REVIEW ARTICLE

Pseudothrombocytopenia and other conditions associated with spuriously low platelet counts

Marco Cattaneo

Fondazione Arianna Anticoagulazione, Bologna, Italy

Correspondence: M. Cattaneo
marco.natale.cattaneo@gmail.com

Received: February 9, 2025.
Accepted: March 18, 2025.
Early view: March 27, 2025.

https://doi.org/10.3324/haematol.2024.286234

Abstract

Accurate measurements of the platelet count are necessary to diagnose thrombocytosis or thrombocytopenia correctly, gauge the severity of clinical risk and identify the most appropriate therapeutic intervention. Despite increased diagnostic accuracy with electronic counters, counting is still unsatisfactory in rare situations. Conditions causing spurious thrombocytopenia include the following. (i) Pre-analytical errors, such as difficult venipuncture, over-/under-filling of blood collection tubes, insufficient mixing of blood with the anticoagulant (EDTA), which may cause fibrin formation. (ii) EDTA-induced, temperature- and time-dependent, antibody-mediated *in vitro* platelet agglutination, with consequent reduction in the number of single platelets in the sample; this condition, referred to as pseudothrombocytopenia, is benign and does not need follow-up or medical interventions; the use of alternative *in vitro* anticoagulants does not prevent formation of agglutinates in all samples; accurate platelet counts could be obtained by testing EDTA-samples immediately after blood collection. (iii) EDTA-induced *in vitro* platelet adherence to leukocytes (platelet satellitism), caused by bridging IgG antibodies binding to GPIIb-IIIa on platelets and the Fc receptor-III on leukocytes; occasionally, leukocytes may phagocytose platelets and/or form platelet/leukocyte clumps. (iv) The presence of large/giant platelets (commonly from patients with congenital or acquired thrombocytopenia) that are not recognized as such by electronic counters, which distinguish platelets from other cells based on their smaller size. (v) Type 2B von Willebrand disease: blood from patients with this disorder may display large/giant platelets and platelet agglutinates. All the above conditions are easily identifiable by microscopic examination of anticoagulated peripheral blood smears, which is an indispensable diagnostic procedure in hematology.

Introduction

Platelets play an important role in hemostasis, as evidenced by the fact that defects in their number or function are associated with bleeding diatheses. In addition, the platelet count has important implications in the evaluation of patients with a variety of disorders. It is, therefore, extremely important for the treating physician to be able to rely on accurate estimations of the number of circulating platelets. For example, the severity of abnormalities of the platelet count guides decision-making on patients' treatment. Despite greatly improved efficiency and performance with the widespread availability of automated hematology analyzers in routine and specialized laboratories, there are still technical and biological variables that hamper their diagnostic accuracy. This review describes the most common and important conditions that are associated with inaccurate platelet counting resulting in spuriously low platelet counts.

Platelet counting techniques

Different techniques for platelet counting are available, which are illustrated very briefly here. Interested readers are referred to very good, more detailed reviews, which also provide information regarding the availability and applicability of the techniques to clinical practice.[1,2] The relative levels of performance in terms of accuracy and precision of these techniques were compared in one of these reviews[1] and are summarized in Table 1.

International Harmonization Protocol

The International Council for Standardization in Haematology (ICSH) and the International Society for Laboratory Hematology (ISLH) proposed an immunological platelet counting method as the reference (the International Harmonization Protocol). Platelets in whole blood samples diluted with phosphate-buffered saline are labeled with fluorescein isothiocyanate-labeled monoclonal antibodies against two distinct epitopes (CD41 and CD61) of the glycoprotein (GP) IIb-IIIa complex (integrin $\alpha_{IIB}\beta_3$) and analyzed by multiparameter flow cytometry. Platelet count is derived using the ratio between fluorescent platelets and the number of red blood cells, which are counted using automated analyzers.

Manual platelet counting

Although the manual method for platelet counting has been largely superseded by automatic techniques, mostly because it is time-consuming and suffers from imprecision, it is still used in laboratories without specialized equipment. In addition, it is useful in the presence of blood samples with platelets of atypical sizes, platelet clumps or platelets adhering to leukocytes. The blood sample, after 1:10 dilution and incubation in 1% ammonium oxalate (which causes red blood cell lysis), is loaded onto a Neubauer counting chamber and examined by phase-contrast microscopy.

Automatic platelet counting techniques

Impedance platelet counting

The principle of this method is based on the property of blood cells in electrolyte solutions to increase the impedance of the current, when passing through a small aperture between two electrodes, which is proportional to the volume of the cell. The first instruments required platelets to be counted in platelet-rich plasma, but more recent, technologically advanced impedance-based hematology analyzers allow platelet counting in whole blood samples. All particles with volumes within a predefined range are counted as platelets, while particles outside that range are not counted, independently of whether they are or are not platelets. Therefore, the resulting platelet count may be overestimated (due to the inclusion of non-platelet particles in the final count, such as cell debris, immune complexes, bacteria, microcytic red blood cells) or underestimated (due to the presence of very large platelets, platelet clumps or platelet adhering to leukocytes).

Optical platelet counting

Optical light scatter methods have been implemented for platelet counting. One-dimensional and two-dimensional platelet analysis instruments are available. In the two-dimensional analysis, detectors for low- and high-angle scattered light allow measurements of the platelet size and density, respectively, which facilitate the distinction between platelet and red blood cell populations. The two-dimensional counting appears to provide more accurate results in thrombocytopenic samples because, if the number of particles is too low, the data acquisition time is automatically extended, thus increasing the number of events counted.

Table 1. Accuracy and precision of six platelet counting methods.

Method	Accuracy	Precision
International harmonization protocol	High	High
Manual platelet counting	Medium	Low
Impedance platelet counting	Medium	High
Optical platelet counting	High	High
Hybrid platelet counting	High	Medium
Optical fluorescence platelet counting	High	Medium

"Low", "Medium", and "High" indicate relative levels of performance. Adapted from Chen Y, *et al.*[1]

Hybrid platelet counting

Hybrid platelet counting combines the detection of small platelets (<10 fL) by an impedance channel (which is distinct from the red blood cell detection channel) with the detection of large platelets (>10 fL) by the white blood cell differential channel, to obtain accurate platelet counting.

Optical fluorescence platelet counting

A fluorescent dye (oxazine) is used to stain mitochondrial DNA and ribosomal RNA, thus increasing the difference in fluorescence intensity between platelets and fragmented red cells, enhancing specificity. The platelet count is measured in the reticulated red channel of automated hematology analyzers. The parallel analysis of fluorescence and scattered light increases the accuracy of platelet counting, especially because it allows better discrimination between large platelets and small red blood cells.

Reference ranges for the platelet count

The reference range of the platelet count is traditionally set at 150-400x10^9/L (with some variations in the higher limit that some guidelines set at 350 or 450x10^9/L) for all subjects, based on historical studies published in the last decades of the 20th century. However, it must be emphasized that some studies showed that the platelet count is higher in women than in men and in younger subjects than in the elderly,[3] and may vary in different ethnic populations. Based on the results obtained in 40,987 subjects enrolled in population-based studies in seven areas of Italy, a sex- and age-adapted reference range has been proposed for the Italian population (Figure 1).[4] The important message of these studies is that platelet count values slightly lower than 150x10^9/L in adult subjects can be considered normal. A population-based study in France,

which enrolled 33,258 individuals, while confirming the sex-related differences, showed that the lower limit of platelet count in adult individuals was higher than in the Italian population (161 and 187x10^9/L in men and women, respectively).[5]

Conditions associated with spuriously low platelet counts

Pre-analytical errors

Several pre-analytical variables may affect the results of laboratory hematology measurements, including the platelet count.[6] Patients should rest in a seated position for at least 15 minutes before phlebotomy. Blood samples should be collected by venipuncture distant from a drip (never from a line!), with no or minimal venous stasis, in tubes containing an anticoagulant (usually ethylenediaminetetraacetic acid, EDTA). Tubes must not be over- or under-filled and must be carefully mixed with the anticoagulant by eight to ten gentle inversions. Difficult venipunctures, over-filling or insufficient inversions of the tubes may cause *in vitro* activation of coagulation, with fibrin formation, platelet activation and entrapment within the fibrin fibers, leading to underestimation of the platelet count. Simple macroscopic evaluation of the blood sample may be insufficient to detect the blood clot.

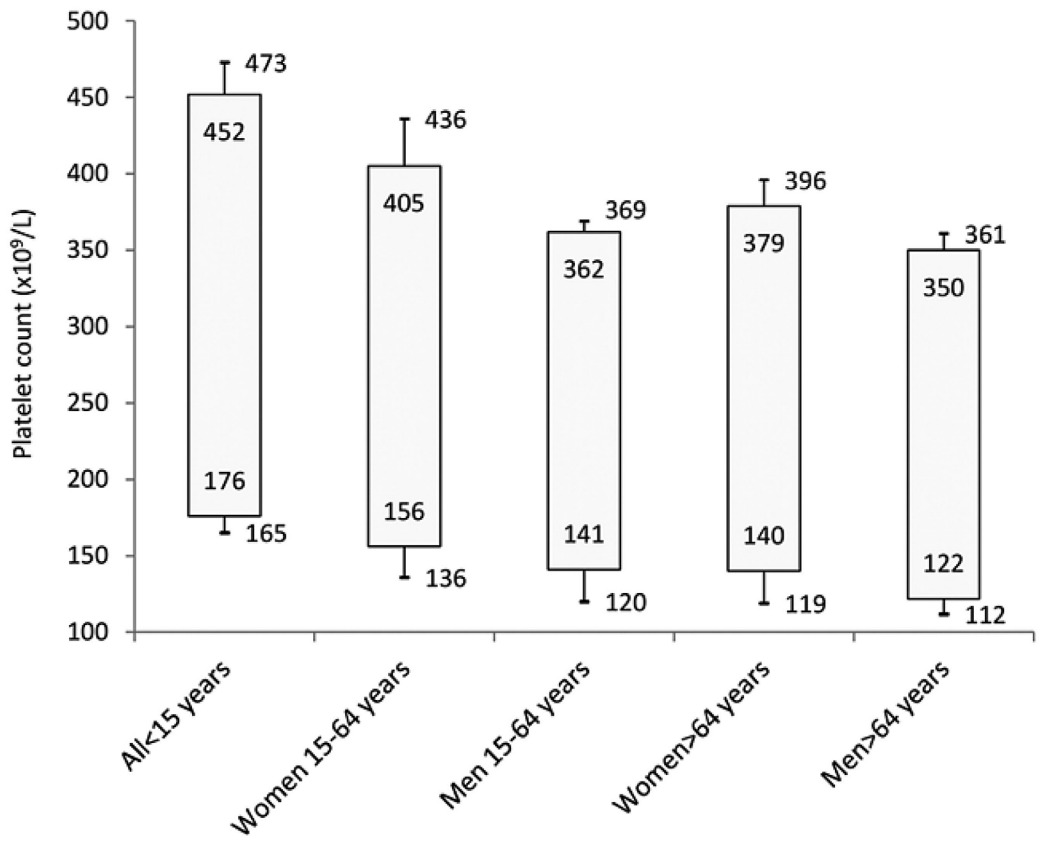

Figure 1. Proposed sex- and age-adapted platelet count reference intervals. Calculations were based on data obtained in 40,987 subjects enrolled in population-based studies in seven Italian areas. Numbers inside bars represent reference intervals estimated on the overall sample; numbers outside bars represent extended reference intervals estimated stratifying by geographical area. Reproduced from Biino G, *et al.*[4]

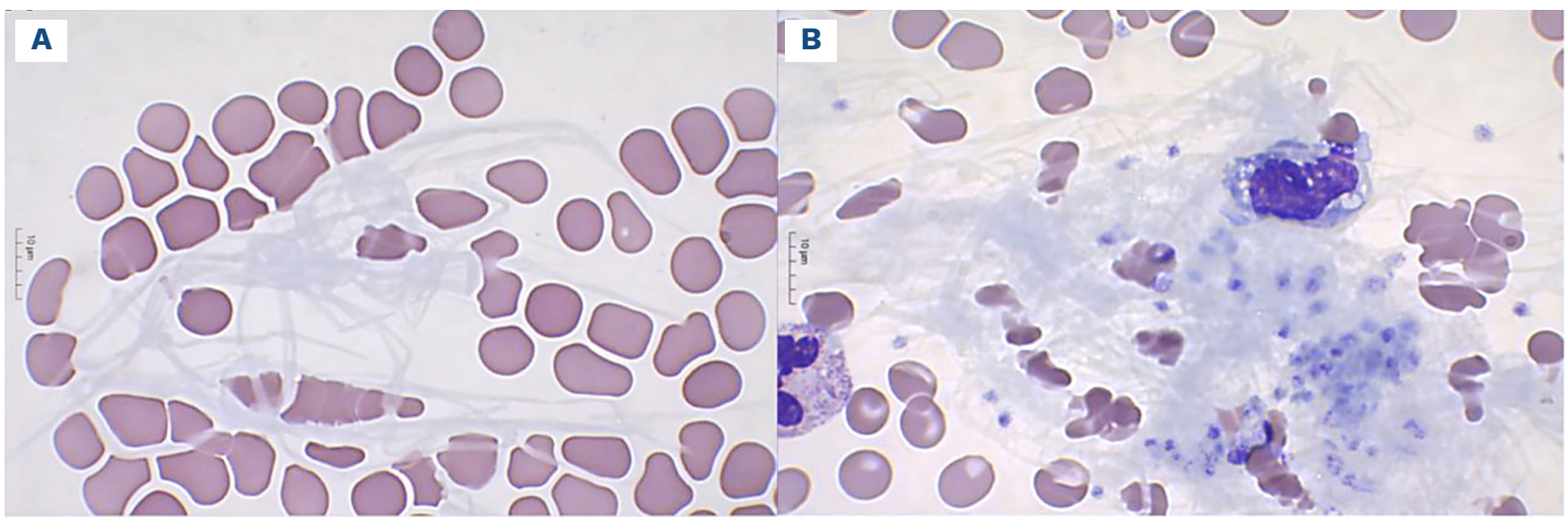

Figure 2. Fibrin strands in a peripheral blood smear formed after partial blood clotting of a blood sample. (A) Fibrin strands surrounded by red blood cells. (B) Fibrin strands entrapping platelet aggregates and white blood cells. Reproduced, with permission, from Sousa S, *et al.*[7]

Therefore, morphological examination of the blood sample film under a microscope is necessary: detection of fibrin strands with or without entrapped platelet aggregates is diagnostic of this artefactual condition (Figure 2).[7]

Pseudothrombocytopenia

Pseudothrombocytopenia (PTCP) is characterized by spuriously low single platelet counts in blood samples collected in tubes containing an anticoagulant to prevent *in vitro* blood clotting, caused by time-dependent *in vitro* formation of platelet clumps during sample storage at room temperature (Figure 3).[8-11] Although the condition is sometimes referred to as EDTA-PTCP because EDTA is the *in vitro* anticoagulant that most often causes PTCP, it is referred to simply as PTCP in this review, in order to avoid confusion potentially generated by the fact that, in a substantial percentage of cases, EDTA is not the only anticoagulant associated with platelet plug formation *in vitro*, as will be documented later in this review.

Platelet counts in PTCP samples may vary widely, from severe thrombocytopenia to mild thrombocytopenia, both among different subjects[9,10] and when tested on different occasions in the same subject.[2,12-14] The platelet count could be within the normal range in mild cases of platelet clumping[12,14] and in cases of concomitant thrombocytosis, which could be masked by PTCP.[15] Although PTCP is an artefact without clinical relevance, failure to distinguish it from real thrombocytopenia may trigger unnecessary investigations and potentially dangerous treatments.[10,14,16-19]

Epidemiology

The prevalence of PTCP in the general population is about 0.1% of all blood cell counts, with uncertain evidence of sex and age differences;[10,12-14,18-24] cases of familial PTCP have been reported.[12] The frequency of PTCP within blood samples with pathologically low platelet counts collected from outpatients varies between 15.3% and 17%, representing the second most frequent cause of detected thrombocytopenia.[16,25] The condition can be persistent or transient.[10]

Mechanism of formation of platelet clumps (platelet aggregates or platelet agglutinates?)

Platelet clumping in PTCP is induced by binding of acquired or, more likely, naturally-occurring autoantibodies against cryptic epitopes that become accessible in the presence of EDTA.[8,10,26,27] Antibodies of the IgG class are more commonly identified, followed by IgM and, very rarely, IgA. In the vast majority of cases, these antibodies behave as cold agglutinins,[28] as they bind platelets most efficiently at 4-20°C, while they are practically inactive at 37°C. In the remaining rarer cases, agglutinins (usually IgM) cause platelet clumping also at 20-37°C.[10] Serum or plasma from PTCP subjects induces platelet clumping and a decrease in single platelet counts in EDTA-anticoagulated blood samples from normal subjects,[9,10,28,29] but not from patients with Glanzmann thrombasthenia,[9,26] whose platelets do not express the GPIIb-IIIa complex. It is now well established that GPIIb-IIIa is the most common target for the agglutinins, which react with an epitope on GPIIb,[26,30] upon dissociation of the glycoprotein complex by EDTA. Other platelet antigens may rarely be targeted by the agglutinins, including a 78-kD membrane glycoprotein[31] and negatively charged membrane phospholipids.[10] The *in vitro* decrease in platelet count consequent to the formation of platelet clumps occurs in the very first minutes after incubation of the blood sample at room temperature, reaches a nadir at about 2 hours, and does not reverse spontaneously[29,32,33] or upon rewarming the blood sample at 37°C.[18,26,28,32] The addition of aminoglycoside antibiotics (such as amikacin or kanamycin) partially reverses the low platelet counts in some, but not all samples.[21,24]

It has been suggested that the platelet clumps in PTCP

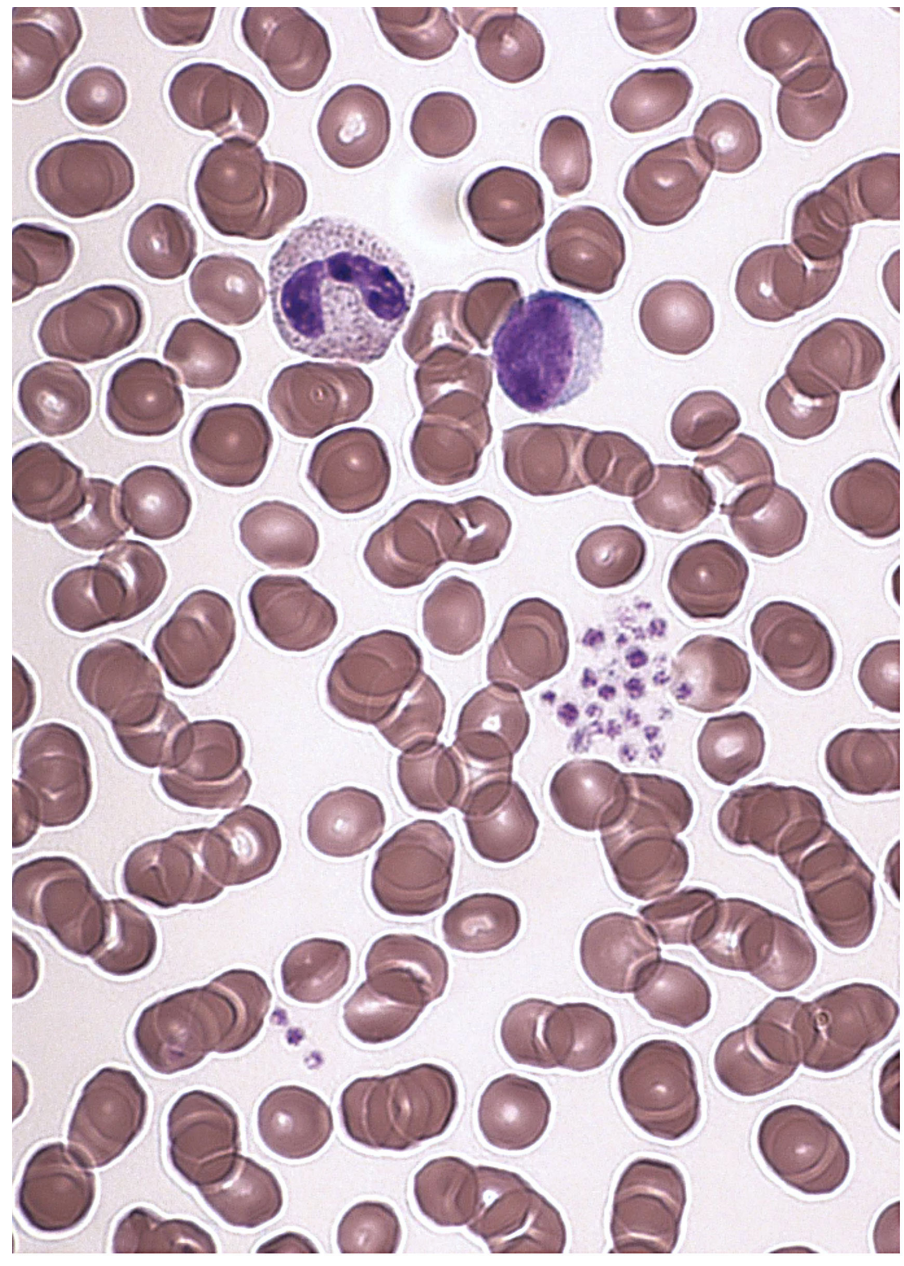

Figure 3. Platelet agglutinates in a peripheral blood smear in EDTA. Blood was taken from a patient with fever and cough. Her platelet count was $38x10^9$/L in EDTA and $176x10^9$/L in CPT. An activated lymphocyte is visible. The final diagnosis was upper airway infection. EDTA: ethylenediaminetetraacetic acid; CPT: citrate, pyridoxal 5′-phosphate, tris. Reproduced, with permission, from Nagler M, *et al.*[11]

are the result of platelet aggregation, rather than platelet agglutination. The hypothesis involving platelet aggregation was based on the finding that, in EDTA-anticoagulated blood, platelets time-dependently express markers of platelet activation, such as CD62 and CD63, which are normally associated with the membranes of intracytoplasmic platelet granules and are exposed on the plasma membrane as a consequence of activation-dependent platelet degranulation.[14,34,35] Platelet aggregation would ensue as a consequence of the action of physiological agonists (such as adenosine diphosphate, ADP), secreted by the platelet granules. However, whatever the mechanism(s) responsible for the expression of activation markers on the plasma membrane by EDTA, it must be argued that platelet aggregation cannot occur in the presence of EDTA, which inactivates and disrupts the platelet GPIIb-IIIa complex, which is essential for platelet aggregation to occur.[29,36] This interpretation is corroborated by the finding that a cold agglutinin of the IgM class, which was isolated from a patient with moderate thrombocytopenia (platelet count of about 100x10^9/L), caused degranulation of normal platelets by binding to GPVI both in EDTA- and citrate-anticoagulated blood samples, but caused an *in vitro* decrease in platelet count at 22°C accompanied by the formation of GPIIb-IIIa- and plasma fibrinogen-dependent platelet aggregates in citrate-anticoagulated, but not in EDTA-anticoagulated samples.[35,37] Indeed, citrate, being a weaker Ca^{2+} chelator compared to EDTA, does not disrupt GPIIb-IIIa and, for this reason, can be generally used as the *in vitro* anticoagulant to study platelet aggregation. Similarly, rare cases of pseudo-gray platelet syndrome or pseudo-storage pool deficiency (which mimic congenital disorders characterized by defects of platelet α-granules and/or δ-granules[38]) have been described, which are characterized by antibody-dependent *in vitro* platelet degranulation of EDTA-anticoagulated samples, usually without formation of platelet clumps and development of spurious thrombocytopenia,[39-41] unless the combined action of the antibody and EDTA also causes PTCP-like platelet agglutination.[42] In contrast, it was shown that aggregates of platelets with normal granularity formed in a citrate-anticoagulated blood sample from one of the patients with EDTA-dependent pseudo-gray platelet syndrome (Figure 4).[40] Platelet aggregates in citrate- but not in EDTA-anticoagulated blood, which possibly formed as a consequence of increased platelet activation, were observed in patients with previous episodes of arterial thrombosis.[43] Based on the aforementioned evidence, it can be safely concluded that PTCP is caused by platelet agglutination and not by platelet aggregation. This conclusion has important implications: for instance, it implies that, in order to prevent the *in vitro* formation of platelet plugs causing PTCP, it is unreasonable to use anticoagulant mixtures containing substances inhibiting platelet aggregation (such as prostaglandin E_1, adenosine, dipyridamole, pyridoxal phosphate) as alternatives to EDTA (see later).

Diagnosis of pseudothrombocytopenia

PTCP must be suspected in subjects with severely decreased platelet counts and no bleeding diathesis. In cases of mildly or moderately decreased platelet counts, which are not usually associated with an overt bleeding diathesis even in patients with true thrombocytopenia, the presence of platelet clumps in blood samples could be suspected based on specific warning flags and histogram patterns provided by electronic counters. However, the accuracy of currently available counters at detecting

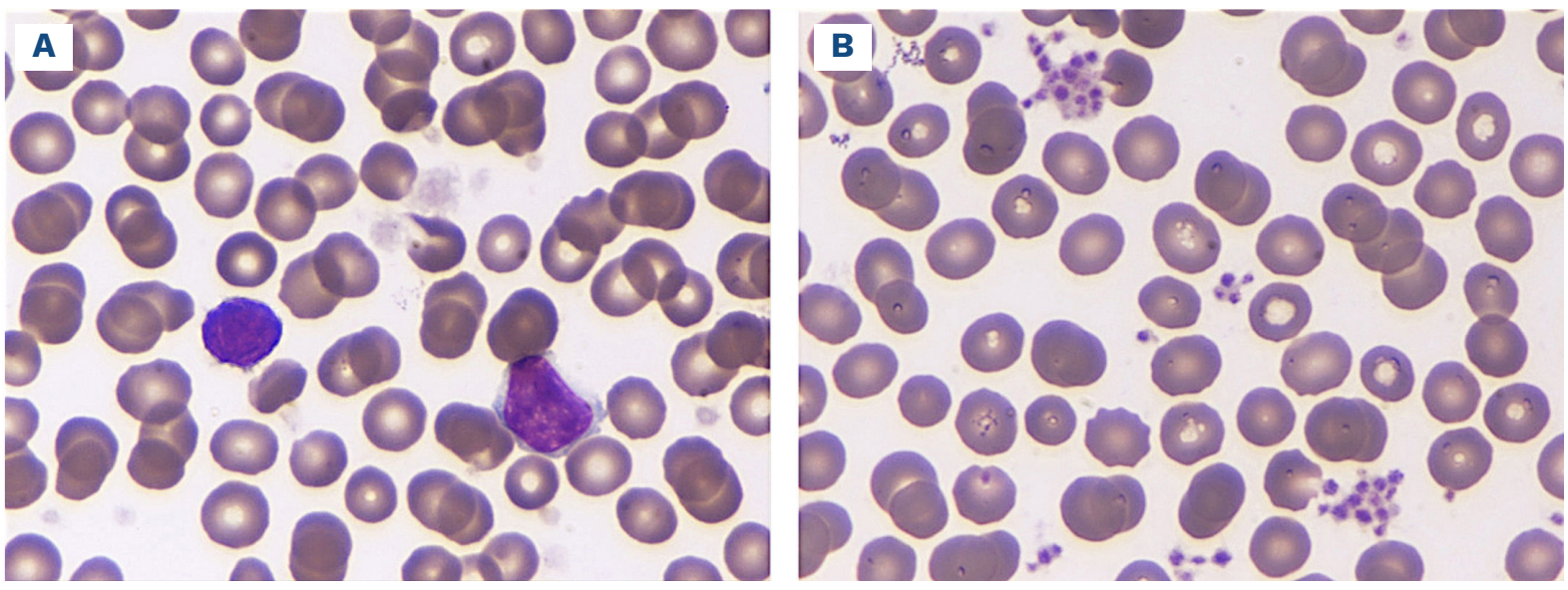

Figure 4. Peripheral blood smears from a patient with EDTA-dependent pseudo-gray platelet syndrome. Blood was taken from a 6-year-old patient with phenylketonuria. The platelet count in EDTA was 167x10^9/L but the electronic counter showed reduced refraction indicating hypogranular platelets. (A) A smear of peripheral blood anticoagulated in EDTA showed agranular and large platelets which, despite having undergone degranulation with consequent secretion of pro-aggregatory molecules such as ADP, did not form aggregates. (B) A smear from peripheral blood anticoagulated in citrate showed platelets that retained normal granularity and size, but formed small clumps. EDTA: ethylenediaminetetraacetic acid; ADP: adenosine diphosphate. Reproduced, with permission, from Pancione Y, *et al.*[40]

platelet clumps has been shown to be inadequate,[14,16,20,44-46] lacking acceptable sensitivity and specificity. For example, small platelet clumps may be mistaken for leukocytes by the counter, resulting in the lack of platelet flags and in spurious leukocytosis,[22,47] Although strategies are being implemented to increase their sensitivity,[48] the absence of platelet flags will likely never allow the certain exclusion of PTCP in a sample with a low platelet count. For these reasons, the diagnosis of PTCP must rely solely on the microscopic examination of blood smears of EDTA-anticoagulated blood samples (possibly the same one on which blood cell counts had been performed), which allows the visual detection of platelet clumps (Figure 3). After all, therefore, the diagnosis of PTCP does not require any specific diagnostic procedure in addition to those recommended for thrombocytopenia and any other kind of abnormalities in the complete blood cell count, which all require the visual inspection of blood smears as the first step in the diagnostic work-up.[49] According to the consensus of the *Groupe Francophone d'Hématologie Cellulaire*, a clump of platelets is defined as a cluster of at least five attached platelets,[2] which are usually easily detected at the margins and the tail of the blood smear. Visual inspection of blood smears carrying platelet clumps in the primary care laboratories that first identify a subject displaying a low platelet count will decrease the undue referral of patients to specialized centers for the diagnostic work-up of thrombocytopenia.[16]

Several alternative *in vitro* anticoagulants (both Ca^{2+}-chelating and non-chelating) have been proposed and tested for collecting blood samples in subjects with PTCP, aiming to avoid the occurrence of platelet clumping. Some of them have been selected for their capacity to inhibit platelet aggregation. One of these is magnesium sulphate ($MgSO_4$),[14,50] which partially inhibits platelet aggregation, probably interfering with the essential role played by Ca^{2+} in this platelet function.[29] $MgSO_4$ did indeed inhibit the full development of PTCP (Figure 5),[14,29,50] but the hypoth-

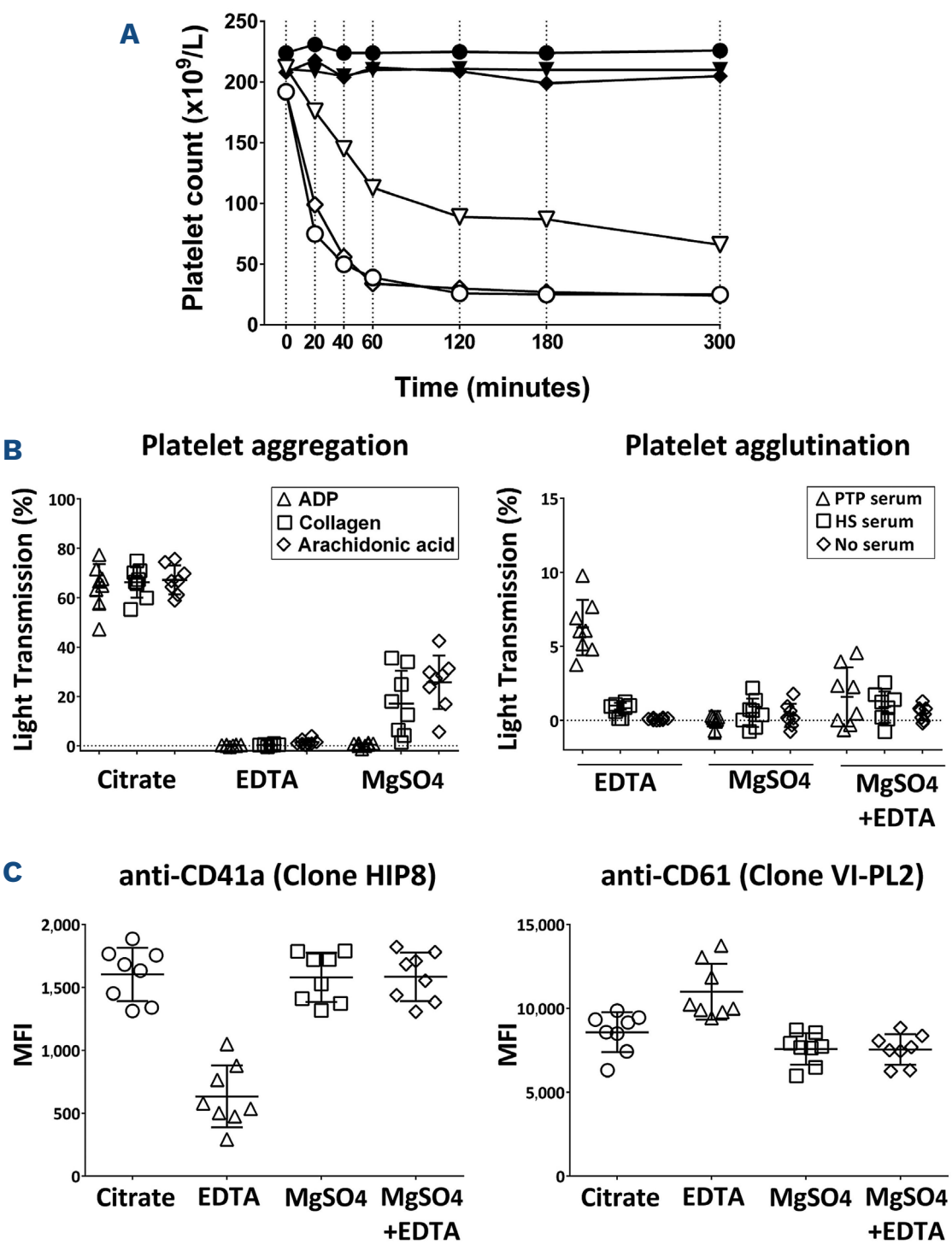

Figure 5. Effects of *in vitro* anticoagulants on the stability of platelet counts in samples from subjects with pseudothrombocytopenia, on platelet aggregation and agglutination, and on monoclonal antibodies binding to platelets. (A) Time-course of median platelet counts in whole blood anticoagulated with EDTA (open symbols) or $MgSO_4$ (closed symbols) from three subjects with pseudothrombocytopenia. (B) *Left graph*. Platelet aggregation was induced by ADP (4 μM), collagen (2 μg/mL) or arachidonic acid (1 mM) in citrate-, $MgSO_4$- and EDTA-anticoagulated normal platelet-rich plasma. *Right graph*. Platelet agglutination was measured in EDTA-, $MgSO_4$- and $MgSO_4$+EDTA-anticoagulated normal platelet-rich plasma in the presence of serum from healthy subjects, from a subject with pseudothrombocytopenia or without added serum. (C) Flow cytometric analysis of binding of FITC-monoclonal antibodies anti-CD41a (which binds to GPIIb when coupled to GPIIIa to form the GPIIb-IIIa complex) and anti-CD61 (which binds to GPIIIa independently of the integrity of the GPIIb-IIIa complex) to platelets in normal platelet-rich plasma anticoagulated by EDTA, $MgSO_4$ or EDTA+$MgSO_4$. EDTA: ethylenediaminetetraacetic acid; $MgSO_4$: magnesium sulphate; ADP: adenosine diphosphate; FITC: fluorescein isothiocyanate; GP: glycoprotein; PTP: pseudothrombocytopenia; HS: healthy subjects; MFI: mean fluorescence intensity. Reproduced from Scavone M, *et al.*[29]

esis that it did so by inhibiting platelet aggregation[14,50] is incongruous, because the anticoagulant that is most effective at inhibiting platelet aggregation *in vitro* is EDTA (Figure 5), which causes PTCP instead of preventing it, thus supporting the interpretation that platelet clumps are not platelet aggregates but, rather, platelet agglutinates. The good performance of $MgSO_4$ in avoiding PTCP[14,29,50] can be explained by the fact that it preserves the integrity of the GPIIb-IIIa complex (Figure 5), thus keeping the cryptoantigen on GPIIb inaccessible to agglutinins.

Although the use of alternative anticoagulants for collecting a second blood sample is still a common policy to test the suspected diagnosis of PTCP of an index subject, it should be abandoned, because these anticoagulants do not always prevent the occurrence of PTCP in all subjects. For instance, sodium citrate and heparin, which are the most commonly used alternative anticoagulants to EDTA because they are available in any routine laboratory, do not prevent platelet clumping in all cases of PTCP (Figure 6).[9,10,21,44,51-53] It has been suggested that the antibodies that are responsible for PTCP developing also in presence of citrate anticoagulant are of the IgM class, most likely the same as those that induce PTCP also at 37°C.[10] PTCP occurring in multiple anticoagulants at low temperatures is caused by IgM agglutinins, which have been referred to as platelet cold agglutinins.[28,54]

It must also be emphasized that, even if alternative anticoagulants could avoid PTCP in all samples, their use would be superfluous, considering the optimal diagnostic accuracy of microscopic inspection of blood smears, which should be considered the diagnostic gold-standard. However, reaching the diagnosis of PTCP does not round off the need for accurate measurement of a subject's platelet count and the assessment of potential coexistence of real thrombocytopenia. Thus, alternative anticoagulants could prove useful to fulfil this unmet need. Ghali *et al.* recently tested the stability of platelet counts in blood samples from ten subjects known to have PTCP, stored at room temperature for up to 180 minutes.[33] EDTA and three alternative anticoagulants that have been recommended for collection of PTCP blood were used: $MgSO_4$,[14] citrate-theophylline-adenosine-dipyridamole (CTAD)[45] and citrate-pyridoxalphosphate-tris (CPT).[55] The median platelet counts measured by the International Harmonization Protocol (taken as the reference standard method[1]) were similar to those measured immediately after blood sampling by a hematology analyzer in samples anticoagulated with any of the four anticoagulants. The median platelet counts in EDTA blood dropped already at the first time point (20 minutes) and reached a nadir within 120 minutes, while the median counts in the alternative anticoagulants remained stable for up to 180 minutes, confirming the efficacy of these anticoagulants at preventing full-blown PTCP (Figure 7).[33] However, a mild decrease in individual platelet counts below $150x10^9$/L was observed in one or two samples already at 20 minutes after sampling and in four or five samples at later timepoints with all the tested alternative anticoagulants, implying that none of them can be recommended in PTCP subjects for obtaining an accurate platelet count (Figure 7).[33] Alternative solutions have been proposed, which, however, may not prove effective in all blood samples, such as: (i) collecting blood by finger stick into ammonium oxalate and performing platelet counting by phase-contrast microscopy, which may prove ineffective in occasional samples;[51] (ii) warming blood samples to 37°C, which may be ineffective in the presence of antibodies of the IgM class;[10] and (iii) adding aminoglycosides to EDTA blood, which is not effective in all samples.[21,24,56] Therefore, measuring the platelet count immediately after blood collection in EDTA is probably the

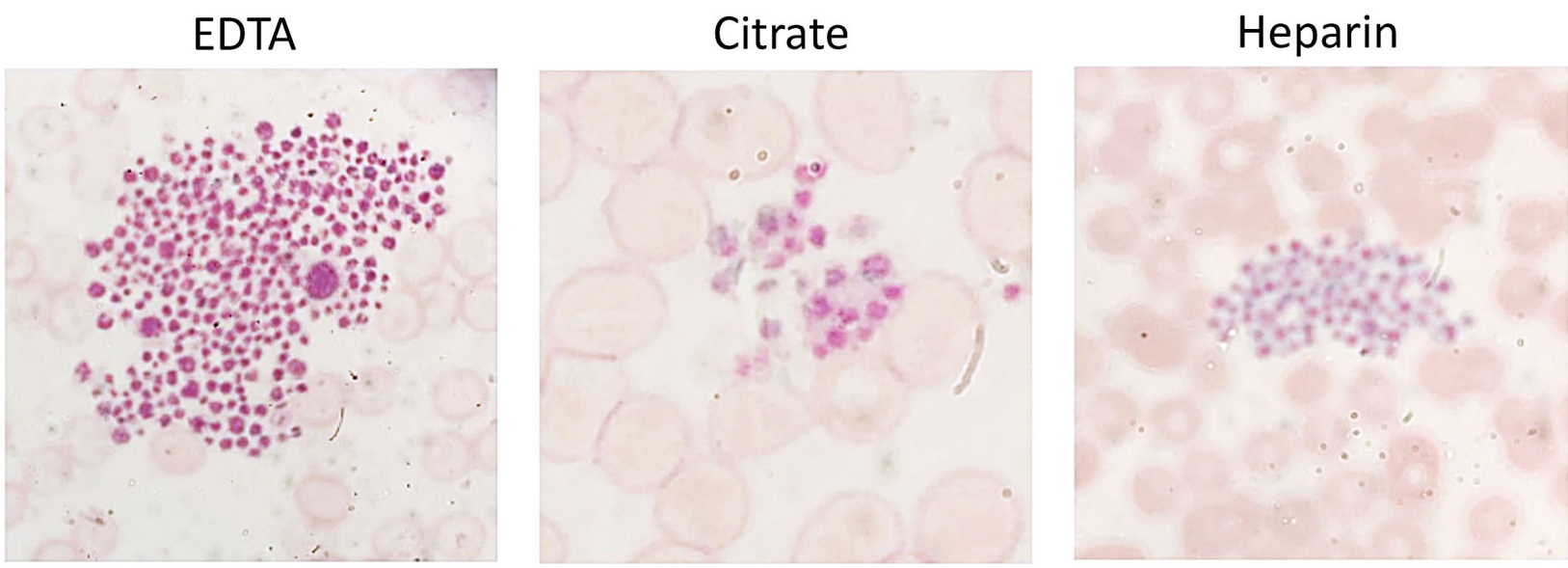

Figure 6. Peripheral blood smears in EDTA, citrate and heparin in a patient with pseudothrombocytopenia. Blood was taken from a 23-year-old female patient with viral gastroenteritis, with no bleeding manifestations. Her platelet count was $10x10^9$/L in EDTA and $53x10^9$/L in citrate, while the electronic counter was unable to report a value for a heparin-anticoagulated sample. Platelet agglutinates, diagnostic for pseudothrombocytopenia, are visible in all the blood samples, collected in different anticoagulants. EDTA: ethylenediaminetetraacetic acid. Reproduced, with permission, from Zhong L, *et al.*[53]

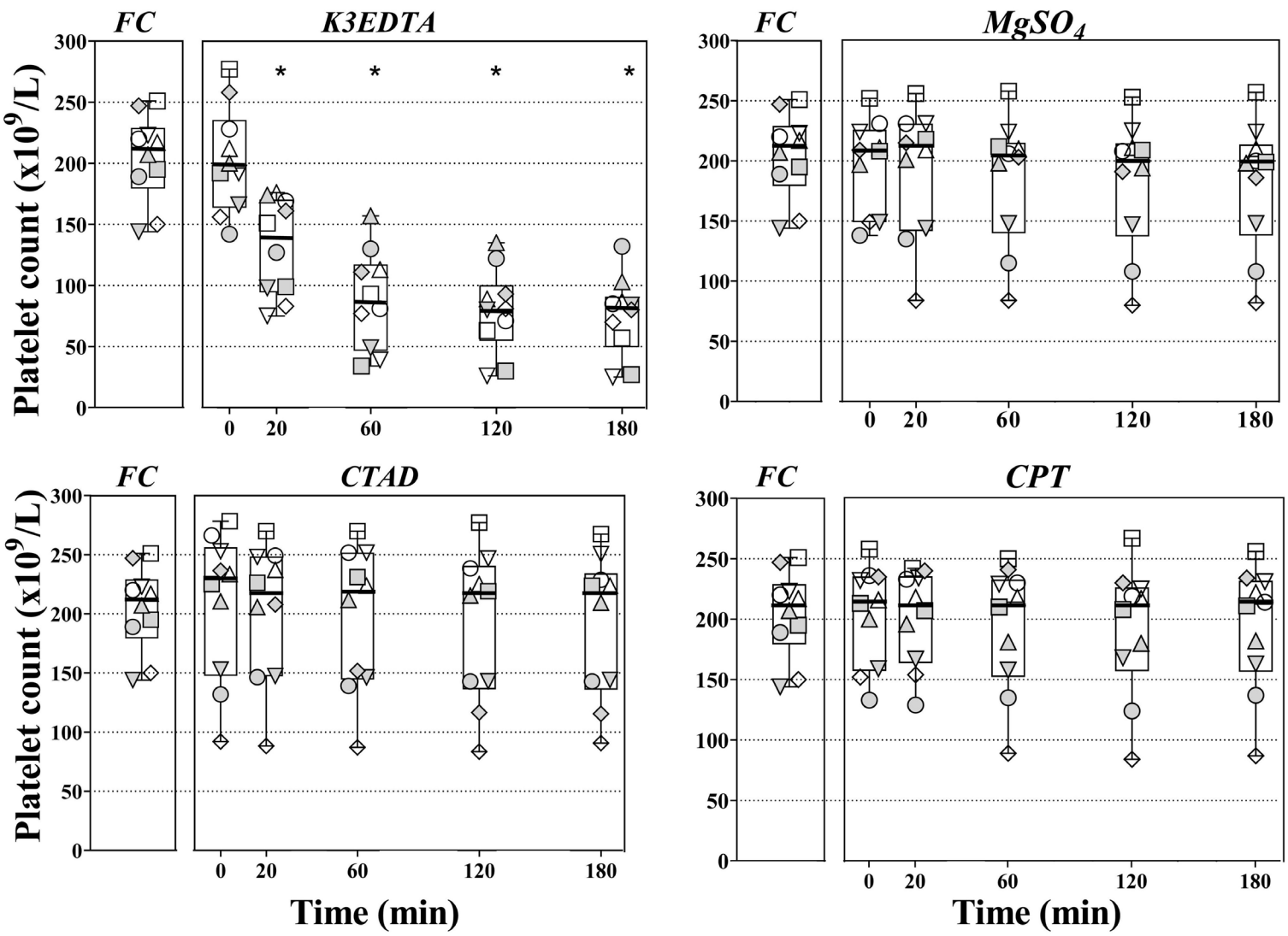

Figure 7. Time-dependent changes of platelet count in whole-blood samples from ten subjects with pseudothrombocytopenia collected in four different anticoagulants and stored at room temperature for up to 180 minutes. Flow cytometry of non-anticoagulated blood samples (first column) was chosen as the reference standard for platelet counting (International Harmonization Protocol). The median platelet counts of ten subjects with pseudothrombocytopenia obtained by a hematology analyzer at different timepoints during storage of the blood samples at room temperature were compared with those measured immediately after sampling (Time 0): statistically significant differences are labeled with (*) (P<0.05). A drop in platelet count below 150x10^{9}L was observed in individual samples anticoagulated with $MgSO_4$, CTAD or CPT, despite the stability of the corresponding median values. FC: flow cytometry; K3EDTA: tripotassium salt of ethylenediaminetetraacetic acid; $MgSO_4$: magnesium sulphate; CTAD: citrate-theophylline-adenosine-dipyridamole; CPT: citrate-pyridoxalphosphate-tris; min: minutes. Reproduced, with permission, from Ghali C, *et al.*[33]

best and simplest strategy to obtain accurate platelet counts in these subjects.[33,57]

Clinical aspects

PTCP is not associated with an increased risk of bleeding, thrombotic events or other relevant clinical complications: no clinical manifestation of disease occurred after up to 10 years (average 4.7 years) of follow-up of 112 PTCP cases, suggesting that they do not need to be followed up with any kind of specific clinical or laboratory monitoring.[10] However, in a retrospective case-control study of 104 PTCP and 208 control subjects, not well balanced in terms of prevalence of neoplastic diseases which were twice as common among the PTCP cases, the mortality rate at a median of 208 days of follow-up was significantly higher at multivariate analysis among PTCP subjects.[58]

PTCP has been described in healthy subjects but also in patients with a vast variety of pathological conditions, including neoplastic, inflammatory, autoimmune, metabolic and infectious diseases. In general, it appears that the association of PTCP with the aforementioned heterogeneous disorders is casual and explicable by the need of affected patients to be repeatedly monitored by laboratory tests, including blood cell counts, thus increasing the chances of detecting this rare condition. However, a specific association with autoimmune disorders was highlighted in a study of 192 patients with PTCP, suggesting that the condition can be assimilated to polyautoimmunity syndromes,[12] but this association was not documented in other reports.[10] One report described a high percentage (35%) of PTCP among patients with myeloproliferative neoplasms, representing a relevant confounding factor for monitoring the effects of cytotoxic treatment in these patients.[59]

Transplacental transfer of a platelet agglutinin from a mother causing PTCP in the newborn has been documented,[60,61] while no transfer from plateletpheresis donors to their recipients has been observed.[62]

EDTA- and time-dependent *in vitro* decreases in platelet

counts were observed more frequently among patients with real thrombocytopenia than among healthy control subjects after storing samples at room temperature for 180 minutes.[57,63] The extent of the observed *in vitro* platelet count decrease would have artefactually switched 16% of thrombocytopenic patients to a higher bleeding-risk category, compared to baseline, as estimated based on the severity of thrombocytopenia.[63] The use of alternative anticoagulants attenuated, but did not completely abolish the *in vitro* decrease in platelet count in thrombocytopenic patients,[63] which was associated with the appearance of platelet clumps in the peripheral blood.[57] Patients with PTCP concomitant with real thrombocytopenia have been reported.[64-66] This observation, together with the risk of underestimating the platelet count in patients with congenital or acquired macrothrombocytopenia (see later), highlights the importance of being aware of potential underestimation of the platelet count in patients with real thrombocytopenia, which might lead to overestimation of their bleeding risk and to unjustified adoption of therapeutic interventions aimed at reducing this risk.

Although cases of EDTA-PTCP have been described in association with the administration of drugs such as valproic acid, insulin, antibiotics, chemotherapeutic agents,[17] clear evidence of a causal association is lacking. In contrast, convincing evidence is available for the association of PTCP with the administration of the platelet aggregation inhibitor abciximab, a human/mouse chimeric Fab fragment against the β3 subunit of the platelet fibrinogen receptor GPIIb-IIIa, approved for reducing the incidence of ischemic complications in patients undergoing percutaneous coronary interventions. A systematic review with meta-analysis of four randomized clinical trials involving 8,555 patients undergoing percutaneous coronary interventions demonstrated PTCP in 2.1% of abciximab-treated patients (117/5,476) compared to 0.6% of placebo-treated patients (17/3,079, $P<0.001$); in 14 patients, PTCP developed also in citrate anticoagulant.[67] True thrombocytopenia was also more frequent in abciximab-treated patients (205/5,476, 3.7%) than in placebo-treated patients (56/3,079, 1.8%, $P<0.001$): therefore, PTCP accounted for 36% and 23% of low platelet counts in the abciximab and placebo arms, respectively.[67] In this meta-analysis, low platelet count was defined as $<100x10^9$/L or a drop from baseline of ≥25%. In a study that adopted different criteria (platelet count $<150x10^9$/L or a drop of ≥40% from baseline) the frequencies of true thrombocytopenia and PTCP in 66 abciximab-treated patients were 12% and 27%, respectively.[68] Although true thrombocytopenia is a common complication observed also with the small molecules tirofiban and eptifibatide inhibiting GPIIb-IIIa, PTCP was documented only in association with abciximab.[69] Hypothetical mechanisms of abciximab-associated PTCP include the presence of naturally occurring anti-Fab antibodies bridging platelets, cooperation of abciximab and EDTA to induce conformational changes of GPIIb-IIIa enhancing the access of agglutinins, and abciximab itself acting as an agglutinin binding to exposed epitopes of GPIIb-IIIa. Failure to identify PTCP, distinguishing it from true thrombocytopenia, may lead to inappropriate discontinuation of antithrombotic treatment and/or transfusion of platelet concentrates, which increase the risk of ischemic complications in patients undergoing percutaneous coronary interventions.[68]

Platelet satellitism

Platelet satellitism is an *in vitro* phenomenon observed in anticoagulated blood samples and is characterized by the adhesion of platelets to the plasma membrane of white blood cells of normal subjects or also, more rarely, of patients with various diseases. Platelets adhere to polymorphonuclear neutrophils in EDTA-anticoagulated blood,[70-72] but not in other anticoagulants (Figure 8). Occasionally, platelets have been observed adhering to lymphocytes in B-cell lymphoproliferative disorders (Figure 9),[71,73] monocytes in heparin-anticoagulated blood in myeloproliferative neoplasms,[74] basophils in chronic myelocytic leukemia[75] and several types of white blood cells, independently of the type of anticoagulant (EDTA, citrate, heparin) in cuta-

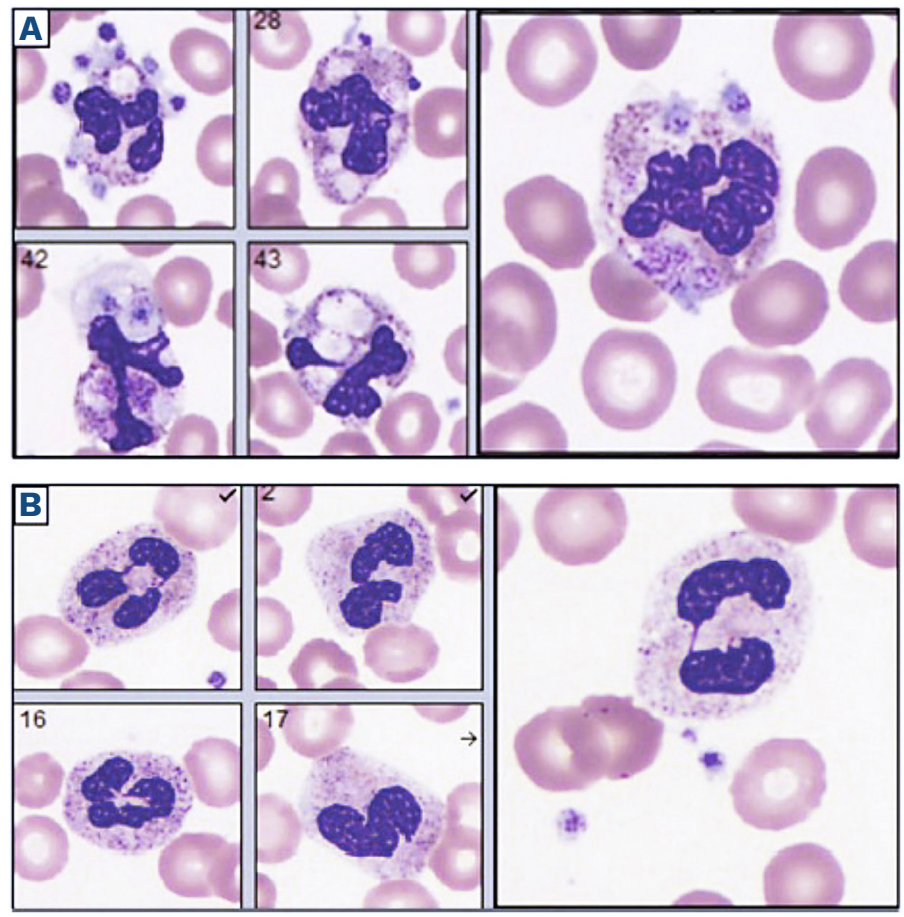

Figure 8. Blood smears in EDTA and citrate from a patient with platelet satellitism and phagocytosis. Blood was taken from a 50-year-old male patient receiving palliative chemotherapy for pancreatic adenocarcinoma and ascites. His platelet count was $83x10^9$/L in EDTA and "normal" in citrate. (A) Neutrophils with platelet satellitism and phagocytosis were observed in a peripheral blood smear in EDTA, (B) but not in citrate. EDTA: ethylenediaminetetraacetic acid. Adapted, with permission, from Sousa SM, *et al.*[72]

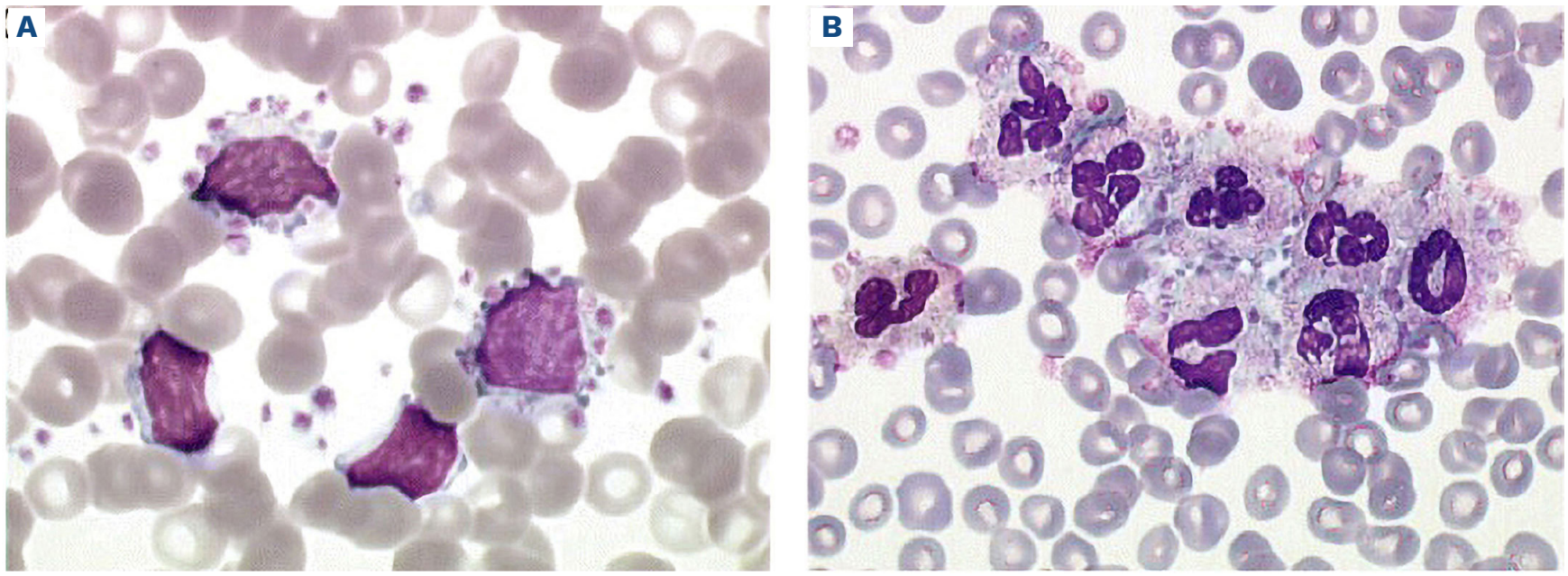

Figure 9. Blood smears in EDTA showing platelets adhering to lymphocytes and platelet-neutrophil agglutinates. (A) Platelets surrounding lymphocytes in a patient with chronic lymphocytic leukemia. (B) Platelet-neutrophil agglutinates, showing platelets bridging platelet-neutrophil rosettes, which are similar to those observed in platelet satellitism. EDTA: ethylenediaminetetraacetic acid. Reproduced, with permission, from Zandecki M, *et al.*[71]

neous vasculitis.[76] Platelet satellitism is much rarer than PTCP, with a prevalence of about 1:12,000 blood counts.[77] Its frequency, however, is most likely underestimated because it often goes unnoticed, as flagging of automatic counters is inconsistent and thrombocytopenia rarely occurs, due to the *in vitro* "consumption" of only a small fraction of platelets. Its diagnosis is necessarily based only on the examination of peripheral blood smears under light microscopy.

The proposed mechanism of platelet satellitism involves the binding of acquired or natural-occurring antibodies of the IgG class to GPIIb-IIIa on platelets and the Fc receptor III on neutrophils, thus bridging the two cells.[70,71,76,77] However, alternative mechanisms have been advocated in some cases, including binding of neutrophils to platelets expressing thrombospondin or other α-granule proteins.[78] Platelet phagocytosis by neutrophils and monocytes has been described (Figure 8).[72,79]

EDTA-dependent platelet-neutrophil agglutination

A condition similar to platelet satellitism is characterized by EDTA- and temperature-dependent platelet-neutrophil agglutination, resulting in spurious thrombocytopenia, leukopenia and alterations of the white blood cell differential.[71,80] Large hetero-agglutinates including several neutrophils and platelets (Figure 9) are formed through an as yet unknown mechanism, involving a transferable molecule in the plasma or serum of the affected subjects. The diagnosis must rely on inspection of peripheral blood smears by light microscopy.

EDTA-dependent platelet-neutrophil agglutination is an *in vitro*, EDTA-dependent artefact that should be clearly distinguished from pathological conditions characterized by the presence in circulating blood of platelet/neutrophil and/or platelet/monocyte aggregates, which are triggered by *in vivo* platelet activation, exposure of P-selectin on the platelet membrane and its binding to the P-selectin glycoprotein ligand 1 (PSGL-1) counter-receptor on the leukocyte plasma membrane.[81] These platelet/leukocyte hetero-aggregates are readily identified by whole blood flow cytometry.[81]

Presence of large/giant platelets

The presence of large (diameter >4 μm) or giant (diameter >7 μm) platelets in the circulation is a common feature of many forms of inherited thrombocytopenia,[82] myeloproliferative neoplasms, post-splenectomy states and myelodysplastic syndromes,[2] and acquired conditions associated with increased platelet destruction by immune or non-immune mechanisms,[83-85] leading to compensatory stimulation of the bone marrow to produce "stress" platelets, which differ from normal platelets by their increased size and function.[86] Morphological examination of blood smears by light microscopy is of utmost value to detect the presence of large/giant platelets (Figure 10),[82] which in some cases rosette around neutrophils (Figure 11).[87] Because electronic counters distinguish platelets from other blood cells based on their smaller size (diameter 1.5-3 μm), the platelet count of these patients may be underestimated because large/giant platelets are not recognized as such. Indeed, platelet counts in these patients measured by electronic counters tend to be lower than those measured manually by optical microscopy (Table 2),[88-91] which is the recommended method for these patients.[1]

Type 2B von Willebrand disease

von Willebrand disease (VWD) is a common congenital

bleeding disorder associated with quantitative or qualitative abnormalities of von Willebrand factor (VWF), comprising a spectrum of many subtypes.[92,93] Type 2B VWD is a rare VWD subtype characterized by gain-of-function mutations in the *VWF* gene that result in heightened interaction of 2B VWF with the platelet GPIbα, spontaneous binding of VWF to platelets, *in vivo* platelet clumping, clearance of the VWF large molecular weight multimers and mild thrombocytopenia.[92,94,95] The platelet count of patients with type 2B VWD may be underestimated because of the presence of large/giant "stress" platelets,[96-99] produced by compensatory bone marrow stimulation consequent to heightened platelet consumption *in vivo*.[86] Conditions characterized by increased synthesis or release of type 2B VWF in the circulation, such as pregnancy[100-102] and infusion of desmopressin,[103] aggravate the clinical picture. In case reports, the presence of platelet clumps in EDTA blood also contributed to underestimation of the platelet count (Figure 12).[93,96,98] Although the involvement of large/giant platelets in EDTA platelet clumps differentiates type 2B VWD from PTCP, the cornerstone of the differential diagnosis between the two conditions must rely on the presence of a bleeding diathesis, which should impose the need for further diagnostic investigations, including the measurement of plasma VWF with immunological and functional tests and determination of the composition of the VWF multimers.

Conclusions

It is of fundamental importance to identify conditions that are associated with spuriously low platelet counts in order to avoid unnecessary, expensive and/or potentially dangerous diagnostic and therapeutic interventions. Four important recommendations can be made in order to reach

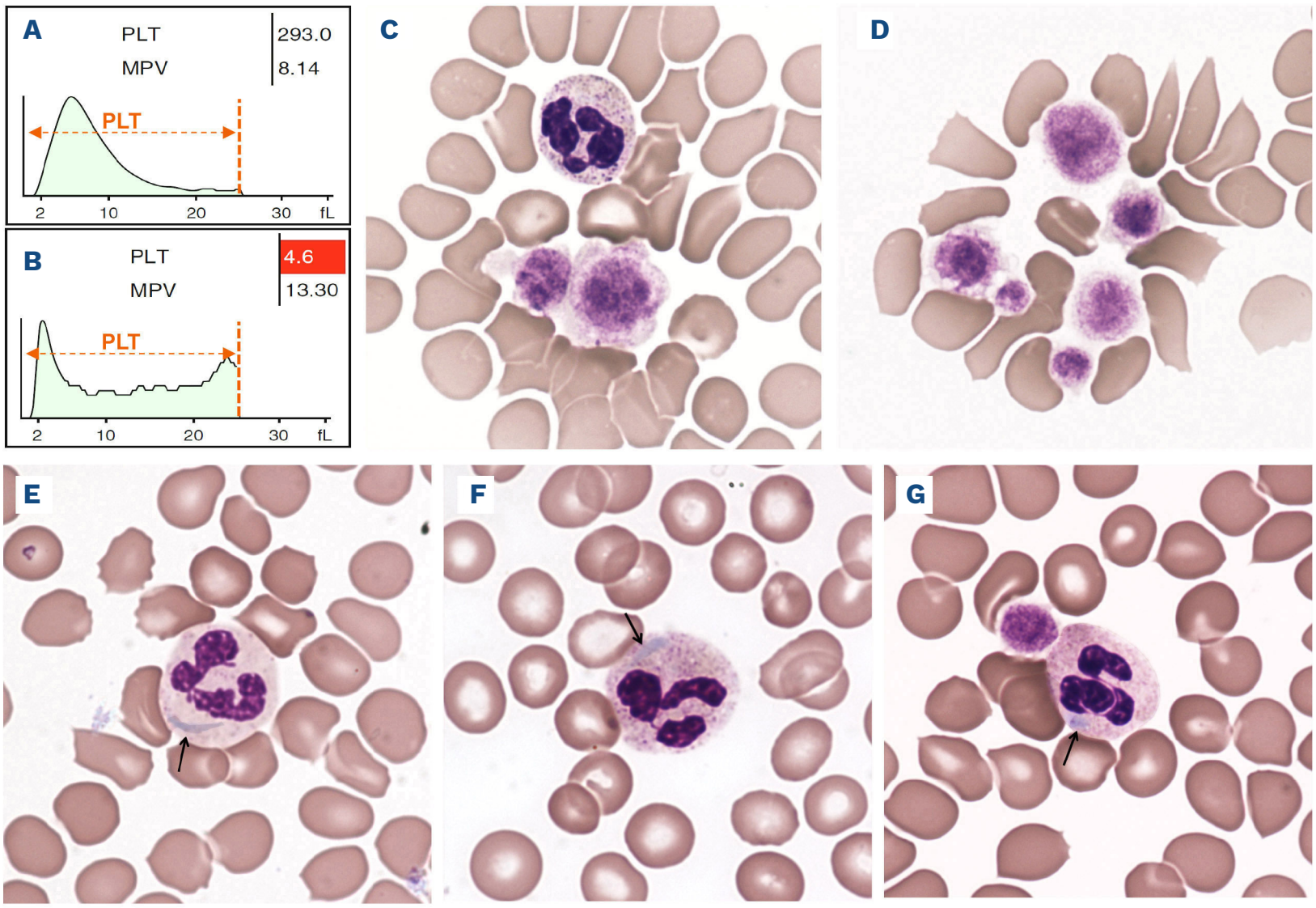

Figure 10. Platelet histogram and peripheral blood smears from patients with inherited macrothrombocytopenia. (A, B) Platelet histograms of a normal subject (A) and a patient with *MYH9*-related macrothrombocytopenia (B). The shape of the patient's histogram indicates the presence of a high percentage of large/giant platelets, some of which extended beyond the platelet size discriminator bar set at 25 fL (vertical dotted line), resulting in underestimation of the platelet count (4.6x10^9/L) compared to the manual counting by optical microscopy (32x10^9/L). Modified, with permission, from Noris P and Zaninetti C.[91] (C-G) Peripheral blood smears from a patient with the classical, biallelic form of Bernard-Soulier syndrome (C) and a patient with *MYH9*-related macrothrombocytopenia (D-G). Platelets larger than red blood cells or even neutrophils are a distinguishing feature of these platelet disorders. Clumps of MYH9 protein (Döhle-like inclusion bodies) can be typically observed (arrows) in a percentage of neutrophils of patients with *MYH9*-related macrothrombocytopenia (E-G). PLT: platelets; MPV: mean platelet volume. Reproduced, with permission, from Balduini CL, *et al.*[82]

a correct diagnosis: (i) strictly control all the pre-analytical and analytical variables of platelet counting; (ii) always consider the possibility of spurious thrombocytopenia when the bleeding history of the subject is negative, even though "true" congenital and acquired thrombocytopenias may not always be associated with a positive bleeding history;[82,104] (iii) bear in mind that spuriously low platelet counts are more frequently encountered in patients with "true" thrombocytopenia than in normal subjects, due to the presence of large/giant platelets and/or platelet agglutinates, which form more commonly than in normal subjects, thus deceptively aggravating the severity of the condition; and (iv) always examine an anticoagulated peripheral blood smear under a light microscope, which

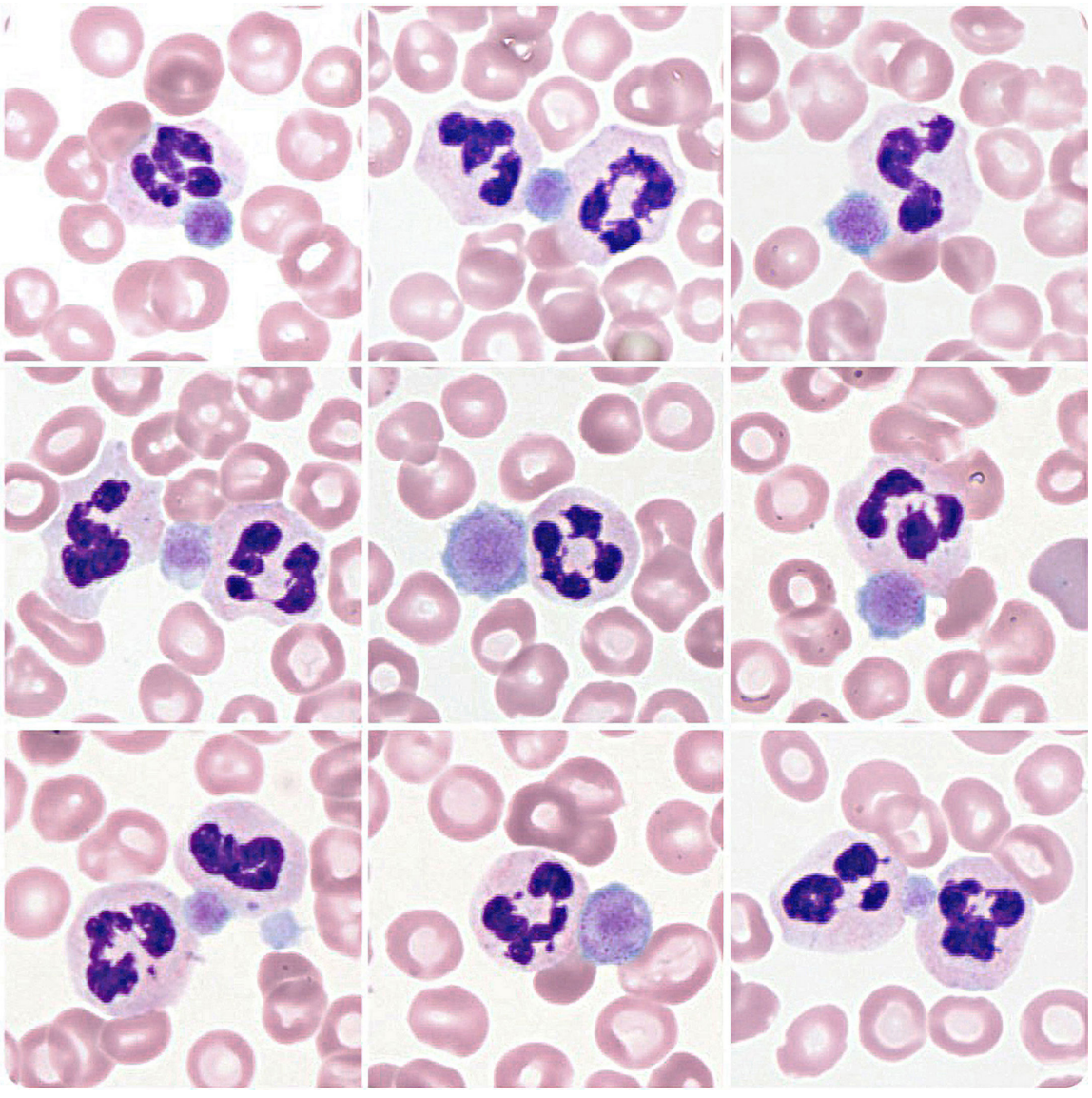

Figure 11. Platelet satellitism to neutrophils in immune thrombocytopenia. Blood was obtained during a relapse of severe thrombocytopenia ($2x10^9$/L) from a 61-year-old man who had been splenectomized 35 years previously for immune thrombocytopenia. Peripheral blood smears showed giant platelets adhering to neutrophils and near total absence of isolated platelets. Reproduced, with permission, from Markewitz RDH and Falk KK.[87]

Table 2. Electronic and manual platelet counts in patients with eight types of inherited or acquired thrombocytopenia with different platelet sizes.

Disease	Ref	N of patients	Electronic platelet count, $x10^9$/L median (IQR)	Manual platelet count, $x10^9$/L median (IQR)	MPV, fL median (IQR)	MPD, μm median (IQR)
MYH9-RD	88	17	47 (19-55)	56 (34-75)	21.2 (19.9-23.8)	4.2 (3.8-4.5)
Biallelic BSS	88	2	12 (6-18)	45 (38-52)	16.6 (16.4-16.9)	4.1 (4-4.2)
Monoallelic BSS	88	11	115 (94-139)	116 (68-144)	15.5 (14.6-16.7)	3.4 (3-3.9)
ITGA2B/ITGB3-RT	88	2	82 (48-116)	85 (56-114)	11.6 (10.2-13.0)	3.5 (3.3-3.6)
ANKRD26-RT	88	34	52 (29-73)	49 (21-62)	8.4 (7.9-9)	2.8 (2.5-3)
Unknown IT	88	12	112 (100-132)	106 (98-121)	11.6 (9.6-13.4)	3 (2.8-3.2)
ITP	88	36	40 (21-59)	44 (23-58)	11.6 (9.7-12.7)	3.1 (2.7-3.4)
Phytosterolemia	89	8	55 (16-94)	101 (68-112)	>20*	NA

*Electronic and manual platelet counts were performed in parallel in patients with phytosterolemia only if their mean platelet volume was >20 fL. Electronic platelet counts were performed in a Cell-Dyn 3700 (Abbott, Lake Forest, IL, USA), using the optical channel of the instrument, by Noris *et al.*,[88] and in either a Sysmex XE-2100 (Sysmex Limited, Milton Keynes, UK) or Bayer Advia counter (Bayer plc, Newbury, UK) by Rees *et al.*[89] Data were assembled from results shown in Tables I-III by Noris *et al.*,[88] and from Table II by Rees *et al.*[89] Ref: reference; IQR: interquartile range; MPV: mean platelet volume; MPD: mean platelet diameter; *MYH9*-RD: myosin 9-related disease; BSS: Bernard-Soulier syndrome; *ITGA2B/ITGB3*-RT: GPIIb-IIIa related thrombocytopenia; *ANKRD26*-RT: ankyrin repeat domain containing 26 related thrombocytopenia; IT: inherited thrombocytopenia; ITP: immune thrombocytopenia; NA: not available.

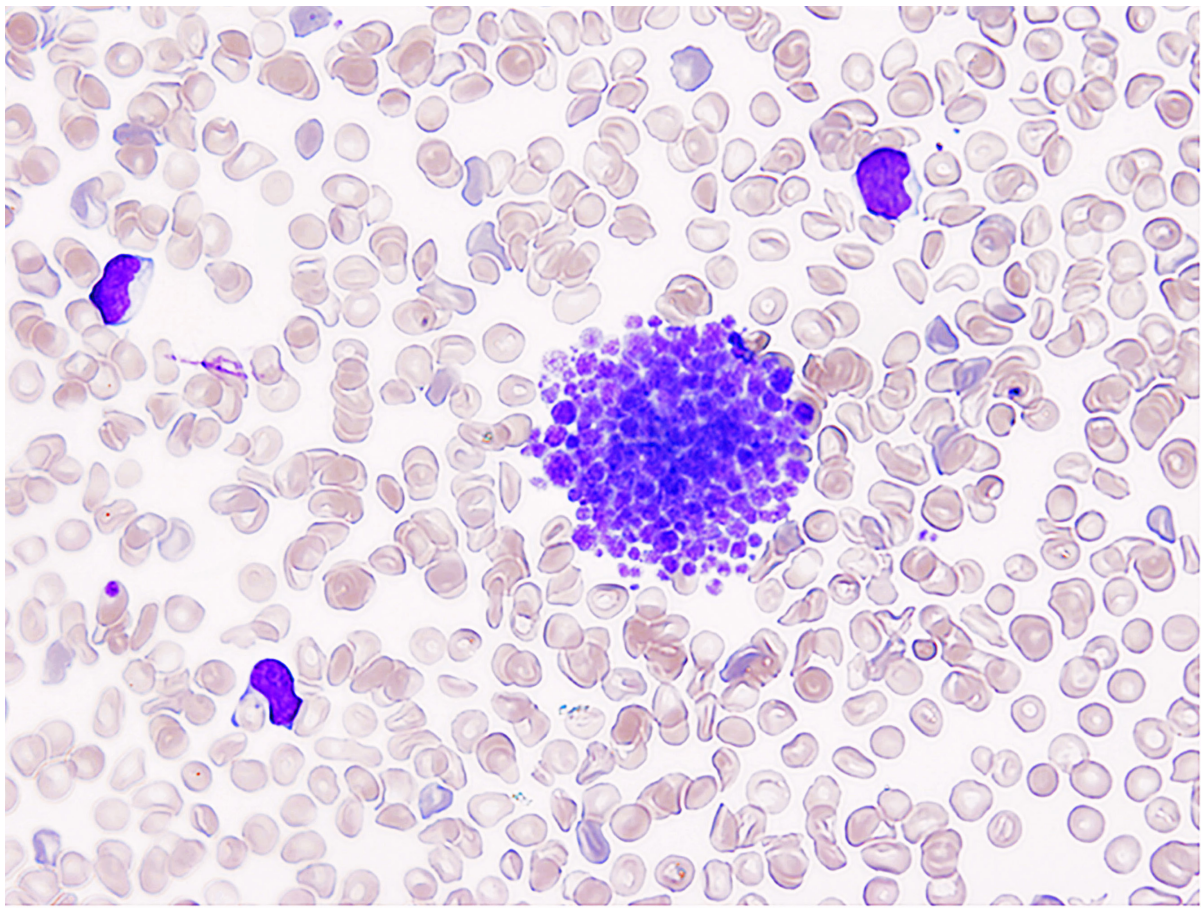

Figure 12. Peripheral blood smear from a patient with type 2B von Willebrand disease. Blood was collected in ethylenediaminetetraacetic acid (EDTA) from a newborn boy, delivered by Cesarean section at the gestational age of 33 weeks. Petechiae were present on his face and body. The blood count was severely reduced ($3x10^9$/L). The peripheral blood smear showed large platelets and platelet agglutinates. The patient, after an initial erroneous diagnostic hypothesis of immune thrombocytopenia, was diagnosed with type 2B von Willebrand disease. Reproduced, with permission, from Hatta K, *et al.*[98]

is the recommended first indispensable step in the diagnostic workup of any hematologic abnormality.

Disclosures

No conflicts of interest to disclose.

Data-sharing statement

Data and protocols can be requested by sending an email to the author.

References

1. Chen Y, Hu Y, Hu H. Advances and challenges in platelet counting: evolving from traditional microscopy to modern flow cytometry. J Lab Med. 2025;49(1):2-13.
2. Baccini V, Geneviève F, Jacqmin H, et al. Platelet counting: ugly traps and good advice. Proposals from the French-speaking Cellular Hematology Group (GFHC). J Clin Med. 2020;9(3):808.
3. Balduini CL, Noris P. Platelet count and aging. Haematologica. 2014;99(6):953-955.
4. Biino G, Santimone I, Minelli C, et al. Age- and sex-related variations in platelet count in Italy: a proposal of reference ranges based on 40987 subjects' data. PLoS One. 2013;8(1):e54289.
5. Troussard X, Vol S, Cornet E, et al. Full blood count normal reference values for adults in France. J Clin Pathol. 2014;67(4):341-344.
6. De la Salle B. Pre- and postanalytical errors in haematology. Int J Lab Hematol. 2019;41 Suppl 1:170-176.
7. Sousa S, Magalhães C, Teixeira C, Eremina YO. Fibrin strands in peripheral blood smear: the COVID 19 era. Clin Chem Lab Med. 2022;60(8):e184-e186.
8. Gowland E, Kay HE, Spillman JC, Williamson JR. Agglutination of platelets by a serum factor in the presence of EDTA. J Clin Pathol. 1969;22(4):460-464.
9. Pegels JG, Bruynes EC, Engelfriet CP, von dem Borne AE. Pseudothrombocytopenia: an immunologic study on platelet antibodies dependent on ethylene diamine tetra-acetate. Blood. 1982;59(1):157-161.
10. Bizzaro N. EDTA-dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. Am J Hematol. 1995;50(2):103-109.
11. Nagler M, Keller P, Siegrist D, Alberio L. A case of EDTA-dependent pseudothrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon. BMC Clin Pathol. 2014;14:19.
12. Pujol-Moix N, Muñiz-Díaz E, Español I, et al. Pseudothrombocytopenia, beyond a laboratory phenomenon: study of 192 cases. Ann Hematol. 2023;102(6):1363-1374.
13. Froom P, Barak M. Prevalence and course of pseudothrombocytopenia in outpatients. Clin Chem Lab Med. 2011;49(1):111-114.
14. Schuff-Werner P, Mansour J, Gropp A. Pseudothrombocytopenia (PTCP). A challenge in the daily laboratory routine? J Lab Med. 2020;44(5):295-304.
15. Dahlqvist SR, Nilsson TK, Norberg B. Thrombocytosis in active rheumatoid arthritis. Relation to other parameters of inflammatory activity and confounding effect of automated cell counting. Clin Rheumatol. 1988;7(3):335-341.
16. Cohen AM, Cycowitz Z, Mittelman M, et al. The incidence of pseudothrombocytopenia in automatic blood analyzers. Haematologia (Budap). 2000;30(2):117-121.
17. Lardinois B, Favresse J, Chatelain B, et al. Pseudothrombocytopenia-a review on causes, occurrence and

clinical implications. J Clin Med. 2021;10(4):594.
18. Payne BA, Pierre RV. Pseudothrombocytopenia: a laboratory artifact with potentially serious consequences. Mayo Clin Proc. 1984;59(2):123-125.
19. Berkman N, Michaeli Y, Or R, Eldor A. EDTA-dependent pseudothrombocytopenia: a clinical study of 18 patients and a review of the literature. Am J Hematol. 1991;36(3):195-201.
20. Bartels PC, Schoorl M, Lombarts AJ. Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. Scand J Clin Lab Invest. 1997;57(7):629-636.
21. Lin J, Luo Y, Yao S, et al. Discovery and correction of spurious low platelet counts due to EDTA-dependent pseudothrombocytopenia. J Clin Lab Anal. 2015;29(5):419-426.
22. Savage RA. Pseudoleukocytosis due to EDTA-induced platelet clumping. Am J Clin Pathol. 1984;81(3):317-322.
23. Garcia Suarez J, Calero MA, Ricard MP, et al. EDTA-dependent pseudothrombocytopenia in ambulatory patients: clinical characteristics and role of new automated cell-counting in its detection. Am J Hematol. 1992;39(2):146-148.
24. Sakurai S, Shiojima I, Tanigawa T, Nakahara K. Aminoglycosides prevent and dissociate the aggregation of platelets in patients with EDTA-dependent pseudothrombocytopenia. Br J Haematol. 1997;99(4):817-823.
25. Silvestri F, Virgolini L, Savignano C, Zaja F, Velisig M, Baccarani M. Incidence and diagnosis of EDTA-dependent pseudothrombocytopenia in a consecutive outpatient population referred for isolated thrombocytopenia. Vox Sang. 1995;68(1):35-39.
26. van Vliet HH, Kappers-Klunne MC, Abels J. Pseudothrombocytopenia: a cold autoantibody against platelet glycoprotein GP IIb. Br J Haematol. 1986;62(3):501-511.
27. Muñiz-Diaz E, Madoz P, Pujol-Moix N, 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):185-191.
28. Watkins SP Jr, Shulman NR. Platelet cold agglutinins. Blood. 1970;36(2):153-158.
29. Scavone M, Bossi E, Podda GM, Cattaneo M. MgSO4 anticoagulant prevents pseudothrombocytopenia by preserving the integrity of the platelet GPIIb-IIIa complex. Br J Haematol. 2021;192(6):e144-e148.
30. Fiorin F, Steffan A, Pradella P, et al. IgG platelet antibodies in EDTA-dependent pseudothrombocytopenia bind to platelet membrane glycoprotein IIb. Am J Clin Pathol. 1998;110(2):178-183.
31. De Caterina M, Fratellanza G, Grimaldi E, et al. Evidence of a cold immunoglobulin M autoantibody against 78-kD platelet glycoprotein in a case of EDTA-dependent pseudothrombocytopenia. Am J Clin Pathol. 1993;99(2):163-167.
32. Asma A, Anissa S, Touhami K. Aggregation kinetic and temperature optimum of an EDTA-dependent pseudothrombocytopenia. Clin Chem Lab Med. 2020;59(1):e31-e33.
33. Ghali C, Scavone M, Clerici B, et al. Time-dependent in vitro variations of platelet count in samples from subjects with EDTA-induced pseudothrombocytopenia: a comparative analysis across different anticoagulants. Br J Haematol. 2024;204(5):2125-2128.
34. Golański J, Pietrucha T, Baj Z, et al. Molecular insights into the anticoagulant-induced spontaneous activation of platelets in whole blood-various anticoagulants are not equal. Thromb Res. 1996;83(3):199-216.
35. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. Clin Chem Lab Med. 2012;50(8):1281-1285.
36. Gogstad GO, Hagen I, Krutnes MB, Solum NO. Dissociation of the glycoprotein IIb-IIIa complex in isolated human platelet membranes. Dependence of pH divalent cations. Biochim Biophys Acta. 1982;689(1):21-30.
37. Sánchez Guiu IM, Martínez-Martinez I, et al. An atypical IgM class platelet cold agglutinin induces GPVI-dependent aggregation of human platelets. Thromb Haemost. 2015;114(2):313-324.
38. Podda G, Femia EA, Pugliano M, Cattaneo M. Congenital defects of platelet function. Platelets. 2012;23(7):552-563.
39. Agarwal S. Pseudo-grey platelet syndrome: a rare artifact. Indian J Hematol Blood Transfus. 2013;29(1):52-54.
40. Pancione Y, Fumi M, Sale S, Rocco V, Bain BJ. Gray platelets - artifact or real? Am J Hematol. 2016;91(5):538.
41. Cockbill SR, Burmester HB, Heptinstall S. Pseudo grey platelet syndrome—grey platelets due to degranulation in blood collected into EDTA. Eur J Haematol. 1988;41(4):326-333.
42. Amoruso M, Alberio L, Nagy M. EDTA-related degranulation mimicking storage pool disease. Am J Hematol. 2018;93(9):1192-1193.
43. Shimizu M, Yamamoto M, Miyachi H, et al. Simple, rapid, and automated method for detection of hyperaggregability of platelets using a hematology analyzer. Am J Hematol. 2003;72(4):282-283.
44. Cunningham VL, Brandt JT. Spurious thrombocytopenia due to EDTA-independent cold-reactive agglutinins. Am J Clin Pathol. 1992;97(3):359-362.
45. Lombarts AJ, Zijlstra JJ, Peters RH, et al. Accurate platelet counting in an insidious case of pseudothrombocytopenia. Clin Chem Lab Med. 1999;37(11-12):1063-1066.
46. Sandhaus LM, Osei ES, Agrawal NN, et al. Platelet counting by the Coulter LH 750, Sysmex XE 2100, and Advia 120: a comparative analysis using the RBC/platelet ratio reference method. Am J Clin Pathol. 2002;118(2):235-241.
47. Solanki DL, Blackburn BC. Spurious leukocytosis and thrombocytopenia. A dual phenomenon caused by clumping of platelets in vitro. JAMA. 1983;250(18):2514-2515.
48. Woo S, Kim B, Heo NH, et al. Definition of significant platelet clumping: should we review all samples with a platelet clumping flag from automated hematology analyzer? Int J Lab Hematol. 2025;47(1):79-86.
49. Adewoyin AS, Nwogoh B. Peripheral blood film - a review. Ann Ib Postgrad Med. 2014;12(2):71-79.
50. Mannuß S, Schuff-Werner P, Dreißiger K, Burstein C. Inhibition of agonist-induced platelet aggregation by magnesium sulfate warrants its use as an alternative in vitro anticoagulant in pseudothrombocytopenia. Platelets. 2020;31(5):680-684.
51. Tan GC, Stalling M, Dennis G, et al. Pseudothrombocytopenia due to platelet clumping: a case report and brief review of the literature. Case Rep Hematol. 2016;2016:3036476.
52. Zhou X, Wu X, Deng W, et al. Amikacin can be added to blood to reduce the fall in platelet count. Am J Clin Pathol. 2011;136(4):646-652.
53. Zhong L, Chadha J, Ameri A. A curious case of pseudothrombocytopenia due to in vitro agglutination. Case Rep Hematol. 2020;2020:6236350.
54. Farkas JC, Brossel C, Boyer JM, et al. Cold-reactive platelet agglutinins. Thromb Haemost. 1993;70(5):879-880.

55. Lippi U, Schinella M, Nicoli M, et al. EDTA-induced platelet aggregation can be avoided by a new anticoagulant also suitable for automated complete blood count. Haematologica. 1990;75(1):38-41.
56. Bokaei PB, Grabovsky D, Shehata N, Wang C. Impact of amikacin on pseudothrombocytopenia. Acta Haematol. 2017;137(1):27-29.
57. Podda GM, Pugliano M, Casazza G, et al. Measurement of platelet count with different anticoagulants in thrombocytopenic patients and healthy subjects: accuracy and stability over time. Haematologica. 2019;104(12):e570-e572.
58. Ohashi-Fukuda N, Inokuchi R, Sato H, et al. Poorer prognosis with ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia: a single-center case-control study. Medicine (Baltimore). 2015;94(15):e674.
59. Norberg B, Nilsson TK. Platelet clumping in Ph-negative myeloproliferative syndromes. Acta Med Scand. 1987;222(5):459-464.
60. Solanki DL, Blackburn BC. Spurious thrombocytopenia during pregnancy. Obstet Gynecol. 1985;65(3 Suppl):14S-17S.
61. Chiurazzi F, Villa MR, Rotoli B. Transplacental transmission of EDTA-dependent pseudothrombocytopenia. Haematologica. 1999;84(7):664.
62. Sweeney JD, Holme S, Heaton WA, et al. Pseudothrombocytopenia in plateletpheresis donors. Transfusion. 1995;35(1):46-49.
63. Podda GM, Pugliano M, Femia EA, et al. The platelet count in EDTA-anticoagulated blood from patients with thrombocytopenia may be underestimated when measured in routine laboratories. Am J Hematol. 2012;87(7):727-728.
64. Bizzaro N, Brandalise M. EDTA-dependent pseudothrombocytopenia. Association with antiplatelet and antiphospholipid antibodies. Am J Clin Pathol. 1995;103(1):103-107.
65. Salama A. Autoimmune thrombocytopenia complicated by EDTA- and/or citrate-dependent pseudothrombocytopenia. Transfus Med Hemother. 2015;42(5):345-348.
66. Pujol-Moix N, Español I, Soler A, Muñiz-Diaz E. Immune thrombocytopenia with associated pseudothrombocytopenia. Am J Leuk Res. 2024;7(1):1034.
67. Sane DC, Damaraju LV, Topol EJ, et al. Occurrence and clinical significance of pseudothrombocytopenia during abciximab therapy. J Am Coll Cardiol. 2000;36(1):75-83.
68. Schell DA, Ganti AK, Levitt R, Potti A. Thrombocytopenia associated with c7E3 Fab (abciximab). Ann Hematol. 2002;81(2):76-79.
69. Huxtable LM, Tafreshi MJ, Rakkar AN. Frequency and management of thrombocytopenia with the glycoprotein IIb/IIIa receptor antagonists. Am J Cardiol. 2006;97(3):426-429.
70. Zeigler Z. In vitro granulocyte-platelet rosette formation mediated by an IgG immunoglobulin. Haemostasis. 1974;3(5-6):282-287.
71. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part I: platelets. Int J Lab Hematol. 2007;29(1):4-20.
72. Sousa SM, Sousa TM, Silva CF, Mendes CC. Pseudothrombocytopenia: a case of platelet satellitism and phagocytosis by neutrophils. Platelets. 2020;31(4):541-543.
73. Lopez-Molina M, Sorigue M, Martinez-Iribarren A, et al. Platelet satellitism around lymphocytes: case report and literature review. Int J Lab Hematol. 2019;41(4):e81-e83.
74. Cohen AM, Lewinski UH, Klein B, Djaldetti M. Satellitism of platelets to monocytes. Acta Haematol. 1980;64(2):61-64.
75. Liso V, Bonomo L. Platelet satellitism to basophils in a patient with chronic myelocytic leukaemia. Blut. 1982;45(5):347-350.
76. Lazo-Langner A, Piedras J, Romero-Lagarza P, et al. Platelet satellitism, spurious neutropenia, and cutaneous vasculitis: casual or causal association? Am J Hematol. 2002;70(3):246-249.
77. Bizzaro N, Goldschmeding R, von dem Borne AE. Platelet satellitism is Fc gamma RIII (CD16) receptor-mediated. Am J Clin Pathol. 1995;103(6):740-744.
78. Christopoulos C, Mattock C. Platelet satellitism and alpha granule proteins. J Clin Pathol. 1991;44(9):788-789.
79. Criswell KA, Breider MA, Bleavins MR. EDTA-dependent platelet phagocytosis. A cytochemical, ultrastructural, and functional characterization. Am J Clin Pathol. 2001;115(3):376-384.
80. Moraglio D, Banfi G, Arnelli A. Association of pseudothrombocytopenia and pseudoleukopenia: evidence for different pathogenic mechanisms. Scand J Clin Lab Invest. 1994;54(4):257-265.
81. Michelson AD, Furman MI. Laboratory markers of platelet activation and their clinical significance. Curr Opin Hematol. 1999;6(5):342-348.
82. Balduini CL, Pecci A. Inherited thrombocytopenias. Haematologica. 2020;105(Supplement 1):237-247.
83. Cooper N, Ghanima W. Immune thrombocytopenia. N Engl J Med. 2019;381(10):945-955.
84. Handtke S, Thiele T. Large and small platelets-(when) do they differ? J Thromb Haemost. 2020;18(6):1256-1267.
85. Jesudas R, Takemoto CM. Where have all the platelets gone? HIT, DIC, or something else? Hematology Am Soc Hematol Educ Program. 2023;2023(1):43-50.
86. Tong M, Seth P, Penington DG. Proplatelets and stress platelets. Blood. 1987;69(2):522-528.
87. Markewitz RDH, Falk KK. Platelet satellitism in immune thrombocytopenic purpura. Blood. 2022;140(19):2091. [Erratum published in Blood. 2023;141(8):962].
88. Noris P, Biino G, Pecci A, et al. Platelet diameters in inherited thrombocytopenias: analysis of 376 patients with all known disorders. Blood. 2014;124(6):e4-e10.
89. Rees DC, Iolascon A, Carella M, et al. Stomatocytic haemolysis and macrothrombocytopenia (Mediterranean stomatocytosis/macrothrombocytopenia) is the haematological presentation of phytosterolaemia. Br J Haematol. 2005;130(2):297-309.
90. Wang L, Huang J, Li X. Marked underestimation of platelet count and a characteristic platelet histogram as clues to MYH9-related disorders. Clin Lab. 2024;70(3):610-613.
91. Noris P, Zaninetti C. Counting and measurement of platelet dimensions. In: Gresele P, Kleiman NS, Lopez JA, Clive P, editors. Platelets in Thrombotic and Non-Thrombotic Disorders. Springer; 2017. pp. 571-588.
92. Sadler JE, Budde U, Eikenboom JC, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand factor. J Thromb Haemost. 2006;4(10):2103-2114.
93. James PD, Connell NT, Ameer B, et al. ASH ISTH NHF WFH 2021 guidelines on the diagnosis of von Willebrand disease. Blood Adv. 2021;5(1):280-300.
94. Othman M, Favaloro EJ. 2B von Willebrand disease diagnosis: considerations reflecting on 2021 multisociety guidelines. Res Pract Thromb Haemost. 2021;5(8):e12635.
95. Saba HI, Saba SR, Dent J, et al. Type IIB Tampa: a variant of von Willebrand disease with chronic thrombocytopenia, circulating platelet aggregates, and spontaneous platelet aggregation.

Blood. 1985;66(2):282-286.
96. Lu X, Yu D, Dai W, et al. Misdiagnosis of type 2B von Willebrand disease as immune thrombocytopenia in a thrombocytopenic patient. Clin Chem Lab Med. 2024;62(9):e212-e214.
97. Loffredo G, Baronciani L, Noris P, et al. Von Willebrand disease type 2B must be always considered in the differential diagnosis of genetic thrombocytopenias with giant platelets. Platelets. 2006;17(3):149-152.
98. Kumar R, Creary S, Varga EA, Kahwash SB. Thrombocytopenia pitfalls: misdiagnosing type 2B von Willebrand disease as ethylenediaminetetraacetic acid-dependent pseudothrombocytopenia. J Pediatr. 2016;175:238-238.e1.
99. Hatta K, Kunishima S, Suganuma H, et al. A family having type 2B von Willebrand disease with a novel VWF p.R1308S mutation: detection of characteristic platelet aggregates on peripheral blood smears as the key aspect of diagnosis. Thromb Res. 2015;136(4):813-817.
100. Pareti FI, Federici AB, Cattaneo M, Mannucci PM. Spontaneous platelet aggregation during pregnancy in a patient with von Willebrand disease type IIB can be blocked by monoclonal antibodies to both platelet glycoproteins Ib and IIb/IIIa. Br J Haematol. 1990;75(1):86-91.
101. van Kwawegen CB, Atiq F, Endenburg D, et al. Genetic variants, thrombocytopenia, and clinical phenotype of type 2B von Willebrand disease: a median 16-year follow-up study. J Thromb Haemost. 2024;22(12):3460-3472.
102. Miljic P, Noureldin A, Lavin M, et al. Challenges in the management of women with type 2B von Willebrand disease during pregnancy and the postpartum period: evidence from literature and data from an international registry and physicians' survey-communication from the Scientific and Standardization Committees of the International Society on Thrombosis and Haemostasis. J Thromb Haemost. 2023;21(1):154-163.
103. Holmberg L, Nilsson IM, Borge L, et al. Platelet aggregation induced by 1-desamino-8-D-arginine vasopressin (DDAVP) in type IIB von Willebrand's disease. N Engl J Med. 1983;309(14):816-821.
104. Altomare I, Cetin K, Wetten S, Wasser JS. Rate of bleeding-related episodes in adult patients with primary immune thrombocytopenia: a retrospective cohort study using a large administrative medical claims database in the US. Clin Epidemiol. 2016;8:231-239.