore, *in vitro* experiments provided evidence that the pro-inflammatory cytokine interleukin-6 (IL-6) increases Tpo gene expression in human hepatocytes (Hep3B and HepG2 cells) and in murine liver endothelial cells (Fig 2A; Cardier, 1999; Wolber & Jelkmann, 2000; Kaser *et al*, 2001). However, other cytokines, including IL-1, IL-11, and tumour necrosis factor-α, do not modulate Tpo gene expression in hepatoma cells (Wolber & Jelkmann, 2000). *In vivo* studies confirmed that RT induced by bacterial lipopolysaccharides results from increased hepatic Tpo production (Wolber *et al*, 2001).

Besides Tpo, other cytokines or haematopoietic growth factors, such as granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3, IL-6, IL-11 and leukaemia inhibitory factor contribute directly to megakaryopoiesis, each of them with a specific activity at certain steps in this process (Begley & Basser, 2000). Most recently, the role of IL-1 in megakaryopoiesis has been further elucidated. IL-1β directly stimulates the formation of megakaryocytic colony-forming units (CFU-Meg) (Yang *et al*, 2000). Moreover, IL-1β stimulates in megakaryocytic progenitors (human CHRF and MegO1 cells) both the expression of Tpo and transcription factors, such as GATA-1 and NF-E2, which are involved in megakaryopoiesis (Chuen *et al*, 2004).

Primary thrombocytosis

Pathophysiology

Primary thrombocytosis is classified as a chronic myeloproliferative disorder of haematopoiesis, resulting in uncontrolled platelet production as the major haematological abnormality (Tefferi, 2001). Primary thrombocytosis can be a monoclonal or polyclonal disorder. The risk of thromboembolic complications seems to be higher in case of monoclonality (Harrison *et al*, 1999a).

As primary thrombocytosis is extremely rare in childhood, data on the pathogenesis have been obtained almost exclusively in adults. Circulating Tpo concentrations are normal or slightly elevated, although decreased Tpo concentrations would be expected as a consequence of the elevated mass of c-mpl bearing cells according to the model of an 'end-cell mediated regulation' of Tpo (Horikawa *et al*, 1997; Wang *et al*, 1998; Harrison *et al*, 1999b; Allen *et al*, 2001). In contrast to the familial forms of thrombocytosis, structural defects in the Tpo or *c-mpl* gene locus have not been found yet in hereditary thrombocytosis (Kiladjian *et al*, 1997; Harrison *et al*, 1998; Wiestner *et al*, 2000). However, the expression of c-mpl on megakaryopoietic progenitors and platelets can be decreased in primary thrombocytosis (Horikawa *et al*, 1997; Li *et al*, 2000). Very recently, a polymorphism in the Tpo receptor (mpl Baltimore, which is

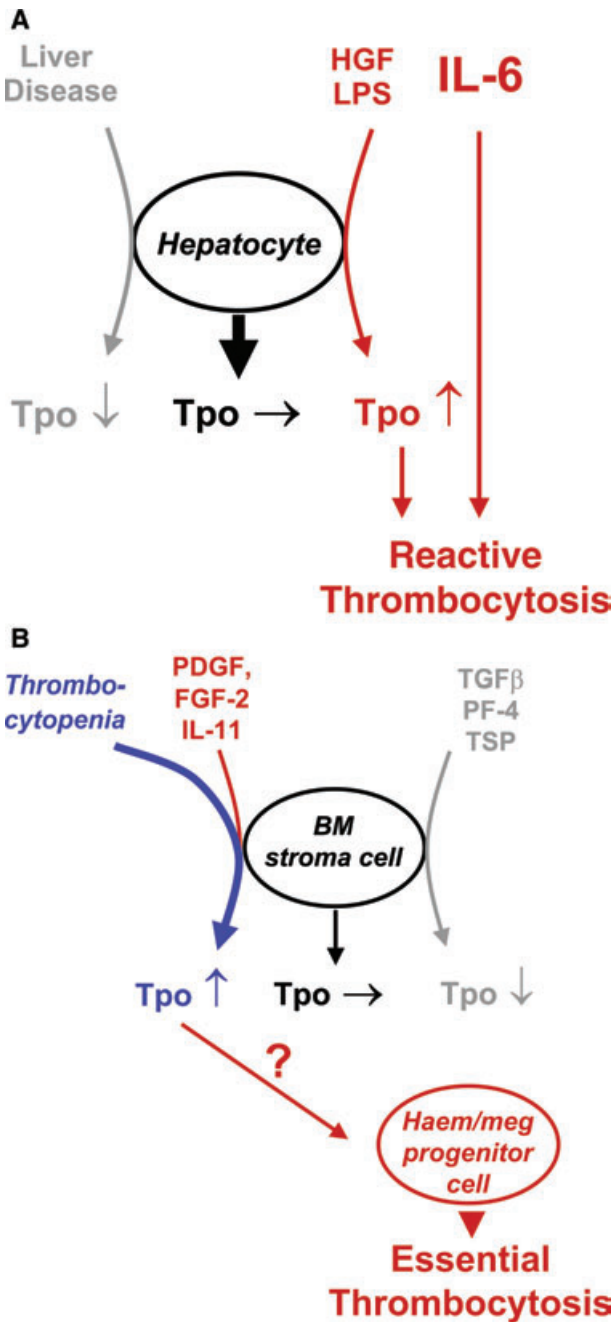

Fig 2. Modification of thrombopoietin (Tpo) expression in the liver and bone marrow. Current data suggest that some of these mechanisms are involved in the pathogenesis of certain forms of reactive thrombocytosis. (A) In the liver, hepatic growth factor (HGF), lipopolysaccharides (LPS) and interleukin-6 (IL-6) induce Tpo production. IL-6 also directly stimulates directly megakaryopoiesis and contributes thereby to reactive thrombocytosis. (B) In bone marrow stroma cells, the expression of Tpo mRNA is inducible by thrombocytopenia and various platelet granular proteins (PDGF, platelet-derived growth factor; FGF2, fibroblast growth factor 2; PF-4, platelet factor 4). The implication of these mechanisms on haematopoietic/megakaryopoietic cells with a clonal disorder leading to primary thrombocytosis is unclear. TSP, thrombospondin; TGFβ, transforming growth factor β.

characterized by a single nucleotide substitution and results in a change from lysine to aspargine (K39N) in the ligand-binding domain of c-mpl) has been found and is associated with a reduced function of c-mpl and thrombocytosis. The polymorphism, which has been found only in the African–American population, is autosomal and appears to have an autosomal dominance with incomplete penetrance (Moliterno *et al*, 2004). If c-mpl expression or ligand-binding is reduced, circulating Tpo can be bound only to a low extent, leading to a stimulation of megakaryopoiesis (Horikawa *et al*, 1997; Li *et al*, 2000). In general, neither 'inadequately high' (or elevated) Tpo concentrations nor reduced c-mpl expression on megakaryocytes and platelets are specific for primary thrombocytosis and are also described in polycythaemia vera (Harrison *et al*, 1999b; Le Blanc *et al*, 2000). Spontaneous formation of CFU-Meg is another well-known phenomenon in serum-free haematopoietic progenitor cell assays from both adult and paediatric patients with non-familial thrombocytosis (Li *et al*, 1994; Randi *et al*, 2000; Florensa *et al*, 2002). Megakaryopoiesis can be additionally stimulated in primary thrombocytosis by an increased sensitivity of CFU-Meg to Tpo (Mi *et al*, 2001). While the proliferation of megakaryopoietic progenitor cells cannot be inhibited by antibodies directed against IL-3, IL-6, GM-CSF, or Tpo, blocking of *c-mpl* reduces the proliferation rate of megakaryopoietic progenitor cells *in vivo* (Kimura *et al*, 1998; Taksin *et al*, 1999). This phenomenon has been discussed as a paradox, Tpo-independent, but c-mpl-dependent modulation of megakaryopoietic progenitors in primary thrombocytosis (Tefferi, 2001). As in adults, 80% of paediatric patients with non-familial primary thrombocytosis show both spontaneous formation of CFU-Meg and erythroid progenitors (erythroid burst-forming units), indicating that at least two different cell lines or a bi-potent erythro-megakaryocytic progenitor is affected (Florensa *et al*, 2002).

The role of the bone marrow microenvironment in the pathogenesis of primary thrombocytosis is still not completely elucidated. In bone marrow stroma cells, Tpo gene expression is regulated by proteins released from platelet α-granules. Specifically, platelet-derived growth factor and fibroblast growth factor-2 stimulate Tpo mRNA expression, whereas platelet factor-4, thrombospondin and transforming growth factor-β suppress Tpo mRNA expression (Fig 2B; Hirayama *et al*, 1998; Sakamaki *et al*, 1999; Sungaran *et al*, 2000). It is still unclear whether stimulated angiogenesis in the bone marrow stroma has a primary pathogenic implication or whether it is a secondary phenomenon of primary thrombocytosis (Mesa *et al*, 2002). Recently published data have indicated that the expression of haematopoietic relevant genes, such as the polycythaemia rubra vera-1 (PRV-1) gene or the transcription factor NF-E2, can be changed in ET (Catani *et al*, 2002; Teofili *et al*, 2002). Adult PRV-1 positive patients with primary thrombocytosis seem to have a significant increased risk for vascular complications compared with PRV-1 negative patients (Johansson *et al*, 2003).

Features of primary thrombocytosis in childhood

The annual incidence of newly diagnosed primary thrombocytosis in childhood is about 1 per 10 million, 60 times lower than in adults (Hasle, 2000; Jensen *et al*, 2000). Based on the criteria of the *Polycythemia Vera Study Group*, about 75 children with primary thrombocytosis have been reported between 1966 and 2000 (Michiels & Van Genderen, 1997; Dror & Blanchette, 1999; Jensen *et al*, 2000; Kudo *et al*, 2000; Randi *et al*, 2000; Yang & Qian, 2000). Familial thrombocytosis has been observed in 40–55% of all children with primary thrombocytosis. Approximately two-third of children with primary thrombocytosis had platelet counts $>1000 \times 10^9$/l. In childhood, the median age at diagnosis of primary thrombocytosis was 11 years. Similar to adults, about 30% of patients experienced thromboembolic or haemorrhagic complications at the time of diagnosis or later; 20% of initially asymptomatic children suffered from these complications later on (Dror & Blanchette, 1999; Jensen *et al*, 2000). Splenomegaly has been found in every second child, hepatomegaly in every fourth. Ten percent of patients died because of the underlying haematopoietic disorder. Another 5% developed other myeloproliferative diseases (Dror & Blanchette, 1999).

Morphological abnormalities of platelets include giant platelets, platelet conglomerates, bizarre forms, megakaryocytic fragments, and hypogranularity. Ultrastructural abnormalities are reduced numbers of pseudopodia and α-granules. Bone marrow examination reveals hypercellularity, mostly with an elevated number of megakaryocytes. These cells also display hyperploidy, dysplasia or giant forms. Histological criteria, such as stimulated angiogenesis, reduced c-mpl expression on megakaryocytes, and increased proliferation of megakaryocytes, are important criteria to distinguish primary from secondary thrombocytosis (Table II).

The determination of reticulated platelets, which are newly released platelets containing residual RNA, seems to be suitable to estimate the turnover of platelets and the prothrombotic risk (Rinder *et al*, 1998). The risk of haemorrhage may increase with extreme thrombocytosis ($>2000 \times 10^9$/l) (Greist, 2002). Haemostasis is altered in about 20% of patients with primary thrombocytosis, as indicated by the prolonged bleeding time, prothrombin time, and partial thromboplastin time. Platelet function is also often diminished (Dror & Blanchette, 1999). In adults, clot lysis is reduced and more plasminogen activator inhibitor-1 is released from platelets (Posan *et al*, 1998). Adult patients with primary thrombocytosis also show an increased prevalence of anti-phospholipid antibodies, which may be associated with thrombosis (Harrison *et al*, 2002). However, pro-inflammatory cytokines or C-reactive protein (CrP) are typically in the normal range or not detectable.

Table II. Characteristics of primary (essential) and secondary (reactive) thrombocytosis in childhood.

Criteria	Essential thrombocytosis (ET)	Reactive thrombocytosis (RT)
Age-dependent occurrence	Mostly 11 years*	Mostly <2 years*
Incidence per year	One per 1 million children	>600 per 1 million children
Duration of thrombocytosis	Months, years, or permanently	Days, weeks or months, temporary
Splenomegaly	Often	Rare
Fever	No	Often
Bleeding disorders and thrombosis	Often in monoclonal ET, rare in familiar thrombocythaemia	Extremely rare
Frequent laboratory findings	Prolonged bleeding time, increased PT and PTT in 20%; increased praevalence of anti-phospholipid antibodies	Increased VWF, fibrinogen, proinflammatory cytokines, and C-reactive protein, if RT is caused by infection
Platelet count	mostly $>1000 \times 10^9$/l	mostly $<800 \times 10^9$/l
Platelet morphology	Large or small, dysmorphic†	Large, but normal morphology
Platelet function	Abnormal	Normal
Bone marrow	Increased megakaryocyte number with abnormal morphology‡	Increased megakaryocyte number, normal morphology
Pathogenic mechanisms	Clonal defect in haematopoietic or megakaryopoietic progenitors, decreased c-mpl expression, and/or hyperreactivity to Tpo. *In some familial forms:* mutations in the Tpo or *c-mpl* gene locus	Increased Tpo production or release of megakaryopoietic growth factors, in particular IL-6

Tpo, thrombopoietin; VWF, von Willebrand factor; PT, partial prothrombin time; PTT, partial thromboplastin time.

*This number is relevant only for primary thrombocytosis in childhood (Dror & Blanchette, 1999).

†Giant forms, conglomerates, fragments of megakaryocytes, hypogranularity. Anomalies of the ultrastructure include decreased pseudopodia and α-granules.

‡Giant megakaryocytes with hyperploid nuclei. Spontaneous formation of haematopoietic and/or megakaryocytic progenitor cells in serum-free/Tpo-free cultures (CFU-Meg assay).

Familial forms of primary thrombocytosis

A few familial, mostly recessive (although they can be dominant or rarely X-linked) forms of primary thrombocytosis have been reported (Kikuchi *et al*, 1995; Stuhrmann *et al*, 2001). They need to be distinguished from hereditary forms of primary thrombocytosis because of their specific pathogenesis. In 25% of adult patients with familial forms of thrombocytosis, mutations in the Tpo gene locus have been found. These mutations occur typically in the 5′ untranslated region. The mutations result in a deletion of untranslated open reading frames (uORF; AUG codon), causing a more efficient translation and overproduction of Tpo (Kondo *et al*, 1998; Wiestner *et al*, 1998; Ghilardi & Skoda, 1999). Recently, a dominant-positive activating domain of the *c-mpl* gene has been identified in a Japanese family with familial thrombocytosis (Ding *et al*, 2004). Currently known germline mutations in the Tpo and *c-mpl* gene loci are summarized in Fig 3.

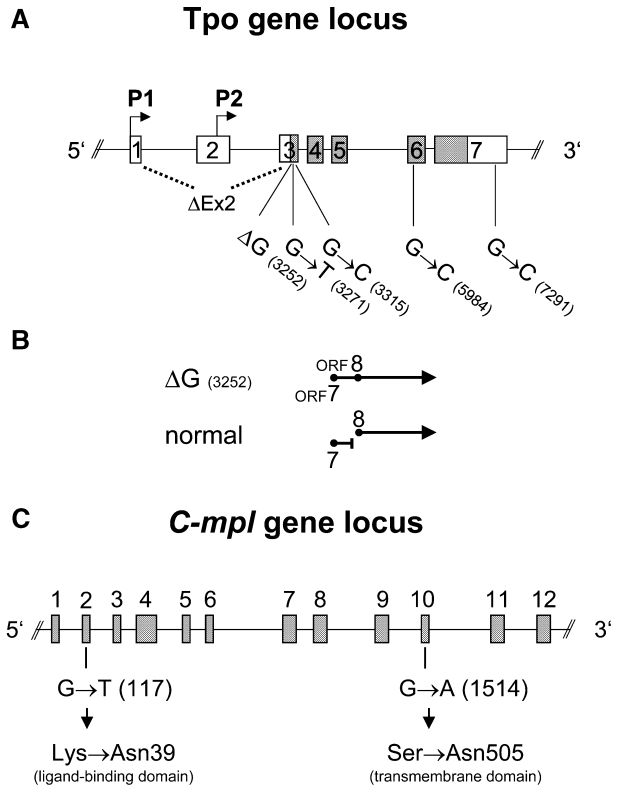

Fig 3. Localization of currently known mutations in the thrombopoietin and *c-mpl* gene locus causing familial thrombocytosis. (A) The human Tpo gene locus. Boxes represent exons, grey areas indicate coding sequences. Arrows indicate the transcription start sites for the P1 and P2 promoter. Various mutations are indicated in the content of the entire gene locus according to GenBank accession no. E12183. Dashed lines depict the deletion of exon 2 (ΔEx2) as consequence of the mutation G → C (3315) (Kondo *et al*, 1998; Wiestner *et al*, 1998; Ghilardi & Skoda, 1999). (B) The ΔG (3252) mutation results in the loss of an open-reading frame in the 5′-untranslated region human Tpo gene locus. Horizontal lines indicate open reading frames (ORF), which are numbered as they first appear in the full-length Tpo mRNA. The short vertical line marks the position of a stop codon (AUG), which is lost in the ΔG (3252) mutation. The mutation results in a more efficient translation of the Tpo gene. (C) Mutations in the *c-mpl* gene cause also primary thrombocytosis. A polymorphism (exon 2, G → T (117); GenBank accession no. M90102) results in the exchange of Lys → Asn in position 39 of the ligand-binding domain and causes thrombocytosis by a decreased c-mpl function. Megakaryopoiesis may be stimulated because of a higher availability of circulating Tpo, which is bound to a smaller amount of platelets as the key factor in regulating Tpo concentrations (Moliterno *et al*, 2004). Moreover, a G → A (1514) mutation in the *c-mpl* gene locus results in the exchange of Ser → Asn in position 505 of the transmembrane domain and causes familial thrombocytosis by the activation of intracellular signalling (Ding *et al*, 2004). Exons are represented as boxes.

In children with familial thrombocytosis, platelet counts are lower than in non-familial primary thrombocytosis, splenomegaly is less frequent, and almost no thrombotic or haemorrhagic complications occur (Dror & Blanchette, 1999). In consequence, treatment strategies can be more conservative in this disorder. Therefore, familial forms of thrombocytosis should be clearly distinguished from hereditary forms. The clinical and pathogenic differences may renew the term 'familial thrombocythaemia', describing a subgroup of primary thrombocytosis.

Treatment options in non-familial, primary thrombocytosis

Treatment for mild (non-familial) primary thrombocytosis, defined as the absence of bleeding or thrombosis in the patient's history and a platelet count $<1500 \times 10^9$/l, is not recommended (Ruggeri *et al*, 1998). In patients at high risk for thrombotic or haemorrhagic complications, hydroxyurea may be a very effective agent. A significant reduction in the incidence of thrombotic complications has been demonstrated in a clinical trial in patients with primary thrombocytosis of all ages (Cortelazzo *et al*, 1995). However, the risk of leukaemic transformation after long-term treatment with hydroxyurea in children and young adults is still a major concern, and its use is controversially discussed (Cheung *et al*, 2004; Randi & Putti, 2004; Schafer, 2004). Two recent studies reported no increased incidence of leukaemic or neoplastic transformation after long-term treatment with hydroxyurea in young adults (19–49 years) with primary thrombocytosis and in children with severe sickle cell disease (Falanga *et al*, 2000; Finazzi *et al*, 2003).

Anagrelide, an oral imidazo-quinazoline derivative, which reduces platelet counts by drug interference with megakaryocyte maturation, may be an alternative agent to treat primary thrombocytosis in children and young adults (Silverstein *et al*, 1988; Lackner *et al*, 1998; Dror & Blanchette, 1999; Storen & Tefferi, 2001). Anagrelide is administered orally and has no known leukaemogenic potential. Therefore, some authors consider anagrelide as first-line therapy for non-familial primary thrombocytosis in children (Randi & Putti, 2004). A long-term analysis of the use of anagrelide in young patients with primary thrombocytosis showed good tolerability, but complications were still

high, with thrombosis in 20%, major bleeding in 20% and anaemia in 24% of patients. It has been suggested that complete normalization of platelet counts may be needed to minimize the residual thrombotic or haemorrhagic risk (Storen & Tefferi, 2001).

Low dose acetylsalicylic acid has been used to reduce platelet aggregation in adults and some children with recurrent thromboses (van Genderen *et al*, 1999; Michiels, 1999; Kudo *et al*, 2000; Randi & Putti, 2004). As previously reviewed, other platelet-lowering agents, such as busulphan, interferon-α, radio-phosphorus or dipyramidol, have been used only in single paediatric patients or young adults, and cannot be recommended in general at this time (Sutor, 1995). Plateletpheresis has been used to reduce platelet counts in adult patients with acute severe thrombotic or haemorrhagic complications or with severe thrombocytosis (>1500 × 10^9/l), but this treatment option has not yet been proven in randomized controlled trials (Greist, 2002).

In children and young adults, the decision on a treatment for ET should be primarily made by weighing the potential risks of all treatment options (particularly the leukaemogenic or myelosuppressive side-effects of cytoreductive therapy) *versus* the expected benefit in terms of preventing thrombotic or haemorrhagic complications (Schafer, 2004). If the indication for a treatment is given, it is highly recommended to enrol these patients in controlled studies.

Secondary thrombocytosis

Pathophysiology

Secondary/RT results from increased megakaryopoiesis and thrombopoiesis, which can be stimulated up to 10-fold (Klinger & Jelkmann, 2002). The most common cause of thrombocytosis in childhood is a reactive process caused by infection, chronic inflammation, tissue damage (trauma/surgery/burns) or neoplasia. Typical laboratory findings of RT are summarized in Table II. IL-6 plays a major part in the pathogenesis of RT, because of its prominent role in the acute-phase response of inflammatory and neoplastic diseases. IL-6 stimulates megakaryopoiesis both directly and indirectly by stimulating hepatic Tpo production (Wolber & Jelkmann, 2000; Kaser *et al*, 2001; Wolber *et al*, 2001). The effects of IL-6, secreted in response to the underlying disease, may explain why circulating Tpo concentrations do not correlate inversely with the mass of c-mpl bearing cells in RT (Wang *et al*, 1998; Cerutti *et al*, 1999; Hsu *et al*, 1999). In neoplasia, thrombocytosis can be caused by increased Tpo production, particularly in hepatoblastoma, and also to a lower extent in neuroblastoma, lymphoma and others (Komura *et al*, 1998; Sasaki *et al*, 1999).

Clinical features of secondary thrombocytosis

Reactive thrombocytosis has an estimated incidence of 6–15% among hospitalized children. As previously reviewed, variations in the reported incidence may result from differences in the definition of thrombocytosis, in the study setting (hospitalized patients, outpatients or both), the epidemic occurrence of infections, or other factors (Sutor, 1999; Matsubara *et al*, 2004). In 72–86% of children with RT, platelet counts range between 500 and 700 × 10^9/l (mild thrombocytosis). Moderate thrombocytosis (platelet count between 700 and 900 × 10^9/l) has been found in approximately 6–8% of children with RT, and only 0·5–3% have a platelet count >1000 × 10^9/l (Vora & Lilleyman, 1993; Yohannan *et al*, 1994; Heng & Tan, 1998; Sutor, 1999; Matsubara *et al*, 2004).

The incidence of RT in childhood shows an age-dependent pattern. The highest incidence has been found in infants aged up to 24 months (Table III). Platelet counts >500 × 10^9/l have been found in 13% of neonates at birth; during the first month, thrombocytosis occurred in 36% of neonates, most of them low birth weight infants (<2·5 kg) and/or neonates who suffered from infection. At this age, 10% of the studied neonates had platelet counts of 600–700 × 10^9/l, 4% had counts >700 × 10^9/l. In the second month, 8% of infants had platelet counts >700 × 10^9/l. The incidence of RT returned to 13% in the group of 6–11-month-old infants. Afterwards, the incidence gradually decreased to only 0·6% in 11–15-year-old children (Matsubara *et al*, 2004).

The higher susceptibility for thrombocytosis during the neonatal period may result from various physiological phenomena: a high Tpo gene expression in the bone marrow during the ontogeny of medullary haematopoiesis (Wolber *et al*, 1999), higher circulating Tpo concentrations in fetuses and neonates than in children and adults (Dame, 2002; Cremer *et al*, 2003), and an increased sensitivity of megakaryocytic progenitor cells to Tpo (Murray *et al*, 1998; Sola *et al*, 2000). In 'small-for-gestational age' infants (birth weight below the 10th percentile), additional factors may be relevant for an overwhelming platelet production and thrombocytosis, particularly after fetal distress or eclampsia with or without initial neonatal thrombocytopenia.

Frequent causes of secondary thrombocytosis in childhood

Bacterial or viral infections (acute or chronic) are the most common cause for RT (37–78%) at any age during childhood. Within this group, infections of the respiratory tract account for 60–80% of RT, followed by infections of the gastrointestinal and urinary tract (Wolach *et al*, 1990; Garoufi *et al*, 2001). No relationship has been found between thrombocytosis and the prognosis or antibiotic treatment of the infection. Kilpi *et al* (1992) found thrombocytosis in 49% of 311 children with bacterial meningitis after the first week of treatment. RT had no influence on the neurological outcome of surviving patients. Patients who died developed thrombocytopenia instead of thrombocytosis (Kilpi *et al*, 1992).

The analysis of circulating Tpo concentrations helped to elucidate the role of Tpo in RT associated with infectious diseases. In the first week, when platelet counts are still normal,

Table III. Clinical features of secondary/reactive thrombocytosis in children. Only studies that included more than 130 children are reported.

	Reference (country)						
	Sutor and Hank (1992) (Germany)	Vora and Lilleyman (1993)(UK)	Yohannan *et al* (1994) (Saudi Arabia)	Heng and Tan (1998) (Singapore)	Chen *et al* (1999) (Taiwan)	Matsubara *et al* (2004) (Japan)
Number of children (platelet count, $\times 10^9$/l)	227 (>500)	458 (>500)	663 (>500)	135 (>600)	220 (>500)	456 (>500)
Age	72% <2 years	Median 13 months	Mostly <5 years†	Mostly <1 year	Mostly <2 years	Mostly <2 years
Boys to girls ratio	1	1.1	1·6	1·7	1·7	1·3
Infections (%)	39	38	37	78	50	68
Trauma/surgery/burns (%)	15	20	15		13	3·3
Anaemia (%)	12	6	14·8 plus 19·3†		3·7	6·4
Gastroesophageal reflux (%)	6·5					
Autoimmune diseases,	4	9	2	*	3·6	2
Kawasaki syndrome (%)					6·4	9
Low birth weight (<2·5 kg) (%)	2·1			3·2	3·2	9·2

Further conditions (<3% of children with thrombocytosis) included neoplasia, drug-associated thrombocytosis, stress, allergies, metabolic disease and others.
*The majority of children with autoimmune disease suffered from Kawasaki syndrome.
†Not including infants; the country reported has a high prevalence of hereditary haemolytic anaemia.

circulating Tpo concentrations peak on day 4 ± 2 and then gradually decrease. When platelet counts peak in the second or third week, Tpo concentrations are back to the normal range. Tpo concentrations are correlated with those of CrP and IL-6 (Ishiguro *et al*, 2002). Usually the blood sedimentation rate is increased, and concentrations of CrP, pro-inflammatory cytokines (such as IL-6), fibrinogen, and von Willebrand factor are elevated (Kutti & Wadenvik, 1996).

In 1–21% of children with RT, megakaryopoiesis is stimulated after tissue damage (major surgery, trauma, burns) (Sutor, 1999; Matsubara *et al*, 2004). As in infections, megakaryopoiesis may be stimulated by cytokines, such as IL-6, and other haematopoietic growth factors. Platelet counts peak usually between the first and second postoperative week. RT is also a frequent finding after splenectomy because of reduced platelet storage and removal in the reticulo-endothelial system (Sutor, 1999).

Various types of anaemia account for 6–12% of RT in childhood. Haemolytic anaemia and anaemia because of iron deficiency are most frequently associated with RT. Their incidence varies with the ethnic origin of the study population and the age of the children studied (Table III). Iron deficiency occurs in 4–6% of children with RT, most frequently in infants. Within a study group of children with iron deficiency, RT was found in up to one-third of them (Dickerhoff & von Ruecker, 1991). The relationship between iron deficiency and RT is likely to be complex, and there is increasing evidence that it is not a consequence of cross-reactivity between erythropoietin and Tpo (Geddis & Kaushansky, 2003). Thrombocytosis has also been observed in infants with bleeding because of vitamin K deficiency or other bleeding disorders, and after severe haemodilution in open-heart surgery (Sutor, 1999).

Autoimmune diseases (juvenile rheumatoid arthritis, inflammatory bowel disease, polyarteriitis nodosa, Kawasaki disease) account for 4–11% of RT in childhood (Table III). Among them, Kawasaki syndrome is the major cause of RT in children under 7 years, while other diseases occur mainly in children aged 11 years or older. As in adults, a direct correlation between IL-6 concentrations and the activity of the disease as well as platelet counts has been described (de Benedetti *et al*, 1991). In active inflammatory bowel diseases, Tpo concentrations are also elevated, but do not correlate with thromboembolic complications. The lack of a direct correlation between Tpo and platelet counts, and the fact that the increase in Tpo concentrations precedes thrombocytosis, again highly suggest that megakaryopoiesis is stimulated by both Tpo and other megakaryopoietic growth factors (Papa *et al*, 2003). Children with Henoch-Schoenlein purpura show only mild thrombocytosis. The coincidence of RT and abdominal pain as a result of haemorrhage and thrombosis is remarkable (Al Mazyad, 1999; Sutor, 1999).

In various studies, about 1–3% of children with thrombocytosis suffered from malignancies, before cytoreductive treatment was initiated (Chan *et al*, 1989; Sutor, 1995). Elevated Tpo concentrations have been reported in malignant Tpo-

producing liver tumours, particularly in hepatoblastoma and hepatocellular carcinoma (Komura *et al*, 1998). Less frequently, RT has also been associated with acute lymphocytic leukaemia (ALL) (3·2% of children diagnosed with ALL) (Blatt *et al*, 1989).

Reactive thrombocytosis can also be related to the treatment with several pharmaceutical agents. Adrenalin, corticosteroids, ciclosporin, vinca-alkaloids, miconazole, penicillamine, imipeneme and meropenem are claimed or known to cause or promote thrombocytosis in children (Oral *et al*, 1998; Sutor, 1999; Hsu *et al*, 2001; Koksal *et al*, 2001). Adrenalin and stress increase the platelet count by shifting stored platelets from the spleen into the circulation (Chamberlain *et al*, 1990). Ninety percent of children treated with corticosteroids and/or vinca-alcaloids because of malignancies (solid tumours, ALL) developed thrombocytosis during therapy (Sutor, 1999).

Transient thrombocytosis has been described in neonates with intrauterine exposure to methadone, hydantoine, or psychopharmaceutical drugs (Sutor, 1999). It has been speculated that RT is caused by a rebound of megakaryopoiesis after suppression of fetal Tpo production. Such a rebound phenomenon is also obvious in neonates who received antiretroviral treatment with zidovudine because of maternal human immunodeficiency virus infection. After initial thrombocytopenia or normal platelet counts, platelets can rise up to $>1000 \times 10^9$/l (Bruel *et al*, 2001).

In children, RT can be also associated with various other diseases, such as allergies, metabolic diseases, myopathies or neurofibromatosis (Sutor, 1999). However, the exact pathomechanisms leading of RT in these disorders are unclear to date.

Complications of RT in childhood

In childhood, RT usually does not result in thromboembolic or haemorrhagic complications. However, such complications occur after splenectomy or if the underlying disease is associated with additional thrombotic risk factors (Sutor, 2003). Thromboembolism, for example, occurs frequently in children with thalassaemia who have an increased platelet reactivity, low protein C and antithrombin concentrations (Shebl *et al*, 1999), or who suffer from sequelae of thalassaemia, such as cardiomyopathy, diabetes, hepatopathy and portal hypertension (Borgna *et al*, 1998).

Furthermore, neonates and infants have a higher thromboembolic risk if central venous catheters are placed or other thrombophilic conditions are present, such as maternal diabetes, maternal antiphospholipid syndrome, septicaemia, intrauterine growth retardation or cardiac malformation (Edstrom & Christensen, 2000).

Indications for treatment of RT

Reactive thrombocytosis in children does not justify general prophylaxis with anticoagulants or platelet aggregation inhibitors, even if the platelet count is $>1000 \times 10^9$/l. There is no evidence for the efficacy of prophylaxis against thromboembolic complications in asymptomatic children with RT. Individually tailored thrombosis prophylaxis should be considered if additional thrombotic risk factors exist. Treatment should be targeted at the basic disease (e.g. iron deficiency) rather on the platelet count. Only if thrombosis occurs repeatedly, a reduction of platelet aggregation and platelet count is indicated (Sutor, 2003).

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