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REVIEW

THE ROLE OF OXIDATIVE STRESS IN ESSENTIAL THROMBOCYTHEMIA

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Abstract

In patients with myeloproliferative neoplasms (MPNs), including essential thrombocythemia, current studies have demonstrated a JAK2V617F-dependent reactive oxygen species (ROS) elevation partially mediated by a decrease in catalase expression associated with DNA damage. Oxidative stress plays a major role in carcinogenesis as well as in genomic instability, disease progression, myelofibrotic and leukemic transformation, and possibly in the development of vascular events in patients with essential thrombocythemia. Further comprehensive studies are needed to establish the role of oxidative stress in the pathogenesis of essential thrombocythemia, disease progression, vascular complications and whether targeting ROS as a therapeutic option could prevent disease progression and the development of vascular events MPNs patients.

Key words: essential thrombocythemia, oxidative stress, ROS, disease progression, vascular events.

Résumé

Le rôle du stress oxydatif dans la thrombocytémie essentielle

Chez les patients avec néoplasies myéloprolifératives, inclusivement la thrombocytémie essentielle, les recherches scientifiques actuelles ont trouvé une augmentation JAK2V617F-dépendante de la quantité d'espèces réactives de l'oxygène partiellement expliquée par la réduction de l'expression de la catalase associée aux lésions de l'ADN. Le stress oxydatif joue un rôle central dans la carcinogenèse, tels que dans l'instabilité génétique, la progression de la maladie, la transformation en myélofibrose et leucémie, et possiblement dans le développement d'***événe***ments vasculaires chez les patients avec thrombocytémie essentielle. Des recherches d'ensemble sont nécessaires dans le futur pour définir le rôle du stress oxydatif dans la pathogénie de la thrombocytémie essentielle, dans la progression de la maladie, dans les événements vasculaires et pour voir si la lutte contre les espèces réactives de l'oxygène comme une option thérapeutique peut prévenir la progression de la maladie et le développement d'événements vasculaires chez les patients aux ***néoplasies myéloprolifératives.***

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Mots-clés: thrombocytémie essentielle, stress oxydatif, stress oxydant, espèces réactives de l'oxygène, progression de la maladie, *événements vasculaires*.

Essential thrombocythemia – state of the art

According to the World Health Organization (WHO) classification system for hematopoietic tumors (revised in 2016), essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) are classified as BCR-ABL-1–negative myeloproliferative neoplasms (MPNs). These disorders are characterized by a stem cell-derived clonal myeloproliferation with mutually exclusive „driver" mutations (JAK2, CALR, and MPL) and a high risk of thrombotic and hemorrhagic complications or leukemic transformation[1,2]. Essential thrombocythemia is characterized by global myeloid proliferation, especially bone marrow megakaryocyte expansion with large megakaryocytes of mature morphology and hyper-lobulated nuclei, persistent trombocytosis >450x10^6/L independent of the thrombopoietin level, leukocytosis, splenomegaly, thrombosis or haemorrhage and a possible evolution to secondary myelofibrosis or acute leukemia[3].

The genetic basis of ET: JAK2, CALR and MPL gene mutations

Characteristic for the chronic myeloproliferative neoplasms is the pathological activation of the JAK-STAT (Janus-Associated Kinase – Signal Transducer and Activator of Transcription) signaling pathway. Approximately 55% of the patients with essential thrombocythemia harbor a JAK2 (Janus-Associated Kinase 2; 9p24) mutation induced by the substitution at codon 617 of valine with phenylalanine[4,5,6]. JAK2 is a tyrosine kinase with an essential role in the cytokine-receptor signaling pathway in physiological myelopoiesis, transmitting signals to erythropoietin (EPO) receptors, to thrombopoietin (TPO) receptors controlled by the MPL gene, and to granulocyte-colony stimulating factor (G-CSF) receptors[7,8].

The JAK2 gene has three structural domains: JH1 – the active kinase domain (functional domain) –, JH2 – the inactive pseudokinase domain (a negative regulator of the JH1 domain) –, and the FERM domain. The point mutation of the JAK2 gene at the level of the JH2 pseudokinase blocks the autoinhibition of the JH1 kinase and leads to the phosphorylation of JAK2 kinase and to the activation of the signaling pathways involved in the proliferation, differentiation and cell survival independently of haematopoietic growth factors[8].

In essential thrombocythemia, the presence of the JAK2V617F mutation has been associated with: old age at diagnosis, higher levels of hemoglobin, leukocytosis, lower platelet counts, increased risk of arterial thrombosis and a lower risk for fibrotic transformation[1,9]. In JAK2V617F-negative patients, other mutations in genes related to the activation of the JAK2/STAT signaling can be found: JAK2 exon 12 mutations, mutations in the calreticulin gene and mutations in the MPL gene. In essential thrombocythemia, mutations of the calreticulin gene (CALR: 19p13.2) can be discovered in 15-24% of cases[10-14]. The CALR gene encodes a multi-functional Ca^{2+} binding protein chaperone, which specifically activates the JAK-STAT5 signaling pathway via the TPO receptor[15]. In patients with ET, CALR gene mutations have been associated with male gender, young age at diagnosis, lower hemoglobin levels and leukocyte counts, higher platelet counts, and a lower risk for thrombosis[1,16]. In 4% of ET patients, mutations in the exon 10 of the MPL (myeloproliferative leukemia virus oncogene; 1p34) gene are associated with old age at diagnosis, female sex, lower hemoglobin levels, and higher platelet counts[17]. ET patients are triple-negative for JAK2, CALR or MPL mutations in 17% of cases[1].

The WHO criteria for the diagnosis of essential thrombocythemia (2016 revised):

According to the WHO criteria for the diagnosis of ET, ET diagnosis requires all four major criteria or first three major criteria and the minor criterion[1,2].

The major criteria are:

1. Platelet count > 450 x 10^6/L.
2. Bone marrow megakaryocyte proliferaton with large megakaryocytes with mature morphology and hyperlobulated nuclei.
3. Exclusion of other myeloid neoplasms (PV, PMF or chronic myeloid leukemia).
4. Presence of JAK2, CALR or MPL mutations.

The minor criterion is represented by:

1. The presence of a clonal marker or absent evidence for reactive thrombocytosis.

Survival and complications

The median survival for ET is approximately 20 years and it seems that it is not influenced by the presence of driver mutations. Overall survival is influenced by advanced age, leukocytosis, thrombosis and the presence of adverse mutations, including SH2B3, SF3B1, U2AF1, TP53, IDH2, and EZH2[1]. Carobbio A et al (2011), in a multivariable analysis on 891 patients with ET, after a median follow-up of 6.2 years, revealed as predictors for arterial thrombosis the age over 60 years, history of thrombosis, smoking, arterial hypertension, diabetes mellitus, leukocytosis over $11x10^9$/L, and the presence of JAK2/MPL mutations. Patients with a platelet count over 1000 x 10^9/L and mutant CALR had a lower risk of arterial thrombosis. Male gender was the only predictor for venous thrombosis. Extreme thrombocytosis might be associated with acquired von Willebrand's syndrome and poses a risk of aspirin associated haemorrhage[18]. The presence of JAK2V617F has been associated with a lower risk of fibrotic progression, whereas MPL mutation has been associated with a higher risk of fibrotic progression. The risk of leukemic transformation was increased by the presence of JAK2V617F[19,20]. Risk stratification in essential thrombocythemia has been associated with age, history of thrombosis and the presence of JAK2/MPL mutations, and includes four categories: very low risk (age below 60 years, no history of thrombosis, JAK2/MPL-unmutated), low risk (age below 60 years, no history of thrombosis, JAK2/MPL mutated), intermediate risk (age over 60 years, no history of thrombosis, JAK2/MPL-unmutated), and high risk (history of thrombosis or age over 60 years with JAK2/MPL mutation)[19,21].

Risk-adapted therapy

The aim of therapy in ET is to prevent thrombosis, bleeding, fibrotic progression and leukemic transformation. Very low risk ET patients do not require any form of therapy, low risk patients require aspirin at least once daily or, in the presence of aspirin-resistant symptoms, a low dose of aspirin twice-daily or, as an alternative, anti-platelet agents such as clopidogrel (75 mg/day) alone or in combination with aspirin; aspirin twice-daily is recommended also in JAK2-mutated patients with cardiovascular risk factors. Intermediate risk patients and high risk patients require cytoreductive therapy (hydroxyurea, interferon-α), and in the presence of marked splenomegaly, therapy with JAK2 inhibitors is recommended[1].

Oxidative stress

Oxidative stress, defined as an imbalance between an overproduction of reactive oxygen species (ROS) and a deficiency of the antioxidant status of the organism, is involved in many pathophysiological processes, such as atherogenesis, alteration of the immune system, diabetes mellitus, aging, cognitive impairment, chronic inflammation or carcinogenesis[22,23,24]. Reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl radicals) are products of aerobic metabolism which in low concentrations act as signaling molecules that maintain physiological functions such as: enzyme activity, protein kinase activity, activation of transcription factors, immune defense or apoptosis. An overproduction of intracellular ROS damages DNA, lipids and proteins and is involved in disease development[24,25].

Cancer cells generate an overproduction of ROS from mitochondria, endoplasmic reticulum, and NADPH oxidases and activate transcription factors such as NF-κB, PI3K, HIFs and MAPKs. These factors are responsible for the activation of signaling pathways involved in proliferation, survival, metabolic adaptation, genomic instability, and promote tumorigenesis[26,27]. On the other hand, cancer cells increase their antioxidant proteins by activating transcription factors such as NRF2, tumor suppressor genes p53, FOXOs and SIRT3, as to prevent oxidative stress – induced cell death[28-32]. This mechanism has not yet been proven in MPNs, but it may be taken into account, especially since the overproduction of ROS in MPNs gives rise to a proliferative advantage to JAK2-positive clones[33,34].

The role of oxidative stress in essential thrombocythemia. Disscusion and perspectives

Several studies reveal an increased level of ROS in patients with MPNs through the activation of the pro-inflammatory pathways NF-kB and NF-E2. Bjorn ME and Hasselbalch HC (2015) sustained the major role of ROS in carcinogenesis and disease progression in MPNs, in which the malignant clone itself produces an excess of ROS, thereby creating a vicious self-perpetuating circle in which ROS activate proinflammatory pathways (NF-kB) which in turn generate more ROS. They also suggested that a potential cure of MPNs could be obtained by targeting the disease also by using agents with antioxidative, anti-inflammatory, antiangiogenic, antiproliferative and proapoptotic properties, such as N-acetilcysteine, statins or interferon-alpha2, in combination with JAK1/2 inhibitors[35].

In MPNs, pro-inflammatory cytokines and excessive ROS partake in a vicious self-perpetuating circle which may also be driven by NF-E2[35-38]. The role of chronic inflammation and ROS in MPN pathogenesis has been highlighted in a mouse model by inhalatory exposure to formaldehyde, a potent inflammatory compound. Formaldehyde induced an increase in ROS levels, inflammation and toxicity of bone marrow with typical MPN-like alterations, increased NF-kB activity at both mRNA and protein levels, and significantly increased the level of inflammatory markers, TNFalpha and IL-1beta[39]. These findings endorse that chronic inflammation, via induction of oxidative stress, and an inflammatory bone marrow microenvironment induce DNA damage and impair stem cell function. On the other hand, in MPNs, the hematopoietic stem cell niche has been associated with decreased catalase and superoxide dismutase activities. Studies have shown that the antioxidant defence systems of catalase, superoxide dismutase and glutathione-peroxidase are negatively influenced by ROS levels. Increased levels of ROS and the presence of JAK2V617F mutation influence the PI3K/AKT signaling pathway which in turn affects FoxO, a regulator of the transcription of the aforementioned antioxidant defence pathways[40,41]. The result is an increase in oxidative stress, oxidative DNA damage, genomic instability and disease progression to bone marrow fibrosis or leukemic transformation. In MPNs, the response to DNA damage is also affected by the negatively regulated p53 pathway and by the presence of CHEK2 germline mutations[42-44].

Recent studies reveal that the overproduction of ROS gives rise to a growth advantage to JAK2-positive clones. Marty C et al (2013) investigated the influence of JAK2V617F on the hematopoietic stem cell compartment in a knock-in JAK2V617F murine model and in MPNs patients and discovered that the presence of the JAK2V617F mutation is responsible for a ROS accumulation in the hematopoietic stem cell compartment, and also for an increased level of 8-oxo-guanines and DNA double-strand breaks. The increase in ROS is explained by a reduced expression of catalase, an antioxidant enzyme, induced via the PI3K/AKT signaling pathway. Therapy with the antioxidant N-acetylcysteine restored blood parameters, reduced DNA damage, splenomegaly and the frequency of the JAK2V617F-positive hematopoietic progenitors in bone marrow and spleen. Overproduction of ROS is a mediator of JAK2V617F-induced DNA damage that promotes genomic instability, myelofibrotic and leukemic transformation. It is yet uncertain if the antioxidant treatment with N-acetylcysteine could prevent the development of JAK2V617F-positive MPNs[41].

Kagoya Y et al (2014) transplanted JAK2V617F-transduced bone marrow cells in mice and demonstrated that JAK2V617F-harboring cells cause paracrine DNA damage accumulation through secretion of lipocalin-2. Thus, the JAK2V617F-harboring cells acquire a proliferative advantage and both JAK2V617F-positive and JAK2V617F-negative clones increase their risk for leukemic transformation. Normal hematopoietic cells showed raised ROS levels through increased intracellular iron levels when treated with lipocalin-2, which led to the activation of the p53 pathway, an increase in apoptosis, and a decrease in cellular proliferation. In contrast, JAK2V617F-positive clones were granted a relative growth advantage because lipocalin-2 did not induce growth suppression in these cells[45].

Iurlo A et al (2015) compared 30 treatment-naïve ET patients with 26 age-matched and gender-matched controls by measuring serum ROS levels, urinary 8-hydroxydeoxyguanosine, full blood GSH levels, and the reduced/oxidized GSH ratio, revealing higher GSH levels in ET patients than in controls as a possible compensatory mechanism for the ROS excess[46].

Durmus A et al (2013) compared 43 ET patients and 20 healthy volunteers by measuring serum levels of oxidative status parameters (total oxidative status, total antioxidant status, oxidative stress index and malondialdehyde) at the time of diagnosis and six months after, at follow-up. Their results highlighted that in ET patients oxidative stress parameters were significantly increased, while the antioxidant capacity was significantly decreased compared to healthy individuals. The authors also measured oxidative status parameters of ET patients with and without a history of vascular events. Oxidative stress may also play a role in the development of vascular events since the total oxidative status was significantly higher in patients with a previous vascular event compared to patients with no prior events. The researchers also reported a decrease in oxidative stress parameters after therapy[47].

In another study, Durmus A et colab (2014) compared oxidative stress parameters (total oxidative status, total antioxidant status, oxidative stress index and malondialdehyde) of 35 patients with polycythemia vera and 20 healthy volunteers at the time of diagnosis and after 6 months of treatment (phlebotomy, 100 mg of acetylsalicylic acid daily ± hydroxyurea). They concluded that oxidative stress parameters were significantly higher in polycythemia vera patients than in the controls, yet no difference was seen regarding the antioxidant capacity. The study also evaluated the influence of vascular events on the oxidative stress markers in patients with polycythemia vera. PV patients that had had a vascular

event registered significantly higher values of oxidative stress levels than those without any history of vascular events, suggesting that there may be a link between oxidative stress and vascular events in PV patients[48]. Leukocytosis and the release of ROS from activated leukocytes and intracellular proteases predisposed to endothelial dysfunction and favoured the appearance of thrombosis[1].

The role of oxidative stress in chronic inflammation and carcinogenesis is well established. Current studies sustain that the elevation in ROS levels is JAK2V617F-dependent and associated with DNA damage in patients with MPNs[49]. ROS are key elements in genomic instability, as well as in disease progression, myelofibrotic and leukemic transformation. It has also been suggested that increased oxidative stress levels may be related to vascular events in patients with polycythemia vera and essential thrombocythemia, in addition to the role played by the JAK2V617F mutation. Further comprehensive studies are needed to establish whether oxidative stress parameters could be used as predictors for the recognition and early treatment of vascular events in MPNs patients. Also, research should be carried out to discover whether targeting ROS may be a therapeutic option that could prevent disease progression in MPNs.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law."

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