

## WHO Clinical Molecular and Pathological (WHO-CMP) Features of Congenital MPL<sup>S505N</sup> and the Acquired MPL<sup>W515L/K</sup> Mutated Essential Thrombocythemia and Myelofibrosis

Jan Jacques Michiels<sup>1\*</sup>, Achille PICH<sup>2</sup>, Hendrik de Raeve<sup>3</sup>, Vitir Camp<sup>4</sup> and Jiri Schwarz<sup>4</sup>

<sup>1</sup>Goodheart Institute & Foundation, Rotterdam and International collaborations and Research on Myeloproliferative neoplasms and related Disorders (ICAR.MPNr).Erasmus Tower, Veenmos 13 3069 AT Rotterdam, The Netherlands

<sup>2</sup>Department of Molecular Biotechnology and Health Sciences, Section of Pathology, University of Turin, Via Santena 7, I-10126 Torino, Italy

<sup>3</sup>Departments of Pathology, OLVG Hospital Aalst and University of Brussels, Belgium

<sup>4</sup>Department of Pathology and Institute of Hematology & Blood Transfusion U nemocnice 1. CZ-128 20 Prague 2, Czechia

\*Corresponding author: Jan Jacques Michiels, Senior Internist & Investigator, Goodheart Institute & Foundation, Rotterdam and International collaborations and Research on Myeloproliferative neoplasms and related Disorders (ICAR.MPNr).Erasmus Tower, Veenmos 13 3069 AT Rotterdam, The Netherlands, Tel: +316-26970534; E-mail: [goodheartcenter@upcmail.nl](mailto:goodheartcenter@upcmail.nl)

Received date: Nov 17, 2014, Accepted date: Dec 18, 2014, Published date: Dec 28, 2014

Copyright: © 2014 Michiels JJ, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

We analysed the clinical and hematological features in 41 patients of seven families, including 21 ET patients with a proven MPL<sup>S505N</sup> mutation and 20 relatives with thrombocythemia reported in the medical records. Out of the 41 MPL<sup>S505N</sup> 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, 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. Fourteen out of 21 well documented MPL<sup>S505N</sup> 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<sup>S505N</sup> 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<sup>9</sup>/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<sup>S505N</sup> family members with thrombocythemia was 50% at 80 years, and 90% at 80 years of non-affected family members without thrombocythemia. The clinical presentation in 30 ET patients with acquired MPL<sup>S505N</sup> mutation (9 males and 21 females, age 22-84 (mean 56 years of whom 18 had the W<sup>515L</sup> and 12 the W<sup>515K</sup>) 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<sup>9</sup>/L in all and slight splenomegaly in 5 (17%). Bone marrow histology from patients ET carrying the MPL<sup>S505N</sup> 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 panmyloid 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 then 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].

The MPL ligand is identical to TpoR and labeled as megakaryocyte growth and development factor (MDGF) [10-13]. Human TPO has all the functions ascribed to MDGF, and all MDGF-like activity can be neutralized by soluble recombinant Mp [1].

Thrombopoietine (TPO) is encoded by the human TPO gene and located on chromosome 3q271. The mature TPO molecule is composed of 32 amino acids. The TPO Receptor (TpoR) is encoded by the myeloproliferative leukemia (MPL) gene, which is located on chromosome 1p34. TPO and its ligand MPL are involved in nearly every step of megakaryocyte development including mobilisation of hematopoietic stem cells, stimulating megakaryocyte proliferation and lineage differentiation. TPO deficient mice show a marked decrease in the number of megakaryocytes and platelets, accompanied by significant reduction of all lineage hematopoietic progenitor cells [14-16]. These animals are the counterpart of congenital recessive amegakaryocyte thrombocythemia (CAMT), a pediatric disease resulting from the loss of function of the MPL gene in newborns with recessive severe thrombocytopenia who later develop severe anemia in

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

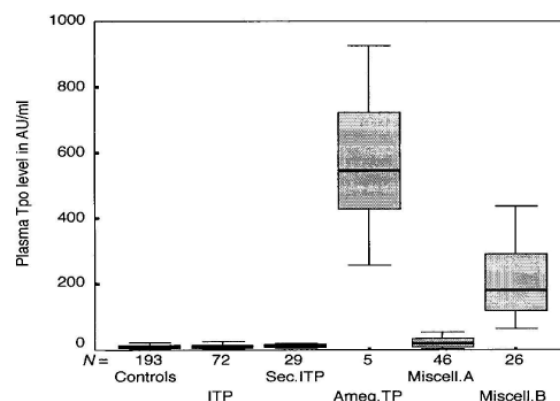
TPO messenger studies showed that the main site of TPO production is in the liver and kidneys, an expression pattern similar to EPO [1]. It is known that hepatoblastoma and nephroblastoma may be accompanied by thrombocytosis due to increased TPO production and increased plasma TPO levels. During megakaryopoiesis 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 (hyperploidy) chromosomes are formed [1]. The last stage I the formation of filamentous extrusions 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].

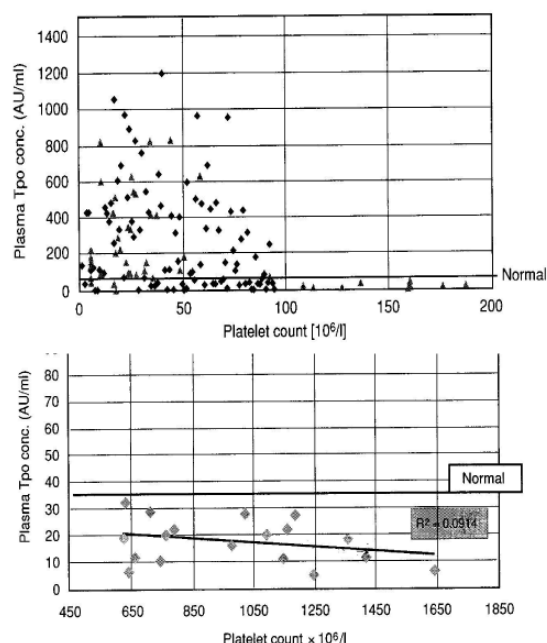
Plasma TPO levels are normal in immune thrombocytopenia due to an increased platelet destruction (Figure 2) and in patients with increased platelet count in reactive thrombocytosis and in essential thrombocythemia. This contrasts with low EPO levels concentrations found in JAK2<sup>V617F</sup> mutated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

Plasma TPO levels in ET (JAK2<sup>V617F</sup> 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.

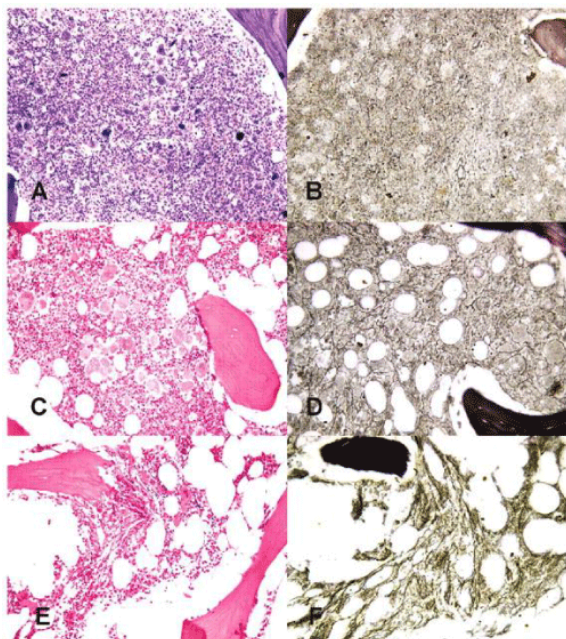
In ET this may lead to the measurement of high serum TPO concentrations in ET. Von den Borne found that plasma TPO levels in essential thrombocythemia are not increased (Figure 2) [1].



**Figure 1:** Plasma TPO levels in controls and in various groups of patients thrombocytopenia: 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; Miscel 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.



**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<sup>S505N</sup> 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<sup>S505N</sup> ET into hypocellular secondary MF.

### Congenital Het Caused by the MPL<sup>SER505ASN</sup> 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 complicated 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 asparagine at position 505 (MPL<sup>S505N</sup>) [22]. Ding et al. demonstrated that the presence of the germ line MPL<sup>S505N</sup> mutation is associated with autonomous activation in the MPL downstream signaling pathways, both *in vitro* in cells transfected with the mutant and *in vivo* in platelets obtained from affected individuals. The authors clearly

demonstrated that cells expressing MPL<sup>S505N</sup> showed autonomous phosphorylation of both Mek1/2 and Stat5 downstream signaling pathways but the clinical course of the disease in terms of vascular complications were not reported in family members carrying the MPL<sup>S505N</sup> mutation.

Teofili et al. described in 2010 eight Italian families positive for the MPL<sup>S505N</sup> mutation and reported on the clinical manifestations and hematological features in 41 patients of seven families, including 21 ET patients with a proven MPL<sup>S505N</sup> mutation and 20 relatives with thrombocytosis [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<sup>S505N</sup> thrombocythemia (Family members with thrombocytosis, Figure 3) was compromised as compared to normal overall and thrombosis-free survival in non-affected family members without thrombocytosis (Family members without thrombocytosis, Figure 4). Clinical manifestation of aspirin responsive microvascular disturbances including erythromelalgia, migraine-like atypical cerebral transient ischemic attacks (MIAs) 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<sup>S505N</sup> mutated ET cases was headache not otherwise specified, and none experienced bleeding complications during follow-up. Fourteen out of 21 documented MPL<sup>S505N</sup> carriers were free of major thrombosis during follow-up at ages between 2 and 76 years (2, 4, 7, 20, 25, 28, 31, 34, 42, 69 and 76 years). There was no splenomegaly in 13 these 14 cases without a history of thrombosis.

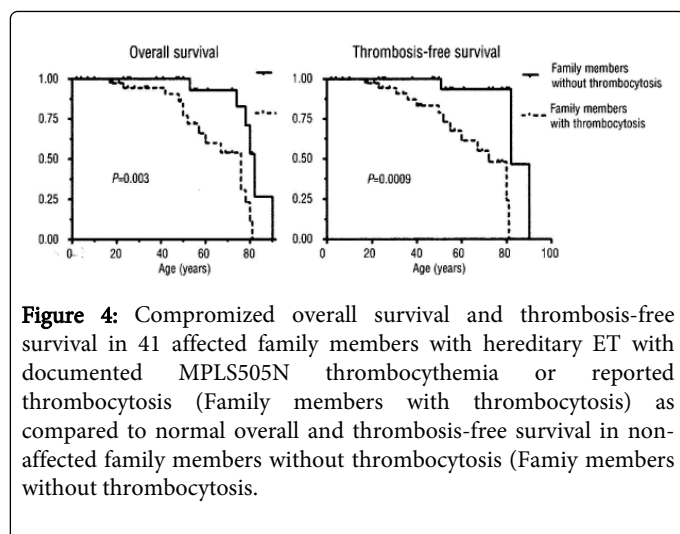
Three stages of atypical evolution megakaryopoiesis and increase of RF in bone marrow biopsies reflecting the natural history of MPL<sup>S505N</sup> ET from normocellular or hypercellular ET into hypocellular secondary MF is shown in Figure 3.

At age 16 years a hypercellular (100%) ET with increased neutrophils and loose clusters of atypical megakaryocytes was associated with 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 an increase of reticulin fibers grade 2 (RF 2=MF 1).

At age 69 years a hypocellular bone marrow with numerous distorted megakaryocytes was associated with diffuse increase in reticulin fibers (RF grade 3 to 4) and focal bundles of collagen myelofibrosis MF-3 with osteosclerosis.





**Figure 4:** Compromized overall survival and thrombosis-free survival in 41 affected family members with hereditary ET with documented MPL<sup>S505N</sup> thrombocythemia or reported thrombocytosis (Family members with thrombocytosis) as compared to normal overall and thrombosis-free survival in non-affected family members without thrombocytosis (Family members without thrombocytosis).

In this cohort of 21 MPL<sup>S505N</sup> 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 MPL<sup>S505N</sup> 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/IFN 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 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<sup>9</sup>/L. At hemoglobin levels above 12 g/dL platelet counts ranged from 605 to 1726 × 10<sup>9</sup>/L. Leukocyte counts were completely normal except leucocytosis 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 3, anemia with hypocellular myelofibrosis (2 cases at age 76 and 80 years, Figure 3), 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 MPL<sup>S505N</sup> mutated thrombocythemia patients. The maximum life expectancy of MPL<sup>S505N</sup> 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 MPL<sup>S505N</sup> cases died from hypocellular myelofibrosis died at old ages of 76 and 80 years.

### Acquired MPL<sup>515</sup> mutated Essential Thrombocythemia

The JAK2 kinase activity in MPN is not only dependent on the amount of heterozygous and homozygous JAK2<sup>V617F</sup> 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 MPL<sup>S505N</sup> mutation [22] and the discovery of the JAK2<sup>V617F</sup> mutation by Vainchenker et al. in 200526 as the cause of ET and PV has led to the discovery of the MPL<sup>W515L</sup> and MPL<sup>W515K</sup> 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 JAK2<sup>V617F</sup> mutated trilinear MPN, patients with JAK2 wild type PT carrying the MPL<sup>515</sup> mutation have no clinical, laboratory and bone marrow features of prodromal PV at diagnosis, do not evolve into overt PV during follow-up, have normal serum EPO and ferritin levels, and show pronounced megakaryocytic proliferation of small and large (giant) megakaryocytes and no increase of erythropoiesis in the bone marrow [29].

Vannucchi et al. studied [30] Essential Thrombocythemia (ET) patients carrying the MPL<sup>515</sup> 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<sup>9</sup>/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<sup>9</sup>/L), slight increase of LDH (459+182 U/L) and splenomegaly in only 5 (17%) of 30 MPL<sup>515</sup> mutated ET cases of whom 18 had the W<sup>515L</sup> and 12 the W<sup>515K</sup> allele mutated. Mutation allele burden was greater than 50% in half of MPL<sup>W515K</sup> patients compared to 17% of MPL<sup>W515L</sup> mutated ET patients. MPL<sup>515</sup> and JAK2<sup>V617F</sup> mutations coexisted in 3 with MPL<sup>W515L</sup> and in 5 with MPL<sup>W515K</sup> allele mutations. General features of bone marrow reports revealed significantly reduced erythropoiesis and decreased cellularity in MPL<sup>W515L/K</sup> 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 MPL<sup>W515L/K</sup> 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 thrombotic during lifelong follow-up.

### Bone Marrow Histology in Acquired MPL<sup>515</sup> Mutated ET and MF

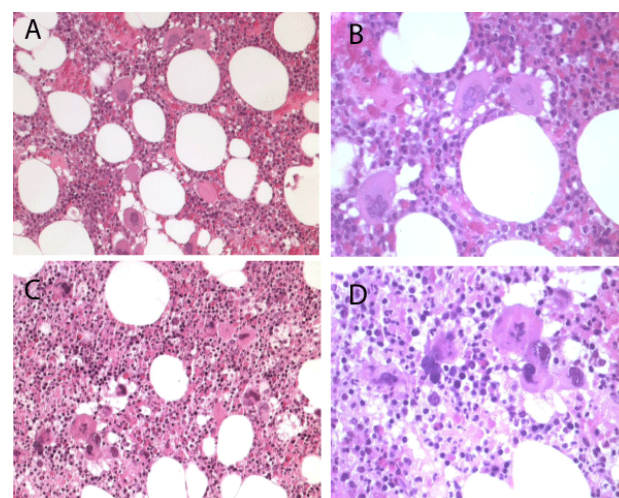
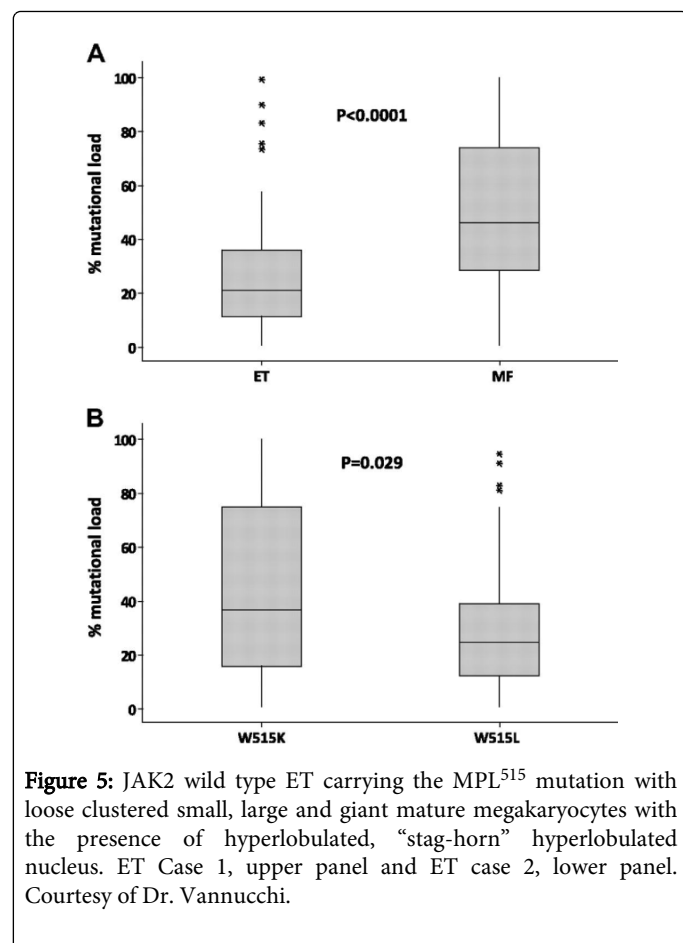
In 2008 we studied bone marrow histopathology in 12 cases with JAK2 wild type ET carrying the MPL<sup>515</sup> mutation kindly provided by the courtesy of Dr. Vannucchi, Florence, Italy. Bone marrow histology from patients with JAK2 wild type ET carrying the MPL<sup>515</sup> mutation

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<sup>515</sup> mutated ET are shown Figures 5 and 6.

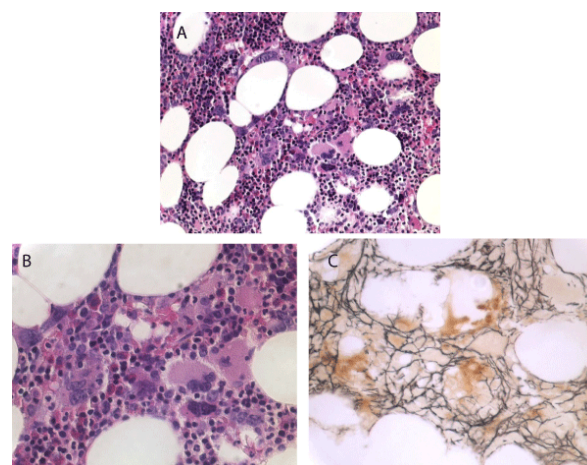
As compared to bone marrow histopathology in JAK2<sup>V617F</sup> mutated ET (Figure 7) there were significant differences on three points. First, the megakaryocytes in MPL<sup>515</sup> mutated PT are larger than in ET and PV (Figures 5 and 6), whereas the megakaryocytes in JAK2<sup>V617F</sup> mutated 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 pleiomorphic megakaryocytes in JAK2<sup>V617F</sup> mutated ET (Figure 7), but not in JAK2 wild type PT carrying the MPL<sup>515</sup> 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<sup>515</sup> mutation respectively by expert hematopathologists remains to be evaluated in prospective clinical and basic research studies.



**Figure 6:** JAK2 wild type ET carrying the MPL<sup>515</sup> 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<sup>V617F</sup> 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<sup>V617F</sup> 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<sup>515</sup> mutations.

## Case Report on the Natural History of MPL<sup>515</sup> Mutated ET

We studied the natural history of a 41-year old woman born in 1995 who presented in January 1996 with MPL<sup>W515L</sup> 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  $7.5 \times 10^9/L$ , normal LDH and spleen size on echogram 12.6 cm (normal

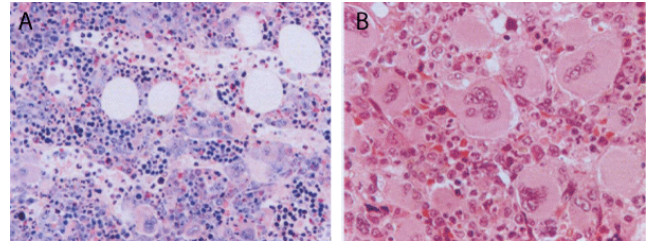


value <12 cm). Platelet counts were  $790 \times 10^9/L$  with highest platelet count of  $1996 \times 10^9/L$  in October 1996. Molecular biology analysis were negative for the JAK2<sup>V617F</sup>, CALR and ASXL1 mutations. Bone marrow histology showed a normal cellularity of about 40%, no increase of erythropoiesis, and prominent increase of large to giant 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 anemia 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 (Busulfan-Cytarabine-ATG) was followed by rapid engraftment and complete hematological remission. She is more than 9 years alive and well in 2014 and beyond.

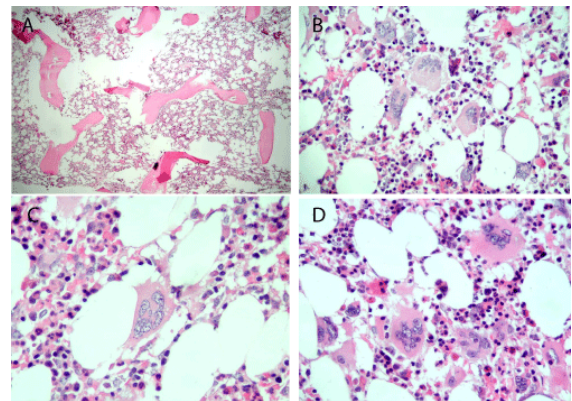
## Discussion

In a European collaborative study, Jones et al. determined the MPL mutation load in 138 W<sup>515K/L</sup> MPN cases: ET, n=99; MF, n=36; unclassified MPN, n=331. The overall median W<sup>515K/L</sup> mutation levels in ET was 21%, which were significantly lower than in MF patients, 46% (P<.001, Figure 9). Twenty nine MPL<sup>515</sup> homozygous cases (mutation load more than 50% in 17 W<sup>515L</sup> and in 12 W<sup>515K</sup> MPN cases) had a diagnosis of ET in 12, MF in 15 and accelerated/transformed MPN in 2. The overall mutation levels in 106 MPL<sup>W515L</sup> cases were lower, 25%, as compared to 37% in 32 MPL<sup>W515K</sup> cases (P=0.02), thereby confirming the observations of Schnittger et al. in 35 cases with four different MPL<sup>515</sup> mutations in ET or MF34. In this German cohort of 324 JAK2 wild type ET, any MPL<sup>W515</sup> mutation was detected in 19 patients (5.9%), and of 104 JAK2 wild type MF, 10 MPL<sup>W515</sup> mutations (9.6%) were detected. In addition two novel MPL mutations W<sup>515R</sup> and W<sup>515K</sup> 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 S<sup>505N</sup>, 2 W<sup>515K</sup> and 9 W<sup>515L</sup> MPN patients [30]. As compared to JAK2<sup>V617F</sup> 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 erythroid 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 JAK2<sup>V617F</sup> positive and JAK2/MPL wild type ET (Figure 10) [37]. Serum EPO levels in MPL mutation patients were significantly higher than in JAK2<sup>V617F</sup> positive ET (prodromal PV) [35,36]. In contrast to JAK2<sup>V617F</sup> positive ET. Beer et al. found no endogenous erythroid colonies (EEC) in any of 5 evaluated MPL<sup>W515L</sup> cases (4 ET and 1 MF). At the basic research level Chaligne et al. demonstrated that the two MPL mutations W<sup>515L</sup> and W<sup>515K</sup> induced a spontaneous megakaryocyte growth in culture with an overall

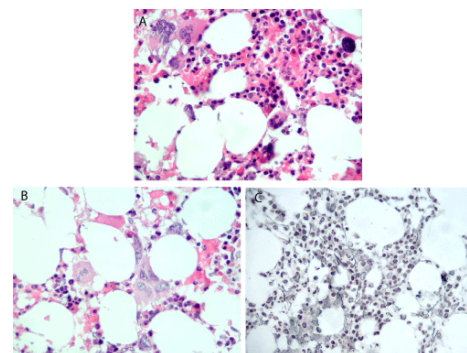
normal response to thrombopoietin (TPO), but the erythroid progenitors remained EPO dependent and did not show spontaneous erythroid colony (EEC) formation (Figure 10) [21,33].



**Figure 8:** Bone marrow histology in normocellular ET carrying the MPLW515L mutation showing giant megakaryocytes with hyperlobulated stag-horn like nuclei characteristic for ET caused by the MPLW515L/K mutation.



**Figure 9:** Evolution of acquired normocellular ET carrying the MPL<sup>W515L</sup> mutation into pancytopenia (anemia and thrombocytopenia) and a hypocellular bone marrow with distorted dysmorphic megakaryocytes and slight increase of reticulin fibers RF grade 1.



**Figure 10:** Isolated megakaryocytic proliferation at diagnosis with a reduction of erythroid and overall cellularity compared to JAK2<sup>V617F</sup> positive and JAK2/MPL wild type ET.

## References

- von dem Borne AE, Folman C, Linthorst GE, Porcelijn L, van den Oudenrijn S, et al. (1998) Thrombopoietin and its receptor: structure, function and role in the regulation of platelet production. *Baillieres Clin Haematol* 11: 409-426.
- Souri M, Vigon I, Penciolelli JF (2013) A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. *Cell* 63: 1137-1147.
- Vigon I, Mornon JP, Cocault L, Mitjavila MT, Tambourin P, et al. (1992) Molecular cloning and characterization of MPL, the human homolog of the v-mpl oncogene: identification of a member of the hematopoietic growth factor receptor superfamily. *Proc Natl Acad Sci U S A* 89: 5640-5644.
- Methia N, Louache F, Vainchenker W, Wendling F (1993) Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryocytopoiesis. *Blood* 82: 1395-1401.
- Mignotte V, Vigon I, Boucher de Crèvecœur E, Roméo PH, Lemarchandel V, et al. (1994) Structure and transcription of the human c-mpl gene (MPL). *Genomics* 20: 5-12.
- Bartley TD, Bogenberger J, Hunt P, Li YS, Lu HS, et al. (1994) Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. *Cell* 77: 1117-1124.
- Sohma Y, Akahori H, Seki N, Hori T, Ogami K, et al. (1994) Molecular cloning and chromosomal localization of the human thrombopoietin gene. *FEBS Lett* 353: 57-61.
- de Sauvage FJ, Hass PE, Spencer SD, Malloy BE, Gurney AL, et al. (1994) Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. *Nature* 369: 533-538.
- Lok S, Kaushansky K, Holly RD, Kuijper JL, Lofton-Day CE, et al. (1994) Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. *Nature* 369: 565-568.
- Kaushansky K, Lok S, Holly RD, Broudy VC, Lin N, et al. (1994) Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature* 369: 568-571.
- Wendling F, Maraskovsky E, Debili N, Florindo C, Teepe M, et al. (1994) cMpl ligand is a humoral regulator of megakaryocytopoiesis. *Nature* 369: 571-574.
- Kaushansky K, Broudy VC, Lin N, Jorgensen MJ, McCarty J, et al. (1995) Thrombopoietin, the Mpl ligand, is essential for full megakaryocyte development. *Proc Natl Acad Sci U S A* 92: 3234-3238.
- de Sauvage FJ, Carver-Moore K, Luoh SM, Ryan A, Dowd M, et al. (1996) Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. *J Exp Med* 183: 651-656.
- Gurney AL, Carver-Moore K, de Sauvage FJ, Moore MW (1994) Thrombocytopenia in c-mpl-deficient mice. *Science* 265: 1445-1447.
- Carver-Moore K, Broxmeyer HE, Luoh SM, Cooper S, Peng J, et al. (1996) Low levels of erythroid and myeloid progenitors in thrombopoietin-and c-mpl-deficient mice. *Blood* 88: 803-808.
- Ballmaier M, Germeshausen M (2009) Advances in the understanding of congenital amegakaryocytic thrombocytopenia. *Br J Haematol* 146: 3-16.
- Wiestner A, Schlemper RJ, van der Maas AP, Skoda RC (1998) An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. *Nat Genet* 18: 49-52.
- Kondo T, Okabe M, Sanada M, Kurosawa M, Suzuki S, et al. (1998) Familial essential thrombocythemia associated with one-base deletion in the 5'-untranslated region of the thrombopoietin gene. *Blood* 92: 1091-1096.
- Kralovics R, Buser AS, Teo SS, Coers J, Tichelli A, et al. (2003) Comparison of molecular markers in a cohort of patients with chronic myeloproliferative disorders. *Blood* 102: 1869-1871.
- Liu K, Kralovics R, Rudzki Z, Grabowska B, Buser AS, et al. (2008) A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. *Haematologica* 93: 706-714.
- Michiels JJ, Jan Stasko J, Peter Kubish P, Achille Pich A, De Raeve H (2014). Hereditary Essential Thrombocythemia due to a gain of function mutation in the thrombopoietin (TPO) and JAK2 gene as the cause of congenital autosomal dominant Essential Thrombocythemia and Aspirin-responsive Sticky Platelet Syndrome.
- Ding J, Komatsu H, Wakita A, Kato-Uranishi M, Ito M, et al. (2004) Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. *Blood* 103: 4198-4200.
- Teofili L, Giona F, Torti L, Cenci T, Ricerca BM, et al. (2010) Hereditary thrombocytosis caused by MPL<sup>Ser505Asn</sup> is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. *Haematologica* 95: 65-70.
- Harrison CN (2006) Management of essential thrombocythemia: implications of the medical research council primary thrombocythemia I trial. *Semin Thromb Hemost* 32: 283-288.
- Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, et al. (2005) European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica* 90: 1128-1132.
- James C, Ugo V, Le Couedic PF, Staerk J, Delhommeau F, et al. (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythemia vera. *Nature* 434: 1144-1148.
- Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, et al. (2006) MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* 108: 3472-3476.
- Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, et al. (2006) MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* 3: e270.
- Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, et al. (2008) Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. *Blood* 112: 844-847.
- Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, et al. (2008) MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. *Blood* 112: 141-149.
- Jones AV, Campbell PJ, Beer PA, Schnittger S, Vannucchi AM, et al. (2010) The JAK2 46/1 haplotype predisposes to MPL-mutated myeloproliferative neoplasms. *Blood* 115: 4517-4523.
- Akkerman JW (2006) Thrombopoietin and platelet function. *Semin Thromb Hemost* 32: 295-304.
- Chaligné R, James C, Tonetti C, Besancenot R, Le Couédic JP, et al. (2007) Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis. *Blood* 110: 3735-3743.
- Schnittger S, Bacher U, Haferlach C, Beelen D, Bojko P, et al. (2009) Characterization of 35 new cases with four different MPLW515 mutations and essential thrombocytosis or primary myelofibrosis. *Haematologica* 94: 141-144.
- Michiels JJ, Berneman Z, Schroyens W, Hebeda K, Bot F, et al. (2013) PVSG and the WHO versus the European Clinical, Molecular and Pathological (ECMP) criteria for the diagnosis, classification and staging of the myeloproliferative neoplasms. *World J Hematol* 2: 71-90.
- Michiels JJ, Berneman Z, Schroyens W, De Raeve H (2015) Changing concepts on the diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms. From Dameshek 1950 to Vainchenker 2005 and beyond. *Acta Haematol* (2014 online) 133: 36-51.
- Pich A, Riera L, Beggiato E, Nicolino B, Godio L, et al. (2012) JAK2V617F mutation and allele burden are associated with distinct clinical and morphological subtypes in patients with essential thrombocythemia. *J Clin Pathol* 65: 953-955.