Retraction Notice

Title of retracted article:	Aspects of Viral Involvement in Chronic Immune Thrombocytopenic Purpura
Author(s):	Mădălina Mocanu, Magda Bădescu, Manuela Ciocoiu, Oana Bădulescu
* Corresponding author.	Email: mada_farm@yahoo.com
Journal:	Journal of Biomedical Science and Engineering(JBiSE)
Year:	2014
Volume:	7
Number:	14
Pages (from - to):	1067-1074
DOI (to PDF):	http://dx.doi.org/10.4236/jbise.2014.714104
Paper ID at SCIRP:	52148
Article page:	http://www.scirp.org/journal/PaperInformation.aspx?PaperID=52148
Retraction date:	2017-02-07

Retraction initiative (multiple responses allowed; mark with **X**):

☐ All authors
X Some of the authors:
☐ Editor with hints from
- ○ Journal owner (publisher)
- ○ Institution:
- ○ Reader:
- ○ Other:

Date initiative is launched: 2017-01-27

Retraction type (multiple responses allowed):

☐ Unreliable findings
- ○ Lab error
- ○ Inconsistent data
- ○ Analytical error
- ○ Biased interpretation
- ○ Other:

☐ Irreproducible results
☐ Failure to disclose a major competing interest likely to influence interpretations or recommendations
☐ Unethical research

☐ Fraud
- ○ Data fabrication
- ○ Fake publication
- ○ Other:

☐ Plagiarism
☐ Self plagiarism
☐ Overlap
☐ Redundant publication *
☐ Copyright infringement
☐ Other legal concern:

☐ Editorial reasons
- ○ Handling error
- ○ Unreliable review(s)
- ○ Decision error
- ○ Other:

X Other:

Results of publication (only one response allowed):

X are still valid.
☐ were found to be overall invalid.

Author's conduct (only one response allowed):

X honest error
☐ academic misconduct
☐ none (not applicable in this case – e.g. in case of editorial reasons)

* Also called duplicate or repetitive publication. Definition: "Publishing or attempting to publish substantially the same work more than once."

History
Expression of Concern:
☐ yes, date: yyyy-mm-dd
X no

Correction:
☐ yes, date: yyyy-mm-dd
X no

Comment:

This article has been retracted to straighten the academic record. In making this decision the Editorial Board follows COPE's Retraction Guidelines. Aim is to promote the circulation of scientific research by offering an ideal research publication platform with due consideration of internationally accepted standards on publication ethics. The Editorial Board would like to extend its sincere apologies for any inconvenience this retraction may have caused.

Editor guiding this retraction: Prof. Hao Lin
(EIC of JBiSE)

J. Biomedical Science and Engineering, 2014, 7, 1067-1074
Published Online December 2014 in SciRes. http://www.scirp.org/journal/jbise
http://dx.doi.org/10.4236/jbise.2014.714104

Scientific Research

Aspects of Viral Involvement in Chronic Immune Thrombocytopenic Purpura

Mădălina Mocanu, Magda Bădescu, Manuela Ciocoiu, Oana Bădulescu

Department of Pathophysiology, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania
Email: magda.badescu@gmail.com

Received 26 September 2014; accepted 11 November 2014; accepted 26 November 2014

Abstract

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 (P.T.I.) 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/mm^3, 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 × 10^3/μL. 80% of patients had positive anti-platelet antibodies. Out of these, 20% carry infections with virus B and C while 30% carry Cytometalovirus infection (CMV). The study found one case of HIV infection. Thus we highlight the involvement of infectious agents in the etipathogenesis of secondary immune thrombocytopenic purpura as well as the way they affect the platelet function.

Keywords

Thrombocytopenic Purpura, Anti-Platelet Antibodies, Infections Agents, Viruses B, C, HIV, CMV

*Corresponding author.

How to cite this paper: Mocanu, M., Bădescu, M., Ciocoiu, M. and Bădulescu, O. (2014) Aspects of Viral Involvement in Chronic Immune Thrombocytopenic Purpura. *J. Biomedical Science and Engineering*, **7**, 1067-1074.
http://dx.doi.org/10.4236/jbise.2014.714104

1. Introduction

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 favor 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 rythematosus, antiphospholipid syndrome, autoimmune thyroiditis or Evans syndrome), mieloproliferative syndroms, 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.

2. Material and Method

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. From the total number of patients included in the study, there are 25 female patients and 15 male patients. 30 patients came from rural areas and 10 were from urban areas. The study was conducted over a period of 10 months (from 01/02/2013 to 01/11/2013). 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 an informed consent. The study respected the law 677/2001 regarding the collection, use and processing of personal data and the free movement of such data. Achieving clinical aspects of the research is conducted in strictly compliance with the rules stipulated by EU legislation bioethics. The study protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy "Gr. T. Popa" Iasi.

The following steps of the investigation protocol are applied to both categories of patients mentioned above in the aim of establishing a correct diagnosis:

- Platelet count by the automatic analyzer with the help of the hydrodynamic focusing method (blood collected in vacutainers containing anticoagulants such as tripotassium/dipotassium/dissodium—K3 EDTA); samples were analyzed within 6 hours after sampling.
- Dosage of anti-platelet antibodies by ELISA method (venous blood without anticoagulant collected in vacutainers), as much as the vacuum allows; in the process of centrifugation serum is being separated, processed as quickly as possible or refrigerated (2˚C - 8˚C) not more than 5 days or frozen at −20˚C) [10] [11].
- Establishing of HCV antibodies and HBs antigens by the immunochemical detection method through electrochemiluminiscence (ECLIA) [12] [13].
- Dosage of anti-HIV antibodies by enzyme-linked immunosorbent assay (ELISA) [14].
- Dosage of CMV antibodies by ECLIA method [15].

The objectives of the above steps are as follows:

1) ITP certification of immune thrombocytopenic purpura diagnosis;

2) Differential diagnosis between primary and secondary immune purpura.

Statistical analysis has shown all the data as mean value ± standard error of the mean (SEM). In order to assess the normal distribution of the groups, Shapiro-Wilk test was performed. Additionally, Levene test was performed to confirm the homoscedasticity of the groups, followed by ANOVA and paired or unpaired t-test to reveal the pairs of groups that differ biostatistical significantly in term of means. Statistical data interpretation considered the corresponding differences for a given significance threshold: $p > 0.05$ statistically insignificant; $p < 0.05$ statistically significant; $p < 0.01$ strong statistical significance; $p < 0.001$ very strong statistical significance.

3. Results and Discussion

3.1. 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%, was within the range of 33 - 87 × 10^3/µL, the mean group being of 60.20 ± 19.75 × 10^3/µL. The results are shown in **Table 1**.

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

3.2. Dosage of Anti-Platelet Antibodies

80% of patients had positive anti-platelet antibodies, with PC counts ranging from 33 to 87 PC × 10^3/µL, with a mean range of 59.13 ± 20.19 × 10^3/µL, slightly reduced as compared to the mean count in patients with negative anti-platelet antibodies ($p = 0.852$). Results are reported in **Table 2** and **Figure 1**.

Table 1. Descriptive indicators PC * 10^3/µL.

Parameter	No.	Mean	Standard Deviation	Standard Error	Confidence Interval 95%		Min	Max
					−95%CI	+95%CI		
Total	40	60.20	19.75	6.25	46.07	74.33	33	87

Table 2. Descriptive indicators PC * 10^3/µL based on anti-platelet Ac.

Anti-Platelet Ac	No.	Mean	Std. Deviation	Std. Error	Confidence Interval 95%		Min	Max	Test F (ANOVA) p
					−95%CI	+95%CI			
Negative	8	64.50	24.75	17.50	−157.86	286.86	47	82	
Positive	32	59.13	20.19	7.14	42.25	76.00	33	87	0.752
Total	40	60.20	19.75	6.25	46.07	74.33	33	87	

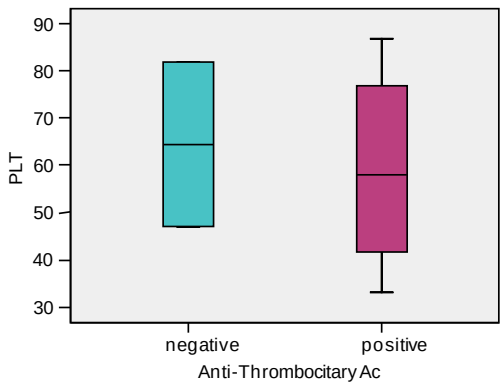

Figure 1. Mean PC * 10^3/µL based on anti-platelet antibodies.

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. 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 antiplatelet antibodies are GP IIb/IIIa and GPIb/IX. Autoantibodies are produced by T-cytotoxic lymphocytes and may lead to the destruction of platelets or to the inhibition of their production [5].

3.3. 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 3**.

3.4. Testing of Anti HCV Antibodies

This test proves the evidence of IgG antibodies which are different from hepatitis C virus. There are six 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 4** and **Figure 2** shown that a percentage of 20% of patients had anti-HCV positive antibodies. These patients had a mean platelet count, that is $80.50 \pm 2.12 \times 10^3/\mu L$. This count was significantly higher compared to the mean count in patients with anti-HCV negative antibodies ($55.13 \pm 18.81 \times 10^3/\mu L$) ($p = 0.046$). 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 3**. The low platelet count can be evidenced in the absence of clinical signs of hepatic disease, that is why the

Table 3. Descriptive indicators of PC * $10^3/\mu L$ based on AgHBs.

AgHBs	No.	Mean	Standard Deviation	Standard Error	Confidence Interval 95%		Min	Max	Test F (ANOVA) p
					−95%CI	+95%CI			
Negative	32	56.25	19.44	6.87	40.00	72.50	33	82	
Positive	8	76.00	15.56	11.00	−63.77	215.77	65	87	0.225
Total	40	60.20	19.75	6.25	46.07	74.33	33	87	

Table 4. Descriptive indicators of PC * $10^3/\mu L$ based on anti-HCV Ac.

Anti-HIV Ac	No.	Mean	Standard Deviation	Standard Error	Confidence Interval 95%		Min	Max	Test F (ANOVA) p
					−95%CI	+95%CI			
Negative	32	55.13	18.81	6.65	39.40	70.85	33	87	
Positive	8	80.50	2.12	1.50	61.44	99.56	79	82	**0.046**
Total	40	60.20	19.75	6.25	46.07	74.33	33	87	

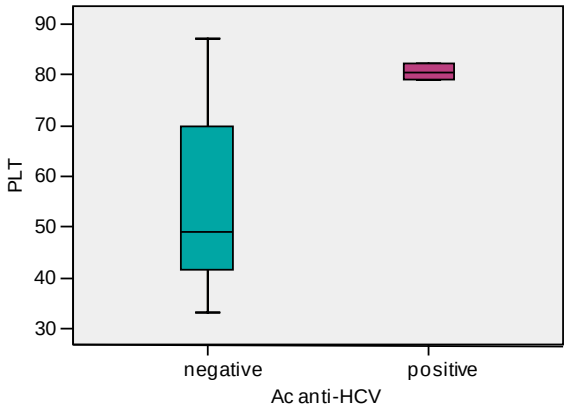

Figure 2. Mean PC * 10^3/µL based on anti-HCV antibodies.

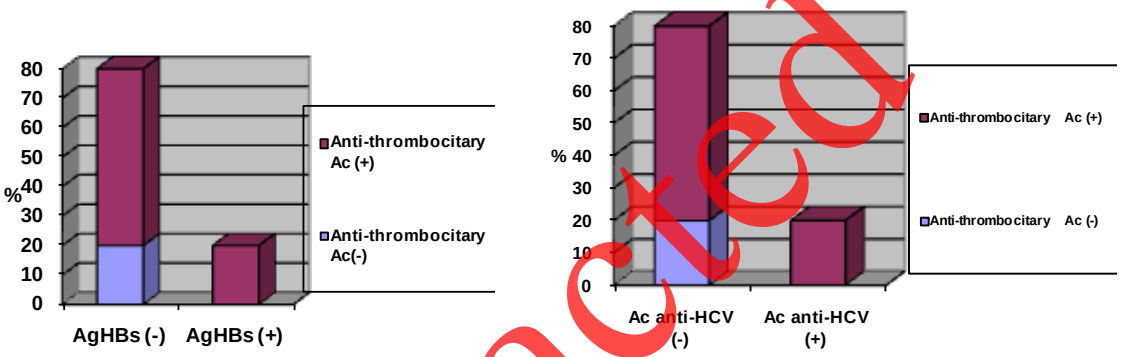

Figure 3. Group distribution based on Ac anti-platelet positivity, AgHBs and Ac anti-HCV.

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. 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. Usually patients show massive bleedings even in the form of moderate thrombocytopenia.

3.5. Dosage of Anti-HIV

One single patient had positive anti-HIV antibodies, reporting a PC of 65 × 10^3/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 5** and **Figure 4**.

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 receiver as well as to other coreceptors located on the megakaryociyte. It leads to dysplasia of infected cells and to peripheral vacuolation of the 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 5**, it 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.

Table 5. Descriptive indicators of PC * 10^3/µL based on Ac anti-HIV.

Anti-HIV Ac	No.	Mean	Std. Deviation	Std. Error	Confidence Interval 95%		Min	Max	Test F (ANOVA) p
					−95%CI	+95%CI			
Negative	39	59.67	20.88	6.96	43.62	75.71	33	87	
Positive	1	65.00	-	-	-	-	65	65	0.815
Total	40	60.20	19.75	6.28	46.07	74.33	33	87	

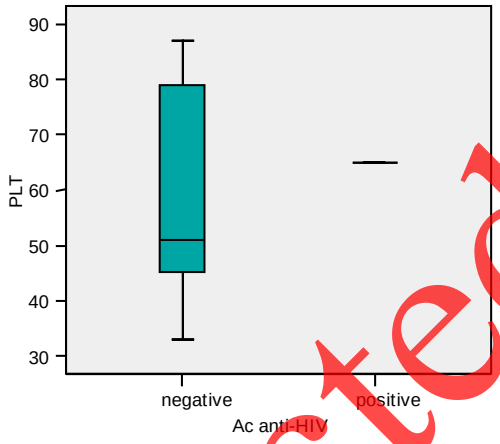

Figure 4. Mean PC * 10^3/µL based on anti-HIV antibodies.

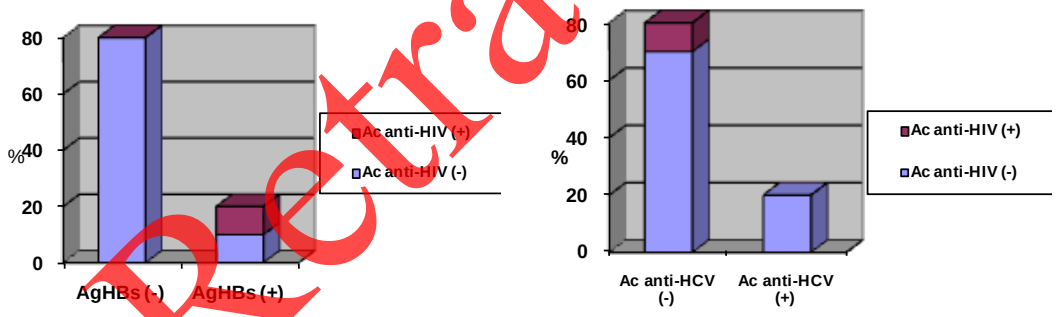

Figure 5. Group distribution based on the presence of AgHBs, anti-HVC and anti-HIV antibodies.

3.6. Dosage of IgM-Anti Cytomegalovirus Antibodies

Cytomegalovirus infection is highly spread while the clinical disease is a rare consequence of this common infection (80% - 100% of adult population has specific antibodies as a main result of asymptomatic infections). The immune response to cytomegalovirus involves the synthesis of specific antibodies of IgM class a few weeks after catching the infection, followed by the appearance of IgG antibodies a week later. IgM antibody level reaches the peak after a few weeks, then decreases within 4 - 6 months. Occasionally, it may persist for several years.

In our study group, 30% of patients had positive Cytomegalovirus IgM antibodies whose PC counts varied from 45 to 82 × 10^3/µL (**Table 6**). The group mean is 58 × 10^3/µL and is not different if compared to the mean count reported in patients with IgM negative antibodies (61.14 × 10^3/µL) (p = 0.833). None of the 12 patients with positive IgM antibodies showed positive HBsAg (p = 0.863) and only 4 showed negative anti-HCV (p = 0.863). We illustrated this in **Figure 6**.

In **Figure 7**, it is shown that in the studied group, the individual values of Cytomegalovirus IgM antibodies did not match PCs (r = 0.075, R2 = 0.0056, p = 0.838).

Table 6. Descriptive indicators of PC * 10^3/Ac µL by Cytomegalovirus IgM.

Cytomegalovirus Ac IgM	No.	Mean	Std. Deviation	Std. Error	Confidence Interval 95%		Min	Max	Test F (ANOVA) p
					−95%CI	+95%CI			
Negative	28	61.14	20.92	7.91	41.80	80.49	33	87	
Positive	12	58.00	20.81	12.01	6.31	109.69	45	82	0.833
Total	40	60.20	19.75	6.25	46.07	74.33	33	87	

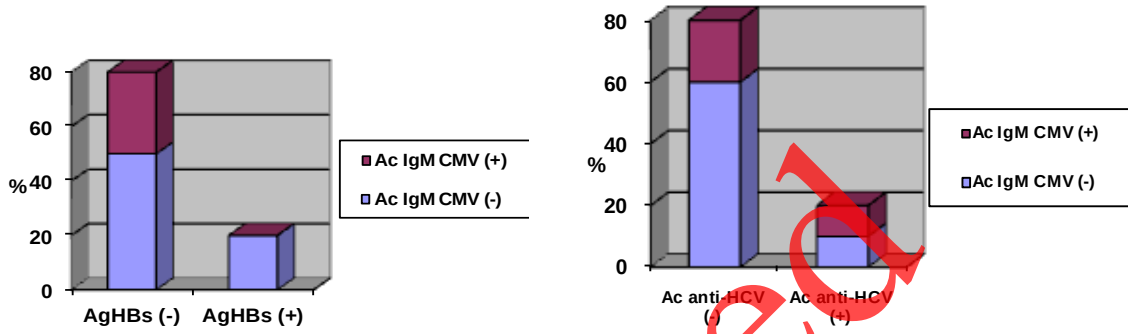

Figure 6. Group distribution based on the presence of Cytomegalovirus IgM Ac, HBsAg and anti-HCVAc.

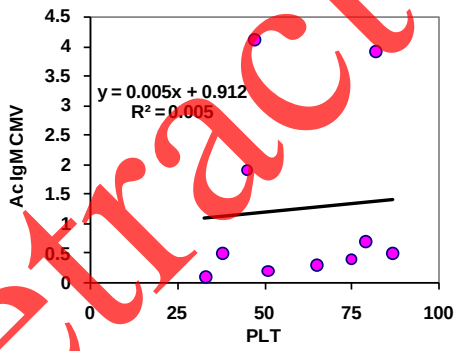

Figure 7. Correlation PC with CMV antibodies IgM.

In the medical literature is specified the involvement of toxic household agents (detergents, paints) or self-medication (non-steroidian anti-inflammatory, aspirin platelet type) in the occurrence of thrombocytopenia. The platelet count could be severely affected by poor patient compliance at home, not only because of platelet pathology diagnosed. Thus, we believe that lack of permanent surveillance of the patient at home for the duration of the study is a limitation of the research activity.

4. Conclusion

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. 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 or CMV and the anti-platelet antibodies produces the thrombocytopenia. Currently

viral and immunological tests in patients with chronic immune thrombocytopenia are essential. Antiviral efficacy was demonstrated to increase the number of platelets. Thus, the determination of etiopathogenic agents is conducive to the establishment of a very good therapeutic scheme.

References

[1] Dănăilă, C. (2004) Hematologie Generală.

[2] Harrison (2008) Principios de Medicina Interna. 15th Edition, McGraw Hill, New York, 882-884.

[3] McMillan, R. (2007) The Pathogenesis of Chronic Immune Thrombocytopenic Purpura. *Seminars in Hematology*, **44**, S3-S11. http://dx.doi.org/10.1053/j.seminhematol.2007.11.002

[4] Thota, S., Kistangari, G., Daw, H. and Spiro, T. (2012) Immune Thrombocytopenia in Adults: An Update. *Cleveland Clinic Journal of Medicine*, **79**, 641-650. http://dx.doi.org/10.3949/ccjm.79a.11027

[5] Anoop, P. (2012) Immune Thrombocytopenic Purpura: Historical Perspective, Current Status, Recent Advances and Future Directions. *Indian Pediatrics*, **49**, 811-818. http://dx.doi.org/10.1007/s13312-012-0195-1

[6] Provan, D. (2009) Characteristics of Immune Thrombocytopenic Purpura: A Guide for Clinical Practice. *European Journal of Haematology*, **71**, 8-12. http://dx.doi.org/10.1111/j.1600-0609.2008.01207.x

[7] Kuwana, M. and Ikeda, Y. (2005) The Role of Autoreactive T-Cells in the Pathogenesis of Idiopathic Thrombocytopenic Purpura. *International Journal of Hematology*, **81**, 106-112. http://dx.doi.org/10.1532/IJH97.04176

[8] Cines, D.B., Liebman, H. and Stasi, R. (2009) Pathobiology of Secondary Immune Thrombocytopenia. *Seminars in Hematology*, **46**, S2-S14. http://dx.doi.org/10.1053/j.seminhematol.2008.12.005

[9] McCrae, K. (2011) Immune Thrombocytopenia: No Longer "Idiopathic". *Cleveland Clinic Journal of Medicine*, **78**, 358-373. http://dx.doi.org/10.3949/ccjm.78gr.10005

[10] Laboratory Corporation of America. Directory of Services and Interpretive Guide (2010) Platelet Autoantibody Panel, Whole Blood. Platelet Antibody Profile, Serum. https://www.labcorp.com/wps/portal/

[11] Laborator Synevo (2010) Referintele specifice tehnologiei de lucru utilizate. https://www.synevo.ro/anticorpi-antitrombocitari-liberi-in-ser/

[12] Laborator Synevo (2010) Referintele specifice tehnologiei de lucru utilizate 2010. https://www.synevo.ro/anti-hcv/

[13] Laborator Synevo (2010) Referintele specifice tehnologiei de lucru utilizate 2010. https://www.synevo.ro/ag-hbs/

[14] Laborator Synevo (2010) Referintele specifice tehnologiei de lucru utilizate 2010. https://www.synevo.ro/anti-hiv12/

[15] Laborator Synevo (2010) Referintele specifice tehnologiei de lucru utilizate 2010. https://www.synevo.ro/citomegalovirus-anticorpi-igg/

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.