Electronic counter-related pseudoleukopenia: more than a rare occurrence

Electronic counter-related pseudoleukopenia can change clinical decisions. By using a comparative analysis between the electronic counter-related white blood cell count and the count obtained by a direct leukocyte count we show that this pseudoleukopenia might be not rare in patients with increased leukocyte adhesiveness/aggregation.

Electronic counter-related pseudoleukopenia has been previously reported.[1-3] We conducted a study to see how increased leukocyte adhesiveness/aggregation (LAA)[4-6] affects the results of the electronic counter white blood cell count (ecWBCC). We investigated 496 patients with pneumonia, urinary tract infection, soft tissue infections, sepsis, upper respiratory tract infections as well as various viral and rheumatic diseases aged 62±24 years and 112 controls with a mean±SD age of 35±14 years. Peripheral venous blood was obtained for ecWBCC in EDTA vacutainer tubes using an electronic (Coulter electronics) counter and for the leukocyte adhesiveness/aggregation test (LAAT) using an image analyzer (imWBCC) (INFLAMET™) as described elsewhere.[7-9] As a standard against which to test the ecWBCC, we used an image analyzer-based estimate of leukocyte count (imWBCC). This estimate has no bias related to leukocyte aggregation. A calibration factor converting the total number of leukocytes counted by the image analyzer to leukocytes/mm^2 is obtained as follows. The median ratio between the ecWBCC and the total leukocyte number counted in each slide is calculated, taking into account only those slides with an aggregation level of up to 10% obtained in young controls who have minimal aggregation. By doing this, it is possible to circumvent the electronic counter underestimated leukocyte count. Multiplying the total leukocyte number by this factor for each slide yields an image analyzer (im)-based WBCC reading (the imWBCC).

To test for possible bias in ecWBCC at high levels of aggregation, we calculated the ratio ecWBCC/imWBCC for samples with high aggregation levels, i.e., an aggregation of >20%. There were 264 such samples in the infection/inflammation group and none in the control group. Figure 1 shows a histogram of the ecWBCC/imWBCC ratio for high aggregation samples (note that the ratio was below 0.5 indicating 50% bias for 12 patients, and that it was below 0.4 indicating 60% bias for 4 patients). Figure 2 shows the corresponding histogram for the 45 samples from patients with infection/inflammation and an aggregation of <10%. We compared confidence intervals for the medians of the underlying distributions. The 95% confidence intervals for the median of ecWBCC/imWBCC for these subgroups were calculated by half population jack-knifing. The results were: (0.78, 0.86) for aggregation >20% and (0.92, 1.18) for aggregation <10%. The significance level (also computed by jack-knifing) for the hypothesis that the median is smaller for the high-aggregation population is 2×10^{-4}. This is a proof that increased LAA causes a negative bias in ecWBCC.

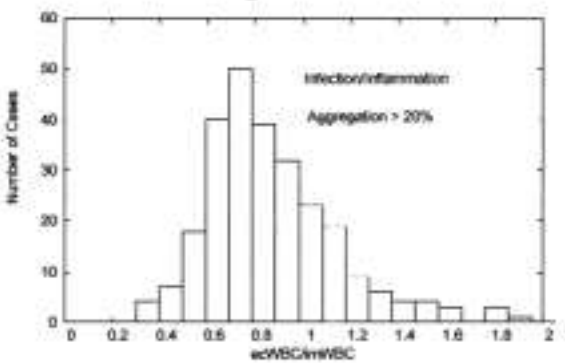

Figure 1. The ratio between the electronic counter white blood cell count and the inflammation meter white blood cell count (ecWBCC / imWBCC) in patients with infection / inflammation and an aggregation level of above 20%.

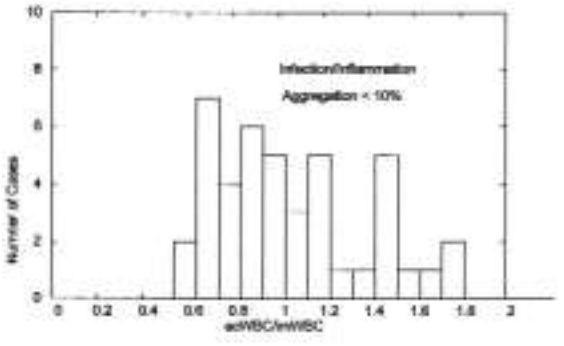

Figure 2. The ratio between the electronic counter white blood cell count and the inflammation meter white blood cell count (ecWBCC / imWBCC) in patients with infection / inflammation and an aggregation level of below 10%.

We conclude that increased LAA in the peripheral blood of patients with acute infection/inflammation is common and might influence the WBCC obtained by using an electronic counter. Physicians should be aware that a WBCC within normal limits obtained by an electronic cell counter in a patient with infection/inflammation, could be an underestimate. These results were recently strengthened in a model in which the patient participated as his or her own control following the infusion of a leukocyte-aggregating agent.[10]

Shlomo Berliner, Renato Fusman, Rivka Rotstein, Daniel Avitzour,§ Itzhak Shapira,* David Zeltser**

**Departments of Medicine "D", at the Tel Aviv Sourasky Medical Center, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv; §Timorim Technologies, Jerusalem, Israel*

Correspondence: Shlomo Berliner M.D., Ph.D., Head, Department of Internal Medicine "D", Tel-Aviv Sourasky Medical Center, 6 Weizman Street, Tel Aviv 64239, Israel. Phone: international +972-3-6973313 - Fax: international +972-3-6974961 - E-mail: shapiraiz@tasmc.health.gov.il

Key words: pseudoleukopenia, leukocyte adhesiveness/aggregation.

Acknowledgments: we thank Esther Eshkol for her assistance with the manuscript.

References

1. Rohr LR, Rivers FM. Spurious leukopenia due to in vitro granulocyte aggregation. Am J Clin Pathol 1990; 93:572-4.

2. Epstein HD, Kruskall MS. Spurious leukopenia due to in vitro granulocyte aggregation. Am J Clin Pathol 1988; 89:652-5.
3. Hillyer CD, Knopf AN, Berkman EM. EDTA-dependent leukoagglutination. Am J Clin Pathol 1990; 94:458-61.
4. Astiz ME, DeGent GE, Lin RY, Rackow EC. Microvascular function and rheologic changes in hyperdynamic sepsis. Crit Care Med 1995; 23:265-71.
5. Yodice PC, Astiz ME, Kurian BM, Lin RA, Rackow EC. Neutrophil rheologic changes in septic shock. Am J Respir Crit Care Med 1997; 155:38-42.
6. Urbach J, Lebenthal Y, Levy S, et al. Leukocyte adhesiveness/aggregation test (LAAT) to discriminate between viral and bacterial infection in children. Acta Pediatr 2000; 89:519-22.
7. Berliner S, Shapira I, Rogowski O, et al. Combined leukocyte and erythrocyte aggregation in the peripheral venous blood during sepsis. A clue to the presence of a commonly shared adhesive protein(s). Int J Clin Lab Res 2000; 30:27-31.
8. Fusman R, Zeltser D, Rotstein R, et al. INFLAMET: an image analyzer to display erythrocyte adhesiveness/aggregation. Eur J Int Med 2000; 11:271-6.
9. Maharshak N, Kassirer M, Zeltser D, et al. The inflammation meter: novel technique to detect the presence of infection/inflammation in patients without leukocytosis but with an increased leukocyte adhesiveness/aggregation. Acta Haematol 2000; 104:16-21.
10. Zeltser D, Fusman R, Chapman Y, et al. Increased leukocyte aggregation induced by gamma globulin: a clue to the presence of pseudoleukopenia. Am J Med Sci 2000; 320:177-82.