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EDTA-associated pseudothrombocytopenia: definition and real-world occurrence

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Abstract

Objectives: To better characterize occurrence and extent of anticoagulant-associated pseudothrombocytopenia (PTCP) in the daily routine of a high-throughput clinical laboratory in order to draw conclusions on a more precise definition of this phenomenon.
Methods: Concomitant platelet counts in both EDTA and citrate whole blood (WB) performed in our laboratory over a period of four years and 9 months, were analyzed, calculating the correlation, as well as the absolute difference in the results obtained from both materials, cross-referencing these measures with automated flags for platelet aggregates and the results of the visual examination for platelet aggregates of peripheral blood smears.
Results: Platelet counts in both materials were strongly correlated (ρ=0.86; $p<0.0001$) but are on average significantly higher in EDTA WB than in citrate WB (median difference: 11 ± 14.8/nL, $p<0.0001$). This is in spite of numerous instances of EDTA-associated PTCP recorded in our data, where the opposite is the case. The automated flag for possible platelet aggregates was shown to be very unspecific, while a machine-learning algorithm suggested the difference in platelet counts between EDTA and citrate WB as a predictor of platelet aggregates.
Conclusions: EDTA-associated PTCP is a regular occurrence. Differences in platelet counts between EDTA and citrate WB appear to be a far better predictor of PTCP than automated flags. A clear and useful definition of PTCP is still missing, however, and cannot be derived from our data either, indicating the need for further research.

Keywords: platelets; pseudothrombocytopenia; thrombocytes.

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Introduction

Pseudothrombocytopenia (PTCP) is the finding of spuriously low platelet counts. First described in 1969 [1], this is a well-known phenomenon that is observed regularly in the hematological laboratory while reports on its prevalence range from 0.01–1 % [2–5]. Most commonly, the presence of PTCP is associated with the use of ethylenediamine tetraacetic acid (EDTA) as anticoagulant in the tube used for collecting the blood sample, although it has also been observed in tubes using different anticoagulants, such as citrate [5]. The most commonly assumed pathomechanism is the exposure of normally “hidden” epitopes of platelets associated with an EDTA-mediated alteration of the platelet membrane. These epitopes, usually associated with the platelet GPIIb/IIIa receptor, then bind to non-pathogenic autoantibodies, leading to *in vitro* platelet aggregation [2, 6]. In some cases, instead of aggregation, the spuriously low platelet counts are caused by platelet satellitism, a related phenomenon in which a rosetting of platelets around neutrophils can be observed *in vitro*. Similarly, this phenomenon is associated with EDTA and likely caused by autoantibodies against the platelet GP IIb/IIIa receptor that react with the neutrophil Fcγ receptor III (FcγRIII) [7]. Furthermore, the artefactual aggregation of platelets in EDTA whole blood (WB) is time dependent with a significant decrease in platelet numbers being observed within the first two hours after sample collection already [2].

Although the underlying causes and mechanisms have been defined relatively well in decades of research, the very definition of PTCP remains surprisingly vague. As stated at the start, it is usually understood to be “finding of spuriously low platelet counts”. The spuriosity of the phenomenon is usually confirmed by the detection of either of the two described *in vitro* phenomena (aggregation and satellitism) in a peripheral blood smear and/or through the detection of a normal (or at least significantly higher) platelet count using an anticoagulant other than EDTA (mainly citrate or magnesium sulfate ($MgSO_4$) for the collection of the blood sample. But several important questions remain: How low is low? The name PTCP suggests that it can only be diagnosed, when the spurious result is that of a thrombocytopenia (and therefore below 150/nL by the most common definition). But what about cases where

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aggregates and/or a significant difference in the platelet count between EDTA WB and citrate WB can be detected but the platelet count in EDTA is within the reference range? Furthermore, what is a "significant difference" between platelet counts in EDTA and citrate WB? There is no common definition of how far apart these two measurements have to be, either in absolute or in relative terms, in order for PTCP to be suspected. This is further complicated by the fact that the duplicated measurement of platelets in both EDTA and citrate WB regularly returns slightly higher results for the former anticoagulant [8–10], partly due to matrix effects caused by the dilution [10], partly due to temporal instability of platelets in citrate WB [11–14]. Therefore, when contemplating the difference between platelet counts in EDTA and citrate WB, one has to bear in mind that the norm for this difference is not zero, or, in other terms: even a difference of zero between two platelet counts could be compatible with a very mild form of PTCP. Finally, to date there is no international consensus on what is to be considered a platelet aggregate in the peripheral blood smear (i.e. how many platelets have to be aggregated) and how many of these aggregates have to be found in how many visual fields for aggregates in the blood smear to be reported. There have been some efforts towards a more unified approach in these two latter questions [12, 15, 16], but no generally agreed consensus.

Therefore, in order to better characterize PTCP as it occurs in real-world, we set out to analyze a large data set of concomitant platelet counts from both EDTA and citrate WB. Our aims were to examine the differences between platelet counts in both materials: Where do they occur and how pronounced are they? Which of these measurements were automatically flagged for potential platelet aggregates by the analyzer and which samples revealed aggregates in the peripheral blood smear (when performed)? Is PTCP really a phenomenon that can be readily detected in a given sample, as the vast amount of literature, mostly comprising of case reports of impressively pronounced cases suggests (some examples: [17–20], and if yes, what are relevant parameters and cutoffs that might reliably distinguish between samples in which PTCP occurs and samples in which it doesn't)?

Materials and methods

Study cohort and sample characteristics

We included all concomitant (i.e. from the same peripheral venous puncture) platelet counts in both EDTA and citrate WB ordered at our high-throughput clinical laboratory at the University Hospital Schleswig-Holstein between January 1st 2018 and February 13th 2023. In all, 10,243 such measurements were included in our analysis. These were collected from 5,957 different individual patients. The median frequency of samples per patient was 1 (±0, range: 1–42). These patients were on average 67.7 ± 16.8 years old at the time of the collection of the first included measurement (range: 0–101). 3,336 (56.0 %) of the patients were male, and 2,621 (44.0 %) were female. As a retrospective analysis of anonymized data, the study was deemed exempt from review by the Institutional Board of the University of Lübeck.

Platelet count

All analyzed blood samples were collected using collection tubes from Sarstedt (Nümbrecht, Germany), specifically the S-Monovette K_3-EDTA tube for EDTA WB and the S-Monovette Citrat 3.2 % for citrate WB. The platelet counts from both EDTA and citrate WB. were performed using the XN-9000 system (Sysmex, Kobe, Japan), which counts platelets via impedance measurement (with the possibility of confirmation via flow cytometry in cases where the impedance measurement yields ambiguous results). Platelet counts from citrate WB are automatically corrected for the dilution by the anticoagulant within the collection tubes. The presented values are already thus corrected. Prior to analysis, all citrate tubes are examined for filling level, with both under- and overfilled tubes being automatically rejected. The data analysis includes the information whether or not the XN-9000 flagged the measurement for the possible presence of platelet aggregates. This flag is generated via an automated algorithm by the XN-9000 system, analyzing different properties of the platelet count. If this flag is generated, a peripheral blood smear is automatically performed in our laboratory which is examined via light microscopy for the presence of platelet aggregates. The result of this examination was also included in the data analysis. Further, in order to avoid performing peripheral blood smears in cases with a low pre-test probability and/or clinical significance, only EDTA WB samples will be examined via microscopy that return a platelet count of <110/nL. Also, platelet aggregates may be an additional finding in a peripheral blood smear performed during the course of a manual complete blood count, even in samples that were not flagged by the XN-9000. Therefore, the amount of flagged samples is not equal to the amount of samples which were examined for platelet aggregates via light microscopy.

The result of the visual examination via light microscopy is semiquantitative, with possible results being: o=no platelet aggregates, (+)=platelet aggregates present in borderline quantity, +=few platelet aggregates present, ++=platelet aggregates present, +++=platelet aggregates abundantly present. Of note, this semi-quantitative assessment is made by a human examiner and is thus subject to a considerable degree of inter-observer variability. Of note, to date, in our laboratory, this quantification is not subject to a fixed set of rules concerning the number of aggregates found in a certain number of visual fields or the number of platelets necessary in order for an aggregate to be defined as such.

Statistical analysis

The absolute difference between platelet counts in EDTA and citrate WB was calculated as platelets (EDTA) – platelets (citrate), meaning

that positive results are indicative of higher counts in EDTA WB and negative results of higher counts in citrate WB. Average values and measures of dispersion are presented as the median and the median absolute deviation, respectively, unless explicitly stated otherwise. Differences in continuous variables (such as platelet counts) between two or more groups are calculated using the Mann-Whitney-*U*-test (with correction for multiple comparisons, using the method proposed by Benjamini and Yekutieli [21], in the case of more than two groups). Correlations between two continuous variables were analyzed calculating Spearman's rho. Statistical relevance was assumed for $p<0.05$. The decision tree for predictors of platelet aggregates in the peripheral blood smear was calculated as a classification tree using the R-package "rpart" [22]. As a preparatory step, the data was randomly partitioned in a training data set (comprising 70 % of the original data set) and a test data set (comprising the other 30 % of the original data set). For the decision tree only the training data was used and the test data set was used to as an unseen sample in order to test the performance of the decision tree's proposed algorithm. All statistical analyses were performed using the open-source software for statistical computing and graphics, R (v4.1.0), with the integrated development environment RStudio (v1.4.1717) [23].

Results

Descriptive statistics

Both platelets in EDTA WB and in citrate WB show an approximately log-normal distribution in the cohort selected with a relatively low median of 85 ± 59.3 platelets per nL in EDTA WB (range: 0–2,566) and 74 ± 48.9/nL (range: 0–1,726/nL) in citrate WB, after correction for the dilution by the sodium-citrate in the tube. Correspondingly, the median absolute difference in platelet counts between both materials was positive with 11 ± 14.8/nL (range: –424–840/nL), indicating a general trend for higher counts in EDTA WB compared to citrate WB and showing a slightly left-skewed distribution with many outliers, some extreme, to either side. Shapiro-Wilks-tests consequently revealed that none of the aforementioned variables are distributed normally. The difference in platelet counts in favor of EDTA WB is statistically highly significant ($W=47{,}104{,}497$, $p<0.0001$).

Of all 10,243 measurements included in the analysis, 780 (7.6 %) were automatically flagged for the possible presence of platelet aggregates. For 599 (5.8 %) a visual examination of a peripheral blood smear for the presence of platelet aggregates via light microscopy was available. "Only" 547 of these 599 (91.3 %) had also been flagged automatically. Of the 599 samples examined visually, 379 (63.3 %) were judged to contain no platelet aggregates in the peripheral blood smear, while 1 sample was reported (+), 107 were reported as +, 56 as ++, and also 56 as +++ for platelet aggregates.

Correlation between platelet counts in EDTA and citrate whole blood

Generally, there was a highly significant correlation of large effect size between platelets in EDTA and platelets in citrate WB ($\rho=0.86$; $p<0.0001$; see Figure 1A). However, there are a number of measurements that deviate markedly from the general trend as indicated by the regression line (which itself deviates markedly from the function y=x, along which the measurements would ideally be scattered were it not for the abovementioned trend of lower platelet counts in EDTA WB compared to citrate WB), especially in the lower left-hand corner of the scatter plot: These measurements, with low platelets in EDTA WB and significantly higher platelets in citrate WB are those that would traditionally be classified as possible evidence of EDTA-associated PTCP. However, the scatter plot also shows that this is no distinct set of measurements, but rather within in continuum with other measurements that are more closely dispersed around the regression line. When the information on automated flags is incorporated, it becomes evident that many, but by no means all, of these discordant platelet counts were flagged for the possible presence of platelet aggregates. On the other hand, many measurements very close to the line y=x were also flagged (Figure 1B). Further incorporating the information of the visual examination of the peripheral blood smear, it becomes evident that these latter measurements tend to be those, for which no platelet aggregates were detected visually (Figure 1C). Combining the information on flags and visual examination, one can further see that by no means all measurements with highly discordant platelet counts between both materials for which aggregates were visually found were flagged (Figure 1D). In detail, 30 of 220 samples (13.6 %) in which platelet aggregates were found via light microscopy were not flagged by the analyzer.

Absolute difference in platelet counts between materials in relation to platelet counts

Plotting the absolute difference between platelet counts in both materials against the platelet count in citrate WB, a striking subset of measurements can be seen that deviate almost linearly towards a greater negative absolute difference (indicating higher counts in citrate WB) with increasing platelet counts in citrate WB (Figure 2A). These are also those measurements, that would be traditionally classified as potential EDTA-associated PTCP, again in a continuum with other, more "normal" measurements.

Automated flags and visual examination for platelet aggregates

Again incorporating the information on flags and visual detection of aggregates, it becomes evident that this subset of measurements is indeed where the flagged measurements are primarily clustered (Figure 2B). However, the visual examination reveals that in most cases, which were flagged, but in which the difference between EDTA and citrate WB was small, no platelet aggregates were found

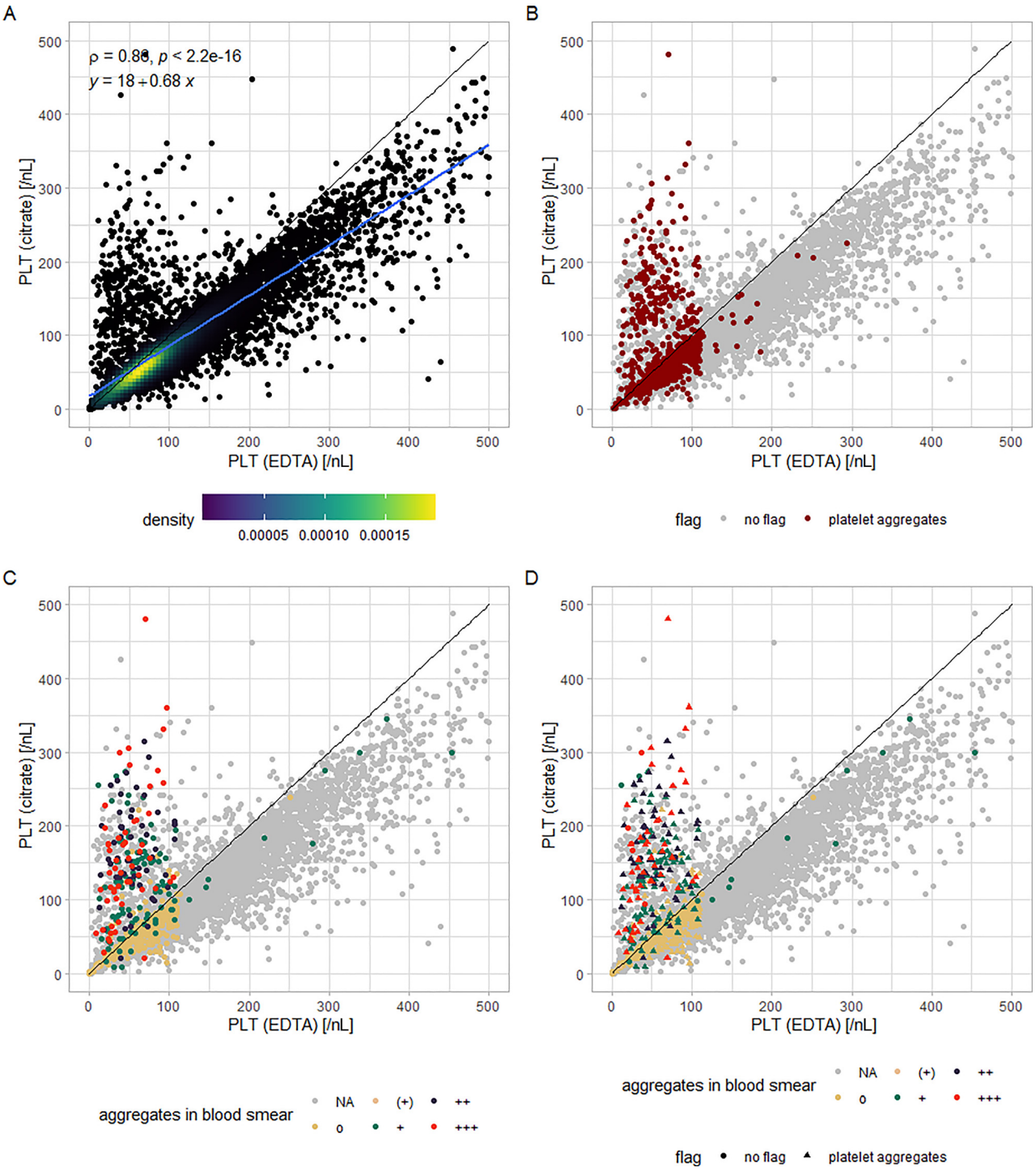

Figure 1: Scatter plots showing the correlation between platelet counts in EDTA whole blood (x-axis) and citrate whole blood (y-axis). Panel (A) shows the general distribution of measurements, including a density plot, panel (B) adds information in automated flags, panel (C) the results of the visual examination for platelet aggregates of peripheral blood smears and panel (D) combines both information.

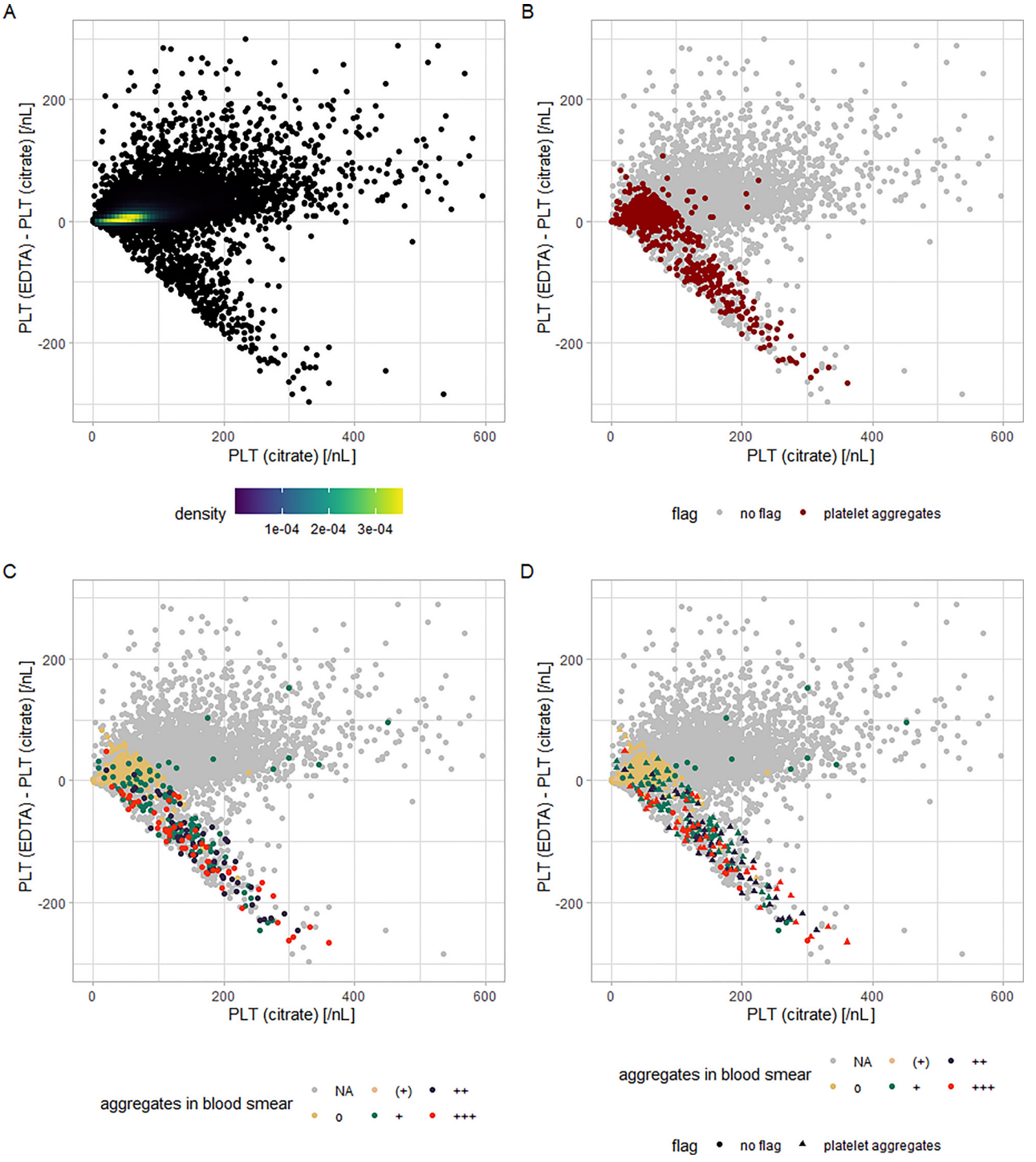

Figure 2: Scatter plots, plotting the absolute difference in platelet counts between EDTA and citrate whole blood (y-axis) against the platelet count in citrate whole blood (x-axis). Panel (A) shows the general distribution of measurements, including a density plot, panel (B) adds information in automated flags, panel (C) the results of the visual examination for platelet aggregates of peripheral blood smears and panel (D) combines both information.

visually, which is quite in contrast to cases with a greater difference (Figure 2C, D). Consequently, pairwise Mann-Whitney-U-tests revealed that the (negative) difference is significantly greater in cases in which aggregates were found visually (and even significantly increasing depending on the amount of aggregates detected visually) compared to cases in which no aggregates were found and cases in which no visual examination was performed (with

no significant difference between these latter two; Figure 3A).

Seeing as many cases that were flagged could not be confirmed via visual examination and that many cases that cluster with confirmed cases of PTCP in the scatter plots were not flagged (in addition to the small number cases that were not flagged but examined visually anyway and found to contain platelet aggregates), we calculated sensitivity and specificity of the automatic flag (compared to the visual examination as gold standard) and found these to be 86.4 % and 5.8 %, respectively. However, these numbers have to be interpreted with utmost caution: Due to the fact that mostly flagged samples were examined visually, the sensitivity is probably greatly overrated as witnessed by the many cases that were not flagged (and therefore mostly not examined visually) but that cluster with confirmed cases of PTCP, at least a subset of which likely would have been classified as PTCP by visual examination had it been performed. Also, even the poor specificity of 5.8 % is likely artificially increased by the rule that only EDTA WB samples containing fewer than 110/nL platelets were allowed to be flagged.

Predictors of platelet aggregates

In light of these results, we aimed to devise a better predictor of the occurrence of PTCP than the automated flag. The above-mentioned significant divergences of the difference in platelet counts between EDTA and citrate WB suggest to use this parameter for this prediction. We used a machine learning algorithm (via a decision tree incorporating the absolute difference as well as the ratio between platelet counts in EDTA and citrate WB, as well as age and gender) to define predictors that separate well between confirmed cases of PTCP and cases that could not be confirmed via microscopy. Thus, we arrived at a difference of −7.5/nL between platelet counts in EDTA and citrate WB which provides the most meaningful prediction of platelet aggregates in light microscopy (while the ratio, age and gender were not used by the algorithm as they did not meaningfully contribute to a correct classification; Figure 3B). Using just this value to classify cases as probable PTCP in an unseen test data set, again using the visual examination as the gold standard, we found sensitivity and specificity of this approach to be at 91.0 % and 93.2 %, respectively. Again, one has to bear in mind that these numbers are not completely unbiased due to the fact that the cases that were examined visually had a somewhat elevated pre-test-probability for platelet aggregates as a consequence of the mechanism causing flagged samples to be examined visually. Plotting the original data filtered for measurements that fulfill the difference < −7.5/nL criterion, most of the unspecifically flagged measurements are eliminated, while at the same time a majority of the resulting measurements was not flagged and therefore in most cases not examined visually for platelet aggregates (Figure 3C, D).

Therefore, it has to be considered that only a minority of 5.8 % (5.5 % in the test data set) of all samples were visually examined for platelet aggregates. If one applies the threshold of −7.5 to all samples of the test data set that were not thus examined and considers the specificity of 93.2 %, one arrives at a number of 4.2 % of samples that might have been subject of PTCP according to the decision tree algorithm but were overlooked by the automated flag, which is in a staggering contrast to the mere 0.02 % of samples in the test data set in which platelet aggregates were confirmed via light microscopy, supporting the above-mentioned assumption that the calculated sensitivity of the automated flag is likely severely overrated in our data. Again, this is assuming that the specificity of 93.2 % of the threshold of −7.5/nL holds true at least to some degree, despite the above-mentioned biases in the data.

Discussion

Our results clearly show that EDTA -associated PTCP is a regular occurrence within the hematological laboratory. However, our results point to an important methodological problem in the detection of PTCP: The automated flags, which can be used either for guiding which samples to examine visually for platelet aggregates via light microscopy or for generating a comment for the clinician warning of the possibility of PTCP, appear to be extremely unspecific and at the same time not very sensitive (at least for the equipment used in this study). This is in notable contrast to recent results by another group, which found markedly better values for sensitivity and specificity of the automated flags generated by Sysmex equipment [24]. This contrast is possibly explained by differences in the evaluation of the peripheral blood smears, with the method used by Lunde et al. possibly being more sensitive in the detection of platelet aggregates. The fact that the absolute difference in platelet counts between EDTA and citrate WB did not differ significantly between cases in which no aggregates were found and cases for which no smear was performed combined with the fact that both of these groups of cases differed significantly in this respect to all cases in which platelet aggregates were found int the peripheral blood smear by us suggests that our methods do not

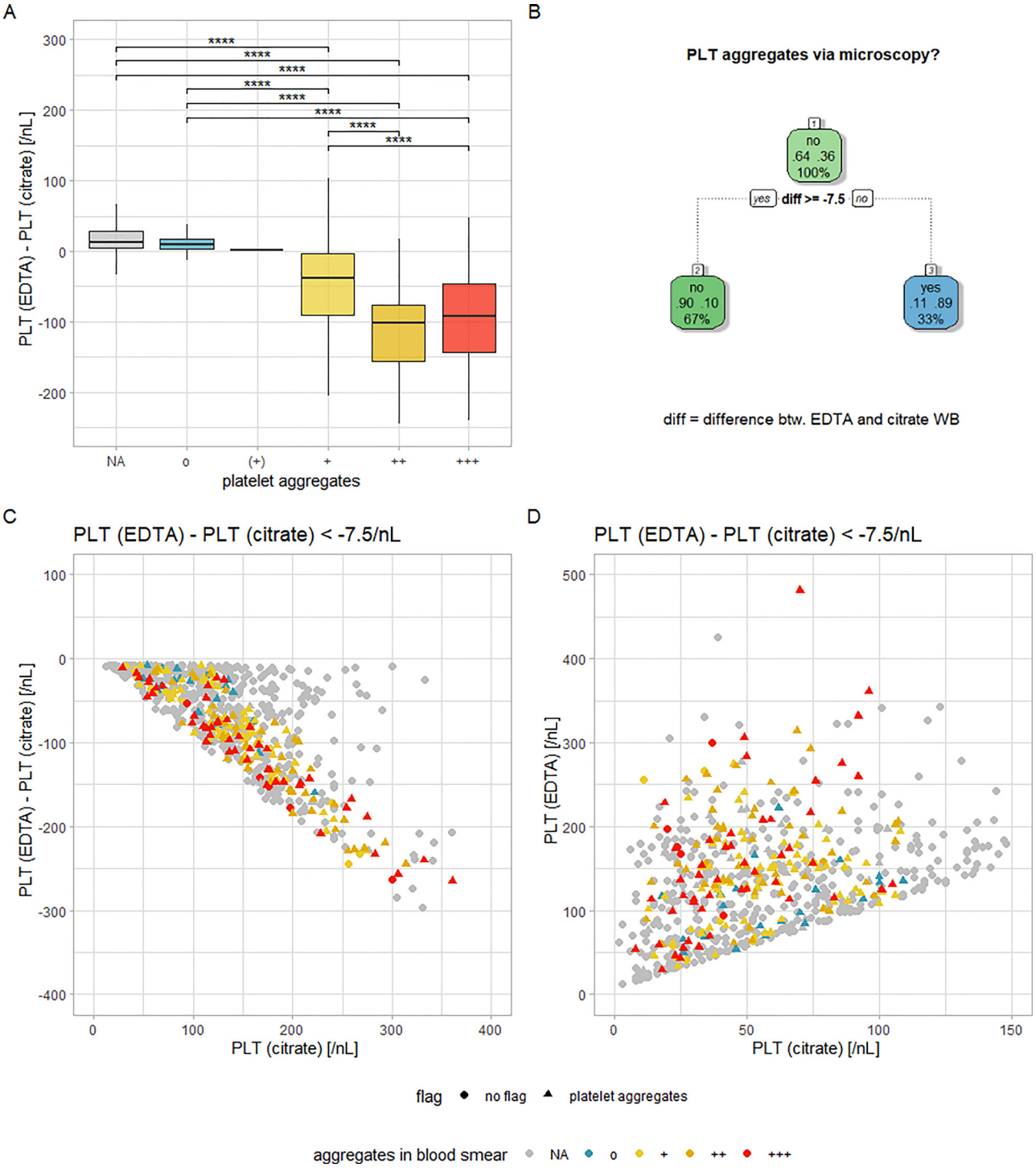

Figure 3: (A) Association between the absolute difference in platelet counts between EDTA and citrate whole blood and the semiquantitative results of the visual examination of peripheral blood smears for platelet aggregates (levels of significance: ****=p<0.0001). (B) Decision tree for the correct classification of samples with respect to the occurrence of platelet aggregates. Each node contains the most common classification in the respective (sub-) population of measurements (no=no aggregates; yes=aggregates found), the distribution of measurements with respect to the occurrence of platelet aggregates (the first number in the second line indicating the fractions of measurements without aggregates) and the share of the whole population represented by the individual node. (C, D) scatter plots of the correlation between platelet counts in EDTA whole blood (x-axis) and citrate whole blood (y-axis; panel (C)), and of the absolute difference in platelet counts between EDTA and citrate whole blood (y-axis) against the platelet count in citrate whole blood (x-axis, panel (D)), using only measurements that fulfill the criterion diff<7.5/nL and incorporating information on flags and the visual examination via microscopy.

overlook a significant number of cases of artefactual platelet aggregation. While ideally every EDTA WB would be examined via light microscopy for platelet aggregates to sensitively and specifically identify samples in which PTCP occurs, this would present an unrealistic work load in the times of automated whole blood counts. Therefore, some other predictor for the occurrence of PTCP is needed. Our data suggest that the difference in platelet counts between EDTA and citrate WB might be a predictor of the occurrence of artifactual *in vitro* aggregation of platelets, with the threshold of −7.5/nL easily outperforming the automated flags in both sensitivity and specificity in our data set. One obvious, but important limitation of this approach is that the concomitant measurement of platelet counts in both EDTA and citrate WB is needed. However, our findings indicate that this approach would lead to a significant increase in the detection of cases of PTCP while it would also significantly reduce the number of peripheral blood smears performed "in vain" for the detection of platelet aggregates, the large majority of which currently reveal no evidence of PTCP due to the poor specificity of the automated flag.

Still, this theoretical approach in the detection raises at least two important questions which lead us back to the initial issue of the definition of PTCP itself:

First: Is it sensible to define cases as PTCP in which the difference between EDTA and citrate WB is just beyond −7.5/nL and for which platelet aggregates were found via light microscopy? Our data suggest that it may be correct, supporting the assumption that PTCP is a phenomenon that occurs in a wide range of pronunciation that is within a continuum with seemingly normal samples. On the one hand, it is dubitable that from a clinical point of view such cases would be considered significant enough to receive the label of PTCP, on the other hand, it is known that the pronunciation of PTCP in EDTA whole blood is time dependent [2]. Therefore, the presence of platelet aggregates, even when associated with a small absolute difference in platelet counts between EDTA and citrate WB, may be indicative of a predisposition towards PTCP, which may be aggravated when the interval between sample collection and measurement is prolonged for whatever reason, making it all the more important that samples from these patients be examined as timely as possible. It has to mentioned, however, that the threshold of −7.5/nL, which was found by the analysis of a huge data set, might be impractical on a single-specimen basis for another reason: Due to the fact that platelet counts in both EDTA and citrate WB each are subject to both biological and analytical variations [25, 26], which necessarily compound when calculating their difference, a difference of −7.5/nL will almost certainly fall within the range of the combined measurement uncertainty of both determinations. Should a meaningful threshold for the absolute difference in platelet counts between both materials ever be proposed, it should in any case also incorporate these uncertainties.

The second question pertains to the cases that the presented approach overlooks: Cases in which platelet aggregates could be found via light microscopy but no meaningful difference between EDTA and citrate WB. There are two possible explanations for these cases: Either, there are cases in which EDTA-associated platelet aggregation can be found as an *in vitro* phenomenon that doesn't influence the platelet count in EDTA WB significantly, or these are cases in which the platelet count in both EDTA and citrate WB is subject to PTCP and therefore similarly spuriously decreased. The latter possibility has certainly been described in the literature [27], but to prove it, one has to perform a platelet count from a $MgSO_4$ anticoagulated tube, which in our laboratory is unfortunately very rarely ordered (only 134 times from 95 patients, none of which in a pertinent case within the time frame of the data analysis; an obvious limitation of the study). If the first possibility were found to be true, the question of the definition of PTCP would become even more complicated (as well as, to a degree, academic): Can a case be defined as a pseudothrombocytopenia even if the associated thrombocytopenia can be confirmed in its severity (therefore not being "pseudo") solely on the basis of platelet aggregates in the visual examination of the peripheral blood smear?

Our proposal would be to define PTCP based on two criteria, which would have to be met: 1) Platelet aggregates in the peripheral blood smear and a defined difference between platelet counts in at least two different materials (EDTA WB and citrate WB or EDTA WB and $MgSO_4$ WB if the first comparison reveals no meaningful difference). However, our data is not suited to propose a meaningful difference in platelet counts between EDTA WB and citrate WB, among other limitations not least because of the bias introduced into the data by the rules guiding the examination of peripheral blood smears that are based on automated flags which, even if the flags perform poorly in terms of specificity and sensitivity, still likely increases the pretest probability of the detection of aggregates in the peripheral blood smear.

Therefore, in order to define a meaningful difference in platelet counts between EDTA and citrate WB, a study would have to be performed that overcomes the several limitations of the current study: During it, peripheral blood smears should be performed indiscriminately for all concomitant platelet counts in EDTA and citrate WB (and ideally in magnesium-sulfate WB, also), with the evaluation

of the peripheral blood smears being performed in a standardized manner, possibly following the protocol proposed by the Groupe Francophone de'Hematologie Cellulaire (GFHC) [12] or that designed by Lunde et al. [24]. A lack of standardization in this regard must certainly be mentioned among the limitations of the current study, as does the aforementioned bias introduced by the process that guided the selection of samples from which peripheral blood smears were performed in our study. Further, the platelet counts would have to be performed in a much more representative cohort than the one examined by us: The low median platelet counts in both materials suggest that our data is subject to a considerable selection bias. Also, to increase the validity of the results obtained, at least a subset of samples with a standardized temporal interval between sample collection and determination of the platelet counts should be included. The lack of standardization of this interval in the current study, owed to its retrospective character, must also be counted among its limitations. As explained above, longer intervals between sample collection and measurement are associated with more pronounced EDTA-associated *in vitro* aggregation of platelets [2] while the platelet count in citrate WB might also decrease spuriously over time, due to a certain temporal instability of platelets in this matrix [11–14], further advocating, by the way, the use of $MgSO_4$ as anticoagulant for the investigation of possible PTCP. At least the fact that platelet aggregates were very rarely found in cases in which platelet counts in citrate WB were lower than in EDTA WB suggests that the temporal delay in our study was sufficiently short in order for PTCP not to be "masked" in the absolute difference in platelet counts by the spurious decline of platelet numbers in citrate WB in a significant number of cases.

During a study performed as outlined above, not only the sensitivity and specificity of the automated flags might be appraised more realistically, but also a more valid predictor of the occurrence of artefactual *in vitro* aggregation of platelets might be defined.

Until such a study is performed, the question of the exact definition of PTCP remains difficult to answer. However, it can be concluded from the current study that automated flags, generated during the platelet count in one material alone, may be insufficient as a predictor of the occurrence of platelet aggregates and, by extension, of the occurrence of PTCP, which may in turn only be detected validly via the performance of platelet counts in at least two different materials in synopsis with the visual examination of peripheral blood smears of at least one of these materials, that returns the lower platelet count.

In the meantime, a change in nomenclature is in our opinion called for: The term pseudothrombocytopenia has two implications which are not universally correct: Firstly, it implies (via the prefix "pseudo") that the finding of platelet counts below the reference range is always spurious, while our results show that there are cases in which platelet aggregation leads to decrease platelet counts in EDTA WB, but the platelet count in citrate WB also shows a thrombocytopenia, albeit with a higher platelet count. Secondly, the term implies, as mentioned above, that the phenomenon described by it always leads to platelet counts below the reference range, which is not necessarily true, as platelet aggregation could also be shown in our data for cases of completely normal platelet counts in EDTA WB. Therefore, the term pseudothrombocytopenia should be regarded as a misnomer and be replaced e.g. by "anticoagulant associated *in vitro* aggregation of platelets", as an umbrella term, with the word anticoagulant optionally being replaced by any specific anticoagulant, such as EDTA or citrate, depending on the specific phenomenon described.

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