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CASE REPORT

Sysmex XT-2000iV scattergram analysis in a cat with basophilia

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Abstract: A 13-year-old female Domestic Shorthair cat was presented to the Veterinary Teaching Hospital of the University of Milan for an interscapular mass suspected to be a mesenchymal malignant tumor. A preoperative CBC performed with Sysmex XT-2000iV showed leukocytosis with neutrophilia and eosinophilia. The Sysmex WBC/DIFF scattergram showed an additional, well-separated cluster of events between the neutrophil, eosinophil, and lymphocyte clusters. Blood smear evaluation revealed the presence of a significant number of basophils; thus, it was hypothesized that the additional cluster could represent the basophilic population. A second CBC, 24 days later, showed the same pattern on the WBC/DIFF scattergram in the absence of leukocytosis and neutrophilia. After surgical excision of the mass, a definitive diagnosis of feline injection site sarcoma was made. To the author's knowledge, there are no previous reports about the identification of feline basophils in the WBC/DIFF scattergram of Sysmex XT-2000iV.

Case Presentation

A 13-year-old, female spayed Domestic Shorthair cat was presented to the Veterinary Teaching Hospital of the University of Milan for an interscapular mass. A diagnosis of malignant mesenchymal cell tumor was already made by the referring veterinarian, based on the cytologic examination of a fine-needle aspirate of the mass. The cat appeared in good general condition and the physical examination revealed the presence of a mobile, solid, nonpainful, and nonulcerated round mass, 1.5 × 1.4 cm in diameter, located in the subcutaneous tissue of the interscapular area. The cat had been regularly vaccinated, and treated for ectoparasites with fipronil-based products. Tests for both feline immunodeficiency virus and feline leukemia virus had never been performed.

Cancer staging by a total-body computer tomography scan did not reveal loco-regional or distant metastasis. A wide-margins excision of the neoplasm was performed, and the histologic examination led to a definitive diagnosis of feline injection site sarcoma. A preanesthesia evaluation of the cat was performed, including a biochemical profile with an automated spectrophotometer (Cobas Mira, Roche Diagnostics, Basel, Switzerland), and CBC using the Sysmex XT-2000iV hematology laser analyzer (Sysmex Corporation, Kobe, Japan). A blood smear was also prepared and stained with a rapid stain (Hemacolor, Merck, Darmstadt, Germany). The biochemistry results were unremarkable except for a mild hyperglycemia, most likely stress-induced. The cat's erythrogram showed no significant alterations, while the leukogram was characterized by leukocytosis due to slight neutrophilia and moderate eosinophilia (Table 1). Interestingly, the Sysmex WBC/DIFF scattergram showed an unusual cluster located between the neutrophil, the eosinophil, and the lymphocyte cluster, and partially overlapping with each of these population gates. This cluster was clearly separated from the other cell populations when the scattergram was switched to the manual analysis frame, a Sysmex feature providing color codes for easier manual gating of the present populations (Figure 1). A double-blind manual differential count of 200 nucleated cells on the blood smear revealed 4.5% basophils ($0.91 \times 10^9/\mu L$), suggesting that the unusual cluster represented in fact basophils (Figure 2).

On the day of surgical tumor removal, 24 days after the first blood sample, another blood sample was collected for a repeat CBC. In addition, a coprological examination was performed to rule out endoparasite

Table 1. Comparison of relative and absolute automated Sysmex XT 2000iV and manual differential leukocyte counts in a cat with basophilia.

	First Blood Sample (Presurgery)		Second Blood Sample Day 24		
Analyte	Sysmex	Manual Count	Sysmex	Manual Count	Reference Interval
Total WBC ($\times 10^9$/L)	20.14	–	13.81	–	6.0–17.0
Neutrophils (%)	66.0	66.7	47.4	53.8	35–75
Neutrophils ($\times 10^9$/L)	13.30	13.43	6.55	7.43	2.5–12.5
Band (%)	–	1.7	–	0.0	< 3
Band ($\times 10^9$/L)	–	0.34	–	0.0	< 0.3
Lymphocytes (%)	12.7	8.5	37.1	34.4	20–55
Lymphocytes ($\times 10^9$/L)	2.55	1.71	5.12	4.75	1.5–7.0
Monocytes (%)	3.3	1.1	2.4	2.5	1–4
Monocytes ($\times 10^9$/L)	0.66	0.22	0.33	0.35	0.0–0.85
Eosinophils (%)	18.0	17.5	13.1	6.8	2–12
Eosinophils ($\times 10^9$/L)	3.63	3.52	1.81	0.94	0.0–1.5
Basophils (%)	0.0	4.5	0	2.5	Rare
Basophils ($\times 10^9$/L)	0.0	0.91	0	0.35	Rare

Manual counts were performed on 200 nucleated cells on a stained blood smear.

infestation. Basophilia typically occurs along with eosinophilia, and frequently accompanying a parasite infestation or an allergic condition. The fecal flotation test was negative for nematodes and protozoa. Moreover, the cat's clinical history reported no episodes of allergic reactions or any other symptoms suggesting hypersensitivity or an allergic condition.

The Sysmex WBC/DIFF scattergram of the second blood sample was similar to the first one, but both automated and manual differential counts revealed normal WBC and neutrophil counts, while a slight eosinophilia was still present (Table 1). As in the previous leukogram, the basophil cluster was still visible, supported by 2.5% basophils ($0.35 \times 10^9/\mu L$) based on blood smear evaluation.

Discussion

Basophils represent the smallest granulocyte population in peripheral blood in most mammalians, representing approximately 0.5% of blood leukocytes in healthy animals.[1] Consequently, both manual and automated counts suffer from imprecision, and only increases above 200–300 basophils/μL should be defined as basophilia.[2,3]

Basophilia is rarely detected in domestic mammals and it is commonly associated with eosinophilia. The conditions responsible for basophilia are mostly IgE-mediated disorders, such as allergic diseases and parasitism, therefore these should be considered first.[1,4] In addition, several neoplastic conditions seem to be associated with basophilia, such as mast cell neoplasia with or without peripheral blood involvement, basophilic leukemia, myeloid leukemia, polycythemia vera, and feline eosinophilic granuloma complex[2,3], and also other types of tumors.[1]

Canine and feline basophils are not reliably measured by most automated instruments, even if equipped with laser technology such as the Sysmex XT-2000iV.[5] This instrument performs the WBC count in 2 different channels, the WBC/DIFF cytogram where WBC are differentiated based on fluorescence (fluorescent light scatter) and complexity (side scatter). Specifically, the cells are stained with a fluorescent polymethine agent after being permeabilized with a surfactant. Polymethine dye binds to nucleic acids and cytoplasmic organelles. The WBC differential count is determined by fluorescence-activated flow cytometry using a red semiconductor laser at a wavelength of 633 nm.[6] The different WBC cell clusters are then separated based on side fluorescence light (SFL) and a laser side scatter light (SSC), and the results are displayed in a differential scattergram. Leukocytes with a high amount of nucleic acids, such as lymphocytes and monocytes, are placed higher on the scattergram *y*-axis, which corresponds with the SFL. Neutrophils and eosinophils, because of their cytoplasmic complexity, are found toward the right side of the *x*-axis, which corresponds to the SSC.[7] In summary, in the DIFF channel, basophils are counted together with neutrophils, so the WBC differential count determines a 4-part differential count.[6,8,9]

A basophil count is also performed in the WBC/BASO channel, where the exposure to a strong surfactant causes the loss of all leukocyte nuclei, except for human basophils.[6] Then the SSC and the forward scattered light (FSC) of lysed cells are determined and displayed on a second scattergram, with the SSC on the *x*-axis and the FSC on the *y*-axis.[6,10]

A previous study about the occurrence and the enumeration of basophils in different species was performed comparing different hematology systems. The Sysmex XT-2000iV failed to detect canine basophils, but the cytograms of several samples showed the presence of a basophil cluster above the neutrophil population.[5] This location is probably due to the cellular characteristics of basophils, showing a complexity similar to that of neutrophils, but a lower affinity for the

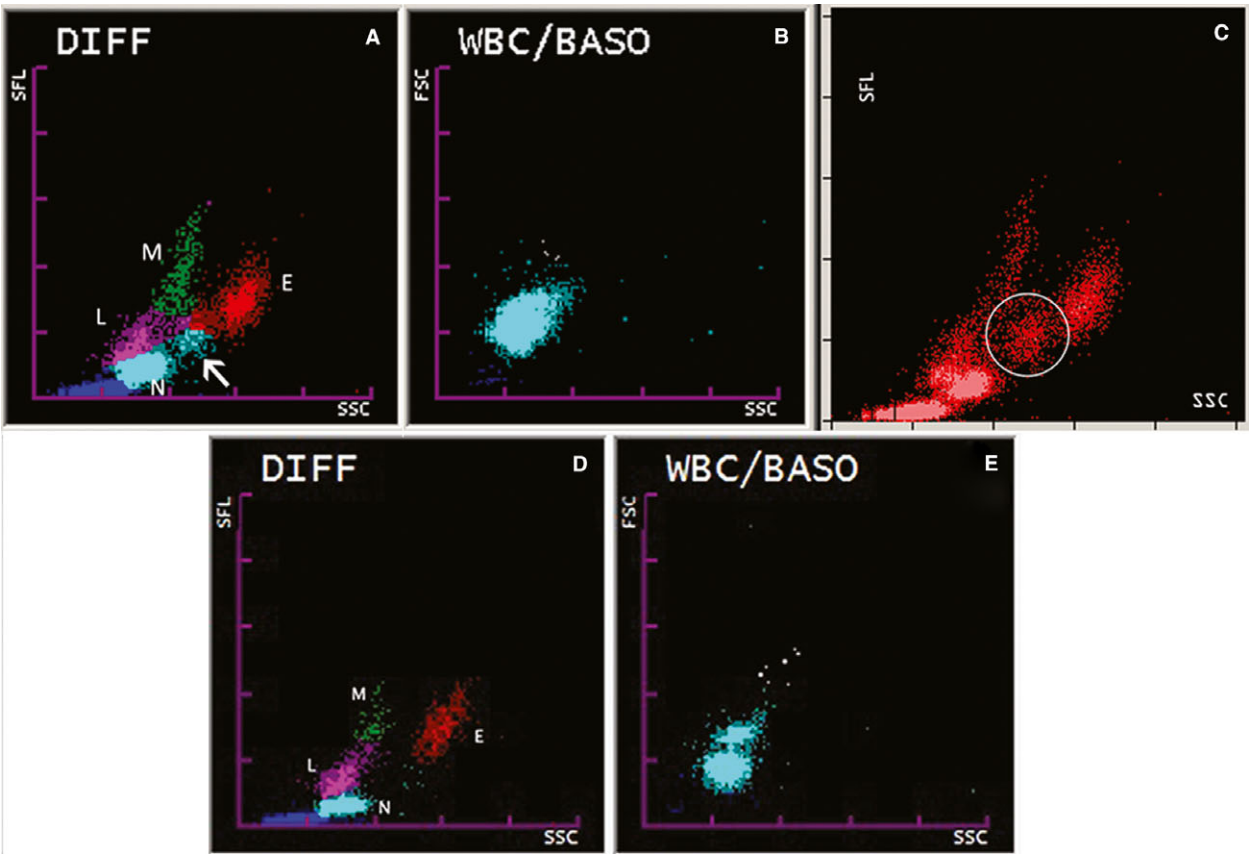

Figure 1. Sysmex XT-2000iV scattergrams of a leukogram in a cat with basophilia. The WBC/DIFF scattergram (**A**) shows an additional cluster (white arrow) between the neutrophil, the eosinophil, and the lymphocyte population. The separation of the "basophils" cluster is more evident when the scattergram is switched to the manual analysis frame, where all dots are red (**C**, white circle). The WBC/BASO channel scattergram (**B**) shows no increased lysis-resistant population, suggesting that feline basophils are subjected to lysis like all other leukocytes, unlike human basophils. A scattergram of a normal feline blood sample shows no additional clusters on the WBC/DIFF channel (**D**) and the WBC/baso channel (**E**), as expected. N indicates neutrophils; L, lymphocytes; M, monocytes; E, eosinophils; FSC, forward scatter; SFL, side fluorescence light; SSC, side scatter.

fluorescent dye compared to eosinophils. Unfortunately, in that study, feline blood samples were not analyzed with the Sysmex XT-2000iV.[5] In our case, based on the manual differential count, there appeared to be a basophil population comparable to the canine basophil clusters described in the study mentioned above.[5]

In another validation study of the Sysmex XT-2000iV, neither canine nor feline basophilis were measured; however, the scattergram of several of the examined feline blood samples showed an additional cluster comparable to the one we describe in this report. This cluster was classified as an extra-eosinophilic cluster.[7]

Recently, a report on the performance of the ProCyte Dx (IDEXX Laboratories, Westbrook, MA, USA), a hematology analyzer based on the same technology as the Sysmex XT-2000iV demonstrated a basophil population.[11,12] Interestingly, the feline basophil cluster in the ProCyte Dx appeared to be located in a similar position as the basophil cluster in the present case. Nevertheless, in that study, the ProCyte Dx generated a high number of false-positive results, detecting basophilia in 37 of the 155 feline blood samples not confirmed by blood smear evaluation, with the exception of one case.[11] Possibly, the erroneous basophilia was due to clumps of platelets appearing in the same area of the scattergram.[13]

In the case described here, platelet clusters were not noticed in blood smear evaluation. Rather, it seems plausible that the additional cluster represented feline basophils, and the position of the cluster could be explained with the cytoplasmic complexity of basophils compared to neutrophils, while binding less fluorescent dye compared to eosinophils.

In conclusion, this case suggests that feline basophils may have physico-chemical properties that allow

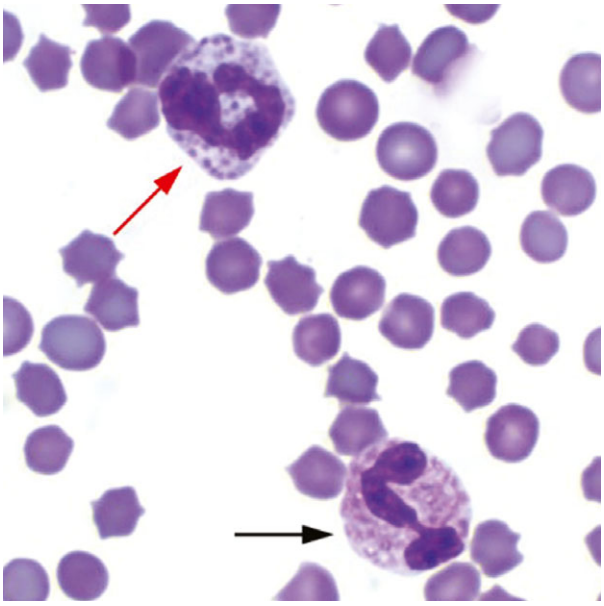

Figure 2. A basophil (red arrow) and an eosinophil (black arrow) in the blood smear from a cat with basophilia (first blood sample). Hemacolor stain, ×100 objective.

their identification in Sysmex scattergrams. In addition, the present report may indicate a possible correlation between basophilia and mesenchymal tumors, but this was not the main purpose of this case report.

Although basophilia is rarely reported, the unreliable identification of basophils with the current generation of veterinary hematology analyzers and their possible misidentification in manual assessments of blood smears, may have contributed to the general underestimation of basophil counts. The presence of an additional cluster in the Sysmex XT-2000iV WBC/DIFF scattergram as the one described in this case report should alert the operators to evaluate the blood smear for possible basophilia. Further studies are needed in order to verify the repeatability of these findings on a larger number of samples, especially in order to manually create a gate for the feline basophil count on the Sysmex XT-2000iV.

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