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PREVENTION OF POLYMORPHONUCLEAR LEUKOCYTE AGGLUTINATION IN VITRO

Michele Schinella, Priyakumari Kojikara, Vito Curci

Laboratorio di Chimica Clinica e Microbiologia Ospedale Civile, Rovereto (TN), Italy

Sir,

recently, Galifi et al.[1] reported a case of EDTA-induced polymorphonuclear (PMN) leukocyte agglutination *in vitro*. The phenomenon was studied on Bayer Technicon H*1, Coulter Counter STKR and STKS analyzers. Leukocyte agglutination is a very rare event (seven cases have been studied up to now) and results in a spurious low white blood cell (WBC) count.[2-7] WBC aggregates may comprise all kinds of cells or be limited to only one type, in particular PMN. Here we report a case of spurious leukopenia due to granulocyte aggregation *in vitro* and the effects of different anticoagulants and time variations on leukocyte and platelet counts.

A 39-year-old man under treatment with phenobarbital and valproic acid for ten years was found to have low WBC (2.5×10^9/L), neutrophil (26%) and lymphocyte (70%) counts during a routine blood test carried out on the 25th of October, 1990. The highest count verified during successive tests was 3.8×10^9/L, and the patient was hospitalized with a diagnosis of *leukopenia in a patient in long-term antiepilepsy treatment*.

During hospitalization several lab checks were carried out and WBC were found to be between 2.6 and 3.1×10^9/L. A bone marrow biopsy was not diagnostic. PMN aggregates were evident in subsequent microscopic examination of stained blood smears. When the count was determined immediately after blood collection, total WBC were 6.1×10^9/L. An attempt to eliminate the aggregates by incubation at 37°C for half an hour proved unsuccessful. On 25th August, 1994, when the patient came to our consulting room, we obtained his consent to conduct further studies on this leukopenia by employing different anticoagulants and by determining counts at various time intervals.

The patient's blood was collected immediately in Vacutainer test tubes (Becton Dickinson, Milan, Italy) containing the following anticoagulants: K3.EDTA (1.5 mg/mL), buffered sodium citrate 0.129 mol/L (cod 606609), and acid citrate dextrose (ACD-cod 367756). At the same time an aliquot (5 mL) of venous whole blood was collected in 55 mg/mL of a mixture containing citrate-pyridoxal 5'phosphate-tris (CPT from Far, Pescantina, Italy).[8,9] All samples were processed immediately and counts were determined later at intervals of 20, 50, 80, 110 and 140 min with Coulter Counter STKR and STKS analyzers (Coulter Electronics, Inc. Hialeah, FL, USA).

Smears were stained with May-Grünwald-Giemsa after each WBC count. This procedure is of fundamental importance because the pseudoleukopenia and pseudoneutropenia resulting from Coulter instruments on blood collected with EDTA are artifacts that may not be suggestive of leukocyte agglutination.[1] This phenomen can be detected only by microscopic observation.

Twenty minutes after the first sampling with EDTA, the initial WBC counts of 5.9 and 6.0×10^9/L dropped to 4.0 and 3.2×10^9/L with STKR and STKS, respectively. After 50 min the number of leukocytes was further reduced to half of the initial count, performed at 0 time. The count obtained after 140 min was 30% lower than the one done 20 min after blood collection in EDTA, showing that there is significant variation in the count during the course of time. This differs from what was described by Rohr et al.

The fact that these counts were similar to those carried out 4 years earlier suggests that

Correspondence: Michele Schinella, Lab. di Chimica Clinica e Microbiologia Ospedale Civile, p.le Santa Maria 6, 38068 Rovereto (TN), Italy.

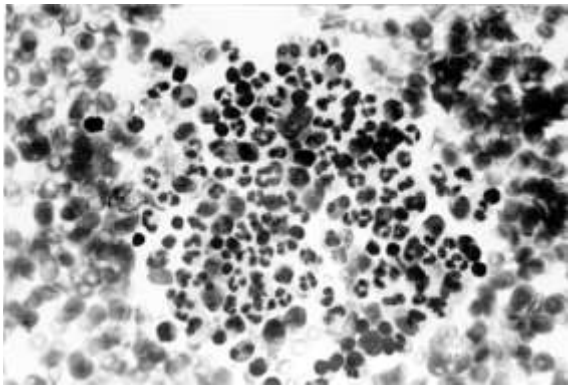

Figure 1. Peripheral blood smear. Clumps consist of very different numbers of white blood cells and entrapped platelets. May-Grünwald-Giemsa stain (×400).

the phenomenon in this patient was neither diminishing nor transient, which is in contrast with what Epstein and Kruskall described in their patients.

The count performed on blood collected with CPT anticoagulant remained constant throughout the observation period (the maximum difference noted between different counts was $\pm$ 0.2×10^9/L leukocytes). While there were no significant variations in the automated leukocyte counts between the two blood analyzers, there were differences in the samples containing anticoagulants EDTA, ACD and citrate.

Surprisingly, in the sample anticoagulated with citrate the platelets gradually decreased overtime. PLT were 171×10^9/L at zero time, 150 after 20 min. 120 at 50 min 100 at 80, 83 at 110 and 95×10^9/L at 140 min; the counts behaved similarly in both analyzers.

Less of a decrease in PLT counts was also recorded in the sample with ACD. Multiple white blood cell aggregates were discovered at microscopic observation of smears from EDTA anticoagulated blood. Clumps were located at the edge of the smears and consisted predominantly of neutrophils. A few entrapped lymphocytes and monocytes were also located within the clumps. Some clumps contained more than 100 elements.

The low PLT counts found in the citrate anticoagulated sample were confirmed by the clumps present on the blood smear (Figure 1).

It will be of great interest to learn whether studies on blood and blood counting can be conducted on other analyzers operating on a different principle from the one utilized by Coulter analyzers and the immunofluorescence test for anti-neutrophil antibodies.

In conclusion, our case of pseudoleukopenia is clear proof that microscopic investigation of WBC agglutinates on blood smears is essential in every patient with unexplained leukopenia or neutropenia; it is also advisable that these blood samples be anticoagulated with CPT to avoid spuriously low WBC counts. Only CPT was able to prevent WBC agglutination in our samples.

References

1. Galifi M, Schinella M, Nicoli M, Lippi G. Instrumental report and effect of anticoagulants in a case of neutrophil agglutination *in vitro*. Haematologica 1993; 78:364-70.
2. Epstein HD, Kruskall MS. Spurious leukopenia due to *in vitro* granulocyte aggregation. Am J Clin Pathol 1988; 89:625-55.
3. Savage RA. Analytic inaccurancy resulting from hematology specimen characteristics. Am J Clin Pathol 1989; 92:295-9.
4. Antonsen S, Beyer JM. *In vitro* granulocyte aggregation. ScandJ Clin Lab Invest 1989; 49:685-88.
5. Rohr LR, Rivers FM. Spurious automated leukopenia due to *in vitro* granulocyte aggregation. Am J Clin Pathol 1990; 93:572-4.
6. Robbins SH, Conly MA, Oettinger J. Cold-induced granulocyte agglutination. Arch Pathol Lab Med 1991; 115:155-7.
7. Lombarts AJPF, de Kieviet W, Franck PFH, Baars JD. Recognition and prevention of two cases of erroneous hemocytometry counts due to platelet and white cell aggregation. Eur J Clin Chem Clin Biochem 1992; 30:429-32.
8. Lippi U, Schinella M, Modena M, Nicoli M, Lippi G. Advantages of a new anticoagulant in routine hematology on the Coulter counter S-Plus STKR analyzer. Am J Clin Pathol 1990; 93:720-4.
9. Lippi U, Schinella M, Modena M, Nicoli M, Lippi G. EDTA-induced platelet aggregation can be avoided by a new anticoagulant also suitable for automated complete blood count. Haematologica 1990; 75:38-41.