A LOOK AT PLATELET COUNT IN CHRONIC HEPATITIS C INFECTION

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Disclosure: The author has declared no conflicts of interest.
Received: 15.02.17 Accepted: 19.04.17
Citation: EMJ Hepatol. 2017;5[1]:97-103.

ABSTRACT

A complete blood count performed in chronic hepatitis C virus (HCV) infected patients can identify thrombocytopenia or an increased number of platelets, the cause of which must be established. Most of these patients are predisposed to develop thrombocytopenia as the disease progresses due to a lower thrombopoietin production, increased platelet pooling in the spleen, viral bone marrow suppression, or interferon-based therapy. However, a severe thrombocytopenia can have an autoimmune aetiology, which is very probable at values <15x10^3/mm^3. Thrombopoietin analogues are useful both in patients with primary immune thrombocytopenia and in those who will begin the treatment with pegylated interferon and ribavirin before surgery. The common causes of an increased number of platelets in chronic HCV infected patients are splenectomy, ribavirin treatment, liver transplantation, and hepatocellular carcinoma. However, thrombocytosis can also be hereditary, reactive, or malignant, especially in essential thrombocythaemia or other myeloproliferative diseases that can be associated. A hepatic blood flow obstruction present in chronic HCV infected patients must draw attention to a possible associated myeloproliferative disorder (which frequently manifests in thrombocytosis) that represents its aetiology in two-thirds of cases and which can evolve with a constant or an intermittent increase in platelet count. The role of the JAK-STAT signalling mechanism is presented in both chronic hepatitis C patients and in those with essential thrombocythaemia. It was suggested that STAT3 could have a role in HCV RNA replication. In addition, the HCV core protein is involved in the modulation of fibrogenetic gene expression in hepatic stellate cells through a JAK2-STAT3 dependent pathway. Ruxolitinib (a JAK1/JAK2 inhibitor) can have beneficial effects in essential thrombocythaemia and is a subject of research in chronic hepatitis C. The discovery of the aetiology of thrombocytopenia or an increased number of platelets can contribute to a more complete diagnosis and appropriate treatment. The identification of associated disorders in chronic HCV infected patients is of vital importance for them.

Keywords: Essential thrombocythaemia, hepatitis C virus (HCV), thrombocytopenia, thrombocytosis.

INTRODUCTION

Many complications can occur during the evolution of chronic hepatitis C virus (HCV) infection. Some of them can be related to thrombocytopenia or thrombocytosis. In addition, some patients may have associated diseases that can evolve with thrombocytopenia or thrombocytosis. The identification of thrombocytopenia or thrombocytosis aetiology and of associated disorders in chronic HCV infected patients can be vital for them. A literature review was carried out in OvidMD Advantage, Ovid MEDLINE® Daily, Ovid MEDLINE, and PubMed using hepatitis C, thrombocytopenia, thrombocytosis, and essential thrombocythaemia as search terms.

THROMBOCYTOPENIA IN CHRONIC HEPATITIS C INFECTION

Thrombopoietin (TPO) is a growth factor produced in the liver that binds to the c-Mpl receptor present on megakaryocytes and platelets. The activation of the JAK-STAT mechanism occurs after TPO binds to its receptor and results in platelets production stimulation. The platelet count rise
occurs after a latency period of 5 days and reaches a peak after 10–12 days. A decreased platelet count leads to an increase of free TPO levels, which induces a higher platelet production by bone marrow megakaryocytes.

Chronic liver disease patients (including those with HCV aetiology) are predisposed to develop thrombocytopenia as the disease progresses. Its aetiology is complex. The most common causes are the lower TPO production and the higher platelet destruction due to hypersplenism. The thrombocytopenia associated with hypersplenism is caused by increased platelet pooling in the spleen. So-called hypersplenism has little clinical effect. In addition, there is no proof showing that correcting the hypersplenism has consequences on patient survival.

HCV itself could also produce bone marrow suppression. In addition, it was found that HCV viraemia was independently associated with lower platelet count after adjustment for liver fibrosis. There are also reports on the improvement of thrombocytopenia after interferon (IFN)-based therapy obtaining sustained viral response. The maximum increase in platelet count was observed after 6 months of antiviral treatment. The patients without virological response to IFN presented with decreased platelet counts.

Treatment-related thrombocytopenia is another cause. Conventional antiviral therapy containing pegylated interferon (PEG-IFN) frequently reduces platelet count in HCV patients. The pooled incidence of clinically significant thrombocytopenia is around 8.8–10.0%. It was observed that a baseline platelet count <100x10^3/mm^3 and a rapid early platelet diminution (>30% decrease in the first 2 weeks) are significantly associated with severe thrombocytopenia, defined as <50x10^3/mm^3, requiring reduction of PEG-IFN dose. A large multicentre study analysed the bleeding risk due to thrombocytopenia in HCV-infected patients with biopsy-proven advanced liver fibrosis (Ishak score 4–6) during IFN-based therapy. Sixteen percent of patients with a platelet count between 75x10^3/mm^3 and 149x10^3/mm^3, and 30% of them with a platelet count <75x10^3/mm^3, required IFN dose reductions, and 3% and 16% of them, respectively, discontinued the IFN due to severe thrombocytopenia. On-treatment bleedings were generally mild despite the advanced fibrosis. Fortunately, current antiviral regimens (IFN-free) comprising direct-acting antiviral agents not only produce excellent sustained virological response rates (>90%) but also carry a very low risk for the development of thrombocytopenia (≤1%, according to prescribing information sheets of daclatasvir/aso-naprevir, sofosbuvir, the association between dasabuvir, ombitasvir, paritaprevir, and ritonavir, and the combination of ledipasvir and sofosbuvir). In other words, the barrier of PEG-IFN-based antiviral therapy in thrombocytopenic HCV patients has been overcome.

But, if thrombocytopenia is severe (<15x10^3/mm^3) in patients with HCV-related liver cirrhosis then an autoimmune aetiology is very probable. A high titre of platelet-associated immunoglobulin G was found in 40 out of 41 such cirrhotic patients and was significantly lower in splenectomised patients compared to those with an intact spleen, as a result of a lower CD4/CD8 ratio in the first group, followed by diminished autoantibody production. Immune thrombocytopenic purpura and thrombocytopenia present in chronic hepatitis C have a common treatment: the c-Mpl receptor agonist, such as eltrombopag, which is a small oral molecule that acts as a thrombopoietic agent able to increase platelet count in thrombocytopenic patients with chronic hepatitis C. Eltrombopag produced a dose-dependent increase of platelet count in HCV-related cirrhosis patients. The antiviral treatment could be initiated in 45 out of 56 of them and some patients completed their 12 weeks of antiviral therapy under concomitant eltrombopag treatment in the study of McHutchison et al. ENABLE-1 and ENABLE-2 are two Phase III randomised, controlled studies that included 1,520 thrombocytopenic patients with HCV and advanced fibrosis and cirrhosis. They were treated with eltrombopag in order to reach a predefined minimal threshold for the initiation of antiviral treatment with PEG-IFN-α and ribavirin. More patients treated with eltrombopag maintained >50x10^3 platelets/mm^3 during the anti-HCV-treatment, could receive higher PEG-IFN-α doses, and reached significantly higher rates of sustained virological response, compared to placebo. Liver decompensation and thrombotic events were more frequently present in the eltrombopag group of ENABLE-2.

Romiplostim, a second-generation c-Mpl receptor agonist, contains four TPO agonist peptides inserted in an immunoglobulin (IgG) heavy chain and is effective in the treatment of primary immune thrombocytopenia. If eltrombopag is the agent widely studied in patients with HCV infection,
only a few reports and one observational study up to the present described the use of romiplostim before PEG-IFN/ribavirin treatment or before surgery in these patients. Romiplostim was given to a splenectomised patient with immune thrombocytopenic purpura before PEG-IFN/ribavirin treatment; the antiviral treatment started at a value of 65x10\(^3\) platelets/mm\(^3\) and led to an early virological response followed by a sustained virological response; in this case, romiplostim was effective and safe.\(^{19}\) Romiplostim also allowed the treatment of hepatitis C in a patient coinfected with HIV.\(^{20}\) A severe thrombocytopenia produced during the antiviral treatment of two HCV-related cirrhosis patients was successfully treated with romiplostim (with a platelet count >50x10\(^3\)/mm\(^3\)), which allowed continuation and completion of the IFN protocol without dose reduction; both patients obtained a sustained virological response.\(^{21}\) A group of 35 thrombocytopenic patients with HCV-related liver cirrhosis received romiplostim at a dose of 2 μg/kg/week for a maximum of 1 month in order to increase the platelet count. Of this cohort, 33 achieved a number of ≥70x10\(^3\)/mm\(^3\) and became eligible for surgery. The maximum peak of platelet count was between 73x10\(^3\)/mm\(^3\) and 24x10\(^4\)/mm\(^3\). They had no postoperative bleeding or thrombotic events.\(^{22}\) TPO also affects the liver. Researchers have previously investigated whether, as well as stimulating liver regeneration, TPO also stimulates hepatocellular carcinoma cell proliferation. Until now, the answer to this question has been no, in both in vitro and in vivo studies.\(^{23}\)

It is useful to look at platelet count in chronically HCV-infected patients who developed hepatocellular carcinoma. Chronic HCV infection is an important aetiological factor for this type of cancer. It was shown that patients with a pretreatment platelet count <118x10\(^3\)/mm\(^3\) have a low risk for extrahepatic metastasis after treatment, while a platelet number >212x10\(^3\)/mm\(^3\) was associated with a higher risk for this type of metastasis.\(^{24}\) This observation may help to improve the therapeutic strategy in patients at high metastatic risk. It is known that tumours can contribute to an increase of platelet production and activation; activated platelets can contribute to tumour growth and metastasis.\(^{25}\) But it seems that HCV-related cirrhotic patients have no activated platelets (assessed by flow cytometry) during hepatocellular carcinoma development or recurrence; they have also an increased level of von Willebrand factor and of ADAMTS13 activity.\(^{26}\)

**THE INCREASE OF THE PLATELET COUNT IN CHRONIC HEPATITIS C**

Common causes of the increase of the platelet count in chronically HCV-infected patients are splenectomy, ribavirin treatment, and liver transplantation (LT). But clinically evident thrombocytosis, usually defined as >45x10\(^3\)/mm\(^3\), is rare in HCV patients receiving splenectomy or ribavirin monotherapy.

A platelet count augmentation can be observed after splenectomy in HCV-chronic infected patients and this increase persists for a long time.\(^{27}\) An increase of platelet count can be found in patients with chronic hepatitis C treated with ribavirin, which induces haemolytic anaemia followed by a rise in serum erythropoietin. A higher endogenous erythropoietin stimulates not only the erythrocytes production but also that of platelets. Such an augmentation was shown after 4 weeks of ribavirin monotherapy (from 14.0x10\(^4\) to 15.8x10\(^4\)/mm\(^3\)) while TPO did not increase.\(^{28}\) IFN-related thrombocytopenia diminished in patients treated not only with IFN, but also with ribavirin, due to its thrombocytotic response.\(^{29}\) It was shown that rs1127354 and rs7270101 (two functional variants in the ITPA gene) produce ITPase deficiency and defend against ribavirin-induced haemolytic anaemia. However, a platelet count reduction appeared in these patients.\(^{29}\) A reactive thrombocytosis (platelet count >45.0x10\(^3\)/mm\(^3\) for at least 7 days), which begins within 8 weeks after LT, was observed especially when LT was made after a seronegative fulminant hepatic failure and was negatively associated with HCV-related liver cirrhosis. This thrombocytosis had a median duration of 25 days and did not raise the hepatic artery thrombotic risk.\(^{30}\)

Thrombocytosis may also occur in hepatocellular carcinoma patients. Fifty-two of 634 biopsy-proven hepatocellular carcinoma patients had a platelet count >40.0x10\(^3\)/mm\(^3\). The patients with thrombocytosis were younger and had a larger tumour size, less cirrhosis, higher serum level of alpha-fetoprotein, and an increased risk of main portal vein thrombosis. They were also less able to receive therapy than those without thrombocytosis and had shorter survival.\(^{32}\) In addition, they had a significantly higher mean serum TPO level than those without thrombocytosis. Thrombocytosis is considered to be a paraneoplastic syndrome in these patients and is due to the overproduction of TPO by hepatocellular carcinoma cells.\(^{32}\)
Thus, although TPO may not have direct effects on cancer cell proliferation, platelets most certainly do, and TPO agonists may therefore, at least in theory, have adverse effects on HCV-infected patients who develop hepatocellular carcinoma.

Unfortunately, no associated diseases pathology has been written at present; such an attempt would be difficult. But it is useful to point out a possible association of two chronic diseases: chronic hepatitis C and essential thrombocytopenia, as they have a common pathway and a possible common treatment. It is estimated that the incidence of essential thrombocytopenia in the European Union (EU) is between 0.38 and 1.7 per 100,000 people per year. When hepatitis C coexists with essential thrombocytopenia, plateletpheresis is indicated if the patient has thrombocytosis (e.g. 1.3 million/mm$^3$) and should be subjected to a surgery procedure (e.g. a cardiopulmonary bypass as a treatment modality for an aortic insufficiency). The common pathway present in these two diseases is represented by the signalling mechanism JAK-STAT. About 53% of patients with essential thrombocytopenia present with the mutation JAK2 V617F (that was discovered in 2005), which is responsible for JAK2 enzyme activation and is involved in the control of several vital cell functions, such as survival, differentiation, and proliferation. It is not entirely clear at present how mutations in the pseudokinase domain (JAK homology 2 domain or JH2 domain) can increase the JAK2 activation but some progress has been made. A rigidification of alphaC-helix contributes to a hyperactivation of the JH1 domain in patients with a JAK2 mutation. The heterozygous JAK2 V617F mutation stimulates megakaryopoesis and patients often have essential thrombocytopenia, while a homozygous JAK2 V617F mutation increases erythropoiesis and decreases megakaryopoesis, often leading to polycythaemia vera. But the essential thrombocytopenia patients can also have other mutations. About 3% have a gain-of-function mutations in the gene that encodes the Mpl receptor (discovered in 2006): another pathway to activate JAK2. Other patients (~32%) have mutations in exon 9 of the calreticulin gene (discovered in 2013) that can also hyperactivate the JAK2-STAT pathway, or are triple negative (~12% of them). The occurrence of disease-initiating mutations in haematopoietic stem cells could be the consequence of genomic instability present in these patients. Each of the three mutations activates the JAK2-STAT signalling mechanism. An important remark must be made: serum TPO levels are normal or slightly elevated in essential thrombocytopenia patients as the c-Mpl receptor is poorly expressed and the uptake and catabolism of TPO is defective; an inverse correlation was found between serum TPO levels and platelet mass.

What pathophysiological implications does the JAK-STAT pathway have in chronic HCV infected patients? STAT3 is activated by non-structural proteins present in HCV structure through oxidative stress mediation; activated JAK2 also influences this process. It was suggested that STAT3 could have a role in HCV RNA replication. HCV core is involved in increasing expression of IFN-γ receptor 2, which can explain the up-regulated JAK-STAT pathway produced by HCV core. In contrast, JAK1/2 and STAT3 activation and STAT3-mediated transcription were impeded by HCV core in the presence of interleukin (IL)-6 stimulation. Blocking the IFN mechanism of action through the inhibition of STAT1 phosphorylation by JAK1 favours a possible hepatitis E virus infection but not with HCV. In addition, HCV core protein is involved in the modulation of fibrogenetic gene expression in hepatic stellate cells through a JAK2-STAT3 dependent pathway.

Apart from IFN-α, a medication used for essential thrombocytopenia treatment is useful also in chronically HCV-infected patients: ruxolitinib, approved by the US Food and Drug Administration (FDA) for the treatment of intermediate or high-risk myelofibrosis. A JAK2 V617F allele burden decrease with >50% was obtained in 23.5% of the 22 essential thrombocytopenia patients treated with ruxolitinib, an oral JAK1 and JAK2 inhibitor, but without complete molecular remission. JAK2 inhibitors proved to be useful for the treatment of patients with myeloproliferative neoplasms. The chronic JAK inhibitor treatment leads sometimes to cell persistence by transphosphorylation of JAK2 through other JAK kinase family members.
Tofacitinib (a pan-JAK inhibitor) could be a solution for the patients who are resistant to ruxolitinib (a selective inhibitor of JAK1/2). But it should be noted that no JAK inhibitor to date has proved beneficial in treating HCV infection. This is only one direction for future research, such as that published by Ma et al.

Chromically HCV-infected patients may have other causes of the increase of the platelet count. A thrombocytosis found in them is rarely hereditary (as a result of mutations of the TPO or MPL genes, or of the JAK2 gene apart from V617F and that of the gelsolin gene) and, more often, can be present in a disease or situation that evolves with reactive thrombocytosis (various infectious or inflammatory diseases, blood loss, iron deficiency anaemia, or just iron deficiency), in prefibrotic myelofibrosis, chronic myeloid leukaemia, BCR positive thrombocytosis, or some types of myelodysplastic syndromes, such as the 5q deletion (5q-syndrome). The MPL Baltimore (Lys39Asn) mutation that manifests with thrombocytosis has to be mentioned, as it can be found in about 7% of African Americans.

An extreme thrombocytosis found in chronic HCV-infected patients (that is often the expression of essential thrombocythaemia or associated with other myeloproliferative diseases) may be clinically suspected not only in patients with various located thromboses (that occurs at a platelet count between 40x10^4 and 10x10^5 platelets/mm³) or bleeding (possible at >10x10^5 platelets/mm³, when acquired von Willebrand disease can occur) but also in those with erythromelalgia, which is the expression of chronic microvascular arterial occlusive disease. There is increasing evidence on the role of thrombotic risk factor for JAK2 V617F mutation. The thrombotic risk is much higher in patients with myeloproliferative neoplasm (including those with essential thrombocythaemia) who also have some inherited thrombophilic single nucleotide polymorphisms.

A hepatic blood flow obstruction present in chronically HCV-infected patients must draw attention to a possible associated myeloproliferative disorder (which frequently manifests as thrombocytosis), that represent its aetiology in two-thirds of cases and which can evolve with a constant or an intermittent increase in platelet count. A Budd-Chiari syndrome or a portal cavernoma (secondary to a single or repeated portal vein thrombosis) can also be a consequence of a myeloproliferative disorder, which can occur with thrombocytosis. The JAK2 46/1 haplotype enrichment is associated with myeloproliferative neoplasm occurrence and with a high risk of splanchnic vein thrombosis in them. The risk of complications is much lower in reactive thrombocytosis, excepting the cases with arterial disease or prolonged immobilisation. A differential diagnosis between reactive thrombocytosis and essential thrombocythaemia can be made using lag time (a parameter useful for thrombin generation studying) and procoagulant phospholipids ratio; high values for these parameters were associated with a high negative predictive value for an essential thrombocythaemia diagnosis.

An important issue present sometimes in patients with high platelet count (including in those splenectomised) is pseudohyperkalaemia. A plasmatic ionogram (not only a serum one) is indicated in such situations in order to make a differential diagnosis between it and a real hyperkalaemia; plasmatic potassium level is normal in these patients. The platelet indices can also be useful. Chronic hepatitis C patients with high liver fibrosis evaluated by transient elastography have higher values of mean platelet volume, platelet distribution width, and platelet large cell ratio compared to those with less expressed liver fibrosis.

**CONCLUSIONS**

The most common causes of thrombocytopenia are the lower TPO production and the higher platelet destruction due to increased platelet pooling in the spleen. If thrombocytopenia is severe (>15x10^9/mm³) in patients with HCV-related liver cirrhosis, an autoimmune aetiology is very probable. TPO analogues are useful both in patients with primary immune thrombocytopenia and those who will begin the treatment with PEG-IFN and ribavirin or before surgery. An increase of platelet count found in chronic hepatitis C patients can be due not only to splenectomy, ribavirin treatment, and LT, but also to an associated disease; it can rarely be hereditary. An associated myeloproliferative disorder (which frequently evolves with thrombocytosis) can produce hepatic blood flow obstruction. The JAK-STAT signalling mechanism is presented both in patients with essential thrombocythaemia and in those with chronic HCV infection. Ruxolitinib (a JAK1/JAK2 inhibitor) has beneficial effects in the first disorder and it is a subject of research for the last.
REFERENCES


