

S1), prompting review of a peripheral smear using digital microscopy (Cellavision DI-60; Sysmex Corporation, Kobe, Japan).

Subsequent microscopic examination of the white blood cells revealed the presence of thrombophagocytosis by the patient's neutrophils and, to a lesser extent, monocytes (Figure 1). Simultaneous platelet satellitism was observed. Similar findings were also observed in blood samples obtained 24 and 48 hours later. As blood values remained within reference ranges, no further follow-up samples were collected. Maternal blood was also analyzed to assess a potential familial factor in the observed thrombophagocytosis, but no platelet phagocytosis could be observed in the mother's blood smear examination.

In order to further explore the phenomenon of thrombophagocytosis in the neonate, we re-analyzed the initial sample in which platelet phagocytosis was first observed via the Sysmex XN-20 analyzer approximately 4 hours after initial measurements [to obtain additional scattergrams (Figure S1)]. Interestingly, despite no clotting or platelet aggregates present in the sample, this repeat measurement illustrated a significant decrease in platelets compared to the initial value (88 vs. $160 \times 10^9/l$), which was not expected based on the stability of platelets in EDTA-anticoagulated blood [8].

Discussion

Several EDTA-related artifacts in hematology, such as platelet clumping and satellitism, are well documented and may falsely lower platelet counts [1,2,7]. These phenomena are related to the chelation of Ca^{2+} ions by EDTA, which in turn induces conformational platelet membrane changes with exposure of cryptantigens on glycoprotein IIb/IIIa, which can subsequently be targeted by autoantibodies [1,2,7]. Thrombophagocytosis by neutrophils may potentially likewise be an *in vitro* EDTA artefact [1,2,7]. The *in vitro* nature of this phenomenon may be confirmed by repeating the analysis in a specimen collected in, for example, sodium citrate or with a capillary blood sample (collected without anticoagulant). Regrettably, such samples were not available for our patient. Likewise, as samples were only obtained up to 48 hours after birth (as there was no clinical indication for further sampling), we could not determine whether the observed thrombophagocytosis was a transient process or rather an enduring phenomenon in our patient.

Thrombophagocytosis has thus far only been described sporadically, most commonly in the setting of a bacterial infection [1-7]. In the current case, no indication of bacterial or other infection was present that warranted laboratory investigation, and the mother's drug regimen (tacrolimus, azathioprine) was the only noteworthy finding. Although it is possible that the mother's immunosuppressive therapies may have played a role in the manifestation of this

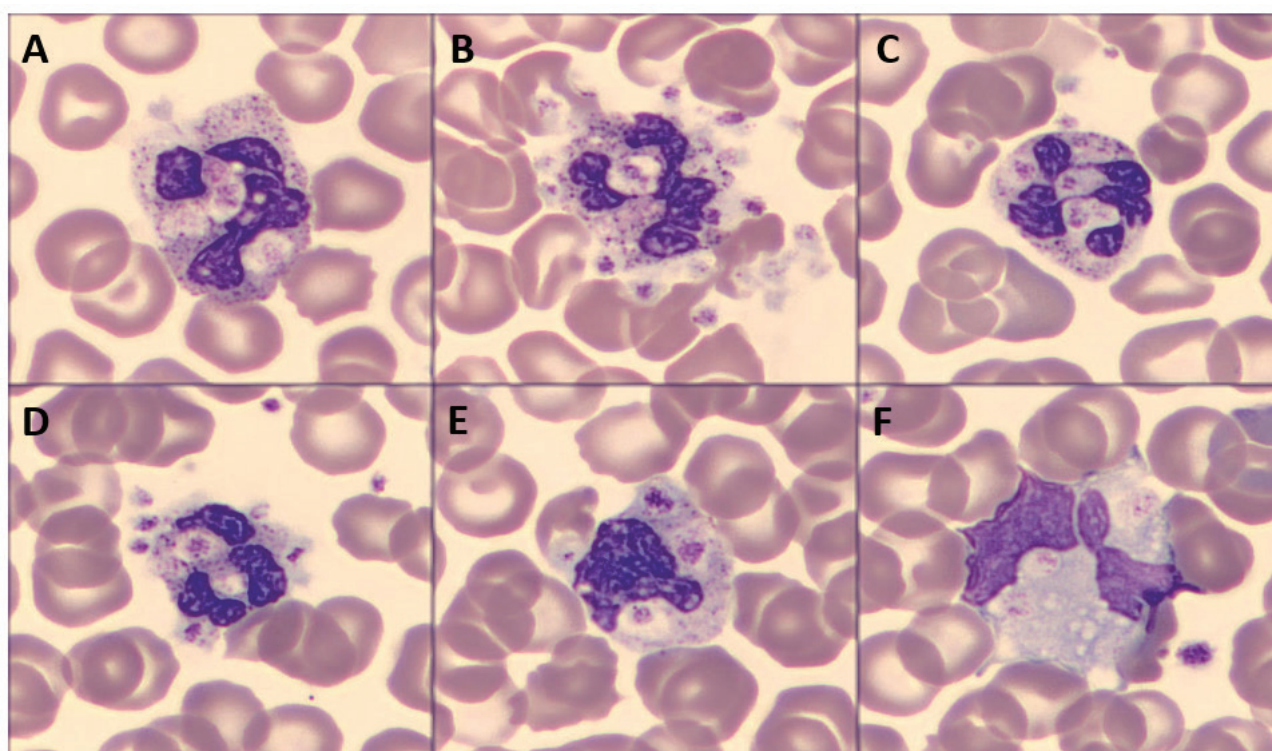


Figure 1. Thrombophagocytosis. Digital micrograph (100x) obtained via DI-60 after May-Grünswald Giemsa staining showing thrombophagocytosis by neutrophils (panels A-E) and monocytes (panel F). Platelet satellitism can also be observed (panels B, D).

phenomenon in the neonate, as tacrolimus and azathioprine have demonstrated (limited) transplacental passage, further study would be required to confirm this hypothesis [9,10]. However, given that platelet phagocytosis could not be observed in maternal blood and that tacrolimus and azathioprine are widely used drugs while platelet phagocytosis is only observed rarely, the role of the immunosuppressive drugs in inducing thrombophagocytosis in the neonate is likely limited.

Interestingly, we were able to demonstrate that a delayed re-analysis of the neonate's initial EDTA-anticoagulated sample resulted in a marked decrease in platelet count despite the absence of visible clotting or platelet aggregates. As such, this finding suggests that ongoing *in vitro* thrombophagocytosis may progressively consume platelets over time, which may have important implications for clinical laboratories and patient care. For example, a delayed processing of samples displaying thrombophagocytosis can yield artificially low platelet values, resulting in pseudothrombocytopenia. If not recognized as an artifact, these falsely decreased counts could subsequently lead to unnecessary diagnostic workup for thrombocytopenia, inappropriate transfusion decisions, or unwarranted therapeutic interventions. Laboratories should therefore implement protocols to ensure the timely analysis of samples. Moreover, when thrombophagocytosis is encountered in a sample, the laboratory should consider alternative anticoagulants (e.g., sodium citrate) or fresh capillary samples to confirm true platelet levels. Clinicians should also be aware of EDTA-dependent changes and interpret low platelet counts with caution when phagocytosis or satellitism is observed, particularly in the absence of clinical signs of bleeding or thrombocytopenia.

Conclusion

This case highlights the phenomenon of thrombophagocytosis, which may not only be limited to infectious settings. Although we could not conclusively determine EDTA anticoagulant as the causative factor for this phenomenon, we illustrated that delayed analysis of samples displaying thrombophagocytosis can result in falsely decreased platelet levels and, hence, pseudothrombocytopenia. Awareness of this artifact and timely processing of blood samples are crucial to ensure accurate platelet counts and avoid misdiagnosis.

What is new

Thrombophagocytosis, the phagocytosis of platelets by neutrophils and monocytes, has previously only been described rarely (e.g., 11 reports since 1994) and mostly in the setting of bacterial infections. In the current case, the authors illustrate that the phenomenon may also present in non-infectious settings, as no infection was apparent in the neonate. Moreover, this case report is also the first to illustrate that delayed analysis of samples displaying thrombophagocytosis may result in falsely decreased platelet

levels (pseudothrombocytopenia), thereby highlighting the importance of timely analysis. As such, this report aims to inform medical professionals about the potential impact of this rarely observed phenomenon.

Acknowledgments

We would like to thank the patient as well as all laboratory personnel of the University Hospital of Leuven.

List of abbreviations

EDTA ethylenediaminetetraacetic acid

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Funding

No funding was received for this manuscript.

Informed consent

No written informed consent was required per institution's policy, as all patients (and/or their guardians) visiting our hospital (University Hospitals of Leuven, Belgium) agree with the use of anonymized data and leftover materials for research purposes via opting-out agreement.

Consent for publication

Due permission was obtained from the parents of the patient to publish the case and the accompanying images.

Ethical approval

Ethical approval is not required at our institution for anonymous case reports.

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Summary of the case

1	Patient (gender, age)	Male, 1 day
2	Final Diagnosis	Healthy, displaying thrombophagocytosis by neutrophils and monocytes
3	Symptoms	None
4	Medications	None, maternal medications: azathioprine, tacrolimus
5	Clinical Procedure	Laboratory analysis via automated hematology analyzer and microscopy
6	Specialty	Laboratory medicine

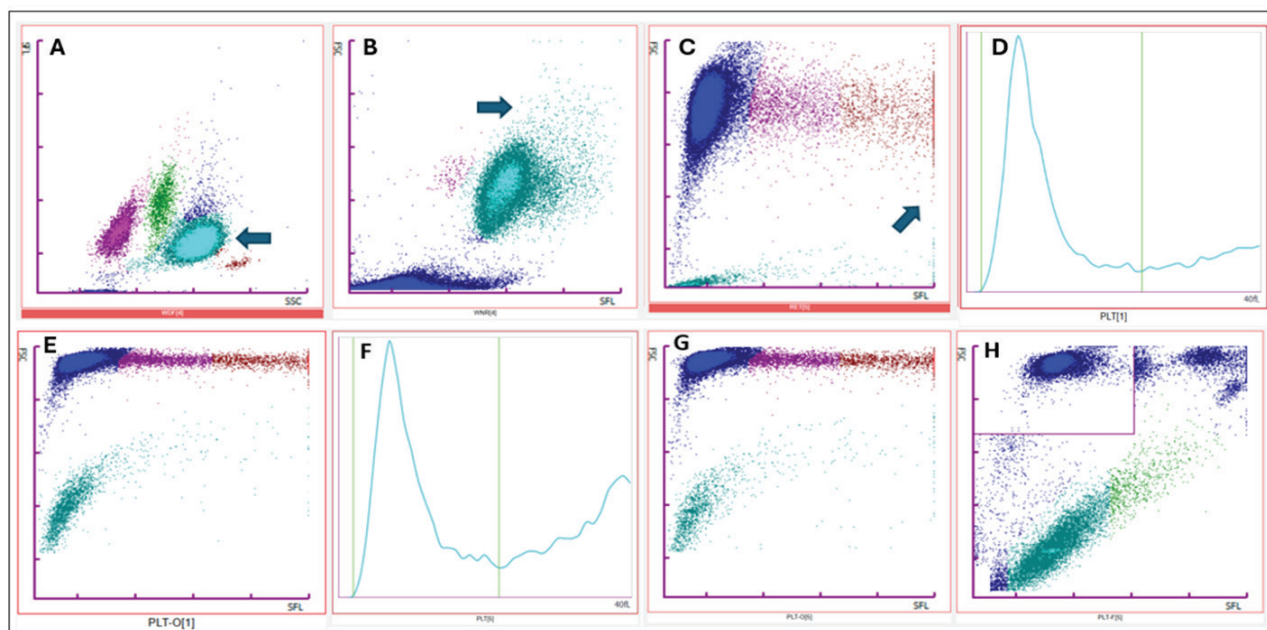


Figure S1. Sysmex XN-20 scatterplots of the neonatal blood sample. The automatic hematology analyzer flagged the sample as suspicious due to an abnormal WBC scattergram [due to i) the clustering location of the light blue neutrophil population in the WDF scattergram (A) which displayed an increased side fluorescence and ii) due to the presence of an increased number of particles on the BASO:WBC border in the WNR scattergram (B)] and an abnormal reticulocyte scattergram (scattergram C; due to the presence of particles with high levels of side fluorescence). Scattergrams: A) WDF channel, B) WNR channel, C) RET-channel, D) PLT (impedance) scattergram of initial measurement (platelets $160 \times 10^9/l$), E) PLT-O scattergram of initial measurement (platelets $159 \times 10^9/l$), F) platelet (impedance) scattergram of second measurement (platelets $88 \times 10^9/l$), G) PLT-O scattergram of second measurement (platelets $103 \times 10^9/l$), and H) PLT-F scattergram of second measurement (platelets $103 \times 10^9/l$). The second measurement of platelets (F-H) occurred approximately 4 hours after the initial measurements (panels A-E).