# Correlations Between Cytomegalovirus Infection and Cutaneous-Mucosal Manifestations of Immune Thrombocytopenic Purpura

OANA VIOLA BADULESCU, MAGDA BADESCU, MANUELA CIOCOIU\*, MADALINA MOCANU

Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, Department of Pathophysiology, 16 Universitatii Str., 700115, Iasi, Romania

Immune thrombocytopenic purpura is a pathological condition in which the decrease in the number of circulating platelets outlines a polymorphic picture. It takes the form of a haemorrhagic syndrome at the facial and mucosal level, manifesting usually through epistaxis, gingivorrhagia, bruising, petechial purpura. Mechanisms of disease onset and symptomatology are not fully known, yet many studies concluded that infectious factors and immunological disorders are involved in the etiopathogenesis of immune thrombocytopenic purpura. Infectious diseases with a possible etiological role in triggering a secondary thrombocytopenic syndrome are predominantly viral: hepatitis virus (B, C) infection, HIV infection, infectious infectious infection. This paper aims to highlight the involvement of Cytomegalovirus infection in the occurrence of antithrombotic antibodies and haemorrhagic manifestations of immune thrombocytopenic purpura. Determination of the anti-Cytomegalovirus viral serology was performed using the immunochemical method of electrochemiluminescence detection (ECLIA).

Keywords: purpura, thrombocytopenia, cytomegalovirus, ECLIA

Immune (idiopathic) thrombocytopenic purpura is caused by a decrease in the number of circulating platelets due to their premature peripheral hyperdestruction. The immunological mechanism involves the appearance of antibodies whose activity is targeted on the platelet membrane [1]. In 75% of patients, anti-thrombotic antibodies are directed against membrane glycoproteins (GP), especially GP IIb/IIIa and GPIb/IX, and only 25% are thought to be involved in other thrombocytopenia epitopes or thrombocytopenia occurs by secondary mechanisms. The identified antibodies were IgG and IgA in most cases (some patients presenting both types) and IgM in several situations but always associated with the other classes [2, 3].

Thrombocytopenic purpura may be primary or secondary, associated with other pathologies, particularly infectious or autoimmune ones, and the safe diagnosis remains one of exclusion. Thrombocytopenic purpura which occurs in the context of banal viral episodes or chronic viral infectious disease is a secondary form and the treatment principles are dependent on the identification of the pathogen incriminated in ethiopathogenesis [4].

In the case of Cytomegalovirus infection, immunological dosing of IgM and IgG antibodies is the most reliable method of detecting chronic or acute viral infection. The presence of anti-platelet antibodies may be correlated with anti-Cytomegalovirus antibodies, and platelet destruction may be explained by this immunological cross-reactivity mechanism [5]. Haemorrhagic clinical manifestations are usually associated by clinical expressions of related pathologies in certain forms of secondary thrombocytopenia [6].

## **Experimental part**

## Material and method

The current study is an observational one and aims to investigate the involvement of Cytomegalovirus infection in the development of antithrombotic antibodies and in the cutaneous mucosal haemorrhagic clinical picture of immune thrombocytopenic purpura. The protocol of the clinical trial was approved by the Commission of Medical Ethics of the Gr.T.Popa University of Medicine and Pharmacy Iasi and was carried out according to the amended Helsinki Declaration (Somerset West Amendment, 1996). The patients were informed of the study and their written consent was obtained. Research strictly adhered to the bioethical EU regulations.

The clinical study was carried out between 01.10.2012 and 01.10.2015, at the Haematology Clinic of the Emergency Hospital Sf. Spiridon in Iasi. The study group comprised 40 patients with immune thrombocytopenic purpura at various stages of evolution. The patients were aged 18-74 years, 25 of them female and 15 male, 30 of the patients came from the rural area and 10 from the urban area.

The patients were divided into two groups:

- asymptomatic, who came for regular analyses;

- with cutaneous-mucosal haemorrhage symptoms: bruises, petechiae, epistaxis, gingivorrhagia.

The common criterion for inclusion of patients in the study was thrombocytopenia.

The stages of the diagnostic algorithm consisted initially in the quantitative and qualitative analysis of platelets, both by counting them using an automated analyser and by microscopically examining the peripheral blood smear. Subsequently, the investigation of the etiology of peripheral thrombocytopenia detected in the first phase by antiplatelet antibody dosing was continued further. Their presence in the entire study group conduced to the investigation of mechanisms of autoimmunity. Possible causative agents of immune thrombocytopenia have been quoted in numerous studies on the cytomegalovirus, which also led in the current study to the dosing of IgG anticytomegalovirus (anti-CMV) antibodies across the study group.

The determination method used is the electrochemiluminescence detection (ECLIA) immunochemistry. Patients did not require special pre-training. The sampled

<sup>\*</sup>email: mciocoiu2003@yahoo.com

specimen was venous blood (at least 0.5 mL of serum), and the vacciner was a vacutainer without anticoagulant, with or without separating gel. The serum was separated by centrifugation and immediately processed. Otherwise, samples could have been stored for 3 days at 2-8°C or 6 months at -20°C. The reference values for the interpretation of IgG anti-Cytomegalovirus antibody results are:

< 0.5 U/mL: Negative 0.5 - 1 U/mL: Equivoque  $\ge$  1U/mL: Positive

#### **Results and discussions**

Quantitative determination of platelets and anti-platelet antibodies revealed thrombocytopenia across the study group, with a mean platelet count of 45.93 x 103 /  $\mu$ L accompanied by positive anti-platelet antibody results in 84.6% of males and 77.8% of the women included in the study. These values justify the uniformity of the study group in relation to the onset of autoimmune thrombocytopenic pathological changes.

Anti-CMV antibodies were determined across the study group to investigate the presence of Cytomegalovirus infection. Markers of chronic CMV infection occur after approximately one month of contact with the pathogen and belong to the immunoglobulin class G.

Determination of anti-CMV antibodies revealed values in the range of 0.10-6.30, with a broad variance (126%). Of the 40 tested patients, 12 had positive anti-CMV antibodies (30%), 3 (7.5%) had equivocal results, and the remaining 62.5% had anti-CMV negative antibodies.

There were no significant differences between the sexes (1.26 vs. 1.30, p = 0.949), age groups (1.29 vs 1.28, p = 0.996) or backgrounds (1.42 vs 0.89, p = 0.365) of the mean values of anti-CMV antibodies. The distribution of positivity by gender, age group and background indicates the predominance of the infection in males (30.8%) compared to females (29.6%). More subjects under 50 have positive anti-CMV antibodies (34.8%) compared to those over 50 years (23.5%). Distribution by background revealed a higher number of cases of CMV infection in urban areas (33.3%) compared to rural areas (20%). We conclude from these statistical data that CMV infection is present in 30%

of patients with thrombocytopenia, the incidence is higher in females and in subjects under 50 from urban areas (fig.1).

The correlation between anti-platelet antibodies and anti-CMV antibodies is statistically significant (p = 0.05). Thus, of the 32 patients with positive anti-platelet antibodies, 25% associated anti-CMV positive antibodies, and of the eight patients with negative anti-PLT antibody results, 50% had positive anti-CMV antibodies (fig. 2).

The correlation of haematological markers with the results of anti-CMV antibody testing revealed a poor correlation with platelet count. They have a low value below the reference limit in patients with positive antibodies (46.67 x 103  $\mu$ L), but the mean value is quite close to that calculated for cases with the same result of anti-CMV testing (54.67 x 103 $\mu$ L). This insignificant correlation could be explained by the presence of the latent virus, activation of which leads to a marked decrease in the number of platelets (table 1).

The purpose of determining anti-CMV antibodies in patients included in the study is to investigate the possible involvement of this virus in decreasing platelet counts. It is vry important to detect Cytomegalovirus infection in thrombocytopenic patients and in the perspective of drug therapy of immune thrombocytopenic purpura. It is known that immune-thrombocytopenia steroid medication has immunosuppressive effects, and on this background, latent CMV virus can be reactivated. Virus activity accentuates the decrease in platelet count by the inhibitory action that the virus exerts on the marrow [7]. Antiviral treatment is imperative in these cases, with a return to normal platelet counts after specific anti-infective treatment. At the same time, it has been demonstrated that standard therapies of purpura immune with immunoglobulins i.v. or splenectomy regains its efficacy once primary viral infection is treated with antiviral agents such as ganciclovir or valganciclovir [8]

The various theories explaining the link between CMV infection and immune thrombocytopenic purpura have been described by Crapnell who considers that the virus has direct cytotoxic action on hematopoietic cells. The virus is able to induce alterations in bone marrow stromal



Ac anti- CMV	N	Mean	Std. deviation	Std. Error	Confidence interval 95%		Min	Max	n
					-95% CI	+95% CI	1.111	IVIDA	Р
PLT x 10 <sup>3</sup> /µL									
Negative	25	44.52	26.48	5.30	33.59	55.45	11	98	0.706
Equivoque	3	54.67	24.50	14.15	-6.20	115.53	30	79	
Pozitive	12	46.67	23.84	6.88	31.52	61.81	8	82	
PDW%									
Negative	25	16.86	5.46	1.09	14.60	19.12	5.60	22.50	0.762
Equivoque	3	18.93	4.00	2.31	8.99	28.87	15.00	23.00	
Pozitive	12	16.58	3.96	1.14	14.07	19.10	9.50	21.30	0.702
MPV, fL									
Negative	25	13.44	4.54	0.91	11.56	15.31	5.70	19.80	
Equivoque	3	16.73	5.89	3.40	2.11	31.35	10.00	20.90	0.389
Pozitive	12	12.88	3.40	0.98	10.72	15.03	7.70	17.80	

Table 1DESCRIPTIVEMARKERS OFHAEMATOLOGICALMARKERSACCORDING TO THEPOSITIVITY OF ACANTI-CMV

cell function and mediate by immune means the destruction of infected medullary cells [9].

CMV infection is a known cause of morbidity and mortality in immunosuppressed patients, and in immunocompromised individuals infection is asymptomatic or manifested by a mononucleosis-like syndrome. The clinical picture consists of symptoms such as: general malaise, myalgia, headache, fever. Clinical syndromes such as pneumonia, encephalitis, retinitis, uveitis, colitis may be associated [10].

By interacting with viral antigens, blood platelets can be directly or indirectly activated as a consequence of virus activity. For example, many viral infections cause systemic inflammation, a phenomenon that induce platelet activation and shorten their life span. In addition to rhinoviruses, Influenza virus and CMV stimulate proinflammatory cytokines, especially IL-6 with noxious effects on platelets [11, 12].

Currently, most of the cases reported in literature highlight the indirect effect between cutaneous-mucosal haemorrhagic manifestations of immune thrombocytopenic purpura and the onset of CMV infection. In fact, this temporal relationship was also seen in previous studies, with thrombocytopenia manifesting several weeks after the onset of the infection. This temporal relationship appears to prove that an indirect mechanism is responsible for decreasing platelet counts. If there was a direct effect of CMV on platelets, it would be expected to be much earlier in the clinical setting. This is the reason that led us to determine in this study that IgG anti-CMV antibodies, not IgM antibodies, indicate acute viral infection [13, 14].

### Conclusions

To conclude, these study results corroborated with those already reported in literature, highlight the importance of testing anti-Cytomegalovirus antibodies in patients with thrombocytopenia, whether or not the symptoms of viral infection are present. Many authors proposed testing anti-Cytomegalovirus antibodies only in immunocompromised patients, but the increased incidence of Cytomegalovirusassociated thrombocytopenia cases in recent years demonstrates the need for antibody dosing in immunocompetent patients, a category in which the damaging effects of Cytomegalovirus on platelet life span have been demonstrated.

#### References

1.TOMIYAMA Y. Current topics in primary immune thrombocytopenia. Rinsho Ketsueki 2017;58(5): 537-541.

2. McMILLAN R. Antiplatelet antibodies in chronic immune thrombocytopenia and their role in platelet destruction and defective platelet production. Hematol Oncol Clin North Am. 2009; 23(6): 1163–1175.

3.McMILLAN R. Autoantibodies and autoantigens in chronic immune thrombocytopenic purpura. Semin Hematol. 2000; 37(3): 239-248.

4.MOCANU, M., BADESCU, M., HANCIANU, M., BADULESCU, O. Role of Immunochemical Detection Methods in the Diagnosis of Immune Thrombocytopenic Purpura, Rev. Chim. (Bucharest), **66**, no.7, 2015, p.997-1000

5.NISHIO Y, KAWADA J.I., ITO Y, HARA S. A case of refractory cytome galovirus - related thrombocytopenia that achieved complete remission without antiviral therapy. J Infect Chemother. 2018; 24(12): 995-997.

6.SIMPSON J.D., MATTHEWS G.V., BRIGHTON T.A. 8. Cytomegalovirusassociated thrombocytopenia treated with thrombopoietin receptor agonist. Intern Med J. 2016;46(9)1096-9.

7.SHRESTHA R, RONDELLI D, SHERPA M.T. Cytomegalovirus: a possible cause of persistent refractory immune thrombocytopenic purpura. J Adv Intern Med. 2014; 3(1): 42–45.

8.DiMAGGIO D, ANDERSON A, BUSSEL J.B. Cytomegalovirus can make immune purpura refractory. Br J Haematol 2009; 146(1): 104-112.

9.YENICESCU I, YETGIN S, OZYUREK E. et al. Virus-associated immune thrombocytopenic purpura in childhood. Pediatr Hematol Oncol 2002; 19(6): 433-437.

10.FLORES-CHANG B.G., ARIAS-MORALES C.E., WADSKIER F.G. et al. Immune thrombocytopenic purpura secondary to cytomegalovirus infection: a case report. Frontiers in Medicine 2015; 79(2): 1-3.

11.BOUWMANN J.J., VISSEREN F.L., BPSCH M.C. et al. Procoagulant and inflammatory response of virus-infected monocytes. EurJClinInvest 2002; 32: 759-766.

12.YEAMAN M.R. Platelets in defense against bacterial pathogens. Cell Mol Life Sci 2010; 67: 525-544.

13.NOMURAA K, MATSUMOTOB Y, KOTOURAB Y. et al. Thrombocytopenia due to cytomegalovirus infection in an immunocompetent adult. Hematology 2005; 10(5): 405-406.

14.SHIMANOVSKY A, PATEL D, WASSER J. Refractory Immune Thrombocytopenic Purpura and Cytomegalovirus Infection: A Call for a Change in the Current Guidelines. Mediterranean Journal of Hematology and Infectious Diseases 2016; 8: 1-5.

Manuscript received: 24.03.2018