American Journal of Leukemia Research

Research Article
Published: 29 Jul, 2024

Immune Thrombocytopenia with Associated Pseudothrombocytopenia

Pujol-Moix N[1,2]*, Español I[1,2,3], Soler A[1,4] and Muñiz-Díaz E[5]

[1]Platelet Pathology Unit, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

[2]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

[3]Department of Hematology, Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain

[4]Department of Hematology, Hospital Universitari Parc Taulí, Barcelona, Spain

[5]Immunohematology Laboratory, Banc de Sang i Teixits de Catalunya, Barcelona, Spain

Abstract

Immune Thrombocytopenia with associated Pseudothrombocytopenia (ITP-PTCP) is a condition combining features of both disorders. Patients exhibit low automated platelet counts which partly reflects the true thrombocytopenia intrinsic to ITP, together with the false thrombocytopenia due to the platelet clumps intrinsic to PTCP. Microscopic platelet counts are higher but, unlike pure PTCP, do not reach normal values. Patients with ITP-PTCP experience less bleeding than expected based on automated platelet counts.

We described nine patients with ITP-PTCP who met the following diagnostic criteria: 1) low automated platelet counts, 2) microscopic platelet counts at least 50% higher than automated counts, but below normal, 3) platelet clumps in blood smears.

The patients, predominantly adult women, exhibited mild or no bleeding. In six cases, the patients and/or their relatives had associated autoimmune disorders. ITP and/or PTCP were detected in the relatives of six patients. Positive platelet autoantibodies and/or cryptoantibodies were found in five patients. Automated platelet counts were consistently low, being below 50×10^9/L in seven cases. Microscopic platelet counts were at least 70% higher than automated counts. Platelet clumps were observed in all cases. Six patients displayed persistent ITP-PTCP during long-term follow-up, another patient evolved to PTCP and the other two progressed to ITP.

Our findings suggest: 1) ITP-PCTP may be more prevalent than previously recognized; 2) Patients with ITP-PTCP share characteristics with both ITP and PTCP patients; 3) The coexistence of ITP and PTCP underlines the strong relationship between these platelet disorders, implying they may represent different phases of the same process.

Keywords: Immune thrombocytopenia; Pseudothrombocytopenia; Platelet clumps; Platelet counts

OPEN ACCESS

Correspondence:
Núria Pujol-Moix, Department of Medicine, Universitat Autònoma de Barcelona, Sant Antoni M. Claret, 167, Barcelona-08025, Spain,

Received Date: *09 Jul 2024*
Accepted Date: *24 Jul 2024*
Published Date: *29 Jul 2024*

Citation:
Pujol-Moix N, Español I, Soler A, Muñiz-Díaz E. Immune Thrombocytopenia with Associated Pseudothrombocytopenia. Am J Leuk Res. 2024; 7(1): 1034.

Copyright *© 2024 Pujol-Moix N. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Introduction

Recently, we published a study on the clinical, biological, and evolutionary characteristics of 192 cases of Pseudothrombocytopenia (PTCP) [1]. In that study, we found several interrelations between PTCP and Immune Thrombocytopenia (ITP). For example, certain Autoimmune Disorders (AD) associated with PTCP were also associated with ITP, particularly autoimmune thyroid diseases. Additionally, we found an enhanced prevalence of familial ITP among individuals with PTCP compared to the general population. Platelet cryptoantibodies were detected in 24.1% of PTCP individuals, and autoantibodies with positive eluate in 14.5% of cases, sometimes together with cryptoantibodies. Furthermore, our observations revealed patients in which PTCP either preceded or succeeded ITP. Another significant finding was the simultaneous occurrence of both conditions. Patients presenting this association were excluded from the PTCP case series since we considered that ITP was the main condition, and now, they are the subject of the present study.

Patients with ITP typically present diminished platelet counts, antiplatelet autoantibodies adhered to the platelet surface, and reduced platelet survival as evidenced by kinetic studies. Clinically, varying degrees of bleeding are observed, commensurate with the severity of thrombocytopenia, and usually respond to immunosuppressive treatments, thrombopoietin receptor agonists, or

splenectomy. Patients with PTCP have reduced automated platelet counts, while microscopic counts remain within normal range, accompanied by the presence of platelet clumping in blood smears. Patients with ITP and concomitant PTCP (ITP-PCTP) exhibit mixed features of both disorders: Thrombocytopenia in automated platelet counts, higher but lower than normal microscopic platelet counts, and platelet clumping in blood smears. Bleeding manifestations in these patients are generally milder than expected based on automated platelet counts.

There is limited literature addressing the ITP-PTCP association. In 2014, Fozza et al. documented a patient of ITP wherein a PTCP component emerged during romiplostim treatment [2]. Later, Salama reported two cases of ITP complicated by PTCP [3]. All these cases displayed the aforementioned characteristics: Patients diagnosed of ITP with microscopic platelet counts significantly higher than automated counts, though not reaching normal levels, along with platelet clumps in blood smear. Prior to these publications, Forscher et al. reported a case of "pseudothrombocytopenia masking true thrombocytopenia" [4]. The patient presented an IgM-type agglutinin that was both EDTA and temperature independent, alongside numerous platelet clumps in blood smear. However, the diagnosis of PTCP cannot be ruled out in this patient since a microscopic platelet count was not performed.

The aim of the present study was to describe the clinical, biological and evolutionary characteristics of nine patients with ITP-PTCP representing the highest number of ITP-PTCP associated cases reported, to our knowledge. This could contribute to the understanding of this condition and enhance the diagnosis and monitoring of patients with ITP accompanied by PTCP. Furthermore, the deepening on the ITP-PTCP association through this study will complement existing knowledge on the various interrelations between PTCP and ITP, which were previously described in our case series on PTCP [1].

The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and was approved by the Institutional Review Board of the Hospital de la Santa Creu i Sant Pau.

Methods

Patients

Nine patients with ITP who met the diagnostic criteria for ITP-PTCP were included in the study. The diagnosis of ITP was established by: 1) low platelet counts without other identifiable causes of thrombocytopenia, and 2) fulfillment of at least one of the following additional criteria: Presence of platelet autoantibodies fixed on the platelet surface, evidence of decreased platelet survival, and/or a notable therapeutic response to ITP treatments. The diagnosis of associated PTCP was established by: 1) low automated platelet counts, 2) microscopic platelet counts at least 50% higher than automated counts, though below normal values, 3) observation of platelet clumps in blood smears.

Clinical and Laboratory evaluation

The clinical evaluation of patients involved assessing the type and severity of bleeding, detecting the presence of any associated AD, and investigating the occurrence of thrombocytopenia and/or AD in first-degree relatives.

Patients underwent general blood tests, including the screening for isolated autoimmunity biomarkers, as well as specific laboratory analyses to characterize platelet numbers and features. The detailed procedures for these specific tests were outlined in our prior study on PTCP [1]. Briefly: 1) Platelet counts conducted on automated hematology analyzers; 2) Microscopic platelet counts performed on blood smears or in a counting chamber; 3) Morphology of platelet clumps and unaggregated platelets on blood smears; 4) Measurement of platelet diameters on blood smears to determine mean platelet diameter, and percentage of giant platelets (diameter ≥ 4 μm); 5) Detection of antiplatelet autoantibodies with the immunofluorescence technique, and characterization of cryptoantibodies using different anticoagulants and temperatures [5,6].

Results and Discussion

The ITP-PTCP is not very common, yet it is more prevalent than is believed. While literature on such cases is scarce [2,3], certain publications indirectly describe this association. For instance, ITP was grouped with other associated AD in a case series of PTCP [7], and another study noted subnormal microscopic platelet counts in some PTCP patients [8] but these findings were not discussed.

From a diagnostic perspective, in patients with ITP, the presence of an associated PTCP should be suspected when a clear discrepancy arises between the severity of the thrombocytopenia on automated counts and the relatively low intensity of bleeding. In ITP with marked hemorrhagic manifestations and severe thrombocytopenia, automated and microscopic platelet counts align, both reflecting the actual low platelet number. However, in ITP with a chronic clinical course and minimal bleeding, it is not uncommon to observe some platelet clumps in blood smear which may slightly lower the automated platelet counts. To state that there is a PTCP associated with the ITP, the clumps must be extensive giving rise to a marked underestimation of the platelets in the automated counts indicating a more pronounced thrombocytopenia than the true one. Therefore, to evaluate the weight of the PCTP component, we used the relative difference between microscopic and automated platelet counts with respect to the automated count. Specifically, we defined a percentage exceeding 50% as a diagnostic criterion for ITP-PCTP together with a microscopic count below normal values. Previous reports on ITP-PCTP patients [2,3] assessed the PCTP component by conducting automated platelet counts in different anticoagulants; in the more common case of EDTA-dependent cryptoantibodies, platelet counts in citrate were higher than those obtained in EDTA and reflected the true number of platelets. However, for clinical and therapeutic monitoring of ITP-PCTP, we consider microscopic platelet counts to be more appropriate than automated counts in citrate since anticoagulant dependence may vary over time or cold agglutinins may also be present.

Recognizing the ITP-PTCP association is of great clinical importance since, similarly to PTCP [9], some patients might receive unnecessary treatments for ITP or stop receiving required therapeutic actions for other diseases, such as surgical procedures.

The main characteristics of patients with ITP-PCTP are described in Table 1. There were eight women aged between 15 and 50 alongside one 50-year-old man. The predominance of adult women aligns with previously reported data, both for ITP and PTCP [1,10,11], as well as for published patients of ITP-PTCP [2,3]. It should be notice that patients 4 and 5 were relatives, specifically mother and daughter respectively. In addition, other patients had family members with either ITP or PTCP, as described in ITP [10,11] and PTCP [1]. All

Table 1: Main characteristics of nine cases of immune thrombocytopenia associated with pseudothrombocytopenia.

Case	Age	Sex	Bleeding	Antiplatelet antibodies	Antiplatelet crypto-antibodies	Associated autoimmune disorders	Isolated autoimmune markers	Relatives with thrombocytopenia	Relatives with autoimmune disorders
1	26	F	EB	IgG		APS, AITD, Di, SLE	APA, ATA		
2	15	F		IgG + IgM	EDTA-PFA -dependent		↑ IgE, RF +	PTCP	Di
3	23	F	EB				ANA	ITP	Di, Sj, Vi
4	42	F	EB				ATA	ITP-PTCP	
5	17	F		IgG	EDTA -dependent		↑ IgE	ITP-PTCP	
6	50	F	EB, SB	IgG + IgM		AITD, SLE		ITP, PTCP	AITD
7	33	F							
8	42	F						PTCP	
9	50	M	EB	IgM	Cold agglutinins	Di			

Abbreviations: F: female; M: Male; EB: Easy Bruising; SB: Excessive Surgical Bleeding; Ig: Immunoglobulin; PFA: Paraformaldehyde; APS: Antiphospholipid Syndrome; AITD: Autoimmune Thyroid Disease; Di: Diabetes; SLE: Systemic Lupus Erythematosus; APA: Antiphospholipid Antibodies; ATA: Antithyroid Antibodies; RF: Rheumatoid Factor; ANA: Antinuclear Antibodies; PTCP: Pseudothrombocytopenia; ITP: Immune Thrombocytopenia; ITP-PCTP: ITP with associated PTCP; Sj: Sjögren syndrome; Vi: Vitiligo

Table 2: Platelet characteristics of nine cases of immune thrombocytopenia associated with pseudothrombocytopenia.

Case	Pt counts × 10^9/L, automated	Pt counts × 10^9/L, microscopic	Pt counts, difference × 10^9/L*	Pt counts, difference %*	Pt clump size	Mean Pt diameter, µm**	Giant Pt, %***
1	41	87	46	112.2	≤ 10 pt	3.11	10
2	39	82	43	110.3	≤ 10 pt	3.85	19
3	57	98	41	71.9	4-30 pt	2.96	8
4	31	86	55	177.4	5- >50 pt	2.95	5
5	45	88	43	95.6	3-15 pt	2.9	7
6	51	95	44	86.3	≤ 10 pt	2.82	4
7	43	79	36	83.7	≤ 10 pt	2.74	4
8	32	70	38	118.8	≤ 10 pt	2.22	0
9	30	75	45	150	≤ 10 pt	2.78	1
mean (SD) range	41 (9) 30-57	84 (9) 70-95	43 (5) 36-55	111.8 (33.7) 71.9-177.4	-	2.92 (0.43) 2.22-3.85	6.4 (5.7) 0-19

Abbreviations: Pt: Platelet/s; SD: Standard Deviation

*Difference between microscopic and automated Pt counts

**The values of pt diameters in healthy individuals were: Mean 2.22 µm, SD 0.16

***Giant pt was defined as this measuring more than 4 µm in diameter

the three relatives with PTCP had been included in our PTCP case series [1].

Although automated platelet counts fell below 60 × 10^9/L, or much lower in some cases, the majority of patients exhibited either mild no bleeding (Table 1). As observed in ITP and PTCP [1,11], some patients with ITP-PTCP presented with associated AD and/or isolated autoimmunity biomarkers. Furthermore, two patients had relatives with AD. The spectrum of AD types and biomarkers detected was diverse, with no clear predominance observed. Patients described by Salama also manifested associated AD, including psoriatic arthritis in case 1 and autoimmune hemolytic anemia in case 2 [3].

In five patients, we detected antiplatelet antibodies with positive eluate (Table 1). The immunoglobulin type of these antibodies was varied, with IgM and IgG plus IgM predominating. Cryptoantibodies of various types were found in three patients. Case 2 presented with EDTA-PFA (paraformaldehyde) cryptoantibodies, a rare occurrence similar to those found in her mother who had pure PTCP [1]. The patient reported by Fozza et al. had an EDTA-dependent cryptoantibody [2]. The two patients reported by Salama presented IgG-type antiplatelet antibodies, and EDTA-dependent cryptoantibodies which, in both cases, became dependent on other anticoagulants several months later [3].

Platelet counts and other platelet characteristics are exposed in Table 2. All patients exhibited automated platelet counts lower than 60 × 10^9/L, with seven of them registering counts below 50 × 10^9/L. Most patients displayed abnormal platelet volume histograms on autoanalyzer's showing similar anomalies to those found in PTCP [1]. However, unlike pure PTCP, the predominant anomaly was the flattening of the curve due to the actual decrease in platelets. Additionally, the autoanalyzer flagged several suspected anomalies, primarily indicating nonspecific irregularities in platelet population and, less frequently, platelet clumps. The mean microscopic platelet count was approximately double the mean automated count and never reached normal values (Table 2). The relative difference between microscopic and automated platelet counts exceeded consistently 70%.

Platelet clumps observed in blood smears were quite variable in size but generally comprising fewer than 10 platelets (Table 2). However, some patients presented larger platelet clumps, reaching to contain more than 50 platelets in case 4. Unaggregated platelets frequently displayed diverse morphological features, such as elongated shape, decreased granulation, and/or intraplatelet vacuoles. Moreover, an

elevated proportion of giant platelets was observed in two patients.

Because of the anomalies in platelet volume histograms from the autoanalyzer's, leading to inaccurate volume parameters, platelet size was assessed by measuring platelet diameters on blood smears. Mean platelet diameters were increased in most patients, particularly in the cases with an elevated number of giant platelets (Table 2). In ITP it is common to observe some increase in platelet size and/or number of giant platelets which has been attributed to a greater proportion of circulating young larger platelets [12]. Additionally, associated PCTP may have contributed to the large platelet size, since in pure PCTP mean platelet diameters and number of giant platelets are also increased [1].

Long-term follow-up was possible for all our patients with ITP-PCTP. In cases 2 to 7, monitored between 14 and 24 years, the ITP-PTP association persisted with minor fluctuations in automated and microscopic platelet counts as well as in the amount of platelet clumps. Patient 8, after four years, transitioned to pure PTCP and, two years later, experienced complete normalization of platelet counts. Patient 9, after eight years, lost the PTCP component and progressed to pure ITP which remained stable during the subsequent two years of follow-up. Patient 1 also transitioned to pure ITP after two years of follow-up; she evidenced poor responses to various treatments, including splenectomy, and died eight years later of pneumococcal meningitis. The patient described by Fozza et al. [2] initially was diagnosed with ITP, and showed similar low counts on different anticoagulants. After insufficient responses to different treatments, the patient's platelets normalized upon romiplostim administration. However, after eight months of optimal response, moderate thrombocytopenia recurred accompanied by numerous platelet clumps in blood smears. Platelet counts conducted in citrate were markedly higher than those obtained with EDTA, indicating a transition from ITP to ITP-PCTP. By contrast, the ITP-PCTP condition did not change in patients reported by Salama [3].

The predominance of adult women, association with AD, familial clustering in some cases and potential transitions between ITP-PCTP, ITP and PTCP are typical traits observed in patients with ITP-PCTP shared with both ITP and PTCP patients. Another similarity among the three platelet disorders is the presence of antiplatelet antibodies with positive eluate, cryptoantibodies, or both. While the characteristics of platelets and platelet clumps appear similar across all three conditions, statistical comparisons were not conducted due to the small patient sample size of ITP-PCTP series.

The findings of the present study indicated a close relationship between ITP-PTCP, ITP and PCTP, suggesting that the occurrence of one condition or another depends on varying degrees of platelet autoimmunity, as both autoantibodies and antiplatelet cryptoantibodies target antigenic epitopes of the IIb-IIIa glycoprotein complex. As we previously suggested in the study on PTCP [1], low-intensity antibodies may necessitate the presence of certain anticoagulants or temperature changes to bind to hidden antigenic epitopes, resulting in a PTCP. Stronger antibodies affecting a larger number of epitopes, including those exposed *in vivo* may lead to ITP. The coexistence of ITP and PTCP may result from antibodies of intermediate intensity.

Conclusions

– Although there are very few reports of patients with ITP associated with PTCP, we have identified nine additional cases, suggesting that this association may be more common than previously thought.

– Diagnosis of ITP - PTCP has relied on two main observations: Firstly, the discrepancy between severe thrombocytopenia and relatively mild bleeding, and secondly, the presence of platelet clumps in blood smears. A relative difference in microscopic and automated platelet counts higher than 50% have validated the diagnosis.

– Certain characteristics observed in ITP-PTCP patients coincide with those in ITP or PTCP patients including predominance of adult women, association with AD, existence of relatives with ITP or PTCP, and indistinct presence of antiplatelet antibodies and/or cryptoantibodies. Furthermore, it is not uncommon to observe patients evolving from PTCP to ITP and *vice versa*.

– The simultaneous presentation of ITP and PTCP also highlights the strong relationship between both platelet diseases suggesting that they may represent different phases of the same process. Further studies are needed to confirm or refute this hypothesis.

Acknowledgment

The authors wish to thank Clara Vosgien for professional language editing.

References

1. Pujol-Moix N, Muñiz-Díaz E, Español I, Mojal S, Soler A, Souto JC. Pseudothrombocytopenia, beyond a laboratory phenomenon: Study of 192 cases. Ann Hematol. 2023;102(6):1363-74.
2. Fozza C, Pardini S, Marras T, Longu F, Isoni A, Contini S, et al. Pseudothrombocytopenia in a patient receiving romiplostim for immune thrombocytopenic purpura. Ann Hematol. 2014;93(5):899-900.
3. Salama A. Autoimmune thrombocytopenia complicated by EDTA and/or citrate-dependent pseudothrombocytopenia. Transfus Med Hemother. 2015;42(5):345-8.
4. Forscher CA, Sussman II, Friedman EW, Solomon V, Spaet TH. Pseudothrombocytopenia masking true thrombocytopenia. Am J Hmatol. 1985;18(3):313-7.
5. von dem Borne AEGK, van der Lelie H, Vos JJE, van der Plas-van Dalen CM, Risseeuw-Bogaert NJ, Ticheler MD, et al. Antibodies against cryptantigens of platelets. Characterization and significance for the serologist. Curr Stud Hematol Blood Transfus. 1986;(52):33-46.
6. Muñiz-Diaz E, Madoz P, Pujol-Moix N, Pastoret C, Arilla M, Ibañez M, et al. Characterization of antibodies directed against platelet cryptantigens detected during the immunological study of 356 consecutive patients with presumed Autoimmune Thrombocytopenia (AITP). Transfus Med. 1995;5(3):185-91.
7. Bizzaro N. EDTA-dependent pseudothrombocytopenia: A clinical and epidemiological study of 112 cases, with 10-year follow-up. Am J Hematol. 1995;50(2):103-9.
8. Zhang L, Xu J, Gao L, Pan S. Spurious thrombocytopenia in automated platelet count. Lab Med. 2018;49(2):130-33.
9. Schuff-Werner P, Mansour J, Gropp A. Pseudo-Thrombocytopenia (PTCP). A challenge in the daily laboratory routine? J Lab Med. 2020;44(5):295-304.
10. Kühne T, Berchtold W, Michaels LA, Wu R, Donato H, Espina B, et al. Newly diagnosed immune thrombocytopenia in children and adults: A comparative prospective observational registry of the Intercontinental Cooperative Immune Thrombocytopenia Study Group. Haematologica. 2011;96(12):1831-7.

11. Díaz-Polo LE, Pujol-Moix N, Jiménez B, Canals C, Barranco-Charris E, Muñiz-Díaz E, et al. Shared Autoimmunity: A case series of 56 patients with Immune Thrombocytopenia (ITP) associated with other autoimmune disorders. OA Lib J. 2016;3(8):e2807.

12. Noris P, Biino G, Pecci A, Civaschi E, Savoia A, Seri M, et al. Platelet diameters in inherited thrombocytopenias: Analysis of 376 patients with all known disorders. Blood. 2014;124(6):e4-e10.