Sysmex scattergram in a cat
WITH DIAGNOSIS

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Signalment
DSH male cat, 2 years old

History
The cat was presented for acute vomiting and diarrhea, with lethargy, inappetence and adypsia for the past 48 hours, and cutaneous nodules in a context of pyodermatitis that had been evolving for months

Cllinical findings
On admission, the cat was in good body condition (SC 4/9) but lethargic, tachypenic (RR = 100 bpm) and had marked ptyalism. Clinical examination also revealed a marked flea infestation associated with extensive crusts and papules. Pulmonary auscultation revealed heavy breath sounds in the dorsal region ; the abdominal palpation was painful.

Diagnostic procedures
Thoracic radiographs showed a moderately increased lung opacity (alveolar pattern) in the right cranial and the left caudal lobes, consistent with brochopneumonia, oedema, hemorrhage or neoplasia. Abdominal ultrasound revealed a mildly thickening of the duodenum wall associated with alteration of the layering and hypomotility. Pancreas appeared thickened and hypoechoic. These abnormalities were primarily consistent with an inflammatory process or a duodenal neoplasia.

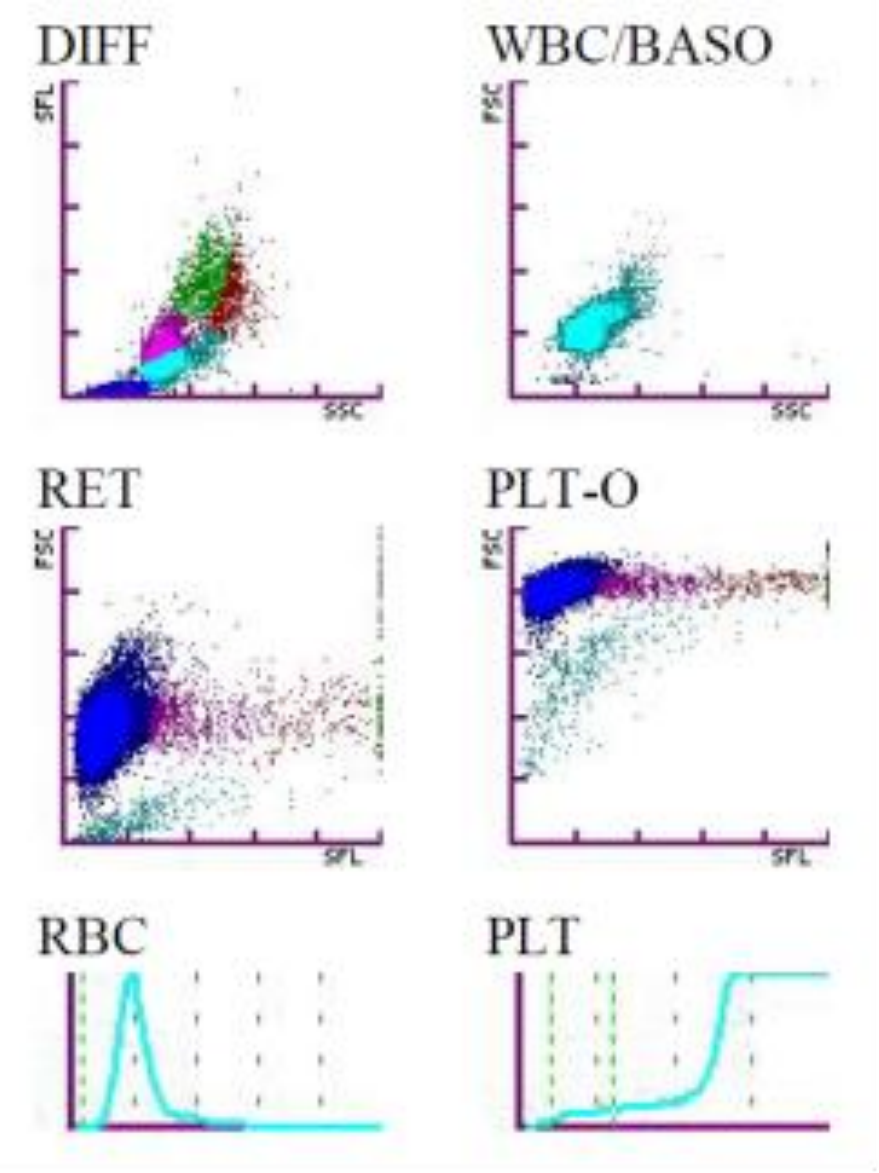
Plasma biochemistry (Na, K, Cl, tCO2, total proteins, albumin, total bilirubin) was unremarkable. Hematology analysis was performed on the Sysmex XT-2000iV, revealing a mild normocytic hypochromic regenerative anemia (Hb 7.7 g/dL [9.8-16.9], MCHC 27.3 g/dL [32.9-39.1], MCV 47.6 fL [33.6-48.3]), a very mild neutrophilia (9.67.10^9/L [1.45-9.62]) and monocytosis (1.20.10^9/L [0.09-0.82]) with no leukocytosis (WBC = 14.5.10^9/L).

Blood smear evaluation revealed a moderate polychromasia, a left shift with toxic neutrophils and an extremely high number of circulating mast cells with heterogeneous granularity, anisocytosis and showing erythrophagocytosis. A manual differential count was performed on the entire blood smear. 24 869 leukocytes were observed, and the differential count showed a neutrophilia [79.67%, 11.55. 10^9/L] and a high mast cell concentration [7.67%, 1.11. 10^9/L].

	Manual Count	Sysmex count	Reference interval
WBC		14.48.10^9/L	3.70-18.66
Neutrophils %	79.67	66.8	-
Neutrophil concentration	11.53.10^9/L	9.67.10^9/L	1.45-9.62
Lymphocytes %	8.75	19.8	-
Lymphocyte concentration	1.3.10^9/L	2.86.10^9/L	1.18-10.36
Monocytes %	2.49	8.3	-
Monocytes	0.36.10^9/L	1.20.10^9/L	0.09-0.82

concentration			
Eosinophil %	0.94	5.0	-
Eosinophil concentration	0.13.10^9/L	0.73.10^9/L	0.16-1.81
Basophil %	0.75	0.1	-
Basophil concentration	0.11.10^9/L	0.02.10^9/L	-
Mast cell %	7.67	-	-
Mast cell concentration	1.11.10^9/L	-	-

Cutaneous nodules were aspirated, and stained with May-Grümwlad-Giemsa. Cytology revealed a necrotic and granular background, a neutrophilic and eosinophilic inflammation associated with numerous large round cells, with a moderate nucleo-cytoplasmic ratio, and numerous small round dark purple granulations within the cytoplasm. These cells were highly suggestive of mast cells, and presented marked atypia such as gigantism, anisocaryosis, multinucleation, and erythrophagocytosis. Neutrophils in the background were phagocytic of mast cells granulations.



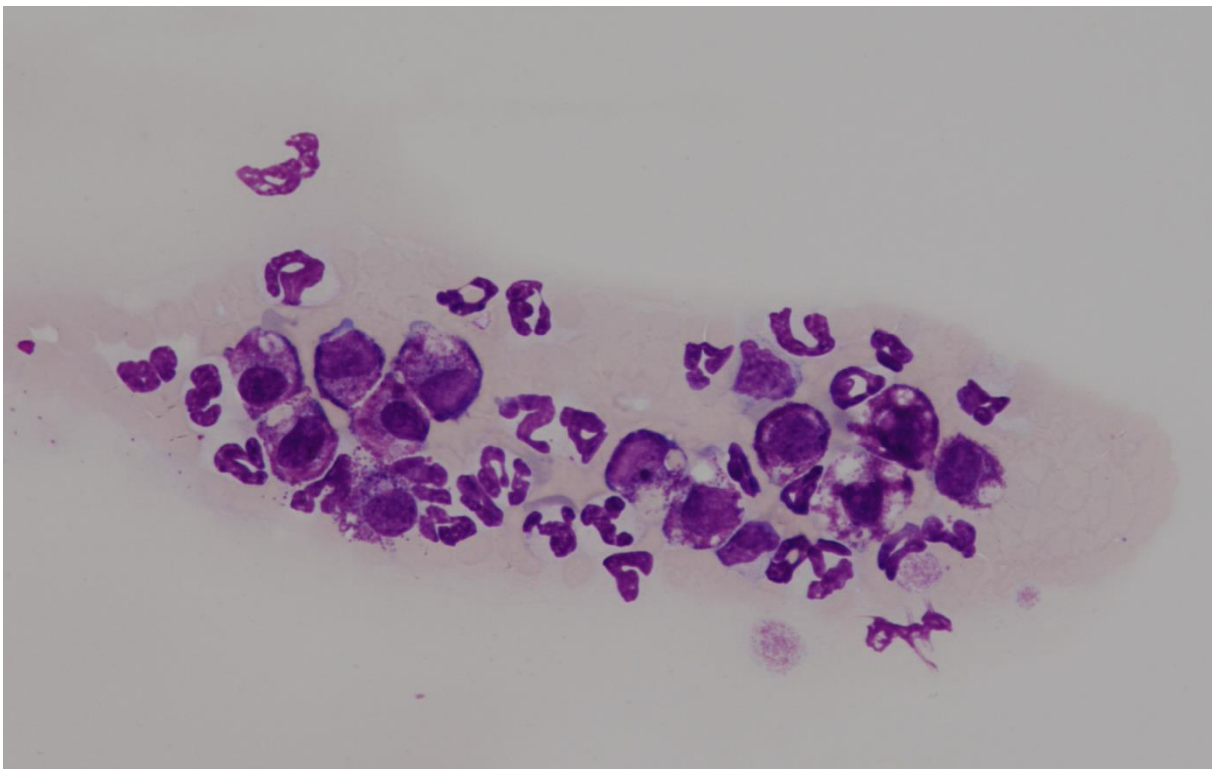


Photo 1 : Peripheral blood. Wright Giemsa stain. Original Magnification x400

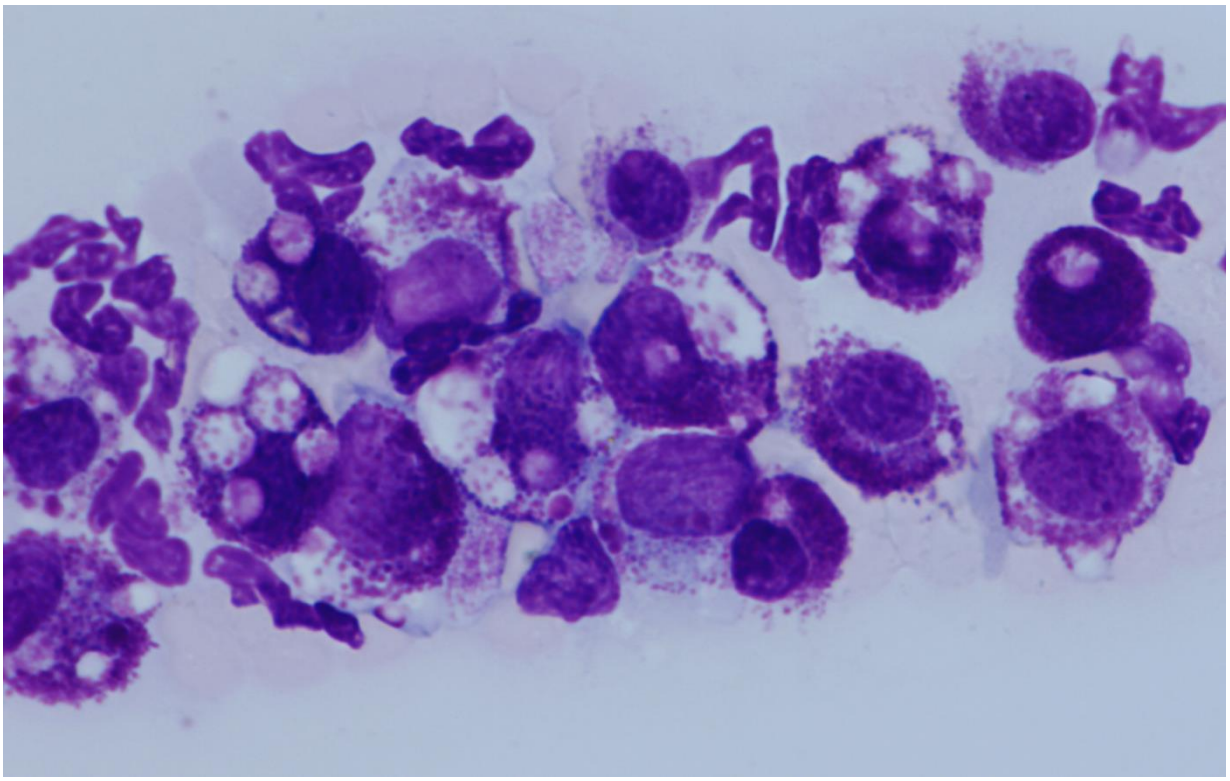


Photo 2 : Peripheral blood. Wright Giemsa stain. Original Magnification x1000

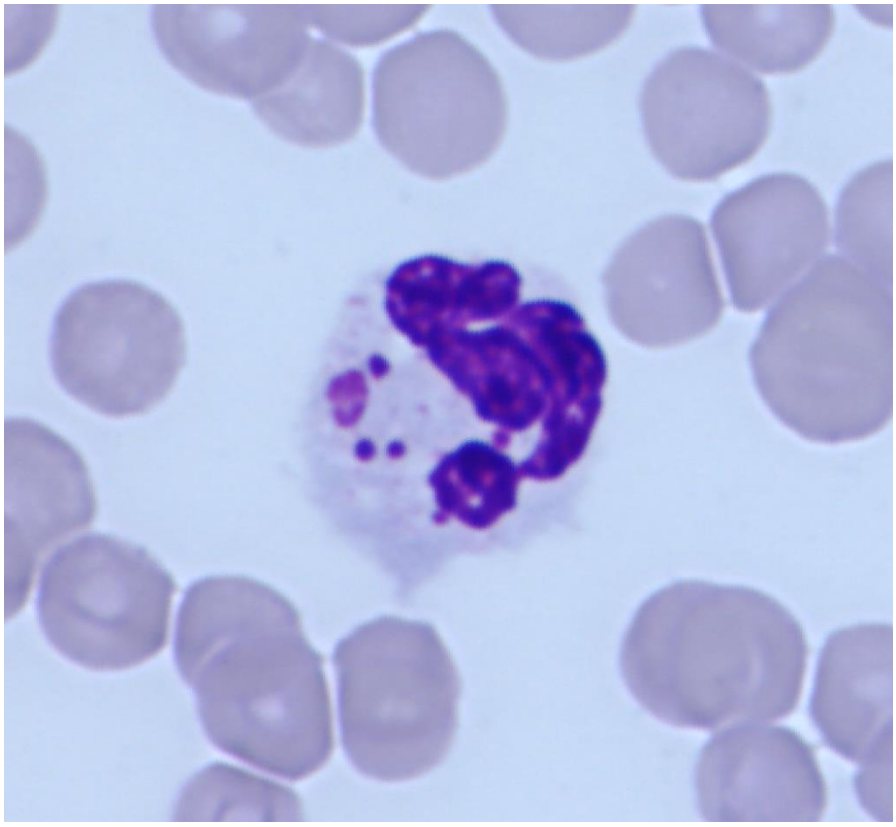
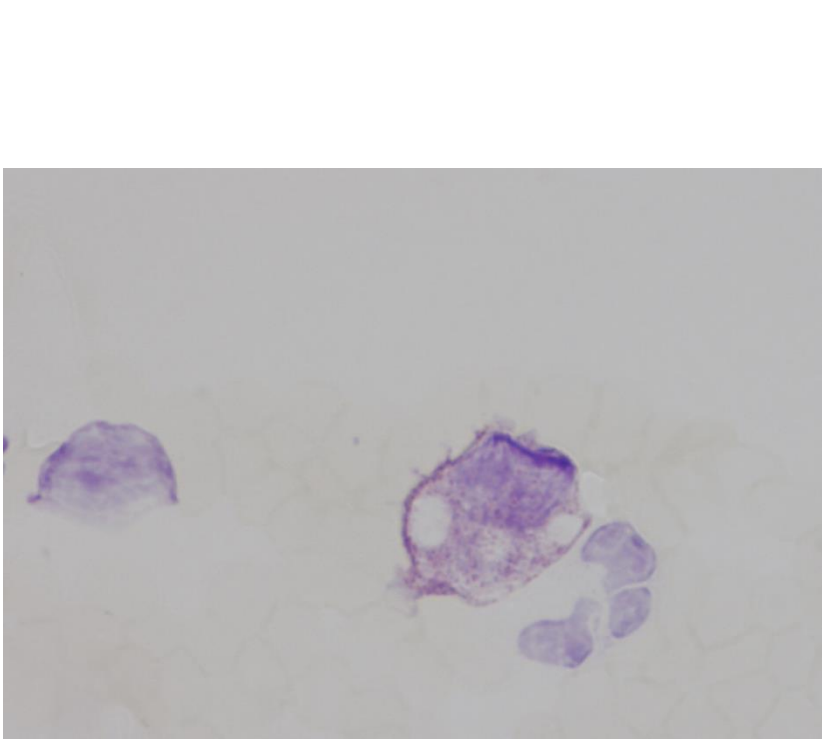


Photo 3 : Peripheral blood. Wright Giemsa stain. Original Magnification x1000



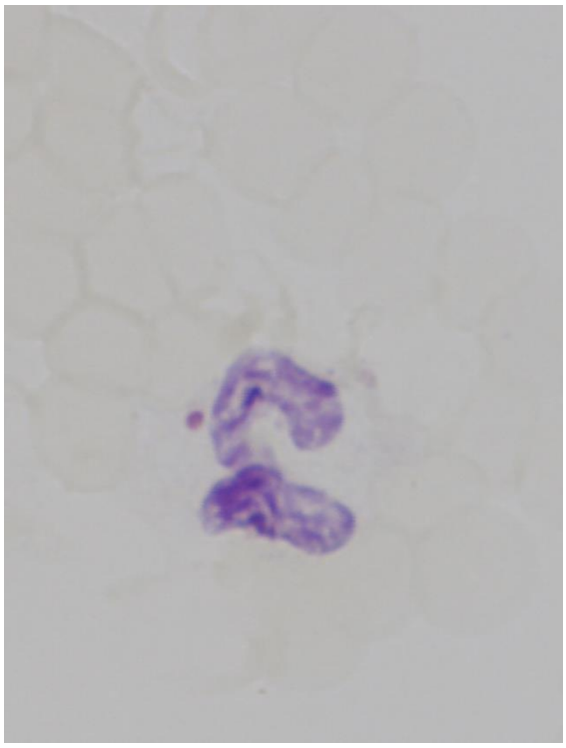


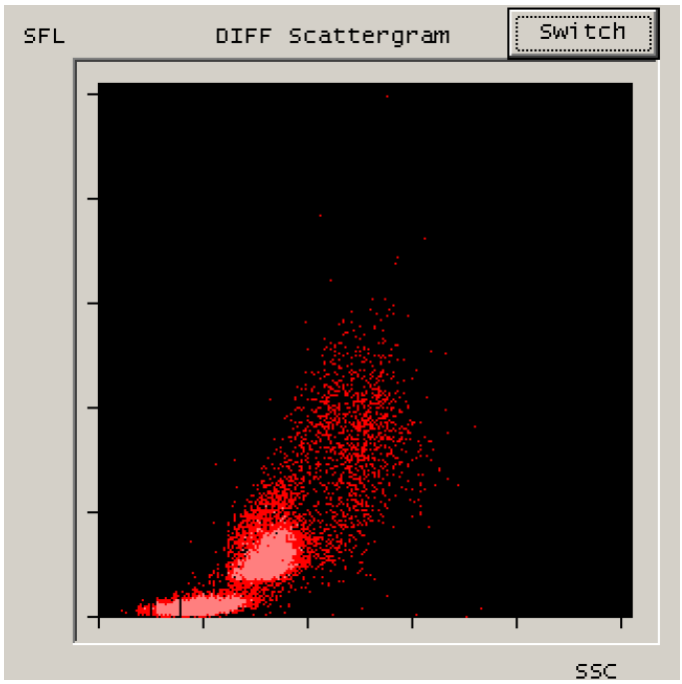
Photo 4a and 4b : Peripheral blood. Toluidine blue stain. Original Magnification x1000

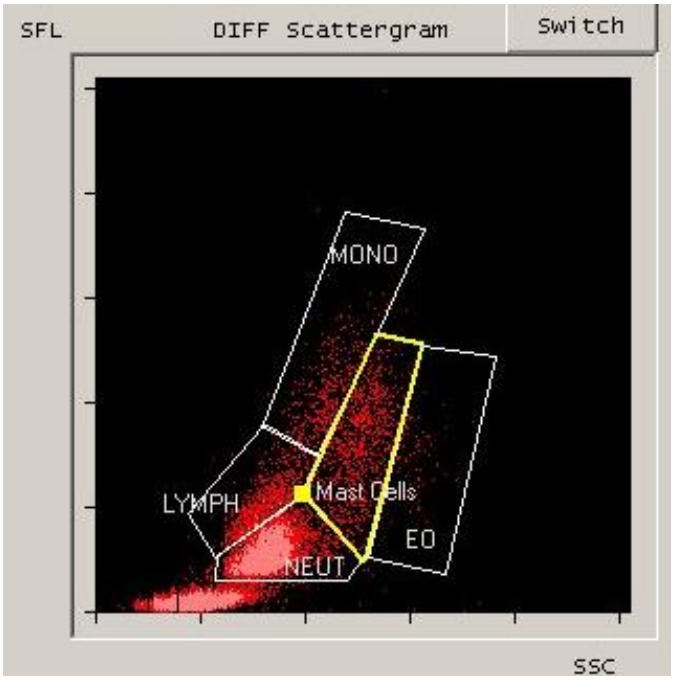
Interpretation

A systemic mastocytosis was highly suspected.

Based on the presence of this very unusual scattergram associated with the high number of circulating mast cells, and the absence of other finding that could have explained the eosinophils-monocytes confusion, we hypothesized that mast cells had been detected by the Sysmex XT-2000iV, and were located in the space above the cat basophil area, and between the normal location of monocytes and eosinophils.

To explore this hypothesis, we regated the DIFF-scattergram, interpreting the cells in the newly defined area as mast cells. With this gating, the differential gave the same percentage of mast cells than the manual count (7.52%; 1.09.10^9/L).





Following investigation

Due to poor prognosis and multiple episodes of spontaneous marked low blood pressure (60 mmHg), the cat was euthanized.

On post-mortem examination, the cat exhibited pale mucous membranes and numerous cutaneous lesions of approximately 2 mm in diameter. Several superficial lymph nodes were hypertrophic, as were the mesenteric and pancreatic lymph nodes and the spleen. Duodenal mucosa was thickened, with multifocal ulcerations. Melena was observed in the colon.

Cutaneous lesions, lymph nodes, the spleen, liver and intestines were taken for histologic examination. The final histologic diagnosis was moderately differenciated cutaneous mast cells tumors with lymph nodes and spleen metastases and possible involvement of the duodenal mucosa and liver. Together, these findings were consistent with systemic mastocytosis.

Discussion

To the best of our knowledge, mastocytemia is a rare finding in cats (1), and is mostly secondary to Mast Cell Tumor (MCT), visceral MCT being the most common disorder reported (2). However, mastocytemia has also been reported in cases of lymphoid neoplasia, hemangiosarcoma and renal failure.

One study pointed out that MCT excluded cases of circulating mast cells seemed to have only a limited number of mast cells compared with MCT cases (2). In our case, the very high number of circulating mast cells presenting marked atypias was consequently extremely suggestive of a MCT. Necropsic examination confirmed a mastocytosis, with splenic, nodal, duodenal and cutaneous involvement. Mastocytosis (mast cell leukemia, systemic mastocytosis, disseminated mastocytosis) is a very rare finding in cats, and often results from the evolution of a hematopoietic system MCT, particularly splenic MCT (3).

The Sysmex XT-2000iV DIFF scattergram is based on flux cytometry and fluorescence to differentiate leukocytes populations. Fluorescence of cells based on their RNA/DNA content in reported on the Y axis and granularity/complexity based on the side scattered light diffraction is reported on the X axis (6). Dot plots are considered acceptable if individual clusters are located in the expected areas, and that all dots in each clusters have the same expected color (4). In our case, several abnormalities were noted on the scattergrams, with a very poor distinction between the different populations with 1/ a poor delimitation between lymphocytes and neutrophils, 2/ a poor delimitation between monocyte and eosinophils and to a lesser extent between neutrophils and eosinophils, leading to an unacceptable dot plot and thus an unacceptable differential count (5)

On the Sysmex XT-2000iV, the poor delimitation between neutrophils and lymphocytes has been reported with the presence of band-cells, toxic neutrophils or both (5). Indeed, band-cells and toxic neutrophils are usually bigger cells and contain more endoplasmic reticulum, and thus have a higher fluorescence. Consequently, immature and toxic neutrophils tend to be located right above the normal location for neutrophils, resulting in a fusion between the neutrophils and lymphocytes areas. In our case, both band-cells and toxic neutrophils were detected on the blood smear, which can explain the absence of correct delimitation between lymphocytes and neutrophils. This is a very common error, with one study reporting up to 15% of error on the lymphocyte-neutrophils ratio among a feline population with the Procyte Dx, which uses the same technology as the Sysmex XT-2000iV(5).

The second main error was the poor delimitation between monocyte and eosinophils and to a lesser extent between neutrophils and eosinophils.

Eosinophils have been reported to be classified as neutrophils by the Sysmex XT-2000iV in Greyhounds with Grey Eosinophils (7). However, first of all, this didn't lead to confusion with the monocyte population but with neutrophils. Second of all, this was responsible for an underestimation of eosinophils, whereas in our case, the DIFF scattergram overestimated the eosinophils. Finally, we didn't see any morphological abnormality concerning the eosinophilic lineage on the blood smear that could have explained such a displacement.

The confusion between eosinophils and neutrophils has also been reported in cases of basophilia in dogs, and one case report also reported that cat basophils were classified as neutrophils (10). In our case, basophils were detected on the blood smear and could have participated in the continuity between neutrophils and eosinophils dots as according to the Procyte, cat basophils are located between the neutrophils and the eosinophils on the diff scattergram (Tvedten 2017 JVIM). However, it doesn't explain the shift of eosinophils and the fusion with the monocytes dots.

Finally, in cases of malaria in humans (8) and in cats with numerous platelets aggregates, the neutrophils dots can be in continuity with the eosinophils. Neither of these was observed on the blood smear.

Overall, the most likely explanation for these scattergram abnormalities was the presence of an unusual population. This hypothesis was backed up by the visualization of the red DIFF scattergram that exhibited a well-delimited area, above basophils and between monocytes and eosinophils.

Moreover, when gating this area as mastocytes, the DIFF scattergrams gave similar results as the manual differential count, further supporting our hypothesis.

To the author's knowledge, this is the second report of circulating mast cells detected by the Sysmex XT-2000iV in a cat (Vajdovitch). Interestingly, mast cells didn't produce the same scattergram modifications in the previous case and in ours.

The previous case and the literature report that mast cells are lysis resistant cells, and are consequently classified as basophils in the BASO channel (5). However in our case, the BASO channel only detected 0.2% of lysis resistant cells classified as basophils, which is lower than the number of mast cells according to the manual count. This could indicate that not all mast cells are lysis resistant, and further studies are needed to understand the reasons why. In the meantime, the lysis-resistant area of the BASO channel should probably not be the only criteria taken into account for a mast cell detection using the Sysmex XT-2000iV.

References

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