Role of Immunochemical Detection Methods in the Diagnosis of Immune Thrombocytopenic Purpura

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The immune chronic thrombocytopenic purpura is an illness characterized by peripheral thrombocytopenia occurred through a mechanism of early hyper destruction of blood platelets or by deficient platelet synthesis in the medulla. The chronic immune purpura can be primary, autoimmune in nature, thrombocytopenic idiopathic or secondary in the context of other associated pathologies. The idiopathic thrombocytopenic purpura (ITP) is an immune-mediated acquired disorder. It is characterized by isolated thrombocytopenia, defined as platelet count assessment from peripheral blood smear of less than 100,000/μL in the absence of a different cause of thrombocytopenia. The secondary immune isolated thrombocytopenia occurs in the context of some associated pathologies. The aim of the study is to highlight the involvement of some infectious agents in the etiopathogenesis of the secondary immune thrombocytopenic purpura. The immune thrombocytopenia can be subordinated to some chronic infections such as infection with virus B or C, infection with virus HIV, infection with Cytomegalovirus (CMV) or the Helicobacter Phylori infection. The study was conducted on a group of 40 patients, distributed into two groups: the first group of patients is the asymptomatic patients who do their common tests while the other group of patients is with bleeding symptoms: petequiae, bruising, epistaxis, gum bleedings. The studied group puts into evidence a thrombocytopenia with a mean platelet count of 60.20 ± 19.75 x10³/μL. 80% of patients had positive anti-platelet antibodies. Out of these, 20% carry infections with virus B and C while 30% carry Cytomegalovirus infection (CMV). The study found one case of HIV infection. Thus we highlight the involvement of infectious agents in the etiopathogenesis of secondary immune thrombocytopenic purpura as well as the way they affect the platelet function.

Keywords: Thrombocytopenic purpura, electrochemiluminescence, Anti-platelet antibodies, ANOVA test

The chronic immune thrombocytopenic purpura is a disease characterized by a low platelet count in peripheral blood [1, 2]. This thrombocytopenia occurs within a mechanism of early hyper destruction of blood platelets caused by some anti-platelet autoantibodies or by some immune complex platelet membrane which causes their absorption by the macrophage [3, 4]. The short lifetime of platelets is the consequence of an autoimmune mechanism. In case of immune primary thrombocytopenia these antiplatelet antibodies occur within an unsolved mechanism [5, 6]. In most cases the antibodies shorten the lifetime of platelets. Antibodies place themselves on platelets and favour their absorption by macrophage. At times these antibodies can place themselves on megakaryocytes leading to an associated megakaryocytic hyperplasia. The evidence based platelets showed a major shortening of platelet lifetime in the blood circulation of ITP patients. The mean survival time is between 2 to 3 days and can be reduced to some minutes. The platelets on which the antibodies are placed most of the times are isolated and destroyed in spleen. The liver and the reticuloendothelial medullary system can have an important role in the platelet sequestration.

The secondary immune thrombocytopenic purpura develops in the context of some other associated diseases such as auto-immune diseases (systemic lupus erythematosus, antiphospholipid syndrome, autoimmune thyroiditis or Evans syndrome), mieloproliferative syndromes, chronic lymphoid leukemia, infections with virus B, C, CMV, HIV or HIV infections [7-9]. The secondary ITP can be induced in pregnancy or drug induced [8].

This study shows the viral pathogens and bacteria in the secondary immune thrombocytopenic purpura. It also analyzes the correlation between these “triggers” and the platelet count assessment from peripheral blood smear.

Experimental part

Materials and methods

The present study is an observational one which aims to highlight the presence of secondary purpura in infections with viral agents and how their presence requires changes in the studied parameters. We studied a group of 40 patients aged between 38-65 years, admitted to the Department of Hematology of the “Sf. Spiridon” Teaching Hospital from Iasi, Romania. Patients were divided into 2 groups:

- asymptomatic patients, on whom common tests were carried out;
- patients with bleeding syndrome: bruising, petechiae, epistaxis, gum bleedings.

Common criterion for inclusion of patients in the study group was thrombocytopenia. Patients included in the study group had no previous medication involved in the occurrence of secondary thrombocytopenia and no active pathology that involves thrombocytopenia.

Patients were informed about the involvement in the study and their participation was possible only after signing
Virological analyses were performed for the studied group. Thrombocytopenia previously certified. Immunological and virological analyses were performed for the studied group.

Results and discussions

Platelet Count

This count pointed out a low platelet count for the studied group.

The blood platelet count (PC), with a variance of 32.8%, were within the range of 33-87 x10^3/μL, the mean group being of 60.20 ± 19.75x10^3/μL.

The next step is to investigate the etiology of thrombocytopenia previously certified. Immunological and virological analyses were performed for the studied group.

<table>
<thead>
<tr>
<th>AgHBs</th>
<th>No.</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard error.</th>
<th>Confidence interval 95%</th>
<th>Min</th>
<th>Max</th>
<th>Test F (ANOVA)</th>
<th>p</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-95% CI</td>
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<td></td>
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<td></td>
<td>+95% CI</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negativ</td>
<td>8</td>
<td>56,25</td>
<td>19.44</td>
<td>6.87</td>
<td>40.00 - 72.50</td>
<td>33</td>
<td>82</td>
<td></td>
<td>0.225</td>
</tr>
<tr>
<td>Pozitiv</td>
<td>2</td>
<td>76,00</td>
<td>15.56</td>
<td>11.00</td>
<td>-63.77 - 215.77</td>
<td>65</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>60,20</td>
<td>19.75</td>
<td>6.25</td>
<td>46.07 - 74.33</td>
<td>33</td>
<td>87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1**

DESCRIPTIVE INDICATORS OF PC*10^3/μL BASED ON AgHBs

Dosage of anti-platelet antibodies

Around 80% of patients had positive anti-platelet antibodies, with PC counts ranging from 33 to 87 PC x10^3/μL, with a mean range of 59.13 ± 20.19x10^3/μL, slightly reduced as compared to the mean count in patients with negative anti-platelet antibodies (p = 0.852). Results are reported in figure 1.

Antiplatelet antibodies are considered the most relevant for ITP diagnosis. The humoral immune response disorder is based on a complex interaction between antigen cells and the lymphocytes T and B. The platelet is an antigen-presenting cell. On the platelet surface physiologically there are membranary glycoproteins that enable the interaction between blood platelets and vascular endothelium of a damaged blood vessel [17-19]. The membranary glycoproteins acquire an antigenic character when the immunological tolerance to its own antigen is lost. Thus, the immune system activation occurs producing antibodies whose main target is to focus on the platelet membrane glycoproteins. In some patients the antibodies recognize antigens deriving from a single glycoprotein, but some other times they recognize multiple glycoproteins. Antigenic targets most frequently identified by anti-platelet antibodies are GP Ib/IIa and GP Ib/IX. Autoantibodies are produced by T - cytotoxic lymphocytes and may lead to the destruction of platelets or to the inhibition of their production [5].

Determination of HBs antigens

It involves highlighting the hepatitis B virus surface antigen. The presence of AgHBs in human serum/plasma is the evidence of infection with hepatitis B virus (up to 40% of infected patients do not show clinical symptoms). Out of the study group, only 20% of patients were HBsAg-positive. The mean PCs were slightly higher in patients with positive HBsAg, but from a statistical point of view, if compared to the patients with negative HBsAg, these counts were not significant (p = 0.225). The results are shown in table 1.

Testing of anti-HCV antibodies

This test proves the evidence of IgG antibodies which are different from hepatitis C virus. There are five major genotypes and 50 serotypes of VHC. The test shows a high sensitivity towards the 6 genotypes coming from different...
geographical areas. Knowing the genotype or the HCV serotype (antibodies specific to the genotype) is helpful in recommending and guiding the treatment. The HCV-RNA test (through PCR) confirms the diagnosis and quantifies the number of viral copies in blood (viremia). Almost all patients with chronic infection had HCV-RNA in their blood. Results reported in table 2 shown that a percentage of 20% of patients had anti-HCV positive antibodies. These patients had a mean platelet count that is 80.50 ± 2.12x10^3/μL. This count was significantly higher compared to the mean count in patients with anti-HCV negative antibodies (55.13 ± 18.81x10^3/μL) (p = 0.046). Even if the platelet count in patients with positive anti-HCV antibodies was higher this count is pathological in nature and proves the evidence of thrombocytopenia.

To some of the studied patients the evidence of anti-platelet antibodies, antigens HBsAg and anti-HCV antibodies proves the involvement of these viral agents in the etiopathogenesis of the secondary immune thrombocytopenic purpura. The group distribution based on AgHBs positivity and anti-HVC presence is shown in figure 2. The low platelet count can be evidenced in the absence of clinical signs of hepatic disease that is why the correct diagnosis is primary ITP. A whole range of physical pathological mechanisms are involved in the development of thrombocytopenia in patients infected with HCV. The response of the immune system to infection can generate antibodies that cross-react with the platelet antigens. Possible mechanisms leading to immune disorders are binding HCV, followed by the appearance of anti-HCV at the platelet membrane level and circulating immune complex level [20]. Platelets are destroyed by phagocytosis. Non-immune mechanisms may also contribute to installation of thrombocytopenia in patients with HCV. Thrombopoietin synthesis can be inhibited by hepatic damage or by antiviral treatment with interferon. Another mechanism is the accelerated destruction of blood platelets through their sequestration in the enlarged spleen. Splenomegaly is secondary to portal hypertension [21, 22]. Usually patients show massive bleedings even in the form of moderate thrombocytopenia.

Dosage of anti-HIV

One single patient had positive anti-HIV antibodies, reporting a PC of 65 x10^3/μL, which was not significantly higher compared to the mean count in patients with anti-HIV negative (p = 0.815). Results are reported in table 3 and figure 3.

We can notice a lower mean platelet count in patients with positive anti-HIV as compared to those patients with negative anti-HIV antibodies. In this case thrombocytopenia derives from the immune thrombocytopenic purpura. It is also caused by megakaryocyte infection and HIV. The virus is connected to CD4 receptor as well as to other coreceptors located on the megakaryocyte. It leads to dysplasia of infected cells and to peripheral vacuolation of the

<table>
<thead>
<tr>
<th>anti-HCV Ac</th>
<th>No.</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>Confidence Interval 95%</th>
<th>Min</th>
<th>Max</th>
<th>Test F (ANOVA) p</th>
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<td>Negative</td>
<td>32</td>
<td>55.13</td>
<td>18.81</td>
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<td>39.40 - 70.85</td>
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<td>87</td>
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<tr>
<td>Positive</td>
<td>8</td>
<td>80.50</td>
<td>2.12</td>
<td>1.50</td>
<td>61.44 - 99.56</td>
<td>79</td>
<td>82</td>
<td>0.046</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>60.20</td>
<td>19.75</td>
<td>6.28</td>
<td>46.07 - 74.33</td>
<td>33</td>
<td>87</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

DESCRIPTIVE INDICATORS OF PC*10^3/μL BASED ON ANTI-HCV Ac

<table>
<thead>
<tr>
<th>Ac anti-HIV</th>
<th>No.</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Confidence Interval 95%</th>
<th>Min</th>
<th>Max</th>
<th>Test F (ANOVA) p</th>
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<tr>
<td>Negative</td>
<td>39</td>
<td>59.67</td>
<td>20.88</td>
<td>6.96</td>
<td>43.62 - 75.71</td>
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<tr>
<td>Positive</td>
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<td>65.00</td>
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<td>-</td>
<td>-</td>
<td>0.815</td>
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<tr>
<td>Total</td>
<td>40</td>
<td>60.20</td>
<td>19.75</td>
<td>6.28</td>
<td>46.07 - 74.33</td>
<td>33</td>
<td>87</td>
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</tr>
</tbody>
</table>

Table 3

DESCRIPTIVE INDICATORS OF PC*10^3/μL BASED ON Ac ANTI-HIV
cytoplasm. The immune component is driven by substances which reproduce anti-HIV antibodies and interfere with the glycoproteins from the platelet membrane. Other factors causing thrombocytopenic purpura in patients suffering from HIV can be opportunistic infections, malignant diseases or certain drugs (chemotherapy, interferon and antiviral agents).

In figure 4 is illustrated an obvious low rate frequency of HIV infection as compared to the frequency of B and C viruses within the same studied group.

Conclusions
Chronic immune thrombocytopenic purpura has two forms, primary (idiopathic) and secondary. The secondary one arises in the context of other associated pathologies. The accurate diagnosis of chronic immune purpura is made by correctly establishing those etiological factors which cause this disease. Primary thrombocytopenia involves the presence of anti-platelet antibodies caused by some associated pathologies. Expert literature reported autoimmune diseases (systemic lupus erythematosus, autoimmune thyroiditis, and antiphospholipid syndrome), myeloproliferative syndromes (lymphatic chronic leukemia), chronic infections with Helicobacter Pylori virus or viruses such as HIV, Cytomegalovirus, and hepatitis B and C virus [23-25].

This study highlights the involvement of viral agents in the pathogenesis of chronic immune secondary purpura. A case study showed that the relationship between viruses B, C HIV and the anti-platelet antibodies produces the thrombocytopenia [26]. Currently viral and immunological tests in patients with chronic immune thrombocytopenia are essential. We noticed the importance of immunochemical method through electrochemiluminescence (ECLA) for determination of anti-HCV antibodies and anti-HIV antibodies in our study [27, 29]. This method is high sensitive and specific, is safe and fast and does not involve high costs [30, 31]. These parameters gives to ECLA method an important role in the establishing of the correct diagnosis. Thus, the determination of etiopathogenic agents is conducive to the implementation of a very good therapeutic scheme.

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Fig. 4. Group distribution based on the presence of AgHBs, anti-HVC and anti-HIV antibodies