# Abnormal population in WDF scattergram (Sysmex XN-V) in a cat

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**Specimen**
EDTA anticoagulated whole blood and fresh blood smears

**Signalment**
9-year-old castrated male domestic shorthair cat

**History**
The cat was presented at the Small Animal Clinic of the Vetsuisse Faculty, University of Zurich (Switzerland) for a re-check one month after a surgical procedure (cystotomy and perineal urethrostomy) due to recurring calcium oxalate urolithiasis. Early in the postoperative period, the cat developed pancreatitis, ileus and bacterial aspiration pneumonia.

**Clinical findings**
The cat was in good general condition and surgical sites were progressing favorably in the recovery process. Clinical examination revealed a previously detected heart murmur. The echocardiographic examination indicated an abnormal movement of the mitral valve, and an early stage of hypertrophic cardiomyopathy was suspected. There was no evidence of cardiogenic decompensation/fluid overload. A follow-up examination was planned within six months. The remaining vital signs were unremarkable. Diagnostic imaging tests, such as abdominal ultrasound and thoracic X-rays, were conducted. Abdominal ultrasound was unremarkable and the pneumonia that developed after surgery resolved completely. EDTA blood was collected for a full hematology and serum for biochemistry evaluation.

Biochemical analyses were run on a Cobas C501 instrument (Roche Diagnostics, Basel, Switzerland), and showed mild hypoalbuminemia (28 g/L; RI 32-42 g/L), and a mild increase in creatine kinase (CK) activity (990 U/L; RI 77-355 U/L). The remaining parameters appeared unremarkable.

Complete blood cell count (CBC) and blood smear evaluation were performed. Results from automated hematology analysis are shown in Table 1 and Figure 1.

| | Units | Sysmex XN-V values | Reference interval |
|---|---|---|---|
| Hematocrit | % | **27** | 33-45 |
| Hemoglobin | g/dL | **8.9** | 11.3-15.5 |
| Erythrocytes | $10^6$/µL | **6.53** | 7.0-10.7 |
| MCH | pg | 14 | 14-17 |
| MCHC | g/dL | 33 | 33-36 |
| MCV | fL | 41 | 40-48 |
| RDW | % | 19.4 | 19.6-33.0 |
| nRBC | % | 0.7 | - |
| nRBC | $10^3$/µL | 0.1 | - |
| Reticulocytes | % | 1.00 | 0.1-1.3 |
| Reticulocytes | $10^6$/µL | 0.0653 | 0.0103-0.1078 |
| Platelets | $10^3$/µL | **46**[(a) (*)] | 180-680 |
| Leukocytes | $10^3$/µL | **14.3** | 4.6-12.8 |
| Neutrophils | % | 55.2 | - |
| Lymphocytes | % | 19.5 | - |
| Eosinophils | % | 19.0 | - |
| Basophils | % | 0.2 | - |
| Monocytes | % | 6.1 | - |
| Neutrophils | $10^3$/µL | 7.92 | 2.32-10.10 |
| Band neutrophils | $10^3$/µL | - | 0.00-0.12 |
| Lymphocytes | $10^3$/µL | 2.79 | 1.05-6.00 |
| Eosinophils | $10^3$/µL | **2.72** | 0.10-0.60 |
| Basophils | $10^3$/µL | 0.03 | 0.00-0.14 |
| Monocytes | $10^3$/µL | **0.88** | 0.04-0.68 |

*Table 1.* Hematological numerical results for EDTA-blood specimens with Sysmex XN-V. Abbreviations: MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean cell volume; nRBC: nucleated red blood cells.

[a] Platelet count was obtained with the optical channel in the Sysmex XN-V

*Flag reported from the Sysmex XN-V analyzer: Platelet abnormal distribution and platelet clumps suspected.

The values highlighted in bold are outside of the reference interval (RI).

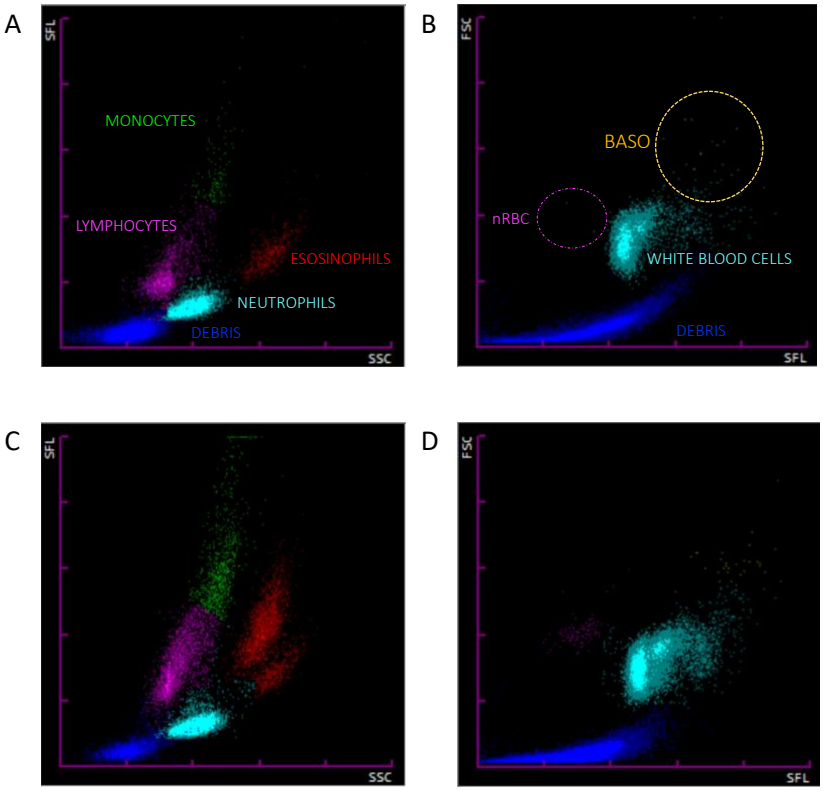


*Figure 1*. **(A)** Sysmex XN-V White Cell Differential Channel by Fluorescence **(WDF)** scattergram and **(B)** White Count and Nucleated Red Blood Cell **(WNR)** scattergram from a healthy 6-year-old domestic shorthair male cat. **(C) WDF** and **(D) WNR** Sysmex XN-V scattergrams from the 9-year-old domestic shorthair cat presented for check-up after a surgery procedure (currently presented case).
*In the WDF scattergram the X-axis shows the side scattered light (SSC; proportional to the granularity) and the Y-axis shows the side fluorescence light (SFL; proportional to the nucleic acid content) of the acquired cells. Each acquired cell is shown as a dot on the scattergrams. In the WDF channel the analyzer categorized the cells as monocytes (green), lymphocytes (magenta) neutrophils (light blue), eosinophils (red) and debris (dark blue). In the WNR, the X-axis shows the side fluorescence light (SFL; proportional to the nucleic acid content) and the Y-axis shows the forward scattered light (FSC; proportional to the size) of the acquired cells. White blood cells are classified in light blue. Debris, platelets, and platelet aggregates are shown in dark blue. Lysis-resistant cells "BASO" are shown in yellow and nucleated red blood cells (nRBCs) are shown in magenta.

## Questions

1. **When comparing the scattergrams from a healthy cat (Figure 1A and B) with those from the present case (Figure 1C and D), what is the main abnormality observed? Describe the abnormality.**

   There is an additional elongated population present in the WDF scattergram between the eosinophil (red dots) and monocyte (green dots) populations, classified by the analyzer as eosinophils (Figure 1C). No abnormal additional population was identified in the WNR scattergram.

2. **How would you further investigate this suspected abnormality?**

   Due to the presence of an abnormal scattergram, a blood smear from the submitted EDTA blood sample needs to be evaluated.

**3. During this additional investigation, what do you expect to observe given the findings present in the scattergrams?**

The additional elongated population present in the WDF (between eosinophil and monocyte, Figure 1C), along with the absence of an additional lysis-resistant population in the WNR scattergram led us suspect an underlying basophilia. In the present case, the blood smear evaluation and 200-cell differential count confirmed a marked basophilia (Table 2). Therefore, the presence of an abnormal population only in the WDF scattergram using the Sysmex XN-V should raise concern of an underlying basophilia in cats which should always be confirmed in blood smear evaluation.

Previous reports have shown that an abnormal additional population in the WDF scattergram above the neutrophils has been identified as basophils in dogs using the Sysmex XN-V [1] and as mast cells or basophils in dogs and cats using the Sysmex XT-2000iV [2-6]. Although, mast cells and basophils, appeared in the same area in the WDF scattergram, they had differential features regarding the appearance in the WNR scattergram. The mast cells were reported to be present as a lysis-resistant cell population above the white blood cell area, whereas the basophils appear to be absent in the WNR scattergram [1-6]. It is important to note that the localization of the additional population in the WDF scattergram in the present case differed from previously reported locations of basophils in dogs and cats using the Sysmex XT-2000iV [2-6] and for dogs using the Sysmex XN-V[1].

Blood smear evaluation of this case showed a mild decrease density of erythrocytes without polychromasia. A moderate anisocytosis, mild poikilocytosis with presence of few elliptocytes were also observed. In addition, some platelet aggregates and few macroplatelets were noted. Several basophils and few reactive lymphocytes were also identified (Table 2). Morphologically, basophils showed segmented nuclei and numerous small lavender to magenta cytoplasmic granules (Figure 2). The presence of these granules makes basophils morphologically clearly distinguishable from neutrophils and eosinophils in cats.

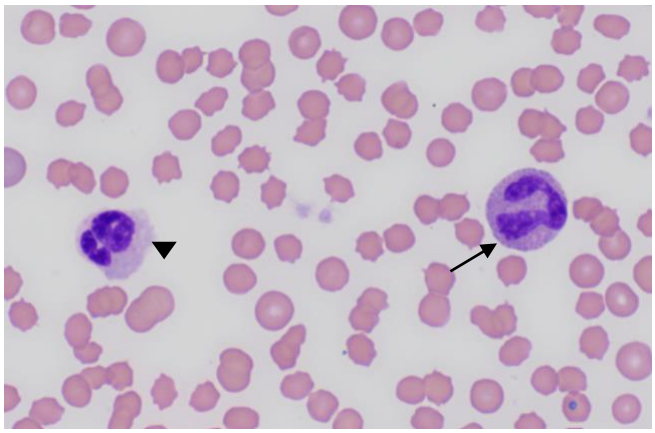

Figure 2**.** Photomicrograph of a peripheral blood smear from a 9-year-old neutered male domestic shorthair cat (current case). Modified Wright's stain. 1000 x magnification (arrow: basophil; arrowhead: neutrophil).

**Clinical outcome**

The underlying cause of the marked basophilia seen in the present case was not confirmed, but parasitic infestation was considered most likely based on clinical evaluation and diagnostic imaging (unremarkable findings in thorax and abdomen). The basophilia indeed completely resolved after administration of antiparasitic drugs. Other causes of basophilia, such as allergic inflammation, neoplasia (i.e., mast cell tumor, basophilic leukemia, myeloid leukemia) or other nonallergic inflammatory conditions, were considered unlikely in this case and were thus not further evaluated.

**Discussion**

Basophilia, though uncommon, is frequently associated with allergic conditions and parasitic infections, often alongside eosinophilia [7]. Basophilia may also occur secondary to other neoplastic disorders such as mast cell neoplasia, feline myeloproliferative disease, polycythemia vera and intestinal T-cell lymphoma [8].

As previously reported, basophil detection by automated methods, such as Sysmex XT-2000iV and CELL-DYN 3500 is not accurate in feline species [5]. In certain species (humans and rabbits, but not cats), the basophil membrane remains intact after exposure to the reagent in the WNR channel which shrinks the white blood cells while permeating their membrane [9]. Therefore, basophil count in the WNR channel is only reliable for humans and rabbits. Manual differential count is still considered the most reliable method for feline basophil enumeration [9]. Nevertheless, due to the low prevalence of circulating basophils, manual differential cell counts are also considered not accurate [9] and basophilia could be missed if blood smear evaluation is not consistently performed.

The present case demonstrates that feline basophils (when present in high numbers) can be suspected using the unique appearance of basophils on the WDF scattergram. The abnormal WDF scattergram obtained with the Sysmex XN-V analyzer was the main finding in this case that raised the suspicion of an underlying basophilia. The WDF scattergram was characterized by an unusual oval elongated population located among monocytes (green dots) and eosinophils (red dots) initially classified as eosinophils by the Sysmex XN-V analyzer (Figure 1C). No flags or alerts were shown regarding the white blood cell population by the Sysmex XN-V analyzer. In addition, no discrepancy between WNR and WDF total white blood cell counts was noted. The initial basophil values obtained from the Sysmex XN-V analyzer were considerably lower compared to the values obtained on blood smear evaluation (Table 2). When the WDF scattergram was re-analyzed and a manual gate on the oval cell population was created, the basophil count was similar to that obtained by the manual count (Table 2).

| | Initial Sysmex XN-V values | | Manual re-gating Sysmex XN-V values | | Manual differential count values | | Reference interval |
|---|---|---|---|---|---|---|---|
| | % | $10^3/\mu L$ | % | $10^3/\mu L$ | % | $10^3/\mu L$ | $10^3/\mu L$ |
| Neutrophils | 55.2 | 7.92 | 55.9 | 8.21 | 51.5 | 7.39 | 2.32-10.10 |
| Band neutrophils | - | - | - | - | **1.5** | **0.22** | 0.00-0.12 |
| Lymphocytes | 19.5 | 2.79 | 20.0 | 2.93 | 18.5 | 2.65 | 1.05-6.00 |
| Eosinophils | **19.0** | **2.72** | 3.7 | 0.54 | 4.0 | 0.57 | 0.10-0.60 |
| Basophils | 0.2 | 0.03 | **14.6** | **2.14** | **18.5** | **2.65** | 0.00-0.14 |
| Monocytes | **6.1** | **0.88** | **5.3** | **0.78** | **6.0** | **0.86** | 0.04-0.68 |

**Table 2.** Percentages and counts of different leukocyte populations obtained from initial Sysmex XN-V data, manual differential count performed during blood smear examination, and Sysmex XN-V data after manual re-gating (as shown in Figure 4). Total WBC count was obtained from the Sysmex WNR channel (14.3 x $10^3/\mu L$; RI: 4.6 – 12.8 x $10^3/\mu L$). Bolded values are outside the reference interval (RI).

Feline basophils were previously identified on the Sysmex XT-2000iV WDF scattergram as an additional cell population situated between the neutrophil, eosinophil, and lymphocyte clusters (Figure 3C) [2]. In the current case, using the Sysmex XN-V, the location of the feline basophils was proved to be different (Figure 4C). Similar to previous findings, feline basophils were also not present in the WNR scattergram using the Sysmex XN-V analyzer [2].

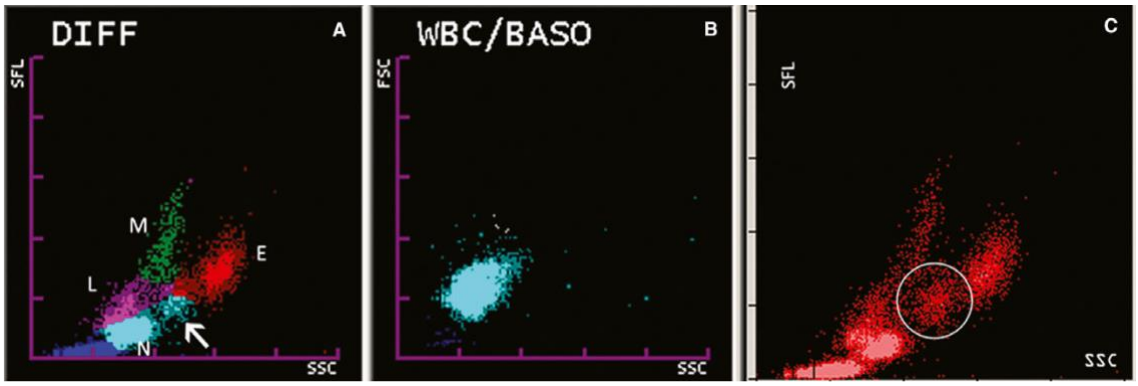


*Figure 3.* **Sysmex XT-2000iV WBC/DIFF(A) and WBC/BASO(B) scattergrams of a leukogram in a cat with basophilia.** Ref. (*Stranieri, A., et al)[2].* **(A)**The cluster representing the basophils (highlighted with a white arrow) is positioned differently to its location now described in the Sysmex XN-V (Figure 4).

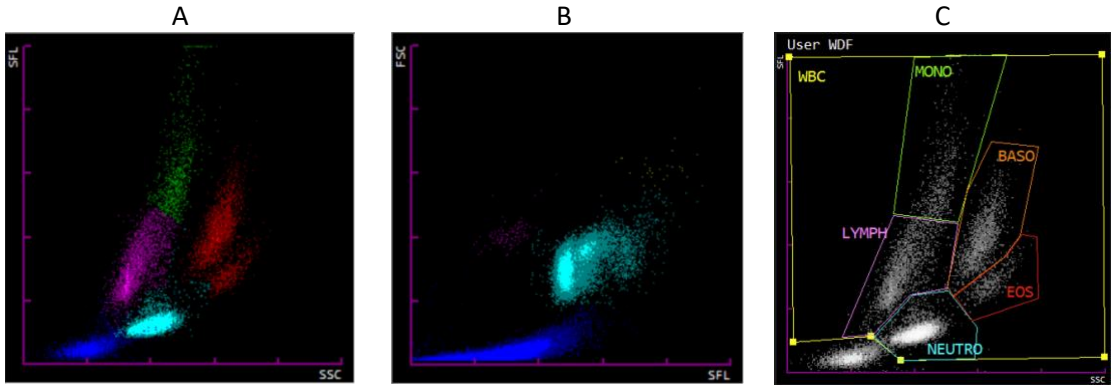


*Figure 4.* **WDF (A), WNR (B) WDF re-analyzed (C) scattergrams from Sysmex XN-V analyzer in a 9-year-old neutered male domestic shorthair cat with marked basophilia (currently presented case). (A)** and **(B)** were previously described, please see Figure 1 (C and D). **(C)** In the WDF re-analyzed scattergram the unusual oval population was separated from the eosinophil population and the cells were reclassified as basophils (after confirmation in blood smear evaluation). The basophil count was similar to that obtained by the manual count (Table 2).

In addition, we decided to screen our laboratory information system for feline cases with basophilia confirmed by blood smear evaluation. Interestingly, we found that 52 cats with basophilia (basophils > 0.5 x $10^3$/µL between April 2021 and October 2023) showed the presence of an abnormal WDF scattergram in the Sysmex XN-V analyzer with an unusual oval elongated population located among monocytes and eosinophils (as identified in the present case, Figure 4). The appearance of this unusual oval elongated population varied based on the severity of basophilia. Mild basophilia was more difficult to recognize compared to marked basophilia as seen in the present case. For these reasons, the presence of an additional cell cluster between eosinophils and monocytes on the WDF scattergram of the Sysmex XN-V, without a corresponding cluster in the WNR scattergram, should raise suspicions of potential basophilia in cats. Consequently, in those cases, the presence of basophils should be carefully evaluated during blood smear examination and manual re-gating on the WDF can be attempted (as shown in Figure 4C).

**References**


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