Marked pseudoreticulocytosis in a leukemic dog

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Signalment:

A 14 years-old, male castrated, 5 Kg, Coton de Tular dog.

Clinical findings:

The dog was presented to a private veterinary practice for clarifying a gastro-intestinal problem. Referred clinical signs were anorexia, five episodes of vomiting, and weakness since one week. Moreover the night before consultation the dog showed an episode of nocturia. On the clinical evaluation, the dog was bright and alert. Auscultation of the heart and lungs was unremarkable. At abdominal palpation the animal seemed mildly painful; spleno- and hepatomegaly were also appreciated.

Diagnostic procedures:

Initial complete blood cell count (CBC), blood smear and biochemical analysis were carried out by the private veterinarian. Biochemistry analysis was unremarkable, only mild increase of alkaline phosphatase (ALP), gamma glutamil transferasi (GGT) and blood urea nitrogen (BUN) was observed. Results of CBC are listed in Table 1. On the basis of blood results a moderate regenerative anemia was present. Total number of white blood cells was severely increased and not measurable by the cell counter. However a first blood smear revealed extreme lymphocytosis with atypical cells.

Table 1: Summary of hematology results obtained with the ProCyte Dx® (IDEXX Laboratories)

Analytes	Data	Unit	Reference Interval
WBC	nd*	K/ µL	5.05-16.76
RBC	2.95	M/ µL	5.65-8.87
HGB	6.80	g/dL	14-19.5
HCT	17.6	%	37.1-57
MCV	72.1	fL	60-77
MCH	21.7	Pg	19.5-24.2
MCHC	30.1	g/dL	31-36
PLT	177	K/ µL	148-484
RET %	12.5	%	0.1-0.9
RET #	305.2	K/ µL	10-110

*nd=not determinable

Survey thoracic radiographs of the thorax were taken, and they did not reveal any morphological alteration of the observed structures. Abdominal ultrasound was subsequently performed and it revealed mild increase in splenic and liver volumes without parenchymal focal lesions. A mild amount of anechoic free abdominal fluid was observed with mild mesenteric reactivity.

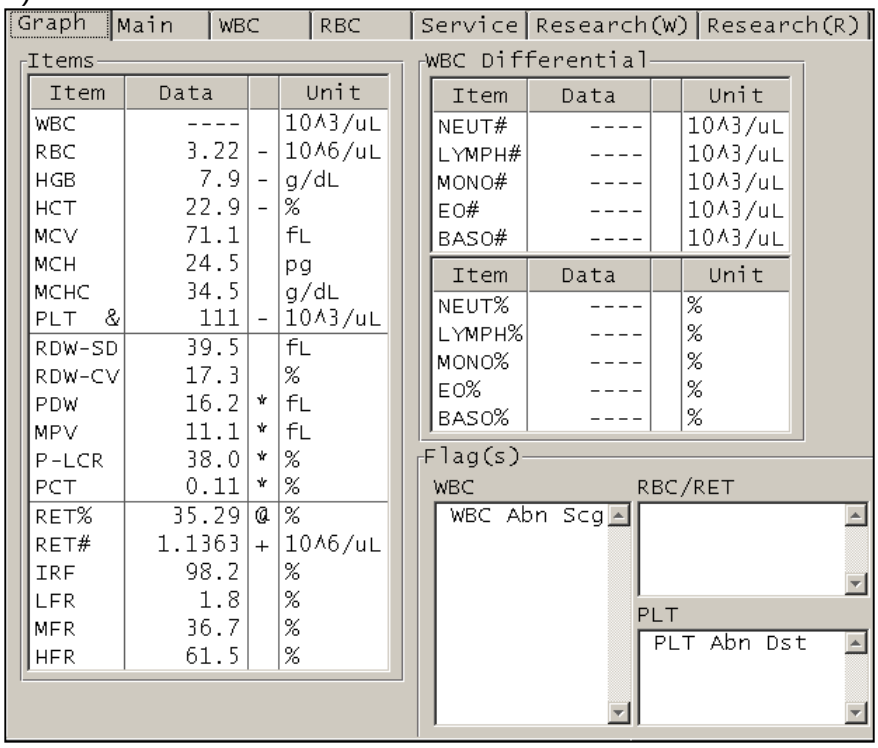
On the basis of the first tests, high suspicion of a neoplastic hematologic disorder was advanced by the private veterinarian that subsequently sent blood samples of the dog to the diagnostic laboratory of the University of Milan for subsequent diagnostic tests because of the high suspicion of hematologic neoplastic disorder. Immunophenotyping by flow cytometry (FC) was required.

Hematology:

A second blood sample was taken and sent for FC and second opinion on the hematological results. Complete blood cell count (CBC) and blood films were performed at the diagnostic laboratory of the University of Milan. CBC was performed with a laser automated analyser (Sysmex XT 2000 iV®). Abnormal leukocyte count was indicated by a flag (see Figure 1). Hematological results are shown in Figures 1, 2, 3 and 4.

Figure 1A and B: Hematology results obtained with the Sysmex XT-2000iV® (Sysmex).

A)



B)

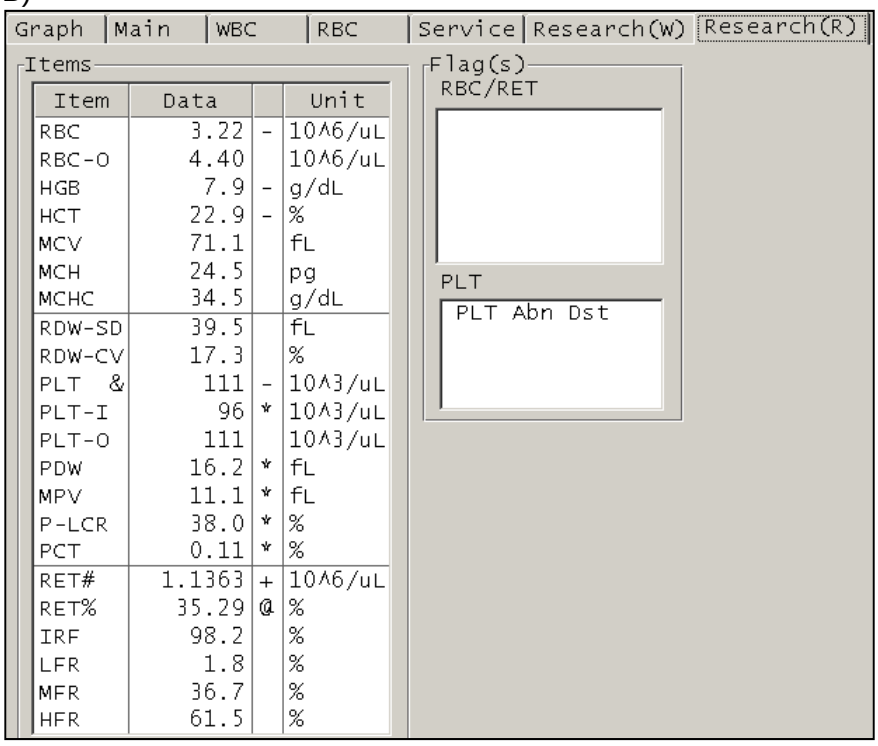


Figure 2: Sysmex XT 2000iV® WBC differential (DIFF) and WBC and basophil (WBC/BASO) scattergrams

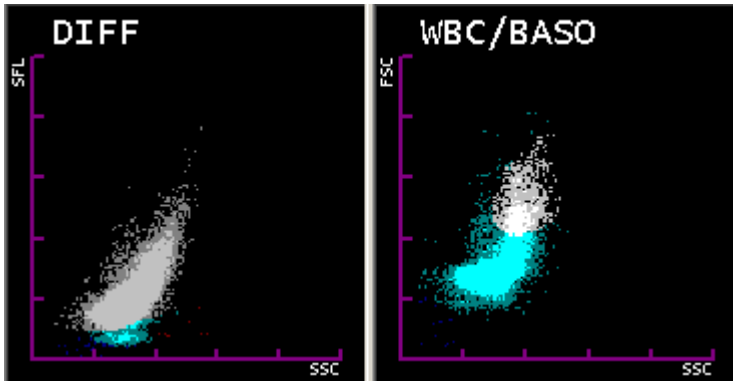


Figure 3: Sysmex XT 2000iV® reticulocyte (RET) and extended reticulocyte (RET-EXT) scattergram

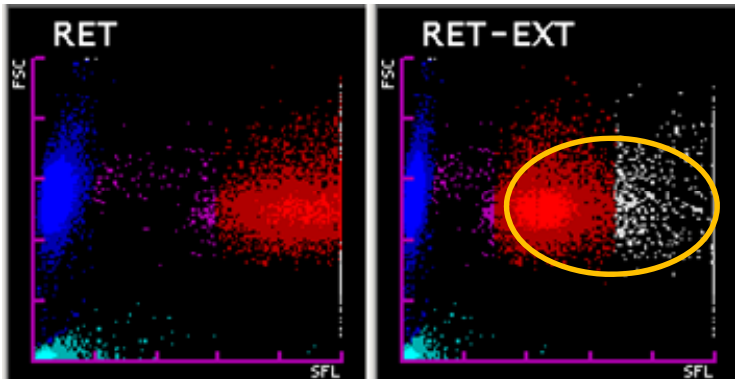
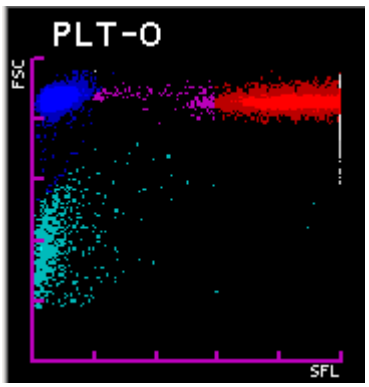


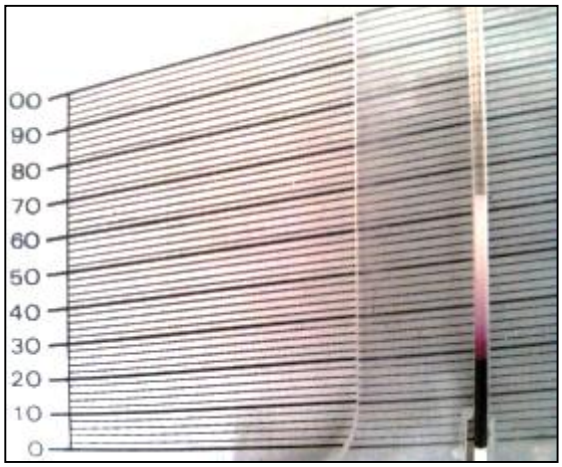
Figure 4: Sysmex XT 2000iV® optical platelet (PLT-O) scattergram



Due to the high leukocyte count, WBC count was repeated after 1:2 dilution in saline solution. After correction for dilution factor, leukocyte counts resulted to be 1’562’680 cells/µL. The reticulocyte percentages were 35.29% and IRF (immature reticulocyte fraction) was 98.2%. In order to confirm the results of the Sysmex XT 2000iV®, manual counts were executed.

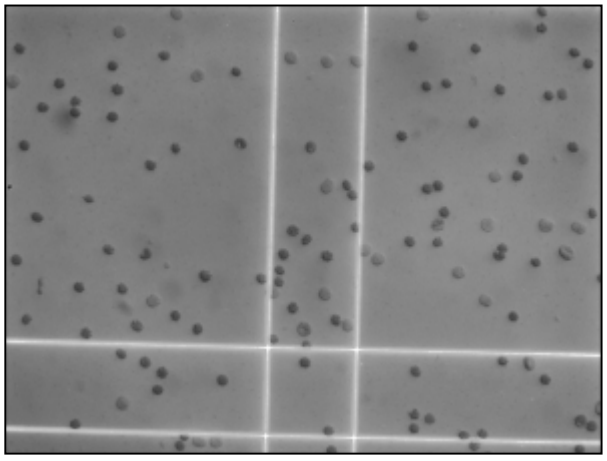
A manual packed cell volume (PCV) was determined by microhematocrit centrifugation. PCV resulted approximately 18%. Pink hue was however seen and the limit of the buffy coat was hardly identifiable.

Figures 5A and B: PCV was determined after sample centrifugation in a microhematocrit tube (A). Pink hue was seen in the white blood cell fraction (B).



Manual cell counts of RBC and WBC (hemocytometer- Burker chamber) were performed in triplicate. Due to the high leukocyte counts WBC count was performed using Turk solution at the same dilution of RBC count (1:200).
RBC= 3.41 (10^6 cells/ µL)
WBC= 1.69 (10^6 cells/ µL)

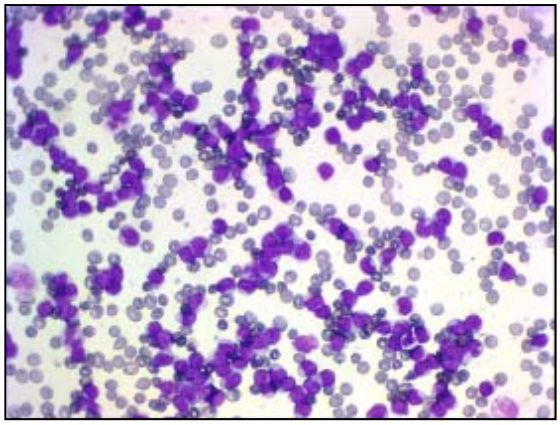
Figures 6: White and blood cells counted in the Burker chamber (1:200 dilution in saline solution)



Erythrocytes indexes were calculated according to manual counts, PCV and hemoglobin concentration (from automated analyzer)
MCV = 52.8 fL
MCHC = 43.9 g/ dL
MCH = 23.2 pg

Blood smears of the sample were stained with May-Grünwald Giemsa. Extreme leukocytosis was recognized. Mild anisocytosis and polychromasia were detected.

Figures 7A and B: Picture of the blood smear, May-Grünwald Giemsa stain, objective 10 x (A) and 40 x (B).



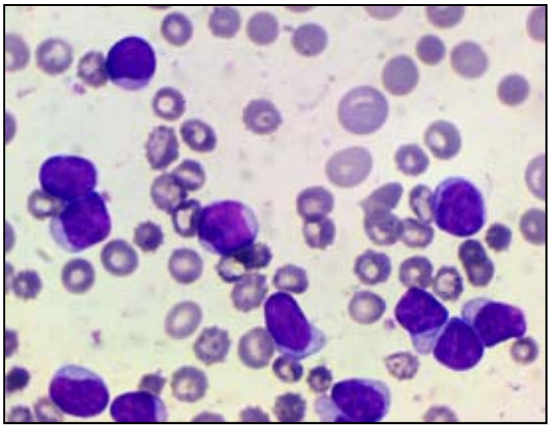


Table 2: Summary table of hematological findings obtained with different analytic techniques:

Analytes	Sysmex electrical impedance	Sysmex optical reading	Manual examination	Reference Interval
RBC	3.22	4.40	3.41	5.7-8.8 (10^6 cells/ µL)
HGB	7.9			12.9-18.4 g/dL
HCT	**22.9**		**18**	37.1-57 **%**
MCV	**71.1**		**52.8**	60-77 fL
MCH	24.5		23.2	19.5-24.2 pg
MCHC	**34.5**		**43.9**	31-36 g/dL
%RET		35.29		< 1
WBC	1’490		1’690	6-19.5 (10^3 cells/ µL)

Questions:

1. Give the most probable diagnosis
2. Give a possible interpretation of the hematological values (marked in bold in Table 2) using different analytical methods. Which is the most accurate method to estimate anemia in the present case?
3. How could the high reticulocyte counts be interpreted in this case?
4. Which is the possible interpretation of the white dots visible in the RET-EXT scattergram (Figure 3, orange circle)?