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CAT
Critically Appraised Topic

Pseudocytothrombopenia

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CLINICAL BOTTOM LINE

The pathophysiology of pseudothrombocytopenia (PTCP) is still not well understood by scientists, we know that it is a rare artifact rare (prevalence of 0.1% in general population) and not symptomatic. The danger is with misdiagnose/misinterpretation which can lead to further unnecessary test, delays in diagnostic or avoidance of therapeutic procedures and discontinuing of treatments, or inappropriate treatments.
In the literature, PTCP is related to different kind of diseases but despite all these, there is still no clinical evidence which associates the presence of PTCP with specific diseases or the use of specific drugs.
Different solutions exist to prevent PTCP. These treatments are not 100% effective, they are expensive and take time to be applied in routine.
In our university hospital, we need to objectify our procedure more. To achieve this goal, we need first to define what a platelet aggregate is and then to define how many aggregates do we need before speaking about pseudothrombocytopenia. Finally we need to find a confirmation test which can help to get a platelet count as close to the real count. An idea would be to evaluate new anticoagulants as S-Monovette® TromboExact from Sarstedt company and so replace platelet count and/or microscopic control on EDTA, Citrate and Heparin samples.

CLINICAL/DIAGNOSTIC SCENARIO

Pseudothrombocytopenia (PTCP) is an artifact problem known for years in clinical biology. Nevertheless the exact mechanism is still unknown. Diagnose can be done by controlling platelet count and performing a microscopic research from EDTA, Citrate and Heparin samples. Solutions are proposed to prevent or to treat PTCP. The best known solution is supplementation of aminoglycosides but this solution and also the other ones are not cost-effective and take too much time to be applied.
In case of PCTP, haematology automate is supposed to warn the technician in presence of platelet aggregates and to make automatically a blood smear. This alarm has to be very sensitive to avoid as much as possible the false negative results. According to our data, sysmex system approaches a negative predictive value of 99.9%. On the other hand, there is a high level of false positive which gives lot of unnecessary control and delays sending of the result.
We are facing two situations: PTCP diagnose for routine samples and confirmation test of PTCP.

1. Rule 15 from XE-5000, Sysmex, is specific for the detection of platelet aggregates and is active for routine samples. When this alarm is positive, the automate makes a blood smear automatically which is reviewed by trained technician. Until the result is given, platelet count and microscopic result are hidden for clinicians. We finally need around 1 or 2 hours to get all results. During this time, patients and clinicians are waiting. This waiting time gives problem with patients from intensive care, urgency department or hematological/oncological department because these kind of patients need the result to be treated as quickly as possible. For instance, when a patient comes to hospital to receive chemotherapy, one criterion is to have platelet count higher than 50.000/mm³. These patients are waiting for the results just to know if they can receive or not their treatment. Most of the time, the "positive alarm" is in fact a false positive results. Therefore patients are waiting for nothing.
2. Pseudothrombocytopenia is now confirmed with the test "pseudothrombocytopenia beoordeling". Problem to diagnose a PTCP is to define what platelet aggregate is and what PTCP is. When you can define it precisely, you need to evaluate and choose the best and most suitable assay to confirm or to exclude PTCP. Exact definition of PTCP is lacking on Pubmed and no consensual confirmation test is described. Fortunately new kind of sample, S-Monovette ThromboExact from Sarstedt was recently available on the market. Would it be a part of the solution?

QUESTION(S)

1) *What is the mechanism of pseudothrombocytopenia?*
2) *How could we decrease patient's waiting time?*
3) *How could we ameliorate diagnose of pseudothrombocytopenia?*

SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: "pseudothrombocytopenia, pseudothrombopenia, thrombocytopenia, sysmex xe, sysmex platelet, platelet clumping, platelet aggregates "*
2) *The American Society of Hematology (www.hematology.org), European Haematology Association (www.ehaweb.org)*
3) *UpToDate Online version 12.2 (2005)*

RELEVANT EVIDENCE/REFERENCES

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SOP-092 - Tabel PB 4 - Bloedcytologie: criteria bloedplaatjes.
http://wiki/download/attachments/92640737/SOP_092_TAB_PB4.htm?version=1&modificationDate=1291651607133 *(2)*

S-Monovette® ThromboExact, Sarstedt. www.sarstedt.com/pdf/prospekte/en/443.pdf. Information obtained on the internet site the 21/04/2013. (27)

Pseudothrombocytopenia in de literature

Studies about pseudothrombocytopenia (PTCP) are pretty rare. We can find a mean of seven articles per year for the last twenty years. With the search item "pseudothrombocytopenia", we got 230 results on Pubmed since 1973.

Before discussing PTCP, we first need to define thrombocytopenia which is usually defined as platelet count below 150.000 / µL (1,2). A classification of mild, moderate and severe or grade I, II, III and IV thrombocytopenia exists but is not universally accepted or used.

Pseudothrombocytopenia (PTCP) was first discovered in 1973 (7) and is defined as laboratory artifact from any automatic analysers given a spuriously reduced platelet count (1,3,4). This artifact occurs only in vitro after the contact between platelets and anticoagulant and has no clinical significances if well recognized. Otherwise it can lead to misinterpretation and may be confused with heparin-induced thrombocytopenia with thrombosis (HITT), disseminated intravascular coagulation or any other hemorrhagic problem (3,13). Moreover it can lead to further unnecessary test, delays in diagnostic or avoidance of therapeutic procedures and discontinuing of treatments (anticoagulant or antiplatelet drugs), or inappropriate treatments as platelet transfusion, steroid therapy and splenectomy,… (1,3-5,12,14-16,22,29,30). In conclusion, PTCP is a trap where you mustn't fall especially when thrombocytopenia occurs without any clinical signs.

PTCP happens always *in vitro*, within 1 or 2 minutes and increases progressively till 2 hours after the blood collection (7). It is mostly described when ethylene-diamine-tetra-acetic acid (K3 or K2 EDTA) is used as anticoagulant. Citrate, oxalate, and heparin anticoagulant have also been implicated in PTCP as well as nonchelating anticoagulants such as hirudin or D-phenylalanine-proline-arginine-chloromethyl ketone (5,12,14,20). EDTA operates especially as a Calcium chelator, in 1:1 complexe (1, 3). By its chelation faculty, EDTA will induce conformational change and will expose platelet antigens which are target for platelet antibodies (1,3). Platelet antibodies recognize the glycoprotein IIb which is only revealed in the dissociated form of glycoprotein IIb/IIIa complex (3,4). This complex dissociates when the calcium concentration is lowered (in presence of EDTA for instance), and it re-associates when calcium is replaced but low calcium concentration alone is not sufficient (6-7). These antibodies (IgM, IgG or IgA ; respectively 43 to 52%, 27 to 32%, and 3%) are identified up to 83% of cases (3,4). Platelet agglutination and clumping in vitro will finally reduce the platelet count for every normal patient but not with patients with Glanzmann disease because they miss the glycoprotein IIb-IIa (6). This suggests that this glycoprotein is involved in the aggregation process of PTCP..
Other study (5) has shown that PTCP due to EDTA doesn't follow physiological aggregation and wasn't affected by inhibitors of the platelet-release reaction (acetylsalicylic acid). It has also been suggested that other autoantibodies could take part in, as anticardiolipin antibodies (3). Other theory is that PTCP was developed in contact with antibiotics but it has not been proven yet. (14) Modification in platelet charge or abciximab-induced conformational changes have also been discussed. (13,14) Moreover cases of EDTA-dependent leukocyte clumping has also been described (22). Then PTCP could also lead to false white blood cell (WBC) and WBC differential when hematology analyzers confound platelet aggregates with leukocyte because of similar size (8,9). Because PTCP is an artifact due to the presence of platelet antibodies, the detection of platelet allo- or autoantibodies using tests based on agglutination or binding of immunoglobulins to platelets should be carefully interpreted because of a risk for false positive result (6). Nevertheless the exact mechanism and the nature of these antibodies hasn't been discovered yet.

What we know is that PTCP is a rare artifact with a prevalence of about 0.1% in the general population (1,3,4,8,10,14) with a range from 0.03 to 1.9% and 0.013% in pols blood donors which was a study over ~77.000 donor but the methodology wasn't clear (9). Abciximab therapy can induce PTCP in about 2% of treated patients with a range from 1.7% to 4.0%. The two other "classic" glycoprotein IIb/IIa receptor antagonists – tirofiban and eptifibatide – are not related to PTCP (13-15). PTCP has been described in neonate (transient PTCP due to transplacental transmission of a maternal serum factor), infants, adults and also in animals such horses and dogs (3,15,16).
Healthy people are involved as well as ill patients under rheumatoid arthritis, malignancies (neuroendocrine carcinoma, thymoma and plasmocytoma), drugs (aminoglycosides, abciximab, valproic acid, sunitinib), viral infection (hepatitis A), cardiac surgery, acute myocardial infarction and HITT (3,5,7,10,13-17,22-24,26,28,29). HITT is a serious complication occurring in 5 days or more after heparin treatment with decreased platelet count. It is mediated by antibodies to heparin-platelet factor 4 (HPF-4) complexes, inducing platelet activation and aggregation which cause arteriovenous thromboembolism. Diagnose can be done with the detection of heparin platelet factor 4 (HPF-4) – induced antibodies. Apparently, cross reaction can happen and give false positive result, especially in certain populations such as cardiac surgery patients, and in patients with antiphospholipid antibodies. Balcik and al (2009) showed the presence of 25% HPF-4 positivity in patients with PTCP. Their conclusion was that if thrombocytopenia occurs in patients receiving heparin therapy, PTCP must be ruled out before considering HITT because of the false positive results. (17) This conclusion was followed by *Martin-Toutain and al. (2009) and also Schwarzinger and al (2000).* Diagnosis of HITT still remains a diagnosis of

exclusion. (18) Despite all these diseases, there is still no clinical evidence that associates the presence of PTCP with specific diseases or the use of specific drugs. (10,25)

Different solutions exist to prevent PTCP, the non-exhaustive list is on table 2 and table 5 (3,7). These treatment are not 100% effective, they are expensive and take time to be applied in routine but they have the merit to exist. But for instance, performing platelet count at 37°C may not be successful because approximately 17% of autoantibodies are reactive at this temperature. (14)
Sane and al (2000) have proposed a gold standard in performing a platelet count on nonanticoagulated blood obtained by finger stick. Pre-supplementation of aminoglycosides to EDTA, before the sample withdrawal, can prevent platelet clumping and supplementation of aminoglycosides to EDTA-samples can induce a dissociation of aggregated platelets. (10-12) A case of multianticoagulant dependent PTCP (NaF, sodium citrate, K2-EDTA and heparin lithium) was corrected by the addition of amikacin but not by vitamin B6, gentamicin, or aminophylline. (11) Another solution is the addition of CaCl2 which replaces Ca-Chelated by EDTA and Heparine which maintains anticoagulation to dissociate platelet clumps in EDTA-PTCP. The result was equivalent to that obtained using kanamycin supplementation (4). Other treatment was also successful with a mixture of heparin and theophylline (21). Last but not least, the Sarstedt company provides a solution with a new kind of anticoagulant but not yet disclosed (27).

Once again, there are not a lot of objective way to diagnose PTCP in the literature. Some of them proposed four of five diagnostic criteria. According to my current knowledge, in practice, any "modern" hematological automate can give an alarm for PTCP and make a blood smear to control microscopically the eventual presence of platelet aggregates. Definition of platelet aggregates was only find in one article from Onder and al, 1980 (56: 177-182). Isik and al (2012) used a minimum of 15 platelet clusters to define a platelet aggregate. (25) Again according to my current knowledge, diagnose of platelet aggregates could be a subjective diagnose and so could differ person per person. More than a blood smear, a new platelet count using an EDTA, citrate and/or heparin anticoagulant is also usually recommended, in second intention, to give a more realistic platelet count (1,3,15,31). In table 1, major criteria have been written to establish a diagnosis of PTCP (3,4). Other criteria were used in study from Sane and al (2000) and requirements are in Table 6. (14)

Pseudothrombocytopenia and Gasthuisberg (UZL) experience

Here in Universitair Ziekenhuis van Leuven (UZL), we use three categories to define trombocytopenia, 100.000 to 150.000/µL as mild, 50.000 to 100.000 as moderate and lower than 50.000 as severe thrombocytopenia (2). To perform platelet count, we use, such as most laboratories in Europe, the K2EDTA form which has been advised by the International Council for Standardization in Hematology (ICSH) (3,4). Platelet count is performed with XE-5000 from Sysmex ® where PTCP are flagged with rules 14, 15 or 16 which most specific is rule 15. This one becomes positive according to the platelet count, the detection of presumed aggregates, an isolated thrombocytopenia, premature platelet form,… We can also see the presence of aggregates on graphic from the IMI and the Diff Channel. The action is then the realization of a blood smear by the SP-1000 from Sysmex which is reviewed microscopically by trained technicians. Finally, the technician writes the result (presence or lack of aggregates) on the LIS and releases the result of the platelet count.

We have collected data from our LIS during 21 days between 13 November 2012 en 03 December 2012 and we obtained 21.474 samples from 10.372 patients after eliminating the test and control samples, the duplicates, samples with technical problems,… Our population was divided in 52% men and 48% women. Our oldest patient was born in 1913 and the youngest in 2012. Our population's mean age was around 65 years old. Out the 21.474 samples, we had 349 samples which had a positive rule 15, so 1.63% of the total samples. Finally, after a microscopic control of these 349 samples, we found 29 samples with platelet aggregates, so 0.14% of total samples.
If we sorted samples per units, we found that haematology and intensive units have the highest number of positive rule 15 (57 samples each) followed by hepatology units (26 samples) and anaesthesiology (25 samples). The sex distribution of these positive rule 15 was 56% men and 44% women.
If we look now to the 29 samples with platelets aggregates, we note that the units repartition has changed. Cardiology unit has now the highest number of samples (6) and is followed by intern and intensive units with 4 samples each. Haematology unit got only 1 positive sample with platelets aggregates. Moreover the sex repartition has also changed with 21% men en 79% women.
Unfortunately these statistical descriptions should be cautiously interpreted because of the little size of samples and much more carefully with positive samples interpretation because of very few data.

When we look to the Sysmex rapport of a positive sample with aggregates, we see (see above) in the IMI channel, the presence of a line (~ y=x). The delimited region for aggregates detection is near the delimited region for immature granulocytes detection. Is there a possible cross-over between these two rules (Ru15 and RU82)? We have researched if there were other rules which were also positive at the same time than the rule 15. Of the 349 samples with positive rule 15, we had 49 samples which were also positive for the rule 82. So a little cross-over could maybe exist between these rules but again more samples are required to conclude.

We have sorted the sample in four categories: true positive (Ru15 positive with platelets aggregates) – false positive (Ru 15 positive without platelet aggregates) – true negative (RU15 negative without platelet aggregates) – false negative (RU15 negative with platelet aggregates). We obtained respectively 18 – 351 – 21473 – 11 samples. When you calculate the sensitivity, specificity, positive predictive value and negative predictive value we had respectively 62.1%, 98.4%, 4.9% and 99.9%. There is a very important bias in this evaluation: the true negative. This category is only negative because we trust every negative results from Sysmex as true negative. But the presence of false negative samples shows that we should not trust the Sysmex ® system all the time. Unfortunately and according to my knowledge, there is no study which have studied the rule 15 to better understand the real performance of this rule. The false negative results came from the rule 17 from which technicians have to control platelets on a smear. This is a non-resolved problem, are there other non-detected false negative samples and if yes, how many?

As I said, diagnose of PTCP is not easy because it is rare and because the diagnostic method lacks of objectivity. Here in Gasthuisberg, we perform a microscopic control by reviewing the edges and the tail of the blood smear because these places give the most chance to see platelet aggregates. During this time, the platelet count is not apparent for the clinician until the answer presence or lack of platelet aggregates. The laboratory took this decision to be sure that the clinician has seen the results of platelet count and also the comment about presence of PTCP or not. The microscopic control is based on an intern procedure which available answers are yes there are aggregates or no there aren't. The clinician has also the possibility to send three different anticoagulant samples (one with EDTA, one with citrate and the last with heparin) what we call the "PTCP test". The microscopic interpretation is also based on an intern procedure which classes PTCP in three categories: lack of aggregates, a few of aggregates or several aggregates. Moreover a platelet count is performed on each sample. We also meet a problem of objectivity here because no exact definition of a platelet aggregate exists (see above). I guess that technicians, scientists and even medical doctor won't give the same answer after microscopic control but this has still to be demonstrated.

Other element to better understand this PTCP test, we collected the data from the last 14 months, from February 2012 to April 2013. We got 318 samples from 293 patients. Like all data from our study, conclusions have to be cautiously interpreted because of multiple bias such as retrospective study, few samples, no correlation with gold standard method, subjective diagnostic method and too many different technicians, scientists or medical doctor who have made the microscopic diagnose. Data about platelet counts are more objective because they were analyzed with identical method except platelet count with citrate anticoagulant because there is no correction after the dilution from volume of anticoagulant. That means that the platelet count with citrate anticoagulant is always underestimated in this study. The mean of platelet count of EDTA, Citrate and Heparin samples were respectively 98.4, 80.8 and 75.6x10^9/L. The median was respectively 80, 64 and 62x10^9/L. We can only conclude that results from EDTA samples are usually higher than samples with heparin. When we compare these results to the microscopic control of platelet aggregates, we see that platelet count corresponds to an inversely tendency with the presence of aggregates. Citrate and Heparin samples would show platelet aggregates more frequently and would have by this way lower platelet count than EDTA samples. This conclusion should also be interpreted carefully because of subjective data and of underestimation of platelet count from citrate samples. Furthermore 15% of samples had platelet count above 150.000 / µL and it were therefore unnecessary to control the presence of aggregates on blood smear.

Conclusion:

The pathophysiology of pseudothrombocytopenia is still not well understood by scientists but we know that this artifact is rare and not symptomatic. The only danger is with misdiagnose/misinterpretation which can lead to further unnecessary test, delays in diagnostic or avoidance of therapeutic procedures and discontinuing of treatments, or inappropriate treatments.
In our university hospital, we need to objectify our procedure more so that we can be sure that everyone works with identical method. To achieve this goal, we need first to define platelet aggregate and pseudothrombocytopenia. Finally we need to find a test which can help us to get a platelet count as close to the real count. We could maybe evaluate new anticoagulants as tromboexact ® from Sarstedt company and so replace platelet count and microscopic control on EDTA, Citrate and Heparin samples. Unfortunately the rules installed on the sysmex automates are not enough understood that to be changed because we are not currently able to predict the consequences of any changes.

COMMENTS

No comments.

TO DO/ACTIONS

1) More objective microscopic definition of platelet aggregate.
2) More objective microscopic definition of pseudothrombocytopenia.
3) Evaluate S-Monovette Thromboexact sample from Sarstedt company and evaluate if it can replace totally or partially the 3 samples (EDTA, Citrate and Heparine) used presently for the test "pseudothrombocytopenia beoordeling".
4) Evaluate thromboexact sample from Sarstedt company to HITT.
5) For citrate samples from the test "pseudothrombocytopenia beoordeling", it should be interesting to know the filling level of this tube and interesting to apply a correction to platelet count because of dilution from anticoagulant volume.
6) Let a trace of diagnosis of PTCP in LIS. By this way, MLT's could know the history of the patients and be more attentive.
7) Evaluate the number of phone call from clinician to get result of platelet count. Discussion with clinician to continue to hide or not the platelet count result while MLT's search the presence of aggregate on blood smear.

ATTACHMENTS

Attachment 1

Table 1 Major criteria for establishing a diagnosis of EDTA-dependent pseudothrombocytopenia.

1. Platelet count typically $<100\times10^9$/L
2. Onset in only EDTA-anticoagulated sample kept at room temperature
3. Time-dependent fall of the platelet count in the EDTA specimen
4. Presence of platelet aggregates and clumps in EDTA-anticoagulated samples
5. Lack of clinical signs or symptoms of platelet disorders

Table 1 : Lippi and al. EDTA dependent PTCP

Attachment 2

Table 2 Additives and other compounds used to prevent EDTA-dependent pseudothrombocytopenia.

- Warming the sample at 37°C
- Buffered sodium citrate
- Lithium or calcium chloride heparin
- Ammonium oxalate
- β-hydroxyethyltheophylline
- Sodium fluoride
- Trisodium citrate, pyridoxal 5′-phosphate and Tris (CPT)
- Antiplatelet agents (e.g., acetylsalicylic acid, prostaglandin E1, apyrase, monoclonal antibodies)
- Potassium azide
- Kanamycin, amikacin and other aminoglycosides

Table 2 : Lippi and al. EDTA dependent PTCP

Attachment 3

Table 3: O. Onder and al, blood 1980 56: 177-182			
0	Single platelets or clumps of less than 5 platelets		
+	Small clumps	5-10 platelets	In less than half of the 10 fields examined
++	Small clumps	5-10 platelets	In over half of the 10 fields exmined

+++	Large clumps	> 10 platelets	In less than half of the 10 fields examined
++++	Large clumps	> 10 platelets	In over than half of the 10 fields examined

Attachment 4

Table 4: UZL classification for PTCP		
Beoordeling pseudo-trombopenie	Vul het absoluut aantal bloedplaatjes in (van de tubes met de verschillende anticoagulantia) gemeten door de celteller in de overeenkomstige vakjes.	
blp.EDTA 10**9/L blp Citraat 10**9/L blp.Heparine 10**9/L	absoluut aantal	
	Beoordeel de bloeduitstrijkjes afgenomen op EDTA,citraat en heparine anticoagulantia op aanwezigheid van bloedplaatjesaggregaten	
Aggr. EDTA Aggr. Citrate Aggr. Heparine	aggregaten afwezig enkele aggregaten aanwezig meerdere aggregaten aanwezig	
Bijzonderheden pseudo-trombopenie	Enkel in te vullen indien bijkomende relevante bevindingen ivm pseudo-trombopenie	textvak

Attachment 5

Table 5 : article 7, Shreiner and al, blood 1973,42:541-549

Table 3. Inhibition of EDTA-Dependent Agglutination

N-ethylmaleimide 0.005 *M*	0
Heparin 100 U/ml plasma	0
α-Tocopherol 0.012 *M*	0
Citrate anticoagulant	0
Oxalate anticoagulant	0
EDTA > 0.1 *M*	+
Excess Ca^{++} ion	+
Excess Mg^{++} ion	+
Anti-IgG sera	+
Anti-IgM sera	+
Anti-IgA sera	0

0, no effect on platelet agglutination.
+, inhibition of agglutination.
Anticoagulant concentrations are given in methods.

Attachment 6

Table 6: PTCP criteria from Sane and al, 2000
1) a difference between the platelet count in 2 anticoagulants, with one having a count at least 20% lower than the count in the comparison anticoagulant.
2) platelet clumping on a blood smear made from anticoagulated blood
3) a normal platelet count on a blood smear made from nonanticoagulated blood
or 4) an unexplained drop in platelet count at 30 min to 4h after abciximab bolus with recovery to a normal count within 4h after the nadir