American Journal of Hematology 50:103–109 (1995)

EDTA-Dependent Pseudothrombocytopenia: A Clinical and Epidemiological Study of 112 Cases, With 10-Year Follow-Up

Nicola Bizzaro
Laboratory of Clinical Pathology, Ospedale Civile, S. Donà di Piave, Venice, Italy

In the past 10 years, we have observed 112 cases of EDTA-dependent pseudothrombocytopenia (PTCP) due to in vitro platelet clumping at room temperature. 93 patients had antiplatelet antibodies (48 IgM, 30 IgG, 3 IgA, and 12 had two different isotypes concomitantly). In 20% of patients, the presence of IgM antibodies characteristically accompanied platelet agglutination also at 37°C, and in citrated blood. The phenomenon was not age or sex related, nor was it associated with any particular pathology or use of specific drugs, and was present in both healthy subjects and patients with various diseases.

Flow cytofluorimetric analysis of CD5-positive B cells, which are responsible for autoantibody production, did not demonstrate any changes in the percentage and absolute number of this lymphocyte subset.

Average follow-up was 5 years (6 months–10 years); however, previous clinical records disclosed that PTCP was present for more than 15 years in four cases, and more than 20 years in three others, with no clinical manifestation of disease.

This study confirms that EDTA-dependent PTCP is a phenomenon related to the presence of natural autoantibodies with antiplatelet activity, devoid of pathological significance. Its clinical interest resides in the need for its prompt and certain recognition in order to avoid unnecessary examinations and therapeutic interventions.

The best and most rapid technique for obtaining accurate platelet counts in PTCP subjects is to collect and examine EDTA blood at 37°C; however, clumping will still be present in about 20% of these cases, and even in citrated blood. To obviate this phenomenon, blood should be collected in ammonium oxalate, and platelets counted in a Burker chamber.

Key words: pseudothrombocytopenia, EDTA, platelet clumping, antiplatelet antibodies, natural antibodies

INTRODUCTION

Patterns of pseudothrombocytopenia (PTCP) detected by automatic instruments for the analysis and counting of blood cells have gained particular attention in recent years, as the use of these machines has become rather widespread in clinical pathology laboratories. PTCP is characterized by a false reduction in the number of platelets in ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood, caused by platelet clumping at room temperature (RT) (Fig. 1A). The phenomenon is due to the presence of antiplatelet autoantibodies that recognize platelet antigens modified by or exposed (cryptoantigens) to the combined action of EDTA and low temperature on the platelet membrane glycoproteins [1–4].

To date there is no clinical evidence that correlates PTCP presence with specific disease (e.g., autoimmune diseases), situations of possible sensitization (i.e., pregnancy or transfusions), or the use of specific drugs; in addition, PTCP has not been associated with hemorrhagic diathesis or platelet dysfunction. Nonetheless, an awareness of this particular condition is necessary, because its lack of recognition may lead subjects with a normal platelet count to be considered, and at times treated, as if they were severely thrombocytopenic.

Received for publication December 13, 1994; accepted May 10, 1995.

Address reprint requests to Dr. Nicola Bizzaro, Laboratory of Clinical Pathology, Ospedale Civile, 30027 S.Donà di Piave, Venice, Italy.

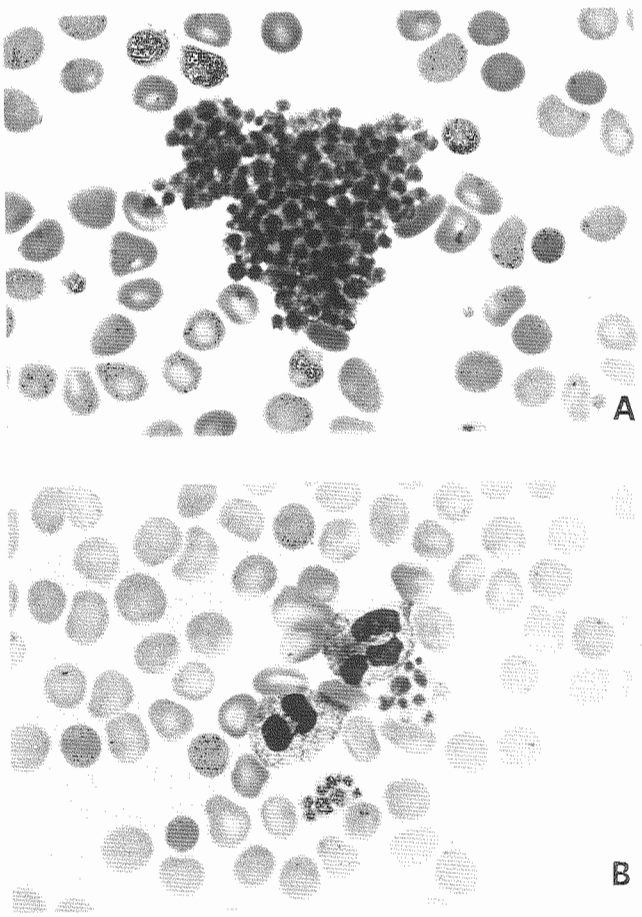

Fig. 1. Platelet clumps in EDTA-anticoagulated blood. A: Large agglutinate made up of 100 platelets (MGG, ×400). B: Some small platelet clumps the size of a leukocyte may be present at times, leading to pseudothrombocytopenia and pseudoleukocytosis (MGG, ×1,000).

Indeed, reports describe patients with PTCP who have been hospitalized and subjected to unnecessary transfusions of platelet concentrates [5,6]; others have undergone bone marrow aspiration and bone biopsy [7], while others still have been administered long-term cortisone therapy or have even been splenectomized [5,8,9]. Late of PTCP recognition may also determine a state of unjustified alarm in the patient, as well as precautionary behaviours for fear of hemorrhage due to thrombocytopenia (e.g., young people may give up sports, and elderly persons may not be administered necessary drug treatments).

The first two cases of PTCP were described in 1969 by Gowland et al. [10], who identified EDTA-dependent platelet clumping as the cause. Although this is the most frequent case, platelet clumping in vitro and consequent PTCP may also be due to improper blood sampling techniques, low anticoagulant concentrations in the test tubes [8] or, more rarely, to the presence of giant platelets (11), or platelet satellitism [12,13].

In EDTA-dependent PTCP, most of the agglutinins show maximum activity at 4–20°C (cold agglutinins). These autoantibodies may be any of the three major isotypes (IgG, IgM, or IgA) indifferently mounting κ or λ light chains [2,3,9,14]. An accurate platelet count may be obtained in this case by collecting and examining the blood sample at 37°C. However, EDTA-dependent PTCPs with agglutinins that are reactive both at room temperature and 37°C (wide heat range agglutinins) have been described [15,16], as well as cases of platelet-clumping, EDTA-independent but citrate-dependent, cold antibodies [2,17–19].

When the clumps are small, a false increase in white cells (pseudoleukocytosis) may be recorded. Indeed, platelet clumps whose size is equal to or slightly larger than a leukocyte, are counted as white cells [7,20,21] (Fig. 1B).

Mant et al. [22] in 1975, and then Manthorpe et al. [23] in 1981 first studied the frequency of the phenomenon, and reported figures of 0.3% and 1.2%, which referred, however, to a small case series. Since then many reports have followed, and this type of alteration is now familiar to the clinical pathologist; consequently, it has been evaluated more accurately, and at present its frequency is considered to be within the range of 0.09–0.11% [8,17,21].

The clinical aspects and follow-up of these patients have instead been described only sporadically. We reviewed the clinical and immunological data of 112 patients with EDTA-dependent PTCP who were seen in our hospital over a 10-year period, from 1985 to 1994. This study analyzes the clinical and epidemiological aspects, as well as the evolution of this phenomenon.

PATIENTS AND METHODS

We studied 112 subjects (43 males and 69 females, mean age 55.7 years, range 6–88 years) with EDTA-dependent PTCP. These cases were detected during routine hematological studies by the finding of a low platelet count (mean value, 72×10^9/L) in EDTA-anticoagulated blood samples at RT, using the continuous flow autoanalyzers, Technicon H6000 and H*2 (Technicon Co., Tarrytown, NY), and the appearance of a characteristic picture on the histogram indicating the presence of platelet clumping. These findings were then confirmed on May–Grünwald–Giemsa (MGG)-stained blood films, on light microscopy.

All patients with suspected PTCP were recalled in order to draw a second EDTA-anticoagulated sample under controlled technical conditions, designed to exclude that platelet clumping might have been caused by microclots due to improper methods of blood drawing and handling. If clumping was still observed on the second examination, the following protocol was carried out; an EDTA-anticoagulated sample and a serum sample were drawn at 37°C,

while a citrate-anticoagulated sample was drawn at RT. In patients who had platelet clumping in EDTA also at 37°C, platelets from capillary blood that had been anticoagulated with ammonium oxalate (Unopette Reagent System, Becton-Dickinson, Rutherford, NJ) were counted in a Burker chamber. All patients were tested more than three times after the diagnosis, and the results were invariably confirmed at subsequent follow-up evaluation.

Among our PTCP cases 76% were outpatients, and the others were hospitalized. In every case, we recorded the patient's age when the platelet clumping phenomenon had manifested or been observed for the first time. In search of a possible common cause that might explain the appearance of the phenomenon, we recorded the following: type of disease in course at the moment of testing; previous medical history; blood pressure; use of drugs; exposure to toxic substances in the work place; parity in women; blood type and transfusions, if any, and biochemical (i.e., cholesterol, triglycerides, glucose, urea), as well as hematological parameters. Every patient was tested for the presence of rheumatoid factor, cryoglobulins, and red cell cold agglutinins; quantitative immunoglobulins were also assayed. When available, previous hematological reports were examined, with reference to the moment the phenomenon appeared. In the first 34 cases, we also addressed the possible presence of PTCP in other members of the family (first-degree relatives).

Antiplatelet Antibodies

The indirect platelet immunofluorescence test (PIFT) was performed on paraformaldehyde-fixed cells according to von dem Borne et al. [24]. Briefly, normal platelets from two healthy donors with blood group O, typed for known platelet-specific antigens, were prepared by differential centrifugation, washed three times in EDTA–phosphate-buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (BSA), and then fixed in 1% (w/v) paraformaldehyde (PFA). The platelets were washed again twice, and resuspended in EDTA–PBS–BSA to a final concentration of 30×10^9/L. Platelets were then incubated with patients' sera and tested for antibody binding by indirect immunofluorescence using fluorescein isothiocyanate (FITC)-labeled polyclonal rabbit antiglobulin conjugates against human Ig, IgG, IgM, and IgA (Dakopatts, Glostrup, Denmark). After another incubation for 30 min at room temperature, the platelets were washed twice, resuspended in a mixture of glycerol-PBS, and examined at the fluorescence microscope.

Expression of CD5+ B Cells

Among the healthy subjects with PTCP, 20 were selected for flow cytometry studies of peripheral blood lymphocytes on lithium heparin anticoagulated blood, using a Cytoron cytofluorimeter (ORTHO Diagnostic, Raritan, NJ), and the standard procedure [25]. A log forward scatter versus log side scatter cytogram was used to gate the lymphocyte population. Lymphocyte gates were validated using FITC-CD45 (pan-leukocyte) and phycoerythrin (PD)-CD14 (monocyte) monoclonal antibodies. In addition to the usual B (CD19, CD20), T (CD2, CD3, CD4, CD8), and natural killer (NK) (CD16) lymphoid markers, CD5+/CD20+ cells were also analyzed by double fluorescence. A minimum of 5,000 cells per sample was analyzed.

TABLE I. Platelet Clumping*

Antiplatelet antibody isotype	EDTA-dependent at RT	EDTA-dependent at 37°C	Citrate-dependent at RT
IgG	30	2	0
IgM	48	16	14
IgA	3	1	1
IgG + IgM	9	0	0
IgG + IgA	2	0	0
IgA + IgM	1	0	0
Negative	19	3	4
Total:	112	22	19

*Antiplatelet antibody isotype distribution and presence of platelet clumping according to the anticoagulant employed and the temperature. Most patients with EDTA-reactivity at room temperature (RT) had IgM antibodies, most of which were active both at 37°C and in citrate.

RESULTS

Effect of Anticoagulants and Temperature

The average number of platelets in the 112 patients studied was 72×10^9/L (range 2.000–282.000) in the presence of platelet clumps in samples collected in EDTA at room temperature; about 30% of the patients had platelet counts below 30×10^9/L. In blood samples collected at 37°C, and in samples anticoagulated with ammonium oxalate, the increase in the number of platelets ranged from three- to 20-fold. In three cases, the platelet count at room temperature, even in the presence of platelet clumps, exceeded 200×10^9/L, and was thus normal; at 37°C, this figure exceeded 400×10^9/L.

In 22 cases (20%), the phenomenon was EDTA dependent, but clearly temperature independent, having occurred at both room temperature and 37°C; indeed, in three cases it was more pronounced at 37°C.

Platelet clumping in citrate-anticoagulated specimens at room temperature was observed in 19 patients, 18 of whom contemporaneously presented EDTA-dependent clumping both at room temperature and at 37°C. Platelet clumping in blood samples anticoagulated with oxalate was not observed.

Antiplatelet Antibodies

EDTA-dependent antiplatelet antibodies were demonstrated in 93 of the 112 (83%) PTCP subjects; 30 positive

sera contained platelet reactive antibodies of the IgG class, 48 IgM, and 3 IgA. Two isotypes were present contemporaneously in 12 cases: IgG and IgM in nine cases; IgG and IgA in two, and IgA and IgM in one (Table I). Citrate-dependent antiplatelet antibodies were detected in 15 cases; 14 IgM, 1 IgA, and no IgG. In four cases of citrate-dependent clumping, no antibodies were detected in the patients sera. None of these antibodies showed specificity for known platelet alloantigens of the HPA system, since they were reactive with all the platelets on the test panel.

Expression of CD5+ B Cells

The lymphocyte immunophenotype in the 20 healthy subjects with PTCP was normal both percentage-wise and in absolute numbers for all the B, T, and NK markers. The percentage of CD5+ B lymphocytes ranged from 0.1% to 0.8% (normal values, 0.1–5.0%) [26].

Clinical Aspects

Among our study subjects, 54 were undergoing routine health examinations, and 49 had various nonautoimmune diseases. Only nine had an autoimmune or a lymphoproliferative disorder: two autoimmune thrombocytopenic purpura (AITP); two hyperthyroidism; one Hashimoto's thyroiditis; one Waldenström's macroglobulinemia; one IgM/κ monoclonal gammopathy of undetermined significance (MGUS); one thymoma, and one systemic lupus erythematosus (SLE). Hypergammaglobulinemia, present in 7 cases, was monoclonal in the subjects with Waldenström's macroglobulinemia and MGUS, and polyclonal in five cases with alcoholic liver cirrhosis or chronic hepatitis. Serologic tests for rheumatoid factor, cryoglobulins, and red cell cold agglutinins were negative. Biochemical profiles, blood coagulation tests, and hematological parameters (apart from platelet counts) were normal in all healthy individuals or correlated with the clinical picture in subjects with disease. In the set of cases in which we also studied first-degree relatives, no platelet clumping was observed.

Study regarding drug intake disclosed that 50.5% of patients were receiving therapy with at least one drug when first examined; the most commonly used drugs were blood pressure lowerers (diuretics, β-blockers), benzodiazepines, and oral blood glucose lowerers. Eight patients were taking oral contraceptives, and one was on long-term therapy with oral sodium warfarin for atrial fibrillation.

Age and Gender

By considering the age of the patients when PTCP was first detected, and then subdividing these ages into 10 year groups, we found that PTCP was uniformly present in every age group. Although the female/male ratio was 1.6:1, we observed a clear prevalence of females up to age 50 years, and a slight male prevalence over 50 years of age.

Follow-Up

With the exception of one patient in therapy with anti-D Ig, 111 subjects continued to present this phenomenon regularly in several subsequent control examinations. Mean follow-up was 4.7 years, with a range of 6 months to 10 years. Moreover, by examining the patients' hemograms and the clinical records, we detected the presence of thrombocytopenia (probably PTCP) up to 20 and 15 years earlier in three and four cases, respectively; all seven subjects enjoy good health.

DISCUSSION

Pseudothrombocytopenia is an immunologically mediated phenomenon caused by the presence of EDTA-dependent cold antiplatelet autoantibodies in blood that cause platelet clumping [1]. The antibodies, usually IgG or IgM, and more rarely IgA, show no platelet group specificity, and are reactive with platelets from normal individuals. They are thus probably directed against cryptic antigens, which are normally undetectable surface antigens, expressed by aged or damaged platelets [2].

Since these antibodies do not react with platelets from patients with Glanzmann's disease [1] that lack the GPIIb/IIIa complex, it was advanced that the platelet membrane glycoprotein complex IIb/IIIa might be involved in the EDTA-dependent antibody reaction. In some cases, it was shown that autoantibody specificity is directed against the gpIIb fraction [27]. This structure is normally hidden in the gpIIb/IIIa complex, but becomes accessible to the cold antibody after dissociation of the glycoprotein complex due to the chelating effect of EDTA on calcium ions [28–30], associated with the alterations in protein conformation induced by low temperature [4,27].

That EDTA might be the cause of the phenomenon is further supported by a body of convincing experimental evidence; indeed, several monoclonal antibodies directed against epitopes on the dissociated gpIIb/IIIa complex, whose binding to platelets was enhanced in the presence of EDTA, have been described [31,32].

Like EDTA, citrate also has a chelating effect on calcium ions, albeit to a lesser extent, and thus might act by modifying the platelet surface with consequent exposure of gpIIb/IIIa-associated cryptoantigens. Nonetheless, this glycoprotein complex might not constitute the only target for these antibodies, because antibodies directed against a 78-kD glycoprotein on the platelet membrane have also been described in EDTA-dependent PTCP [33]. Moreover, EDTA-dependent antibodies with a different specificity surely exist because EDTA-dependent leukocyte clumping has also been observed [34,35].

The first description of this phenomenon, and subse-

quent studies to specify its prevalence or define the underlying pathogenetic mechanisms, understandably coincide with the introduction and widespread use of automatic blood cell counters in the laboratories; the platelet clumps that form in PTCP produce warning and/or error flags that allow a diagnosis of PTCP if correctly interpreted. In this way, we identified 112 cases of EDTA-dependent PTCP during the past 10 years, out of a total of 430,000 blood counts that were performed. As our laboratory serves an area with almost 100,000 inhabitants, we can conclude that at least one blood count/resident was performed in this period. Thus, our prevalence rate would be 112/100,000, which is equal to 0.11%, similar to figures found in the United States by Payne and Pierre [8] (0.09%) and Savage [21] (0.11%), in Italy by Vicari et al. [36] (0.07%), and in Spain by Garcia Suarez et al. [17] (0.11%).

Ninety-three of the 112 subjects (83%) had detectable serum levels of antiplatelet antibodies, most of which were IgM or IgG; IgA were found in three cases. In the 22 cases in which platelet clumping was also observed at 37°C, 16 had IgM antibodies, two had IgG, and one had IgA; antiplatelet antibodies were not detectable in the other three cases.

Platelet clumping occurred in sodium citrate-anticoagulated blood in 19 cases (16.9%). Here as well, the antibodies were most frequently IgM (14 cases), with no cases of IgG, and one of IgA; in four cases, antibodies were not found. It is noteworthy that 18 of these 19 cases were also positive in EDTA at 37°C, and in 93% IgM antibodies were present, thus confirming previous reports [9] that citrate-dependent antibodies are almost always IgM.

Our findings thus show that all the major antibody classes are involved in the platelet clumping phenomenon; IgM antibodies are much more frequent than IgG, and IgA are rarely involved. When this event also occurs at 37°C (20% of our cases), IgM antibodies are almost always present. Citrate-dependent antibodies are also almost invariably IgM, and, at least in our series, are probably the same antibodies responsible for EDTA-dependent clumping at 37°C. Therefore, based on our experience, the use of citrate-anticoagulated samples is contraindicated. The most reliable and rapid method for obtaining accurate platelet counts in PTCP patients is to collect and examine EDTA blood at 37°C. On the rare cases that show clumping at 37°C as well, blood should be collected in ammonium oxalate, and platelet counted in a Burker chamber.

The meaning of antiplatelet antibody presence in both healthy subjects and persons with various diseases, however, remains to be clarified. The most likely hypothesis is that we are dealing with natural autoantibodies with no pathological significance, and a physiological role in the removal of senescent circulating platelets [2,37].

Several studies during the past 25 years have described natural autoantibodies in the sera of healthy subjects [38–44]. It was shown that natural autoantibodies, which are the product of normal germline genes [45], actively participate in the general homeostasis of the organism with the clearance of altered self-constituents [46,47]; furthermore, in normal individuals, at least 20% of all the immunoglobulins correspond to polyreactive natural autoantibodies able to recognize self-structures [47–49]. Indeed, these natural autoantibodies would be directed against self-cryptoantigens (i.e., antigens normally not present on the platelet membrane surface that are exposed following an alteration in the cell membrane). This alteration may be the result of an aging process, or, as in the case of PTCP, the product of EDTA action. Therefore, only the quantity of natural autoantibodies directed against autologous platelets would be greater in subjects with PTCP, compared to other individuals.

None of the family members of our PTCP patients showed clumping. Therefore, unlike the familial occurrence of autoantibodies in first-degree relatives of patients with autoimmune disease, there is no familial tendency for natural autoantibody occurrence; this indicates that the finding of autoantibodies in an apparently healthy subject does not mean a higher risk of the future development of an overt autoimmune condition [50,51].

As CD5+ B cells are responsible for the production of low-avidity polyautoreactive natural antibodies without antigenic challenge [47,49,52,53], we studied 20 of our healthy patients to see whether CD5+ B cell expression was increased. Very low CD5+ B-cell percentages that were less than 1% of the total lymphocytes were found, thus demonstrating that natural agglutinins are not the product of a clonal expansion.

Clinical and follow-up analyses disclosed no correlation between PTCP and state of health, or type of disease. Nonetheless, the two cases of AITP associated with PTCP are interesting, because this previously described combination [2,54], further lowers the platelet count, and presents a characteristic picture of PTCP in true thrombocytopenia [55]. This possibility should be kept in mind for a correct evaluation of the platelet count in subjects who are already thrombocytopenic.

Another interesting observation was antiplatelet activity by IgM antibodies in two patients with monoclonal gammopathy (MG). Although it was clearly demonstrated that sera from patients with MG may have antibody activity against a large number of self-antigens [56–58], cases of PTCP are extremely rare; in addition to our two patients, only one other has been described [59].

Fetomaternal incompatibility due to erythrocyte antigens occurred in a group O Rh-negative patient who had given birth to a group O Rh-positive male child. PTCP first appeared in this patient a few days after the immunoprophylactic administration of 250 μg of human anti-D immunoglobulin (Partobulin, Immuno AG, Vienna, Austria). The antiplatelet antibody involved was IgM class,

and was active both at room temperature and at 37°C, but not in citrate. This patient was checked every month, and the picture was unchanged for 5 months; after this time, it disappeared, and is still absent after 4 years. As Rh antigens are not present in the platelet membrane [60], we might have been dealing with either a cross-reaction between other erythrocytic and platelet antigens, or more likely, antiplatelet alloantibodies present in the immune serum (and, therefore, natural antibodies in the donor serum).

Also worthy of note was the case of a patient on oral anticoagulant therapy (warfarin); numerous examinations in the months preceding the start of warfarin administration documented the absence of PTCP, whose onset exactly coincided with the start of the anticoagulant therapy. Because of ethical considerations, we did not interrupt this therapy, and thus cannot determine if the PTCP, which is still present, is indeed related to it.

In reference to follow-up, it is noteworthy that in some of our patients PTCP presence was documented for years, and in most cases was considered a chronic AITP. Follow-up in four and three of these subjects exceeded 15 and even 20 years, respectively.

In one-third of healthy subjects, it was definitely determined that PTCP had not been present in previous years but had appeared suddenly with no apparent cause. Nonetheless, excluding the patient undergoing anti-D prophylaxis, after PTCP appeared, in none of our cases did it disappear with time; all subjects continue to present this phenomenon at every checkup, even if the entity of the clumping, and subsequently the platelet count, varies in intensity. Indeed, there is general agreement regarding the persistence of the phenomenon, even though none of the reported follow-up evaluations exceeds 2 years.

This study, which comprehends a vast epidemiological survey and the longest follow-up on the occurrence of EDTA-dependent PTCP, demonstrates that the phenomenon is absolutely not correlated with gender, age, and age of onset, nor a particular disease, hemostasis alterations (hemorrhage or thrombosis) or ingestion of specific drugs.

Follow-up management, which exceeds 20 years in some cases, confirms that EDTA-dependent PTCP is an in vitro phenomenon with no pathological significance and is related to the presence of antiplatelet antibodies that most likely recognize platelet membrane antigens that have been altered or exposed due to the effect of EDTA. Further studies and a greater understanding of the mechanisms that regulate natural autoantibody synthesis and function will explain other aspects of this interesting phenomenon.

REFERENCES

1. Pegels JG, Bruynes ECE, Engelfriet CP, von dem Borne AEGKr: Pseudothrombocytopenia: An immunologic study on platelet antibodies dependent on ethylenediaminetetraacetate. Blood 59:157–161, 1982.
2. von dem Borne AEGKr, van der Lelie J, Vos JJE, van der Plas-van Dalen CM, Risseeuw-Bogaert NJ, Ticheler MDA, Pegels HG: Antibodies against cryptantigens of platelets. Characterization and significance for the serologist. Curr Stud Haematol Blood Transf 52:33–46, 1986.
3. Veenhoven WA, van der Schans GS, Huiges W, Metting-Scherphuis HE, Halie MR, Nieweg HO: Pseudothrombocytopenia due to agglutinins. Am J Clin Pathol 72:1005–1008, 1979.
4. Pidard D, Didry D, Kunicki TJ, Nurden AT: Temperature-dependent effects of EDTA on the membrane glycoprotein IIb/IIIa complex and platelet aggregability. Blood 67:604–611, 1986.
5. Nilsson T, Norberg B: Thrombocytopenia and pseudothrombocytopenia: A clinical and laboratory problem. Scand J Haematol 37:341–346, 1986.
6. Berkman N, Michaeli Y, Or R, Eldor A: EDTA-dependent pseudothrombocytopenia: A clinical study of 18 patients and review of the literature. Am J Hematol 36:195–201, 1991.
7. Solanki DL, Blackburn BC: Spurious leukocytosis and thrombocytopenia. A dual phenomenon caused by clumping of platelets in vitro. JAMA 250:2514–2515, 1983.
8. Payne BA, Pierre RV: Pseudothrombocytopenia: A laboratory artifact with potentially serious consequences. Mayo Clin Proc 59:123–125, 1984.
9. Onder O, Weinstein A, Hoyer LW: Pseudothrombocytopenia caused by platelet agglutinins that are reactive in blood anticoagulated with chelating agents. Blood 56:177–182, 1980.
10. Gowland E, Kay HEM, Spillman JC, Williamson JR: Agglutination of platelets by a serum factor in the presence of EDTA. J Clin Pathol 22:460–464, 1969.
11. Kjeldsberg CR, Hershgold EJ: Spurious thrombocytopenia. JAMA 227:628–630, 1974.
12. Greipp PR, Gralnick HR: Platelet to leukocyte adherence phenomena associated with thrombocytopenia. Blood 47:513–521, 1976.
13. Bizzaro N: Platelet satellitosis to polymorphonuclears: Cytochemical, immunological, and ultrastructural characterization of eight cases. Am J Hematol 36:235–242, 1991.
14. Imai H, Nakamoto Y, Miki T, Miura AB: Pseudothrombocytopenia and IgA-related platelet agglutinin in a patient with IgA nephritis. Nephron 34:154–158, 1983.
15. Shreiner DP, Bell WR: Pseudothrombocytopenia: Manifestation of a new type of platelet agglutinin. Blood 42:541–549, 1973.
16. Chang YW: A case of thrombocytopenia. Lab Med 12:508–510, 1981.
17. Garcia Suarez J, Calero MA, Ricard MP, Krsnik I, Rus GP, Perera F, Merino JL: EDTA-dependent pseudothrombocytopenia in ambulatory patients: Clinical characteristics and role of new automated cell-counting in its detection. Am J Hematol 39:146–147, 1992.
18. Cunningham VL, Brandt JT: Spurious thrombocytopenia due to EDTA-independent cold-reactive agglutinins. Am J Clin Pathol 97:359–362, 1992.
19. Kuijpers RWAM, Ouwehand WH, Peelen W, Michiels JJ, Engelfriet CP, von dem Borne AEGKr: Thrombocytopenia due to platelet glycoprotein IIb/IIIa-reactive autoantibodies non-reactive with platelets from EDTA blood. Vox Sang 63:119–121, 1992.
20. Lombarts AJPF, De Kieviet W: Recognition and prevention of pseudothrombocytopenia and concomitant pseudoleukocytosis. Am J Clin Pathol 89:634–639, 1988.
21. Savage RA: Pseudoleukocytosis due to EDTA-induced platelet clumping. Am J Clin Pathol 81:317–322, 1984.
22. Mant MJ, Doery JCG, Gauldie J, Sims H: Pseudothrombocytopenia due to platelet aggregation and degranulation in blood collected in EDTA. Scand J Haematol 15:161–170, 1975.
23. Manthorpe R, Kofod B, Wiik A, Saxtrup O, Svehag SE: Pseudothrombocytopenia. In vitro studies on the underlying mechanism. Scand J Haematol 26:385–392, 1981.
24. von dem Borne AEGKr, Verheugt F, Oosterhof F, von Riesz E, Brutel de la Riviere A, Engelfriet CP: A simple immunofluorescence test for the detection of platelet antibodies. Br J Haematol 39:191–207, 1978.
25. Calvelli T, Denny TN, Paxton H, Gelman R, Kagan J: Guideline for flow

cytometric immunophenotyping: A report from the National Institute of Allergy and Infectious Diseases, Division of AIDS. Cytometry 14:702–715, 1993.
26. Kipps TJ, Vaughan JH: Genetic influence on the levels of circulating CD5+ B lymphocytes. J Immunol 139:1060–1064, 1987.
27. van Vliet HHD, Kappers-Klunne MC, Abels J: Pseudothrombocytopenia: A cold autoantibody against platelet glycoprotein IIb. Br J Haematol 65:501–511, 1986.
28. Howard L, Shulman S, Sadanandan S, Karpatkin S: Crossed immunoelectrophoresis of human platelet membranes. The major antigen consists of a complex of glycoproteins GPIIb and GPIIIa, held together by Ca^{++} and missing in Glanzmann's thrombasthenia. J Biol Chem 257:8331–8336, 1982.
29. Kunicki ThJ, Pidard D, Rosa JP, Nurden AT: The formation of Ca^{++}-dependent complexes of platelet membrane glycoproteins IIb and IIIa in solution as determined by crossed immunoelectrophoresis. Blood 58:268–278, 1981.
30. Ginsberg MH, Lightsey A, Kunicki ThJ, Kaufmann A, Margurie G, Plow EF: Divalent cation regulation of the surface orientation of platelet membrane glycoprotein IIb. J Clin Invest 78:1103–1111, 1986.
31. Khasperova SG, Vlasik TN, Byzova TV, Vinogradov DV, Berndt MC, Mazurov AV: Detection of an epitope specific for the dissociated form of glycoprotein IIIa of platelet membrane glycoprotein IIb-IIIa complex and its expression on the surface of adherent platelets. Br J Haematol 85:332–340, 1993.
32. Kouns WC, Wall DC, White MM, Fox CF, Jennings LK: A conformation-dependent epitope of human glycoprotein IIIa. J Biol Chem 265:20594–20601, 1990.
33. De Caterina M, Fratellanza G, Grimaldi E, Varriale V, Scopacasa F, Di Maro G, Formisano S: Evidence of a cold immunoglobulin M autoantibody against 78-kD platelet glycoprotein in a case of EDTA-dependent pseudothrombocytopenia. Am J Clin Pathol 99:163–167, 1993.
34. Bizzaro N: Granulocyte aggregation is edetic acid and temperature dependent. Arch Pathol Lab Med 117:528–530, 1993.
35. Hillyer CD, Knopf AN, Berkman EM: EDTA-dependent leukoagglutination. Am J Clin Pathol 94:458–461, 1990.
36. Vicari A, Banfi G, Bonini A: EDTA-dependent pseudothrombocytopaenia: A 12-month epidemiological study. Scand J Clin Lab Invest 48:537–452, 1988.
37. Kunicki ThJ, Newman PJ: The molecular immunology of human platelet proteins. Blood 80:1386–1404, 1992.
38. Goodwin JS, Searles RP, Tung KS: Immunological responses of healthy elderly population. Clin Exp Immunol 48:403–410, 1982.
39. Hawkins BR, O'Connor KJ, Dawkins RL: Autoantibodies in an Australian population: Prevalence and persistence. J Clin Lab Immunol 2:211–215, 1979.
40. Avrameas S: Natural autoantibodies: From "horror autoxicus" to "gnothi seauton." Immunol Today 12:154–158, 1991.
41. Cairns E, Germain J, Bell DA: The in vitro production of anti-DNA antibodies by cultured peripheral blood or tonsillar lymphoid cells from normal donors and SLE patients. J Immunol 135:3839–3844, 1985.
42. Yadin O, Sarov B, Naggan L, Shoenfeld Y: Familial studies on the occurrence of natural autoantibodies. Nat Immunol Cell Growth Regul 8:325–330, 1989.
43. Tchernia G, Morel-Kopp MC, Yvart J, Kaplan C: Neonatal thrombocytopenia and hidden maternal autoimmunity. Br J Haematol 84:457–463, 1993.
44. Morel-Kopp MC, Tchernia G, Lambert T: Autoantibodies directed against the platelet GP complex Ib/IX in normal individuals. Blood 80(10):49a, 1992.
45. Amital-Teplizki H, Lymberi P, Tomer Y, Shoenfeld Y: The idiotypic network, autoimmunity, and natural autoantibodies. In Shoenfeld Y, Isenberg DA (eds): "Natural Autoantibodies. Their Physiological Role and Regulatory Significance." Boca Raton, FL: CRC Press, 1993, pp. 175–204.
46. Grabar P: Autoantibodies and immunological theories: An analytical review. Clin Immunol Immunopathol 4:453–466, 1975.
47. Avrameas S, Dighiero G, Lymberi P, Guilbert B: Studies on natural antibodies and autoantibodies. Ann Immunol 134:103–113, 1983.
48. Berneman A, Ternynck T, Avrameas S: Natural mouse IgG reacts with self antigens including molecules involved in the immune response. Eur J Immunol 22:625–633, 1992.
49. Dighiero G, Lymberi P, Guilbert B, Ternynck T, Avrameas S: Natural autoantibodies constitute a substantial part of normal circulating immunoglobulin. Ann NY Acad Sci 475:135–145, 1986.
50. Yadin O, Sarov B, Naggan L, Slor H, Shoenfeld Y: Natural autoantibodies in the serum of healthy women—a five year follow up. Clin Exp Immunol 75:402–406, 1989.
51. Shoenfeld Y, Isenberg D (eds): "Natural Autoantibodies: Their Physiological Role and Regulatory Significance." Boca Raton, FL: CRC Press, 1993.
52. Buskila D, MacKenzie LE, Shoenfeld Y, Youinou P, Lydyard PM: The biology of CD5+ B cells. In Shoenfeld Y, Isenberg DA (eds): "Natural Autoantibodies. Their Physiological Role and Regulatory Significance." Boca Raton, FL: CRC Press, 1993, pp. 125–142.
53. Tomer Y, Shoenfeld Y: The significance of natural autoantibodies. Immun Invest 17:389–424, 1988.
54. Forscher CA, Sussman II, Friedman EW, Solomon V, Spaet TH: Pseudothrombocytopenia masking true thrombocytopenia. Am J Hematol 18:313–317, 1985.
55. Bizzaro N, Brandalise M: EDTA-dependent pseudothrombocytopenia: Association of anti-platelet and anti-phospholipid antibodies. Am J Clin Pathol 103:103–107, 1995.
56. Dighiero G, Guilbert B, Fernand JP, Lymberi P, Danon F, Avrameas S: Thirty-six human monoclonal immunoglobulins with antibody activity against cytoskeleton proteins, thyroglobulin and native DNA. Immunologic studies and clinical correlations. Blood 62:264–270, 1983.
57. Grunebaum E, Buskila D, Shoenfeld Y: Serum immunoglobulins (monoclonal gammopathies) as natural autoantibodies. In Shoenfeld Y, Isenberg DA (eds): "Natural Autoantibodies. Their Physiological Role and Regulatory Significance." Boca Raton, FL: CRC Press, 1993, pp. 81–108.
58. Watts R, Williams W, LePage S: Analysis of autoantibody reactivity and common idiotype PR4 expression of myeloma proteins. J Autoimmunol 2:689–700, 1989.
59. Hoyt RH, Durie BGM: Pseudothrombocytopenia induced by a monoclonal IgM kappa platelet agglutinin. Am J Hematol 31:50–52, 1989.
60. Dunstan RA, Simpson MB, Rosse WF: Erythrocyte antigens on human platelets: Absence of Rhesus, Duffy, Kell, Kidd and Lutheran antigens. Transfusion 24:243–246, 1984.