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REVIEW

IMMUNE THROMBOCYTOPENIC PURPURA: NEW PERSPECTIVES FOR DIAGNOSIS AND TREATMENT

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Summary

Immune thrombocytopenic purpura (I.T.P) is a bleeding disorder that associates purpura and thrombocytopenia. Purpura is the form of manifestation of this pathology, caused by the extravasation of red blood cells in the dermis due to reduction in peripheral platelets. Trombocytopenia involves the decrease of blood platelets under 100,000 elements/mm^3 blood. This is due to either a premature hyper-destruction of peripheral blood or to a bone marrow deficient synthesis. ITP involves anti-platelet antibodies whose action induces morphological and functional changes at the platelet level. ITP has two forms: primary, idiopathic or secondary, emerging in the context of associated pathologies. Differential diagnosis is one of exclusion. It involves exclusion of autoimmune diseases, viral or bacterial infections, lymphoproliferative syndromes likely to induce secondary thrombocytopenia. The accurate establishment of the factors involved in chronic immune purpura etiopathogeny leads to a correct diagnosis and to the establishment of the optimal therapeutic regimen.
Key words: immune thrombocytopenia purpura, anti-platelet antibodies, differential diagnosis, treatment

Résumé

Le purpura thrombocytopénique immun: de nouvelles perspéctives de diagnostic et de traitement

Le purpura thrombocytopénique immun (P.T.I.) représente une affection hémorragique, qui associe le purpura et la thrombopénie. Le purpura est la forme de manifestation de cette pathologie, déterminée par l'extravasation des hématies dans le derme à cause de la réduction du nombre de plaquettes. La thrombocytopénie périphérique suppose la diminution du nombre de plaquettes sanguines en-dessous de 100.000 éléments/mm^3 sang. Cette chose est due soit à une hyper-destruction prématurée dans le sang périphérique, soit à une synthèse déficitaire au niveau médullaire. Le purpura thrombocytopénique immun implique la présence des anticorps antiplaquettaires dont l`action induit de modifications morphologiques et fonctionnelles remarquables au niveau du thrombocyte. Le purpura thrombocytopénique immun revête deux formes: l'une primaire, idiopathique ou secondaire issue dans le contexte de certaines pathologies associées. Le diagnostic différentiel est l'un d`exclusion. Il suppose l'exclusion de certaines maladies auto-immunes, des infections virales ou bactériennes, des syndromes lympho-prolifératifs susceptibles à induire une thrombocytopénie secondaire. L'établissement avec précision des facteurs impliqués à l'ethiopathogenie du purpura immun chronique conduit à l'établissement correct du diagnostic et à l'institution du croquis thérapeutique optimal.
Mots clé: thrombocytopénie immune, purpura, anticorps anti-plaquettaires, diagnostic différentiel, traitement.

ITP is an autoimmune disorder characterized by low platelet counts in peripheral blood. The premature hyper-destruction of platelets is due to anti-platelet antibodies or to immune complexes fixed on platelet membrane, causing their phagocytosis by macrophages. Sometimes these antibodies have the ability to attach to megakaryocytes leading to an associated megakaryocyte hypoplasia (1, 2). Anti-platelet antibody testing is a method whose usefulness and compulsoriness for diagnosis of ITP is certain. Acknowledgement of the usefulness in practice relates to the exclusion of an eventual myelodysplasias in conjunction with data from bone marrow morphology.

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Anti-platelet antibodies effect is now countered in most patients with chronic ITP by means of the therapy with the thrombopoietin (TPO) receptor agonists. Thrombopoietin agonists of the second generation are products of peptide (Romiplostim) or non-peptide nature (Eltrombopag), as well as agonist antibodies of the TPO. Under International Consensus on the management of ITP, TPO receptor agonists benefit from grade A recommendation both as second-line therapy, as well as the preferred option for patients who have failed in at least two treatment lines (3, 4).

Etiopathogeny

The presence of anti-platelet antibodies is specific to immune thrombocytopenic purpura. In the case of primary immune thrombocytopenia these anti-platelets occur by a mechanism that is still unclear. Secondary immune purpura of certain associated pathologies such as viral infections with virus B, C, CMV or bacterial infection (Helicobacter pylori) also requires the presence of anti-platelet antibodies. In this case, most likely a cross-reactivity phenomenon takes place between anti-platelet antibodies and anti-hepatitis C virus (HCV) antibodies or anti-Helicobacter pylory (HP) (5, 6). In the diagnosis compulsory screening serological tests for HIV, HCV, CMV, HP are included due to increased incidence of these infections at the population level and to their frequent involvement in the secondary immune thrombocytopenia etiopathogeny. It is also recommended to investigate the possible autoimmune diseases (lupus erythematosus, collagen diseases, autoimmune thyroiditis) which may induce the decrease in blood platelets by the phenomenon of cross-reactivity between autoantibodies.

The mechanism of anti-platelet antibodies is still unknown. Studies with marked platelets have shown a major shortening of the life span of platelets in the circulation of patients with ITP. The median survival time is between 2-3 days and can be reduced to minutes. The platelets on which antibodies are fixed are seized and destroyed in their majority in the spleen, but the liver and bone marrow reticuloendothelial system can also play an important role in platelet sequestration. The shortening of the life of platelets is the consequence of an autoimmune mechanism (7, 8).

Disorder of the humoral immune response is based on a complex interaction between antigen presenting cells and T and B lymphocytes. The role of antigen presenting cell belongs to the platelet. On its surface there are physiologically membrane glycoproteins which mediate the interaction between blood platelets and the vascular endothelium of a damaged blood vessel. Any disturbance in the morphology and function of the membrane glycoproteins causes platelet dysfunction and blood clogging disorders. Membrane glycoproteins acquire antigenic time when the loss of immunological tolerance to its own antigens takes place. In this way occurs the activation of the immune system which will produce antibodies whose main target is the glycoproteins on the platelet membrane. In some patients the antibodies recognize antigens derived from only one glycoprotein, but at other times can recognize multiple glycoproteins. The antibodies are directed against surface structures such as glycoproteins type IIb / IIIa and Ib / IX, glycosphingolipid or cardiolipins (common). Glycoproteins IIb / IIIa are the main target of autoantibodies.

They have recently identified autoreactive T cells in patients with ITP, but they are all present in the peripheral blood of healthy individuals. They are active in vivo only in patients with ITP demonstrating that the mechanisms of peripheral tolerance are essential to prevent T-cell activation. Maintenance of tolerance at the periphery is provided by regulatory T-cells, by suppressing the activation and proliferation of many cell types such as: T and B lymphocytes, dendritic cells, natural killer cells. In patients with ITP the spleen biopsy has shown a reduced number of regulatory T-cells (9). The spleen is considered an important site for the synthesis of anti-platelet auto-antibodies (as evidenced by pathological studies of parts of splenectomy). The initial antigen response takes place in the spleen, and then in the bone marrow. Pathological studies and of immunohistochemistry performed on spleen from patients with ITP have demonstrated that in the red pulp of the spleen there is an increased number of B-lymphocytes and T-cytotoxic lymphocytes that allow immunological attack on platelets.

Clinical forms

The acute phase of ITP occurs equally in men and women, with a peak incidence in children. Most patients have a viral infection in recent history. Onset is sudden, with signs and symptoms of varying intensity depending on the number of platelets. Bleeding is usually mild, except in the cases in which platelet count falls below 20,000/mm^3 blood. With a platelet count of 20,000 / mm^3 to 50,000/mm^3 we noticed petechiae and bruising after mild trauma. If the number of platelets falls below 10,000 / mm3 generalized petechiae, bruising, mucosal bleeding and at the level of the apparatus occur. In case of platelet counts below 2000 / mm3 blood we can observe widespread bruising, bleeding bubbles, sometimes even retinal haemorrhage. Hepatosplenomegaly or lymphadenopathy are absent. High-dose prednisone therapy and infusion of purified IgG are used. The persistence of pathology for more than six months leads to chronic installation of ITP (10, 11).

Chronic ITP is frequent in adults aged 20-50 years. Predominant in females (F: M = 3: 1). Installation is insidious, progressive with mild to moderate hemorrhagic phenomena, with fluctuated evolution, in a few days-weeks outbursts. The most common manifestation in ITP is mucosal bleeding (epistaxis), gingival bleeding and menorrhagia. Viral recent history may be missing, bleeding symptoms usually occur only in case of severe ITP (platelet count below 30,000/mm^3 blood. During the intercritical period the platelets can climb to normal counts, but often remain below 100,000/mm^3. Spontaneous remissions are rare, incomplete, and relapses may recur anytime. Diagnosis is usually one of exclusion. One should examine the peripheral blood smear to differentiate ITP from any pseudo-thrombocytopenia resulting from the aggregation or from thrombotic thrombocytopenic

purpura (TTP). Often, the marrow smear shows marrow hyperstimulation, increased number of megakaryocytes and giant platelets (12, 13).

Clinical manifestations

Epistaxis (nose bleed) is a common manifestation of the diseases whose pathogenic mechanism is primary haemostasis disorder. Epistaxis severity rating is based on the frequency and duration of bleeding and on possible previous treatments (burns, transfusions, anticoagulant medication).

Gums bleeding is present in most patients with primary hemostasis disorders. Bleeding can occur spontaneously or after dental extraction or tooth brushing.

Hemoptysis, hematemesis, hematuria, hematochezia and melena are rarely the initial symptoms of a bleeding disorder. However, these symptoms may be exacerbated in patients with ITP.

In the physical examination we can notice bruises which occur spontaneously in the absence of trauma. Arterioles and venules bleeding leads to bruising (14). Often, in ITP bruising is accompanied by petechiae. Petechiae and ecchymotic purpura is predominant in the lower limbs and in the areas of friction and pressure. Petechiae are red skin lesions, of small sizes (less than 2 mm), located in the dermis and caused by the loss of red blood cells through capillaries. Purpura is the result of spontaneous extravasation of erythrocytes in blood vessels. Purple lesions of ITP are not tangible, unlike the vasculitis ones.

Positive diagnosis

Thrombocytopenia is confirmed by the blood count which will reveal a low platelet count (less than 100,000 members/mm^3 blood). It can be associated with an anemia proportional to blood loss through bleeding. WBC and differential blood count are usually normal.

Peripheral blood smear examination shows the presence of large platelets (3-4 mm diameter) associated with small platelets or platelet fragments. Platelet anisocytosis and megathrombocytes occur due to platelet bone marrow compensatory hyper-production.

Bleeding time is prolonged, clot retraction is deficient, coagulation tests are normal and the Rumpell-Leede test can be positive.

Coombs test shows the presence of anti-platelet antibodies on thrombocytes and in patient serum.

Bone marrow punction (myelogram) shows a bone marrow with normal cells, with the presence of an increased number megakaryocytes, some with increased volume, certifying the peripheral origin of thrombocytopenia. Rarely, the megakaryocytes number is low due to their destruction by autoantibodies. It is necessary especially in patients older than 60 and in those with systemic symptoms for the exclusion of primary bone marrow diseases associated with thrombocytopenia. In classic ITP the myelogram aspect is most of the time, normal.

In order to preclude the occurrence of other conditions which may be associated with thrombocytopenia (viral infection with virus B, C, HIV, CMV, bacterial infections, autoimmune diseases) immunoassays are carried out.

Specific testing is recommended to detect pregnancy (pregnancy thrombocytopenia) and it should be investigated whether the patient received treatment with substances that can induce thrombocytopenia. Anti-platelet antibodies are dosed to make the differential diagnosis between immune thrombocytopenia and non-immune thrombocytopenia.

Differential diagnosis

ITP diagnosis is based on patient history, clinical and laboratory examination. ITP must be distinguished from a false thrombocytopenia due to vitro agglutination of blood collected on EDTA, then the hypothesis of the presence of vascular purpura should be eliminated (clinical, platelet count).

Correct diagnosis is based on determining the primary, idiopathic origin of thrombocytopenia, or of the secondary one occurring in the context of associated pathologies. In this regard, investigation of possible pathologies is recommended.

Viral diseases are investigated through the research of recent history of measles, chickenpox, infectious mononucleosis, hepatitis with viruses B, C, cytomegalovirus infection or HIV.

Immunological disorders (systemic lupus erythematosus, anti-phospholipid syndrome, Evans syndrome) are diagnosed by determination of specific antibodies, anti-duble-stranded DNA, anti-cardiolipinic, anti-nuclear.

The possible presence of consumption diseases such as

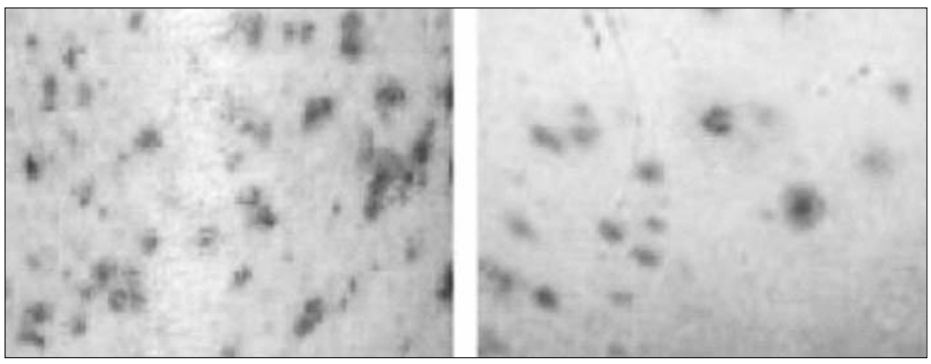

Figure 1 - Skin Petechiae in ITP. From the Collection of the Hematology Clinic St. Spiridon Iasi

neoplasia or myeloproliferative syndromes is investigated (15, 16).

Treatment

Maintenance under observation and periodical checks (12.2 weeks) are recommended in patients with platelets > 30,000 / mm3 who must not be treated, in the absence of active bleeding manifestations and in the absence of aggravating factors (eg gastroduodenal ulcer, metropathies). Patients aged over 60 and those with a history of bleeding require special attention and can represents exceptions.

Secondary prevention is based on non-steroidal anti-inflammatory and antiplatelet agents avoidance. Anticoagulants (oral and parenteral) may be administered in case there is a history indicating such treatment (thrombosis, atrial fibrillation). In general, anticoagulants shall be interrupted if platelets fall below 40,000 / mm3 blood.

1st line therapy is initiated if the platelets are below 30,000 / mm3 blood or in case of higher values if there is manifest bleeding.

Corticosteroid therapy is administered in the absence of contraindications. The mechanism of action involves the decreased activity of mononuclear phagocytes and prevents attachment of antibodies on platelets. They have direct haemostatic action at the level of vascular wall (58, 59). Prednisone 0.5-2 mg / kg / day for several days to several weeks is the initial first-line standard therapy for patients with ITP. Alternatively one can use methyl-prednisolone (Medrol) 0.4 to 1.8 mg / kg / day or dexamethasone, 40 mg / day for 4 days, 4 cycles every 14 days.

Intravenous immunoglobulins (IVIg) are indicated in severe bleeding forms resistant to corticosteroids, when corticosteroids are contraindicated and when a rapid increase in platelets is required (surgery, major bleeding risk, age above 60 years, severe clinical signs, associated pathologies). 1 g / kg (1-2 infusions for 2 days) or 400 mg / kg for 5 days is administered. The answer is usually transient. IVIg are indicated especially in bleeding emergencies or prior splenectomy.

Anti-D immunoglobulins is a therapy proposed instead of immunoglobulins in patients with non-splenectomised Rh +. It determines a saturation of macrophages as well as in the case of immunoglobulins. The answer is usually slow and transient. Anti-D therapy is not approved in Europe and is used mainly in the United States.

Second line therapy addresses refractory patients or relapsing after first line treatment.

Splenectomy consists of elimination of the organ which is the main place of platelet destruction and of synthesis of antiplatelet antibodies. Splenectomy increases the survival time of platelets. Splenectomy can be performed in the classical manner or laparoscopically. Patients proposed for splenectomy require vaccination with anti-Streptococcus pneumoniae, anti-Neisseria meningitis and anti-Haemophilus influenzae.

The timing of the vaccination is with 4 weeks before intervention. Splenectomy can be performed in conditions of relative safety to a level of platelets > 50,000 / mm^3. This level is usually achieved after re-challenge with corticosteroids (1-2 weeks) or in case of resistance to corticosteroids after intravenous immunoglobulins, anti-D immunoglobulins, thrombopoietin receptor agonists (1-3 weeks). In case of emergency or failure to the above ways preoperative platelet transfusions are performed.

In case of contraindications for splenectomy or patient's refusal to perform splenectomy Azathioprine is administered in doses of 100-150 mg / day on the long term, Cyclosporin A- 2.5-3 mg / kg / day alone or in combination with small doses (10-30mg) of prednisone for long periods. It is contraindicated in elderly patients and in those with renal impairment. Another option is Cyclophosphamide - orally 2.1 mg/kg daily for at least 16 weeks or IV 0.3-1 g/m^2 in 1-3 doses in every 2-4 weeks. Vinca alkaloids - Vincristine administered 1-2 mg or Vinblastine 5-10mg in long infusion (3-6 hours) is administered, weekly, 3-6 infusions. Danazol-200 mg is recommended 2-4 times a day (10-15 mg / kg / day). Contraindicated in patients with liver diseases. It should not be associated with cyclosporine A. Rituximab 375 mg/m^2 administered weekly for 4 weeks. Rituximab is contraindicated in patients with active infection with hepatitis viruses B or C.

Thrombopoietin receptor agonists (TPO) act by increasing megakaryocytopoiesis and thus by increasing platelet counts. In this way the effect of the antibodies contained in most patients with chronic ITP is countered. Treatment is made indefinitely, their cessation being associated with decrease in platelets count. An alternative is short-term treatment (2-3 weeks), aiming to increase platelet counts to a level to allow the safe performance of splenectomy. Two of these agents are currently approved on the market: romiplostim and eltrombopag. Romiplostim is a recombinant protein, eltrombopag is an oral -hydrazone. Romiplostim is recommended 1-10 μg/kg/week s.c., and eltrombopag starts with 50 mg/day p.o. Depending on the response the dose may be increased to 75 mg/day or decreased to 25 mg/day.

Third-line therapy is applied to retracting patients who relapse after first line and second line therapies. TPO receptor agonists are administered in the dosages mentioned above, but on the long term. Combination chemotherapy regimen is recommended, repeated at 21-28 days, 3-6 cycles (17,18).

CONCLUSIONS

Chronic immune thrombocytopenic purpura has two forms, primary (idiopathic) or secondary, arising in the context of other associated diseases. Accurate diagnosing of chronic immune purpura is based on definitely establishing the etiologic factors underlying the appearance of this pathology. Primary thrombocytopenia involves the appearance of anti-platelet antibodies at the level of the platelet membrane, without knowing the exact causes of this immune disorder. Secondary immune thrombocytopenia also implies the existence of anti-platelet antibodies, yet their presence is considered to be secondary to associated pathologies. Among them, in the research literature autoimmune diseases are reported (lupus erythematosus, autoimmune thyroiditis, antiphospholipid

syndrome), myeloproliferative syndromes (chronic lymphocytic leukemia), chronic infections with Helicobacter pylori, or those with viruses such as HIV, cytomegalovirus, hepatitis B and C. The accurate establishment of the factors involved in autoimmune thrombocytopenia etiopathogeny leads to the correct establishment of the type of thrombocytopenia and to the establishment of the optimal therapeutic regimen.

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