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Identification of MPL-W515L -W515K - W515R -W515A -S505N mutations in thrombopoietin receptor of essential thrombocythemia patients

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Background: Essential thrombocythemia (ET) is a clonal BCR-ABL1-negative myeloproliferative neoplasm (MPN) characterized by a sustained elevation of the circulating platelet count and bone-marrow hyperplasia with excessive proliferation of megakaryocytes. The neoplasm could be associated with thrombosis, hemorrhage, hepatosplenomegaly, and the risk of transformation to acute myeloid leukemia [1]. To prevent a significant number of early vascular events is still one of the most important challenges in the disease management because thrombosis could be a major cause of morbidity and mortality. Thus early detection of ET is important as without adapted treatment, recurrent thromboses are also frequent.

The discovery of the Jak-STAT signaling pathway in the background of MPN has led to an improvement in establishing the diagnosis. JAK2 V617F positivity detection has become an important part in setting up the correct diagnosis. However 30–40% of ET patients are JAK2 V617F negative, thus, further mutation analysis could be important to help in establishing the proper clinical diagnosis. Mutations of the myeloproliferative leukemia gene (1p34) encoding the thrombopoietin receptor, that works in concert with thrombopoietin affect platelet production. Gain-of-function mutations causing the amino acid substitutions in the thrombopoietin receptor gene (MPL) have been found in 1% to 2% of those with ET [2].

Our aim was to evaluate the frequency of acquired MPL mutations MPL W515L, MPL W515K, MPL W515R and inherited forms of the mutation MPL S505N, MPL S505N in JAK2 V617F-negative ET patients diagnosed at our regional centre. Our purpose was also to identify a type of MPL mutation screening which can be measured easily in everyday medical practice.

Patients and methods: Between 1999 and 2011, 96 patients with essential thrombocythemia were selected randomly. Among them 38 JAK2 V617F-negative ET patients could be found (11 male ; 27 female) with the mean age of 55.52 years [range: 14–95 years]. Median follow-up was 72 months [range: 12–156 months]. The average platelet count was 695.47 G/L at the time of diagnosing ET. Splenomegaly was reported in 5 cases. Hepatomegaly and hepatosplenomegaly were reported in one case each. Patients' distribution according cardiovascular risk factors was as follows: 17 patients (44.73%) had reported high blood pressure, 2 patients (5.26%) had hyperlipidemia, 2 patients (5.6%) had diabetes mellitus, all

of them were medically controlled. Four patients (10.52%) were smokers (more than 10 cigarettes per day).
DNA was isolated from EDTA stabilized peripheral blood samples, which were screened for the MPL mutations by 4 allele-specific PCR reactions and subsequent agarose gel electrophoresis. The method has 1% to 5% sensitivity in terms of allele frequency.

Results: From the acquired MPL mutations, we could detect MPL W515L in 16 patients (42.10% of JAK2 V617F-negative ET patients and 16.66 % of all revised ET patients).
MPL W515K could be detected in 2 cases (5.26 % of JAK2 V617F-negative ET patients and 2.08 % of all revised ET patients), whereas MPL W515R was detected in 1 case (2.56 % of JAK2 V617F-negative ET patients and 1.04 % of all revised ET patients). We could not detect the MPL S505N and S505A mutation.

Next we investigated whether the MPL mutation status has any influence on the appearance of thrombotic events. Acute myocardial infarction was reported in 9 cases (23.68% of JAK2 V617F-negative ET patients). Stroke and transient ischemic attack event could be observed in 7 cases (18.42% of JAK2 V617F-negative ET patients), deep vein thrombosis was reported in 3 cases (7.89% of JAK2 V617F-negative ET patients), whereas pulmonary embolism was reported in 2 cases (5.26% of JAK2 V617F-negative ET patients). The presence of MPL mutations did not correlate with the clinical appearance of the disease, and its prognostic value could not be detected in our group of patients. Acute myocardial infarction, transient ischemic attack, stroke, deep vein thrombosis and pulmonary embolism were present in the same rate in the other MPL negative patients. Mean cell counts (white blood cells, platelets, red blood cells, and hemoglobin) measured at the time of establishing the diagnosis were not highly different in the two studied groups.

Conclusion: In the relevant literature, MPL W515L is also the predominant MPL mutation [3]. However, in our study group, the prevalence of it was much higher than in studies with a larger population, in which MPL W515L or MPL W515K mutations are reported in a lower frequency, approximately 1–2% [4]. However, we could find few summaries which have indicated that the prevalence of MPL W515L/K mutation in ET is underestimated, and it is higher than it has originally been described [5]. In those cases when JAK2 V617F mutation was proven to be negative, the assessment of MPL W515L mutation may help in establishing the diagnosis.

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