WHO Clinical Molecular and Pathological (WHO-CMP) Features of Congenital MPL S505N and the Acquired MPL W515K Mutated Essential Thrombocythemia and Myelofibrosis

Jan Jacques Michiels1*, Achille PICH2, Hendrik de Raeye3, Vitr Camp4 and Jiri Schwarz4

1Goodheart Institute & Foundation, Rotterdam and International collaborations and Research on Myeloproiferative neoplasms and related Disorders (ICAR.MPN); Erasmus Tower, Veenemos 13 3069 AT Rotterdam, The Netherlands
2Department of Molecular Biotechnology and Health Sciences, Section of Pathology, University of Turin, Via Santena 7, 1-10126 Torino, Italy
3Departments of Pathology, OLVG Hospital Aalst and University of Brussels, Belgium
4Department of Pathology and Institute of Hematology & Blood Transfusion Univerzita nemocnice 1. CZ-128 20 Prague 2, Czechia

Keywords: Myeloproliferative Neoplasm; Essential Thrombocythemia; Myelofibrosis; Congenital or acquired MPL mutations.

Abstract

We analysed the clinical and hematological features in 41 patients of seven families, including 21 ET patients with a proven MPL S505N mutation and 20 relatives with thrombocytemia reported in the medical records. Out of the 41 MPL S505N mutated individuals 15 major thrombotic episodes in 14 members (34%) were reported as Budd-Chiari syndrome age 17 in 1, deep vein thrombosis leg age 41 in 1, ecclampsia and fetal in 1, stroke at ages 43, 72, 76 and 80 in 4 and myocardial infarction at ages between 31-81 years, median 52 years. Fourteen out of 21 well documented MPL S505N mutated ET patients had no splenomegaly and were free of major thrombosis during follow-up at ages between 2 and 76 years (mean 31 years). Eight MPL S505N mutated patients had myelofibrosis (MF) from grade MF1 in 5 to grade MF2 in 3 at ages between 28-80 years (mean 48 years), which was associated with mild to moderate splenomegaly (spleen length diameter 14.5 to 18 cm). Six anemic cases at hemoglobin levels between 10 and 11.9 g/dL had platelet counts between 317 and 963 × 10^9/L. Among 15 family members 9 died from thrombosis in 3, hypocellular myelofibrosis (two of them at age 76 and 80 years) in 3, and cancer or undefined in 3 cases. The maximum life expectancy of MPL S505N family members with thrombocytemia was 50% at 80 years, and 90% at 80 years of non-affected family members without thrombocytemia. The clinical presentation in 30 ET patients with acquired MPL S505N mutation (9 males and 21 females, age 22-84 (mean 56 years of whom 18 had the W515L and 12 the W515K) was featured by a high incidence of major arterial event in 23%, venous thrombosis in 10%, aspirin responsive microvessel disturbances in 60%, and major hemorrhage in 7%. The only abnormal laboratory finding in MPL mutated ET was increased platelet counts, 956+331 × 10^9/L in all and slight splenomegaly in 5 (17%). Bone marrow histology from patients ET carrying the MPL S505N mutation consistently displayed a normocellular bone marrow with clustered small and large to giant megakaryocytes with hyperlobulated stag-horn nuclei and no features of polycythemia vera (PV) in blood and bone marrow.

Keywords: Myeloproliferative Neoplasm; Essential Thrombocythemia; Myelofibrosis; Congenital or acquired MPL mutations.

Introduction

In the 1990s, studies on murine leukemia and oncogenes led to the recognition of a new member of the hematopoietin receptor super family [1], which was discovered as the product of the gene c-mpl, the normal cellular homologue of the oncogene v-cmpl, the transforming principle of a murine myeloproliferative leukemia virus, responsible for a pannymloid transformation [2]. This was followed by the molecular cloning and characterisation of Mpl, the human homologue of the c-mpl and v-mpl [3-5]. The new receptor was than rapidly recognized as being the thrombopoietin receptor (TpoR) by the demonstration that antisense oligonucleotides of c-mpl inhibited the colony-forming of megakaryocyte progenitors by Wendling and Vainchenker [4]. The Mpl ligand became the key to the identification of TPO, which was cloned in 1994 by five independent groups [6-11].
early childhood. TpoR or MPL exists as a transmembrane receptor in megakaryocytes and platelets, that has no intrinsic kinase activity, but associates with the cytoplasmic tyrosine kinase JAK2. Upon TPO ligand binding the TpoR/MPL receptor undergoes a conformational shift followed by cross-activation of two JAK2 molecules. TPO stimulation results in phosphorylation of MPL-bound JAKs and the subsequent activation of several down-stream pathways.

**Megakaryopoiesis Plasma TPO, TPOR/CMPL Receptor and Platelet Production**

TPO messenger studies showed that the main site of TPO production is in the liver and kidneys, an expression pattern similar to EPO [1]. It is known that hepatoblastoma and nephroblastoma may be accompanied by thrombocytosis due to increased TPO production and increased plasma TPO levels. During megakaryopoiesis the hematopoietic stem cell pass through a stage that they are still bipotent and have the capacity to become either erythroid cells or megakaryocytes under the influence of EPO and TPO respectively. The megakaryoblasts undergo a number of endomitotic reduplications and mainly megakaryocytes with 16–32N chromosomes and only a few 64–128N (hyperploidization) chromosomes are formed [1]. The last stage I the formation of filamentous protrusions called proplatelets, which fragment into platelets. TPO levels in serum are about three to four times higher than in EDTA plasma, because TPO is stored in platelets in high concentration and released during coagulation.

Plasma TPO levels in various blood and coagulation disorders reveal an inverse relationship between amegakaryocytic thrombocytopenia due to loss of function mutation of cMPL (Figure 1) [1]. In patients with pancytopenia and thrombocytopenia caused by acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) TPO levels are high (Figure 2) very likely due megakaryocytopenia in the bone marrow [1].

Plasma TPO levels are normal in immune thrombocytopenia due to an increased platelet destruction (Figure 2) and in patients with increased platelet count in reactive thrombocytosis and in essential thrombocythemia. This contrasts with low EPO levels concentrations found in JAK2\(^{V617F}\) mutated ET, prodromal PV and classical PV.

Plasma TPO levels in ET (JAK2\(^{V617F}\) positive and JAK2 wild type) are not down regulated. This difference reflects the fact that EPO is regulated at the level of production, while TPO is regulated largely by binding to platelets and their rate of peripheral consumption. In most clinical studies serum is used thus not only free plasma TPO, Serum TPO measures the total of plasma TPO plus TPO released from platelets during blood coagulation.

In ET this may lead to the measurement of high serum TPO concentrations in ET. Von den Borne found that plasma TPO levels in essential thrombocythemia are not increased (Figure 2) [1].

**Figure 1:** Plasma TPO levels in controls and in various groups of patients thrombocytopenia thrombocytopenia: ITP=idiopathic autoimmune thrombocytopenia; Sec ITP secondary autoimmune thrombocytopenia; Ameg TP=amegakaryocytic thrombocytopenia; Miscell A=acquired thrombocytopenia of non-immune cause but with a normal bone marrow; Miscell B=acquired thrombocytopenia of non-immune cause with a depressed bone marrow. Plasma TPO levels in the normal controls are 2.2-35 AU/ml. Von Dem Borne et al. 1998.

**Figure 2:** Relationship between plasma TPO level and decreased platelet count in patients with pancytopenia caused by acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). No increased Plasma TPO levels in 18 patients with acquired essential thrombocythemia. Normal=upper limit of normal.
Congenital Het Caused by the MPL \textit{SER505ASN} Mutation

Congenital gain of function mutation in the TPO gene on chromosome 3q27 results in increased levels of plasma TPO levels, which induce a physiological activation of the TPO-TPOR/MPL signalling pathway in three reported families with hereditary essential thrombocythemia [17-20]. This results in hyperproliferation of large mature megakaryocytes and platelet count complications by platelet-mediated microvascular complications. In a previous report we reviewed the presenting features and the natural history of two families with hereditary ET (HET) and secondary myelofibrosis caused by a gain of function mutation in the TPO gene [21]. In 2004 Ding et al. described the first case of congenital ET in the pedigree of a Japanese family caused by a G to A nucleotide substitution at position 1073 in exon 10 of the MPL gene leading to the exchange of serine for thrombocythemia [17-20]. This results in hyperproliferation of large in platelets obtained from affected individuals. The authors clearly demonstrated that cells expressing MPL\textit{S505N} showed自主性磷酸化 of both Mek1/2 and Stat5 down signaling transduction pathways but the clinical course of the disease in terms of vascular complications were not reported in family members carrying the MPL\textit{S505N} mutation.

Teofili et al. described in 2010 eight Italian families positive for the MPL\textit{S505N} mutation and reported on the clinical manifestations and hematological features in 41 patients of seven families, including 21 ET patients with a proven MPL\textit{S505N} mutation and 20 relatives with thrombocytoysis [23]. The family history of 41 individuals reported 15 major thrombotic episodes in 14 members (34%): Budd-Chiari syndrome age 17 in 1, deep vein thrombosis leg age 41 in 1, eclampsia and fetal in 1, stroke at ages 43, 72, 76 and 80 in 4 and myocardial infarction at ages between 31-81 years, median 52 years. These patients were not on aspirin at time of the occurrence of major thrombotic event and after a major thrombotic event they were treated with hydroxyurea or pegylated interferon (IFN) according to Italian guidelines (high thrombotic risk ELN) [24]. The overall survival and thrombosis-free survival in 41 affected family members with hereditary ET with MPL\textit{S505N} thrombocythemia (Family members with thrombocytoysis, Figure 3) was compromised as compared to normal overall and thrombosis-free survival in non-affected family members without thrombocythemia (Family members without thrombocytoysis, Figure 4). Clinical manifestation of aspirin responsive microvascular disturbances including erythromelalgia, migraine-like atypical cerebral transient ischemic attacks (MIAEs) and visual ischemic disturbances usually precede the occurrence of major thrombosis in ET and PV when not on aspirin. The indication of aspirin in 15 out of 21 MPL\textit{S505N} mutated ET cases was headache not otherwise specified, and none experienced bleeding complications during follow-up. Seventeen out of 21 documented MPL\textit{S505N} carriers were free of major thrombosis during follow-up at ages between 2 and 76 years (2, 4, 7, 20, 25, 28, 31, 42, 69 and 76 years). There was no splenomegaly in 13 these 14 cases without a history of thrombosis.

Three stages of atypical evolution megakaryopoiesis and increase of RF in bone marrow biopsies reflecting the natural history of MPL\textit{S505N} ET from normocellular or hypercellular ET into hypocellular secondary MF is shown in Figure 3.

At age 16 years a hypercellular (100%) ET with increased neutrophils and loose clusters of atypical megakaryocytes and normal erythropoiesis associated with and normal erythropoiesis and increase of reticulin fibers RF grade 0/1. At age 43 years moderate hypercellularity (70%) in ET and dense clusters of atypical megakaryocytes was associated with diffuse increase in reticulin fibers with focal bundles of collagen RF grade 3/4. At age 69 years a hypocellular bone marrow with numerous distorted megakaryocytes associated with diffuse increase in reticulin fibers (RF grade 3 to 4) and focal bundles of collagen myelofibrosis MF-3 with osteosclerosis.

Figure 3: Hematoxylin-eosin (H&E) and silver staining of poor quality bone marrow biopsies from 3 patients with autosomal dominant hereditary thrombocythemia caused by MPL\textit{S505N} mutation at young, adult and old age. A and B, age 16 years. Hypercellular (100%) ET with increased neutrophils and loose clusters of atypical megakaryocytes and normal erythropoiesis and increase of reticulin fibers RF grade 0/1. C and D, age 43 years. Moderately hypercellular (70%) ET with dense clusters of atypical megakaryocytes and increase of RF 2. E and F, age 69 years. Hypocellular with numerous distorted megakaryocytes and diffuse increase in reticulin fibers with focal bundles of collagen RF grade 3/4, MF-3 with osteosclerosis. These 3 stages of atypical evolution megakaryopoiesis and increase of RF reflect the natural history of MPL\textit{S505N} ET into hypocellular secondary MF.
In this cohort of 21 MPLS505N patients four cases presented with major thrombosis (19%); stroke at age 76 and 80 in 2, DVT/TIA at age 41/43 in 1 and myocardial infarction at age 31 in 1. Eight MPLS505N patients had myelofibrosis (MF) [25] grade MF1 in 5 and grade MF2 in 3 at ages 76, 55, 80, 28, 23, 33, 67, which was associated with mild to moderate splenomegaly (spleen length diameter 14.5 to 18 cm). Five of these MF patients were treated for several years with hydroxyurea (HU) in 3, interferon (IFN) in 1 and HU/IF in 1, and four of these five MF cases had anemia as a side effect of hydroxyurea and/or myelofibrotic transformation. All MF1 or MF2 patients had normal leukocyte counts counts except one female at age 72 years. Six anemic cases at hemoglobin levels between 10 and 11.9 g/dL had platelet counts between 317 and 963 × 10^9/L. Leukocyte counts were completely normal except leukocytosis above 10 in 3 young affected children (age 4 to 7 years) and one female at age 72. Among 15 family members 9 died prematurely of major thrombosis in 80 years, Figure 4), and liver cirrhosis, gastric cancer or undefined in 1 case each. Comparing all family members without thrombocythemia the overall survival and thrombosis-free survival was significantly shortened in MPLS505N mutated thrombocythemia patients. The maximum life expectancy of MPLS505N family members with thrombocythemia was 50% at 80 years, and 90% at 80 years of non-affected family members without thrombocythemia (Figure 4). This analysis strongly suggest that the loss of life expectancy is mainly due to major thrombosis and myelofibrotic transformation since 2 of 3 MPLS505N cases died from hypocellular myelofibrosis died at old ages of 76 and 80 years.

Acquired MPL515 mutated Essential Thrombocythemia

The JAK2 kinase activity in MPN is not only dependent on the amount of heterozygous and homozygous JAK2V617F mutant protein, but may also be influenced by the various steps upstream or downstream the signalling pathways including MPL, JAK2, STAT-3. This has been demonstrated in animal models overexpressing c-MPL [8,9]. MPL transgenic mice manifested with typical features of ET with a fourfold increase of platelet count, increased colony formation of megakaryocytes, and increase of clustered enlarged megakaryocytes in the bone marrow. The ET animals appeared healthy, had a very slight decrease of hematocrit (0.39 versus 0.42 in controls) despite an increase of bone marrow EEC, and survived normally with no evidence of myelofibrosis in the bone marrow [8,9]. The first case of congenital ET by Ding et al. in 2004 in a Japanese family caused by the germline MPLS505N mutation [22] and the discovery of the JAK2V617F mutation by Vainchenker et al. in 200526 as the cause of ET and PV has led to the discovery of the MPLW515L and MPLW515K somatic mutations as the cause of acquired ET and secondary myelofibrosis [26-29]. Within the JAK2 wild type MPN, there is a small subgroup who carry an acquired gain of function mutation of the MPL receptor as the cause of ET: 3% in the Vannucchi study [29], and 8.5% in the UK studies [30,31]. In contrast to JAK2V617F mutated trilinear MPN, patients with JAK2 wild type PT carrying the MPL515 mutation have no clinical, laboratory and bone marrow features of prodomal PV at diagnosis, do not evolve into overt PV during follow-up, have normal serum EPO and ferritin levels, and show pronounced megakaryotic proliferation of small and large (giant) megakaryocytes and no increase of erythropoiesis in the bone marrow [29].

Vannucchi et al. studied [30] Essential Thrombocytopenia (ET) patients carrying the MPL515 mutation, 9 males and 21 females, age 22-84 (mean 56) years [29]. The clinical presentation at diagnosis and follow-up was remarkable with a high incidence of major arterial event, 23%, venous thrombosis, 10%, microvessel disturbances, 60%, and major hemorrhage, 7%. The only abnormal laboratory finding was increased platelet counts, 956+331 × 10^9/L, together with hemoglobin values in the lower range of normal (13.4+1.3 g/l), normal white blood cells (8.8+3.1 × 10^9/L), slight increase of LDH (459+182 U/L) and splenomegaly in only 5 (17%) of 30 MPL515 mutated ET cases of whom 18 had the W515L and 12 the W515K allele mutated. Mutation allele burden was greater than 50% in half of MPLW515K patients compared to 17% of MPWL515L mutated ET patients. MPL515 and JAK2V617F mutations coexisted in 3 with MPLW515K and in 5 with MPWLW515K allele mutations. General features of bone marrow reports revealed significantly reduced erythropoiesis and decreased cellularity in MPLW515/K patients, associated with increased number of clustered small and large megakaryocytes, but no significant increase in reticulin fibrosis (RF). Activation of MPL by thrombopoietin enhances normal platelet function and abnormal activation of ET platelets by thrombopoietin preincubation has been described by Akkerman et al. [32]. Consequently, Vannucchi et al. hypothesized that platelets from MPLW515L/R mutated ET patients present constitutively enhanced reactivity (hypersensitive) of the mutated platelets to explain the high incidence of aspirin responsive microvascular disturbances and major arterial thrombotic events in acquired MPL mutated ET [29]. The Dutch family with hereditary ET due to a gain of function mutation in the TPO gene had life-long increased plasma TPO levels and presented at young and adult age recurrent erythromelalgia complicated by acrocyanosis of a few toes followed by gangrene and amputation of toe, which typically responded to low dose aspirin but not by Coumadin [21] thereby preventing the occurrence of major thrombosis during lifelong follow-up.

Bone Marrow Histology in Acquired MPL515 Mutated ET and MF

In 2008 we studied bone marrow histopathology in 12 cases with JAK2 wild type ET carrying the MPL515 mutation kindly provided by the courtesy of Dr. Vannucchi, Florence, Italy. Bone marrow histology from patients with JAK2 wild type ET carrying the MPL515 mutation
consistently displayed clusters small and large megakaryocytes with a greater number of giant megakaryocytes with hyperlobulated stag-horn nuclei in a normal cellular bone marrow and no increase of erythropoiesis. Bone marrow histology of two representative cases of MPL \textsuperscript{515} mutated ET are shown Figures 5 and 6.

As compared to bone marrow histopathology in JAK2 \textsuperscript{V617F} mutated ET (Figure 7) there were significant differences on three points. First, the megakaryocytes in MPL \textsuperscript{515} mutated PT are larger than in ET and PV (Figures 5 and 6), whereas the megakaryocytes in JAK2 \textsuperscript{V617F} mutated ET are not larger than in PV and show similar pleomorphic megakaryocytes morphology as in PV (Figure 7).

Second, there was local increase of erythropoiesis in areas of loose clustered pleomorphic megakaryocytes in JAK2 \textsuperscript{V617F} mutated ET (Figure 7), but not in JAK2 wild type PT carrying the MPL \textsuperscript{515} mutation.

Whether such differences of megakaryocyte morphology in bone marrow biopsies are characteristic enough to distinguish normocellular ET with low JAK2 mutation load from JAK2 wild type ET carrying the MPL \textsuperscript{515} mutation respectively by expert hematopathologists remains to be evaluated in prospective clinical and basic research studies.

**Figure 5:** JAK2 wild type ET carrying the MPL \textsuperscript{515} mutation with loose clustered small, large and giant mature megakaryocytes with the presence of hyperlobulated, “stag-horn” hyperlobulated nucleus. ET Case 1, upper panel and ET case 2, lower panel. Courtesy of Dr. Vannucchi.

**Figure 6:** JAK2 wild type ET carrying the MPL \textsuperscript{515} mutation with features of ET (WHO-ET) with the presence of clustered large and giant megakaryocytes and increase of reticulin fibrosis grade 2/3 (RF 2/3). Such increase of RF in a rather normocellular (65%) bone marrow is not seen in ET and PV carrying the JAK2 \textsuperscript{V617F} and also not in JAK2 wild type hypercellular ET due to PMGM ET case 3, Courtesy of Dr. Vannucchi.

**Figure 7:** Bone marrow histology in 59 cases of JAK2 \textsuperscript{V617F} mutated ET versus 44 cases of JAK2 wild type ET according to Pich et al. JAK2 wild ET patients in the study of Pich et al. are predicted to carry one of the CALR or MPL \textsuperscript{515} mutations.

**Case Report on the Natural History of MPL \textsuperscript{515} Mutated ET**

We studied the natural history of a 41-year old woman born in 1995 who presented in January 1996 with MPL \textsuperscript{W515L} mutated ET and a one year history of tingling prickling sensations in fingers and hand, vertigo and attacks of frontal headaches. Laboratory features at time of diagnosis were, hemoglobin 12.8 g/L, hematocrit 0.39, leukocytes 7.5 \times 10\textsuperscript{9}/L, normal LDH and spleen size on echogram 12.6 cm (normal
value <12 cm). Platelet counts were 790 × 10^9/L with highest platelet count of 1996 × 10^9/L in October 1996. Molecular biology analysis were negative for the JAK2V617F, CALR and ASXL1 mutations. Bone marrow histology showed a normal cellularity of about 40%, no increase of erythroidopoiesis, and prominent increase of large megakaryocytes with hyperlobulated nuclei even stag-horn forms with fine chromatin (Figure 8). Sporadically, small megakaryocytes with less lobulated nuclei were present. Fine perivascular reticulin fibers (RF grade 1) consistent with prefibrotic myelofibrosis MF 0. Treatment consisted of low dose aspirin for the relief of microvascular disturbances, pegylated interferon (IFN) was not tolerated and subsequent treatment consisted of hydroxyurea from 1996 to 1999 was followed by anagrelide from January 1999 until she developed refractory pancytopenic anaemia and thrombocytopenia in 2004. Bone marrow histology in 2004 showed a hypocellular bone marrow with dysmorphic small to large megakaryocytes and only slight increase of reticulin fibers RF grade 1 to 2 (Figure 9). Hematopoietic stem cell transplantation (HSCT) in February 2005 of a matched (10/10) unrelated donor and non-myeloablative conditioning (Buflan-Cytarabine-ATG) was followed by rapid engraftment and complete hematological remission. She is more than 9 years alive and well in 2014 and beyond.

Discussion

In a European collaborative study, Jones et al. determined the MPL mutation load in 138 W515K/L MPN cases: ET, n=99; MF, n=36; unclassified MPN, n=331. The overall median W515K/L mutation levels in ET was 21%, which were significantly lower than in MF patients, 46% (P<0.001, Figure 9). Twenty nine MPL515 homozzygous cases (mutation load more than 50%) in 17 W515K and in 12 W515K MPN cases had a diagnosis of ET in 12, MF in 15 and accelerated/transformed MPN in 2. The overall mutation levels in 106 MPL515K/L cases were lower, 25%, as compared to 37% in 32 MPLW515K cases (P<0.02), thereby confirming the observations of Schnittger et al. in 35 cases with four different MPL515 mutations in ET or MF34. In this German cohort of 324 JAK2 wild type ET, any MPLW515 mutation was detected in 19 patients (5.9%), and of 104 JAK2 wild type MF, 10 MPLW515 mutations (9.6%) were detected. In addition two novel MPL mutations W515R and W515K were found. We request in 2013 and 2014 the German MPN investigators Schnittger, Reiter and Kvasnicka to collect BM biopsies for expert evaluation of the WHO defined clinical molecular and pathological (WHO-CMP) [33-36] features in their cohort of 35 MPL mutated ET and MF patients. Beer et al. described bone marrow biopsies at diagnosis of 13 patients with MPL mutations: 2 S505N, 2 W515K and 9 W515L MPN patients [30]. As compared to JAK2V617F positive ET and JAK2/MPL wild type ET, the bone marrow biopsies from the MPL mutant group were less cellular (P<0.001 and P<0.003 with age). Both elytroid and granulocytic cellularity were reduced in the MPL mutant group. (P<0.001) indicating the absence of PV features. Overall, bone marrow histology in MPL mutant patients revealed more isolated megakaryocytic proliferation at diagnosis with a reduction of erythroid and overall cellularity compared to JAK2V617F positive and JAK2/MPL wild type ET (Figure 10) [37]. Serum EPO levels in MPL mutation patients were significantly higher than in JAK2V617F positive ET (prodromal PV) [35,36]. In contrast to JAK2V617F positive ET, Beer et al. found no endogenous erythroid colonies (EEC) in 5 evaluated MPLW515L cases (4 ET and 1 MF). At the basic research level Chaligne et al. demonstrated that the two MPL mutations W515L and W515K induced a spontaneous megakaryocyte growth in culture with an overall normal response to thrombopoietin (TPO), but the erythroid progenitors remained EPO dependent and did not show spontaneous erythroid colony (EEC) formation (Figure 10) [21,33].
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