

How Much Does the Platelet Aggregate Influence the Total Leukocyte Count? Comparison Between Manual and Automated Impedance Methods in Domestic Cats

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ABSTRACT

Background: Automated hematology analyzers have been developed to optimize the time between analyses and have promising precision and accuracy. Complete blood count (CBC) is often requested as part of veterinary clinical examination. Automated analyzers are often used to determine CBCs, since processing as well as container-related errors may occur owing to variable sizes, aggregates, white or red blood cell fragments, and effects of EDTA on cell morphology. Platelet aggregates frequently occur in felines, with studies reporting a prevalence of approximately 71%. The aim of the present study was to evaluate the influence of exercise aggregates on the global white blood cell count of domestic cats using automated hematological counters with the impedance method.

Materials, Methods & Results: Blood samples of 140 cats, irrespective of age, sex, and breed, were collected into EDTA-containing tubes. The samples were obtained via routine clinical examinations at the Veterinary Hospital of the Federal Rural University of Rio de Janeiro (UFRRJ) and processed at the Veterinary Parasitology Experimental Chemotherapy Laboratory (LQEPV), belonging to the same institution. All the samples were processed on the Sysmex pocH-100iV Diff automated hematology apparatus according to the manufacturer's recommendations. Leukocyte counts were also manually determined using a duplicate Neubauer chamber. Standard dilutions were prepared immediately after the automated analysis. To identify the occurrence of platelet aggregates, a blood smear was made and visualized under a brightfield microscope at a magnification of 10× and scored 0 to 3 (G1, G2, G3, and G4) based on the aggregation intensity. In case of changes, the groups were subdivided according to the intensity of occurrence. Of the 140 samples analyzed, 76.4% (107/140) showed some degree of platelet aggregation. The maximum variation in leukocyte counts determined by the automatic and the manual technique in G1 was 2,500 cells. In G2, it was possible to identify a variation of 6,500 nucleated cells, whereas in G3, this value was 7,100 cells. In G4, where platelet aggregation was intense, the variation between counts was up to 15,000 nucleated cells. A significant difference of variation in total white blood cell count between manual and automated methods was observed when compared to animals that did not show any degree of platelet aggregation ($P < 0.05$). Of the total samples, 23.57% (33/140) comprised G1, 24.28% (34/140) G2, 22.14% (31/140) G3, and 30% (42/140) G4. Of the 140 samples analyzed, 107 showed aggregates, pseudo-thrombocytopenia, and changes in the total number of leukocytes.

Discussion: Samples with higher platelet aggregate formation showed greater interference in global leukometry when analyzed using the hematological counter. White blood cell counts determined by automated analyzers should be interpreted with caution and compared to manual counts when there is significant platelet aggregation in the sample. The findings reinforce the importance of reconfirming the results obtained using an automated equipment in order to avoid misinterpretations that may influence diagnosis and therapy. It is essential to re-check the values obtained from an automated equipment with traditional methods in order to minimize possible errors generated by the equipment, since such errors may affect the clinical diagnosis and subsequently, the therapeutic approach chosen.

Keywords: impedance, leukocytes, thrombocytopenia, Sysmex pocH-100iV Diff.

DOI: 10.22456/1679-9216.105557

Received: 10 October 2020

Accepted: 5 March 2021

Published: 28 April 2021

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INTRODUCTION

The complete blood count (CBC) is commonly ordered as part of the clinical examination. The CBC results can be influenced by the site of collection, the type of tube used, and sedation as well as by the patient's clinical condition [16,17].

Automated analyzers are frequently used to perform CBC's as they reduce processing time. There are 2 methodologies used by these machines to generate the information on white cells, red cells and platelets. These include impedance (electrical interference) or laser technologies (flow cytometry). Errors can occur with platelet counts as a result of variable platelet sizes, platelet aggregation, white or red cell fragments, and EDTA effects on morphology [16,17].

The presence of platelet aggregates is frequently described in felines [6,12,16], with studies reporting a prevalence of approximately 71% [10]. Platelet aggregate formation can provide falsely decreased platelet counts when processed on an automated analyzer [2,12,17,18]. Regardless of the analyzer, misleading results related to platelet count can be observed in cat blood samples taken from standard tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant [3,16,17]. Despite the recognition of these changes, there are few studies evaluating the errors in white blood cell counts due to the presence of platelet aggregates in domestic cats processed in hematology devices using impedance method [18].

Thus, the present study aimed to evaluate how the influence of the presence of platelet aggregates can increase the global white blood cell count of cats when performed on hematological counters automated by the impedance method.

MATERIALS AND METHODS

Samples

Blood samples of 140 cats, without restrictions on age, sex, and breed, were taken in anticoagulant tubes (EDTA). Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work for the procedure(s) undertaken.

The samples were obtained from routine clinical examinations of the Veterinary Hospital of the Federal Rural University of Rio de Janeiro (UFRRJ) and processed at the Veterinary Parasitology Experimental

Chemotherapy Laboratory (LQEPV), belonging to the same institution.

All samples were processed on the Sysmex pocH-100iV Diff automated hematology¹ apparatus according to a manufacturer's recommendations.

Global white blood cell manual counting

Counts by manual leukocyte method were performed in a duplicate Neubauer chamber. Standard dilution was prepared soon after automated analysis.

Classification of platelet aggregates

To assess the occurrence of platelet aggregates, the blood smear was made and visualized under a bright field microscope at 10× magnification. When there was confirmation of change, the groups were subdivided into scores according to the intensity of occurrence. They are (Figure 1): G1: No platelet aggregate formation; G2: Light presence of aggregate formation. Small platelet lump formation at random points on the fringe or when it was verified to be a single small formation; G3: Moderate presence of platelet aggregate formation. When there were larger and more frequent platelet aggregates during observation that did not exceed 50% of the entire fringe extension. G4: Intense presence of platelet aggregate formation, exceeding 50% of the total fringe extension.

Statistical Analysis

The difference between total leukocyte count by the automatic counter and that by manual count was compared based on the presence or absence of platelet aggregation. From the confirmation of the difference, the animals were separated according to degree of aggregation (mild, moderate, and intense), compared to the group without platelet aggregation, and were considered significant when $P < 0.05$. All statistical analyses were performed using the BioEstat 5.0 program [1].

RESULTS

Of the 140 samples analyzed, 76.4% (107/140) showed some degree of platelet aggregation. A significant difference of variation in total white blood cell count between manual and automated methods was observed when compared to animals that did not show any degree of platelet aggregation ($P < 0.05$). Of the total samples, 23.57% (33/140) comprised G1, 24.28% (34/140)

G2, 22.14% (31/140) G3, and 30% (42/140) G4. Regarding platelet quantification, 85.7% (120/140) of the animals had thrombocytopenia, with values below 300,000/ μ L [8].

An increase in leukocyte number variation was observed as platelet aggregate scores increased ($P < 0.05$). When the groups were evaluated separately, no significant difference was observed between G1 and G2 ($P > 0.05$). On the other hand, when the difference in the total number of leukocytes was compared to G3 and G4, it was possible to confirm that platelet aggre-

gation influenced the count of these cells ($P < 0.05$), as shown in Table 1.

The maximum variation between leukocyte counts by automatic and manual techniques in G1 was 2,500 cells. In G2, it was possible to identify a variation of 6,500 nucleated cells, whereas in G3, this value was 7,100 cells. In G4, where the presence of platelet aggregation was considered intense, the variation between counts was up to 15,000 nucleated cells. The variation in the total number of leukocytes in the different groups is shown in Figure 2.

Table 1. Variation in the number of leukocytes, total from automatic and manual counting, in relation to platelet aggregate type.

Group	Median	Average	Standard deviation	Minimum	Maximum
G1 (n=33)	200 ^a	257,6	1.160	-1.900	2.500
G2 (n=34)	950 ^a	900	2.238	-3.900	6.500
G3 (n=31)	2.000 ^b	2.703	2.678	-2.200	7.100
G4 (n=42)	6.450 ^b	6.831	3.651	-400	15.000

Values followed by the same letter in the same column do not differ significantly by the ANOVA at 5% significance ($P < 0.05$).

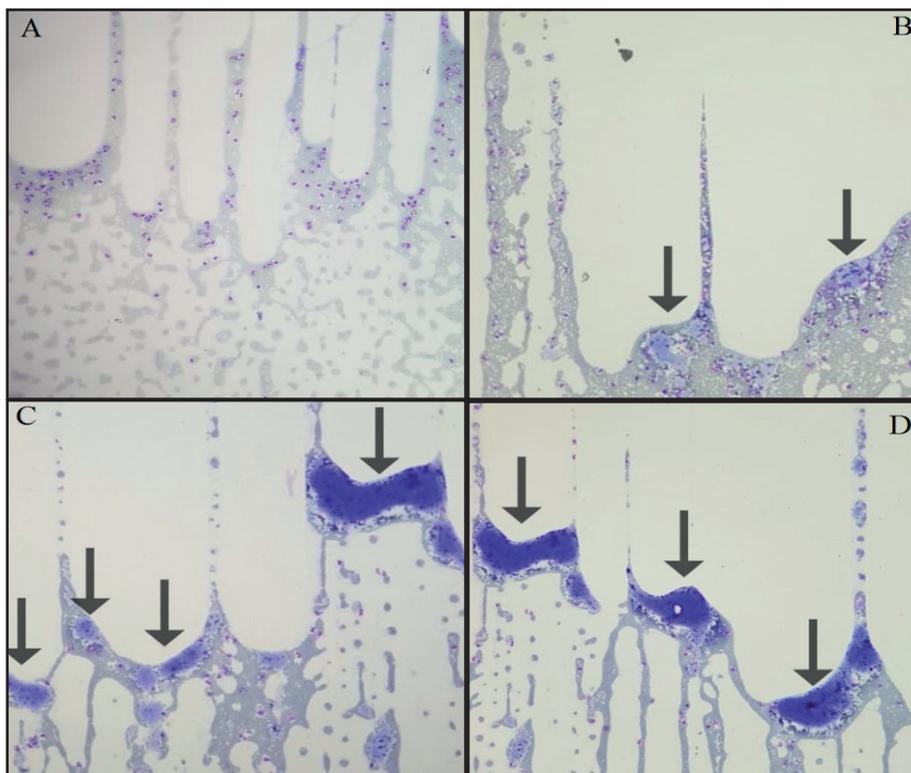


Figure 1. Photomicrograph of the fringe region of the hematological extension of cats, showing the severity of platelet aggregation formation under optical microscopy at 10 \times magnification. A- Absence of platelet aggregate formation. B- Arrows showing mild platelet aggregate formation. C- Arrows showing moderate platelet aggregate formation. D- Arrows showing intense platelet aggregate formation.

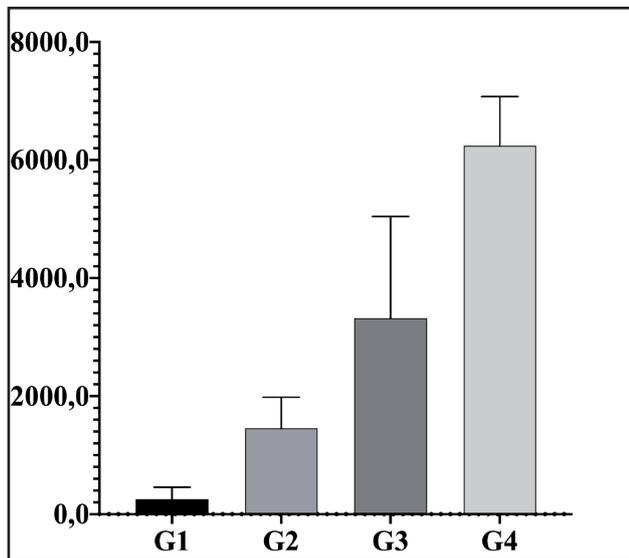


Figure 2. Determination of total number variation of leukocytes in feline patients submitted to the impedance-automated leukocyte counting methods and manual counting, according to the presence of platelet aggregation.

DISCUSSION

Automated hematology analyzers originated with the purpose of optimizing time between analyses promising precision and accuracy to results. However, the observed results suggest that significant variations may occur in these patients [2,11], generating situations with unreliable cell counts [7,10,13,17]. This misconception can occur due to a variety of reasons, such as presence of a substantial quantity of interfering particles in the sample, including red cell fragments [15], leukocytes [17], immune complexes, bacteria, lipid droplets, or platelet aggregates [7].

In vitro platelet aggregation in feline blood samples is common, resulting in pseudothrombocytopenia, with reported prevalence ranging from 52% [9], 62% [18] to 71% [10], where the results of the latter were similar to those observed in the present study. The pathophysiological nature of EDTA-induced pseudothrombocytopenia is still uncertain [14]. Nevertheless, it has been proposed that autoantibodies present in plasma recognize and bind to an epitope of glycoprotein IIb (GPIIb), which is part of the platelet surface GPIIb /IIIa complex, promoting platelet agglutination [4].

Misleading results of leukocyte counts have also been reported. False or falsely elevated counts may occur when platelet aggregates are present in the sample [2,10,17]. Despite the knowledge about possible changes found in the white blood cell count of

these patients, the real alteration that could be present in these animals was not described. In the present study, the global white blood cell count of 107/140 samples analyzed was corrected.

Wrongly decreased total leukocyte counts are believed to be due to platelet satellitism, where platelet neutrophil entrapment occurs. The number of platelets involved in rosette formation is variable and the mechanisms leading to satellitism are not fully elucidated [4,5]. However, it is believed that auto-antibodies bind by the Fab portion to the exposed platelet GPIIb /IIIa epitope, and by the Fc portion to the neutrophil receptor III forming bridges between platelets and neutrophils [5].

Pseudoleukocytosis may be secondary to several factors, such as the presence of erythroblasts. They can be found in the bloodstream under physiological or pathological conditions, and can sometimes be much more numerous than leukocytes [12]. The presence of cryoglobulin can also lead to mistaken elevations of leukocytes [17]. Platelet aggregates may be similar to the size of a leukocyte and thus be mistakenly counted by the apparatus as leukocytes [12,17]. The present study demonstrates the inaccuracy in leukocyte count when intense platelet aggregate formation is present.

As observed in the present study, it is important to emphasize the need for a thorough evaluation by a qualified professional to compare the results generated by the equipment. Values of leukocytes not consistent with the patient's clinic, in view of the confirmation of platelet aggregates considered moderate to intense should be reviewed, to reduce their misinterpretation. Thus, a previous blood smear evaluation will be satisfactory to identify and correct possible errors generated by the equipment. The occurrence of platelet aggregation was an important finding in the patients included in this study, corresponding to more than 2/3 of the population evaluated. Samples with higher platelet aggregate formation showed greater interference in global white blood cell count when analyzed by the hematological counter. These findings reinforce the importance of confronting the analysis expressed by the equipment to contribute to the clinical diagnosis, avoiding misinterpretations that may influence the therapeutic conduct. It is essential to challenge values expressed by automated equipment with traditional methods to minimize possible errors generated by the equipment. There are important changes that may lead

to an error in the clinical diagnosis, and consequently in the therapeutic approach chosen.

CONCLUSION

The formation of platelet aggregates, especially those considered moderate to intense, increases the total leukocyte count when performed on hematological counters automated by the impedance method. Thus, a careful assessment of hematological changes should be performed, since errors in clinical conduct may occur due to false differences observed in the leukometry of these patients.

MANUFACTURER

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Acknowledgements. We wish to thank Dr Fabio Scott (DVM, PhD) for his assistance during the study.

Ethical approval. This work involved the use of non-experimental animal(s) only (owned or unowned), and followed established internationally recognised high standards ('best practice') of individual veterinary clinical patient care. Ethical approval from a committee was not necessarily required.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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