ANOTHER HINT FROM THE SYSMEX XT-2000IV SCATTERGRAM

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Signalment

A 9-year-old, male, neutered domestic short-haired cat.

History and diagnostic procedures

The cat was admitted to the Veterinary Teaching Hospital of the University of Milan for history of dysorexia, vomiting and lethargy during the last two weeks. At the clinical examination, the only evident alteration was the presence of few masses perceived with abdominal palpation. A complete blood count (CBC) with the Sysmex XT-2000iV hematology laser analyzer (Sysmex Corporation, Kobe, Japan) and biochemical analysis with automated spectrophotometer (BT3500, Biotecnica Instruments, Rome, Italy) were performed.

To investigate the presence of abdominal masses, an abdominal ultrasound was also performed, revealing a slight hepatomegaly, a diffuse thickening of the jejunum and marked lymphadenomegaly of all the mesenteric lymph nodes. Fine needle aspirate (FNA) of liver, spleen and mesenteric lymph node were performed for cytologic examination (Figure 1).

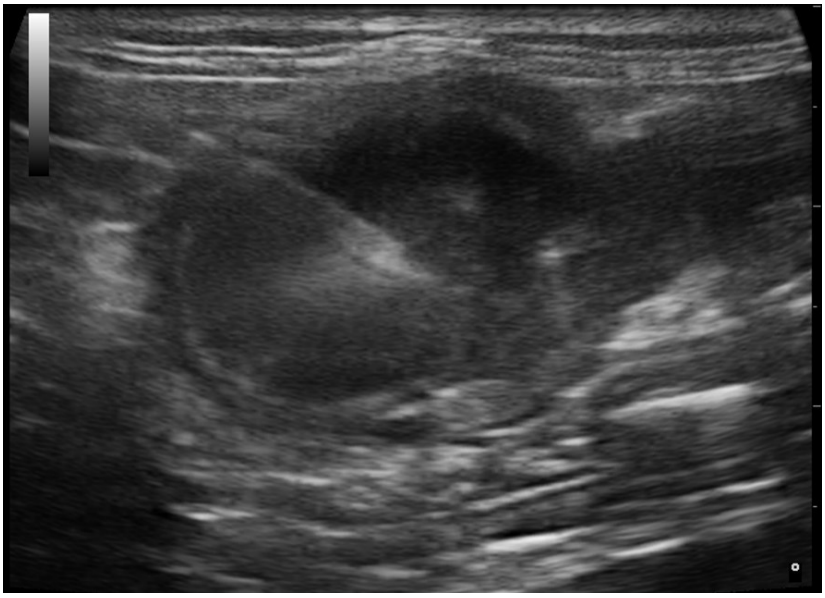

Figure 1. Abdominal ultrasound images of the cat. FNA of one enlarged mesenteric lymph node.

Serum was jaundiced and serum biochemistry revealed a slight hyperglycemia, probably stress-induced, and a marked increase of the liver enzymes ALT and ALP activity (Table 1).

The cat's CBC showed moderate lymphopenia (Table 2), while all the other parameters were within the reference intervals.

ANALYTE	RESULT	REFERENCE INTERVALS
Urea (mg/dL)	43	(20-60)
Creatinine (mg/dL)	1,2	(<1,8)
Glucose (mg/dL)	**196**	(95-130)
Total protein (g/dL)	7,8	(5,4-8,5)
Albumin (g/dL)	2,9	(2,1-3,3)
A/G	0,6	(0,8-1,7)
ALT (U/L)	**2437**	(6-83)
ALP (U/L)	**348**	(25-93)

Table 1. Biochemical results obtained with the automated spectrophotometer BT3500 (Biotecnica Instruments, Rome, Italy). Slight hyperglycemia and marked increase of ALT and ALP are present.

Analyte	Result	Reference intervals
RBC ($x10^6$/µL)	5,43	5,7-10
HGB (g/dL)	10,2	8-15
HCT (%)	30,5	24-45
MCV (fL)	55	39-55
MCH (pg)	18,8	14-19
MCHC (g/dL)	33,4	26-35
PLT ($x10^3$/ µL)	205	200-600
RDW (%)	18,1	14,4-19,4
Total WBC	9,38	6-17
Neutrophils (%)	76,6	35-75
Neutrophils ($x10^3$/µL)	7,18	3-13,4
Lymphocytes (%)	**7**	20-55
Lymphocytes ($x10^3$/µL)	**0,66**	2-7,2
Monocytes (%)	9,6	1-4
Monocytes ($x10^3$/µL)	0,9	0-1
Eosinophils (%)	6,8	2-12
Eosinophils ($x10^3$/µL)	0,64	0,3-1,7
Basophils (%)	0,0	0-1
Basophils ($x10^3$/µL)	0,00	0-0,1

Table 2. Cat's hematological results obtained with the Sysmex XT-2000iV (Kobe, Japan) analyzer. Only a moderate lymphopenia is present.

The Sysmex XT-2000iv WBC/DIFF channel showed an additional population located between the lymphocytes/monocytes clusters and the eosinophils cluster (Figure 2).
The WBC/BASO channel showed an unusual extension on the right of the total WBC cluster.

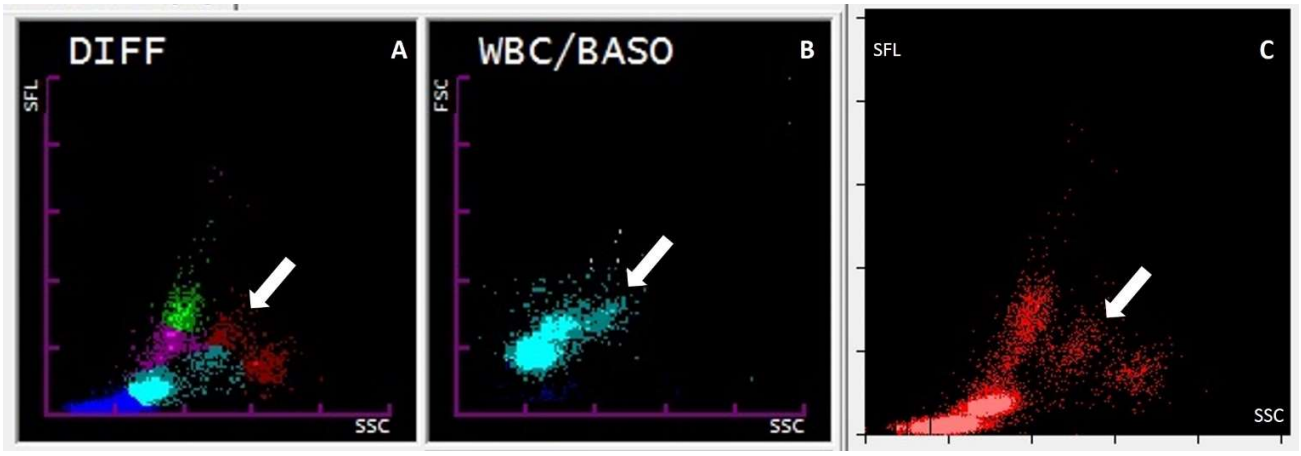

Figure 2. Sysmex XT-2000iV scattergrams. The WBC/DIFF scattergram showed an additional cellular cluster between the lymphocytes/monocytes clusters and the eosinophils cluster (A, white arrow). On the WBC/BASO channel scattergram, an elongation of the total WBC cloud is visible on the right (B, white arrow), but it is not classified as lysis resistant cellular population. On the manual analysis frame, the additional population appears clearly separated from the others (C, white arrow). [FSC: Forward scattered light; SFL: Side fluorescence light; SSC: Side scatter light]

A blood smear was prepared and stained both with a rapid stain (Hemacolor®, Merck, Damrstadt, Germany) both with May-Grunwald-Giemsa, revealing the presence of medium-sized cells with abundant, slightly basophilic cytoplasm containing several large vacuoles and paracentral/eccentric nuclei with prominent nucleoli and coarsely clumped to ropy chromatin, depending on the stain. The cytoplasmic vacuoles were weakly stained with Hemacolor®, while intensively magenta stained with May-Grunwald-Giemsa (Figure 3). These cells were classified as neoplastic large granular lymphocytes (LGL).[1]

The cytological examination of the FNAs resulted inconclusive for the lymph node sample, while neoplastic LGLs were present in the spleen and in the liver samples (Figure 4). A diagnosis of large granular lymphocytes lymphoma was made.

Since the additional cluster on the WBC/DIFF scattergram was then speculated to be associated with the presence of neoplastic LGLs in the peripheral blood, a double-blind manual differential count on 500 cells was performed on a May-Grunwald-Giemsa stained blood smear. Moreover, five new gates were manually created on the Extend-DIFF scattergram area of the manual analysis frame. The percentages obtained with the manual count and with the regated Sysmex differential count were compared (Table 3).

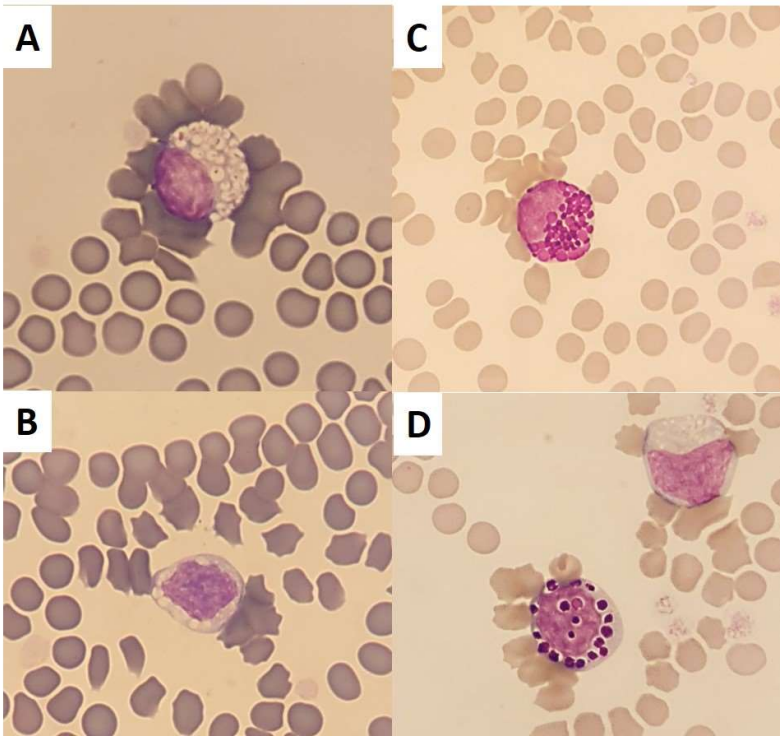

Figure 3. Neoplastic large granular lymphocytes (LGL) on the cat blood smear stained with Hemacolor® (A, B) and May Grunwald-Giemsa (C, D). A, B. The nucleus is eccentric or paracentral, with coarsely clumped chromatin and prominent nucleoli. The cytoplasm contains numerous vacuoles which are weakly stained (A) or not stained at all (B). C, D. The nucleus is eccentric or paracentral, with ropy chromatin and less prominent nucleoli. The cytoplasm contains numerous large vacuoles intensively magenta stained (100x).

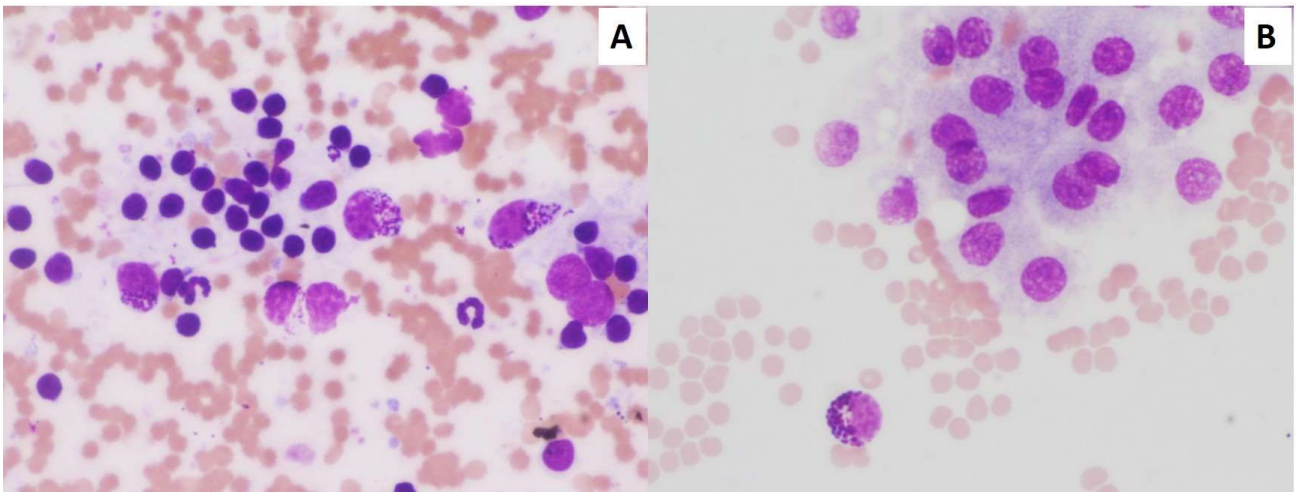

Figure 4. Neoplastic LGL on cytology samples obtained from (A) spleen and (B) liver. May Grunwald-Giemsa, 60x.

Analyte	Sysmex Result	Manual count*	Sysmex Regated	Reference intervals
Total WBC	9,38	-	-	6-17
Neutrophils (%)	76,6	76,7	76,1	35-75
Neutrophils (x10^3/µL)	7,18	7,19	7,1	3-13,4
Lymphocytes (%)	7	2,2	3	20-55
Lymphocytes (x10^3/µL)	0,66	0,2	0,3	2-7,2
Monocytes (%)	9,6	11,1	10,8	1-4
Monocytes (x10^3/µL)	0,9	1,04	1,01	0-1
Eosinophils (%)	6,8	4,1	4,5	2-12
Eosinophils (x10^3/µL)	0,64	0,38	0.42	0,3-1,7
Basophils (%)	-	0,1	-	0-1
Basophils (x10^3/µL)	-	0,09	-	0-0,1
Ne-LGL (%)	-	5,8	5,6	-
Ne-LGL (x10^3/µL)		0,54	0,52	

Table 3. Leukocytes relative and absolute values obtained with Sysmex XT-2000iV, manual differential count and Sysmex XT-2000iV following WBC/DIFF manual regating. *The percentages refer to the mean values obtained from the two operators.

With the new gates, the manual and the Sysmex neoplastic LGLs percentages were superimposable (5,8% and 5,6%, respectively).

Chemotherapy with 10 mg of Lomustine every four weeks was initiated. At the follow up, the cat was in good conditions except for moderate lethargy. A CBC was performed, showing the same additional cluster and the presence of neoplastic LGLs on the blood smear. The percentages obtained manually and with Sysmex after regating were again very similar (11% and 9%, respectively).

Discussion

Feline large granular lymphocyte lymphoma is a relatively rare lymphoma subtype.[2] Clinical data are scarce, but consistent. In cats with this type of lymphoma clinical signs are in fact similar and begin approximately two weeks before presentation. Anorexia, vomiting and lethargy are the most common described symptoms, along with the presence of palpable abdominal masses.[3] Feline LGL lymphomas most probably arise from the lymphocytes residing in the intestinal epithelium. Subsequently, the organ most often involved is the small intestine, typically the jejunum.[4] Extra-gastrointestinal neoplastic involvement is present in the majority of the cats, and the most affected organs are the abdominal lymph nodes, followed by liver, spleen and kidneys.[3,5] From a clinicopathological point of view, common biochemical abnormalities are the increase of hepatic enzymes activities, along with hypoalbuminemia and urea concentration increase. Regarding the CBC, anemia and neutrophilia are relatively common findings, while lymphopenia is less frequently detected.[2,3] A tendency of this lymphoma to involve peripheral blood is often reported and 18% of 109 cats affected by LGL lymphoma

were found to be positive for circulating neoplastic cells. From the same study, a positive association between the presence of circulating neoplastic cells and a worse prognosis was found.[3]

For these reasons, and for the fact that clinical signs occur only when the disease is at advanced stage, it seems important to be able to identify neoplastic LGLs in peripheral blood. Furthermore, rapid stains poorly or not stain the granules and the concentration of these cells may be too low to be found by routine evaluation of blood smears.[1,5]

Sysmex XT-2000iV is an automated hematology analyzer that performs the leukocyte count in two different channels. The WBC/BASO channel uses a strong acidic surfactant that lyses the erythrocytes and shrink all the leukocytes, except for basophils. The side scattered light (SSC) and the forward scattered light (FSC) of these shrunk cells are determined and displayed on the x- and on the y-axis, based on dimension and cytoplasmic complexity, respectively. Basophils should remain intact and appear higher in the y-axis. Anyhow, canine and feline basophils appear to be shrunk with the other leukocytes. Therefore, the WBC/BASO channel provides the total leukocyte count.[6,7]

In the WBC/DIFF channel, leukocytes are differentiated based on their fluorescence and cytoplasmic complexity. A nucleic acids specific fluorescent dye penetrates leukocytes after their permeabilization with a surfactant, then fluorescence flow cytometry with a red semiconductor laser at a wavelength of 633 nm is used to perform the differential count. Then, the fluorescence intensity (side fluorescence, SFL) of the nucleus and of the cytoplasmic nucleic acids, and the analysis of the SSC categorizes leukocytes in lymphocytes, monocytes, neutrophils (with basophils) and eosinophils. On the scattergram, the SFL is plotted on the y-axis and the SSC on the x-axis. Therefore, leukocytes with high DNA and RNA content (lymphocytes and monocytes) will appear high on the y-axis, while neutrophils and eosinophils will appear on the right of the x-axis.[8] When the Sysmex is not able to accurately differentiate leukocyte populations, a ‘‘WBC Abn Scattergram’’ flag appears. Cells that are not categorized because of differences in their characteristics, are stained with grey on the scattergram or either counted as one of the preset populations, even without triggering any “abn scattergram” flag.[7,8] For example, feline basophils in a cat with basophilia were classified as neutrophil, eosinophils and lymphocytes in a well differentiated cluster between these three populations.[9] Indeed, knowing the meaning of some typical changes in the Sysmex DIFF and BASO scattergrams can be helpful in orienting the clinical pathologist on which changes to expect or to look for when examining the blood smear. For example, left shift is often represented as a shift of neutrophils cluster higher on the y-axis, while mast cells or neoplastic lymphoid elements can be shrinkage resisting in the WBC/BASO channel, being therefore counted as basophils.[8,10]

In the case here discussed, the neoplastic LGL cells were located higher than neutrophils on the y-axis and more on the right on the x-axis compared with lymphocytes and monocytes. This location can be explained by the properties of these cells. In fact, a high nucleic acid content is expected because of the nuclei dimension, as

well as a high cytoplasmic complexity due to the large cytoplasmic granules. In the BASO channel, the total WBC cluster showed an elongation directed more on the right, but not lysis resistant. The position could be again explained by the Ne-LGL characteristics, with a complex granulated cytoplasm which influences the side scattered light.

The fact that the additional cellular cluster on the WBC/DIFF channel was caused by the presence of Ne-LGLs was confirmed after the regating of the WBC/DIFF populations and by comparing the resulting Ne-LGLs percentage with the percentage obtained manually on 500 cells.

Finding this additional cluster on the WBC/DIFF scattergram could be a useful hint for the clinical pathologist to evaluate or at least suspect the presence of neoplastic LGLs on the blood smear and could consequently be helpful to the clinician to investigate for the presence of this relatively rare feline lymphoma.

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