Leukocytosis in a dog

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Signalment:

8 year old, female spayed, mixed breed dog.

History and Diagnostic procedures:

The dog was presented to an external clinic for anorexia approximately one month before contacting Auburn University Clinical Pathology Laboratory (AUCPL).

At the external clinic three complete blood counts (CBCs) were performed over a three week period, which showed marked and progressively increasing leukocytosis with neutrophilia. The clinic used an impedance hematology analyzer (Abaxis VetScan HM5 Hematology System, Abaxis North America, Union City, CA, USA).

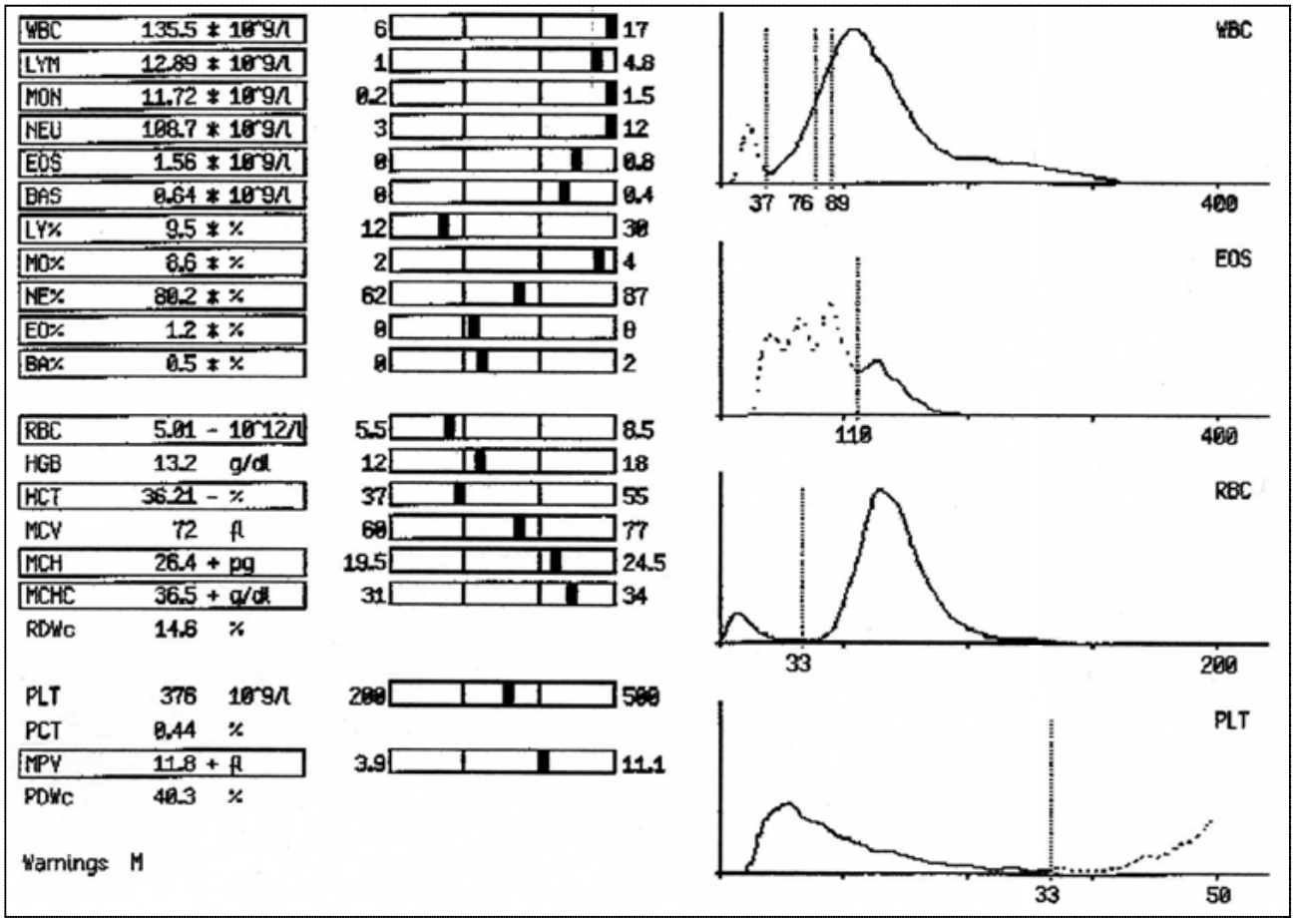
The differential diagnoses for extreme neutrophilia includes severe inflammatory processes (e.g., peritonitis, pleuritis, pyometra, abscess), certain infectious agents (e.g., *Hepatozoon americanum*, *Ehrlichia canis*, *Rickettsia rickettsii*, *Babesia canis*), neutrophilia associated with a neoplastic proliferation or granulocytic leukemia. Pyometra can be ruled out because this dog is spayed. Pleuritis, peritonitis and abscess can be ruled out or confirmed with diagnostic imaging and cytological evaluation of effusion if present. Infectious agents could be ruled out or confirmed using serology tests or PCR and in some cases reviewing a peripheral blood smear. Granulocytic leukemia and paraneoplastic neutrophilia associated with cancer are diagnosed by exclusion of all other causes of neutrophilia.

Table 1: Patient CBC data from HM5.

Analyte	Patient day 1	Patient day 7	Patient day 11	Patient day 21	Reference interval
RBC (/µL)	4,860,000	5,010,000	4,960,000	4,990,000	5,500,000-8,500,00
Hgb (g/dL)	11.7	13.2	14.2	14.1	12-18
Hct (%)	34.98	36.2	34.6	35.2	37-55
MCV (fL)	72	72	70	70	60-77
MCHC (g/dL)	33.4	36.5	41.1	40	31-34
WBC (/µL)	74,090	135,500	153,600	---	6,000-17,000
Neutrophils (/µL)	65,890	108,700	124,300	---	3,000-12,000
Lymphocytes (/µL)	5,330	12,890	12,520	---	1,000-4,800
Monocytes (/µL)	460	11,720	15,530	---	200-1,500
Eosinophils (/µL)	1,570	1,560	1,590	---	0-800
Basophils (/µL)	830	640	680	---	0-400
Platelets (/µL)	485,000	376,000	390,000	388,000	200,000-500,000

--- no values were provided by the HM5 because the total WBC of the patient exceeded the maximum reportable value.

Figure 1: Example patient CBC report (day 7) from impedance hematology instrument with histogram.



After three CBCs with progressively increasing total WBC the leukocytosis worsened and the impedance analyzer could not give a value for the white blood cells (total WBC exceeded the maximum reportable value for HM5). A peripheral blood sample and the patient's hematology reports (Table 1 and Figure 1) performed with the impedance hematology analyzer were submitted to the AUCPL. At AUCPL, a CBC using a flow-cytometry analyzer (ADVIA 120, Siemens Healthcare Diagnostics, Deerfield, IL, USA; software version 3.1.8.0-MS) was performed and a peripheral blood smear was examined (Table 2, Figures 2-5).

Table 2: Patient CBC data from ADVIA 120 hematology instrument and manual differential leukocyte counts.

Analyte	Patient's value	Reference interval
RBC (/µL)	4,600,000	6,020,000 – 8,640,000
Hgb (g/dL)	11.3	13.1 – 20.1
Hct (%)	37.6	38.7 – 59.2
MCV (fL)	81.8	60.5 – 73.8
MCH (pg)	24.6	20.4 – 25.7
MCHC (g/dL)	30.0	32 – 37.2
WBC (/µL)	164,700	5,090 – 17,410
Neutrophils (/µL)	29,800	2,600 – 10,400
Lymphocytes (/µL)	53,500	390 – 6,730
Monocytes (/µL)	4,700	160 – 1,160
Eosinophils (/µL)	900	12 – 1,160
Basophils (/µL)	1,800	rare
Platelets (/µL)	280,000	152,000 – 518,000

Figure 2: Peroxidase cytogram from ADVIA 120 hematology analyzer

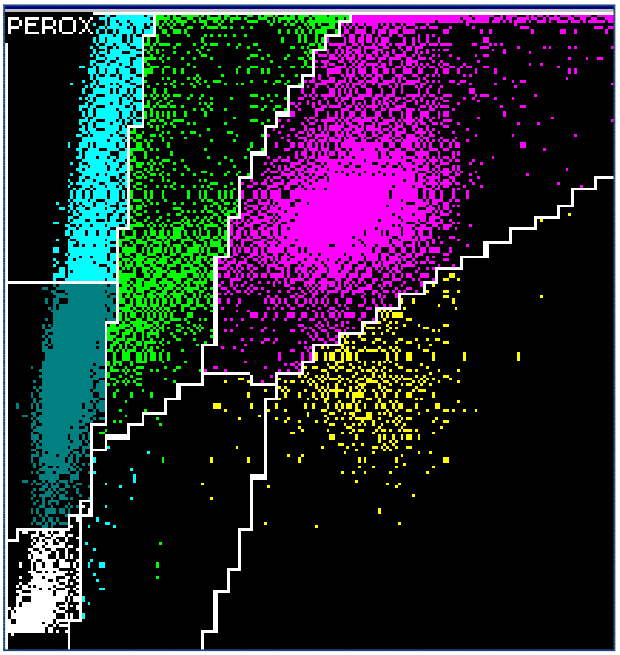


Figure 3: Basophil cytogram from ADVIA 120 hematology analyzer

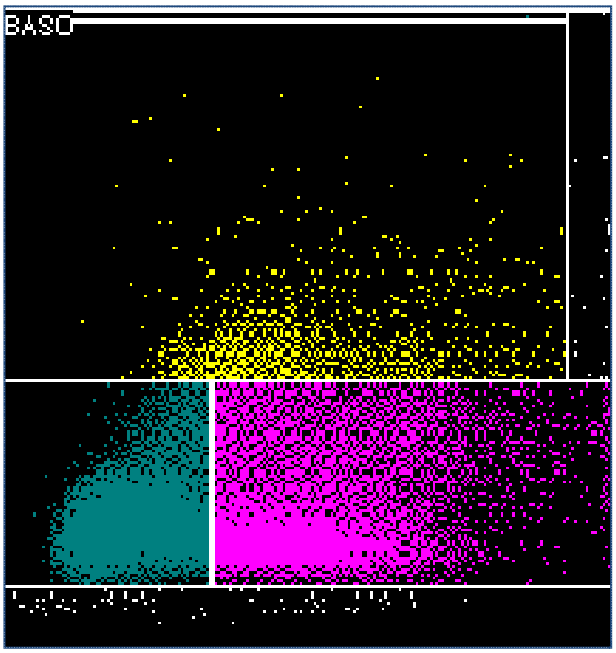


Figure 4: Peripheral blood smear, modified Wright's stain, 10x objective; shows extreme leukocytosis

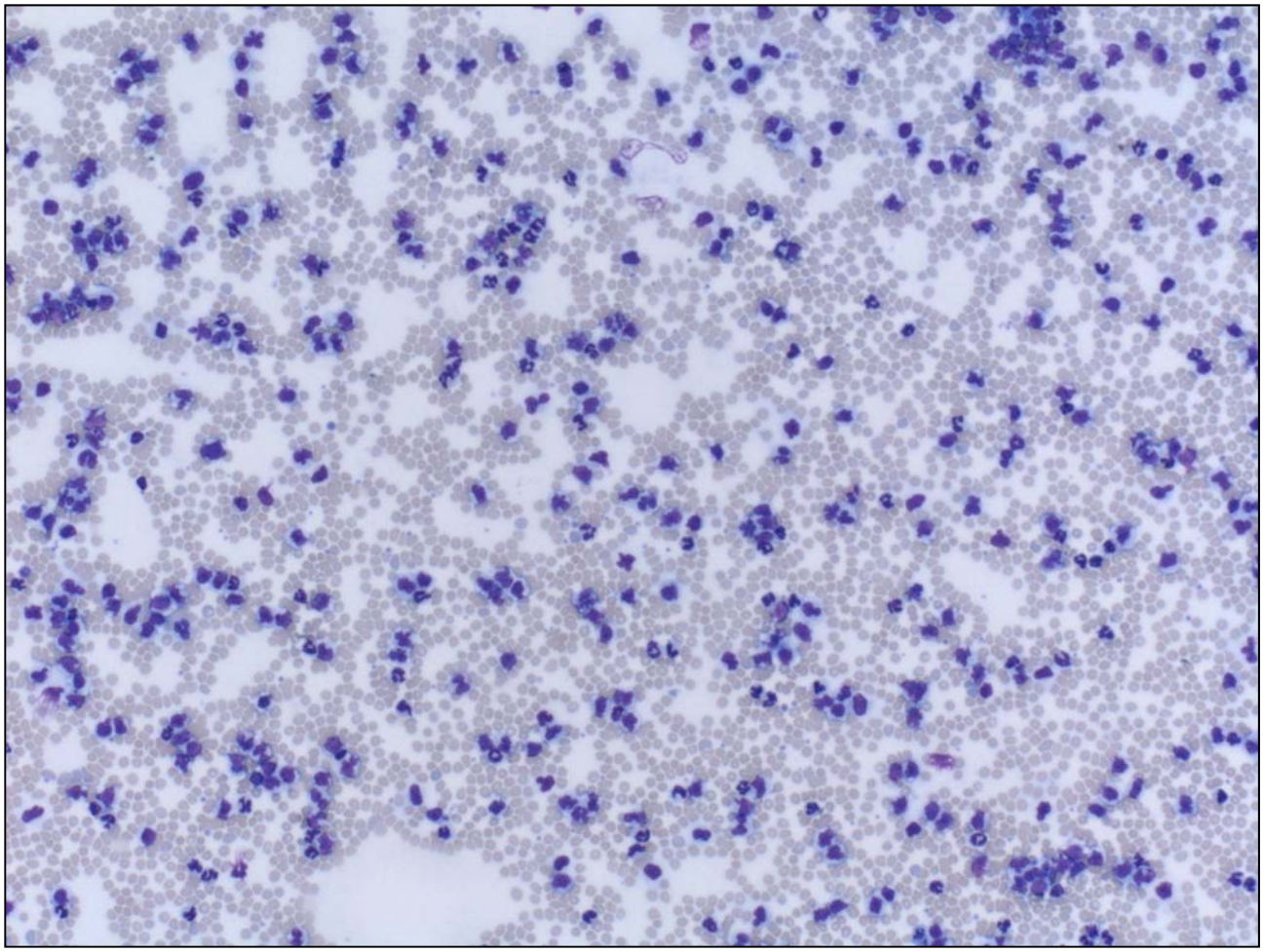
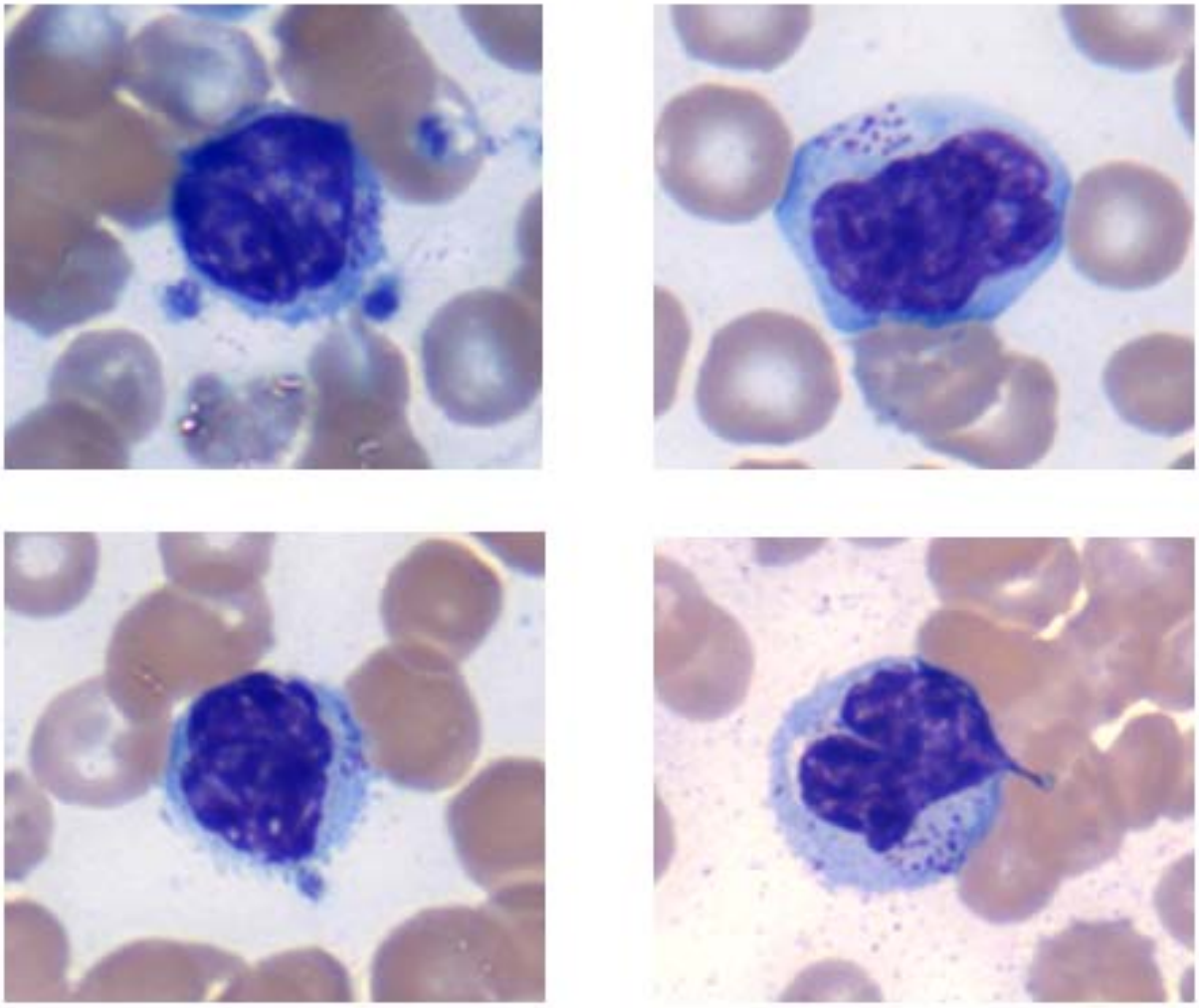


Figure 5: Peripheral blood smear, modified Wright's stain, 100x objective; majority of lymphocytes are large cells, with variably shaped nuclei, inconspicuous nucleoli, abundant cytoplasm and multiple paranuclear azurophilic granules



Questions:

What are your differential diagnosis?

What is your explanation for the differences in the reports from the two analyzers?

What other tests would you suggest to do?

Case discussion:

Peripheral blood smear findings

Estimated WBC from a blood smear is 150,000-200,000 cells/µL. There are approximately 75% lymphocytes, 22% mature neutrophils and occasional monocytes and eosinophils. The lymphocytes are large cells (1.5-2x the size of a neutrophil) with variably-shaped nuclei (round, oval, folded, indented, cleaved, floriform), coarse chromatin and inconspicuous nucleoli. They have pale basophilic cytoplasm that often contains multiple fine paranuclear azurophilic granules. Erythrocytes show minimal anisocytosis. Platelets are adequate in numbers with several large platelets.

Opinion: Large granular lymphocyte (LGL) leukemia or stage V LGL lymphoma.

Outcome:

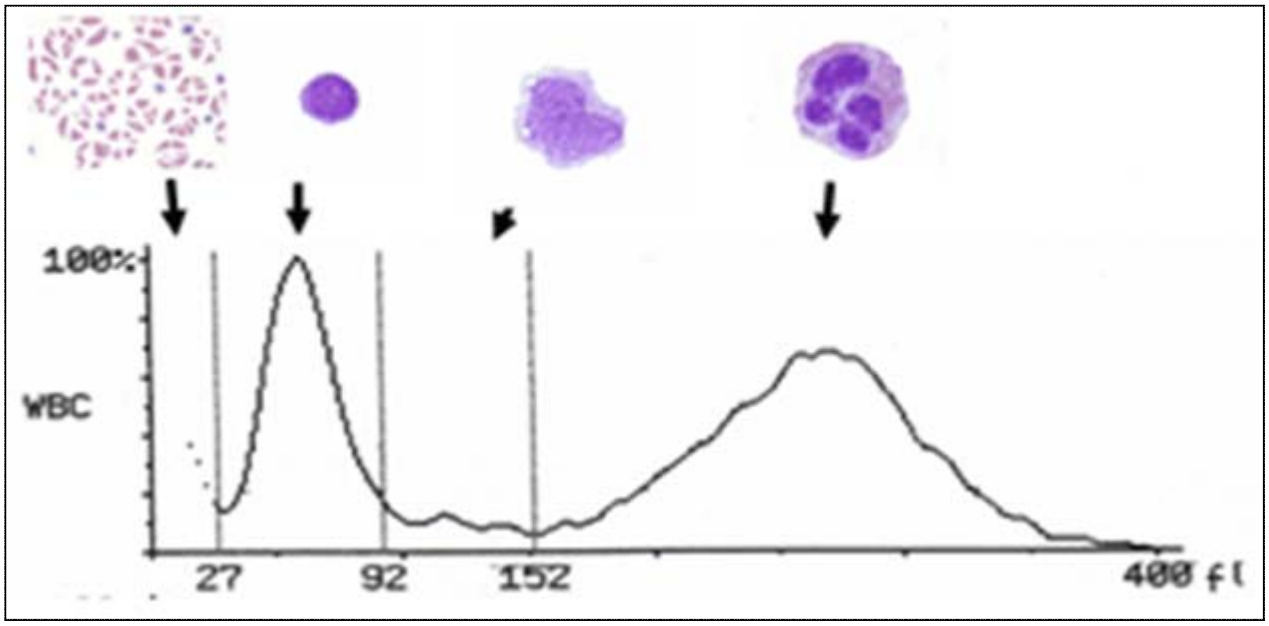
After receiving the CBC report, the owner elected for humane euthanasia due to poor prognosis and critical worsening of the dog's condition.

Discussion:

VetScan HM5 is one of the fairly numerous impedance hematology analyzers that are commercially available in veterinary medicine. These analyzers work based on the Coulter principle and can count and size cells.[1] The cells pass one at a time through a small opening placed between two electrodes that are connected by a continuous electrical current. When a cell passes between the two electrodes, it causes a change in the electric current and a voltage pulse is measured. Each change in voltage is recorded and counted and will determine the cell count. The degree of change in the voltage pulse is directly proportional to the size of the cells and enables the analyzer to perform a classification of the cells.[2] WBC are counted after the sample is mixed with a solution that lyses the erythrocytes. The WBC differential count performed by the impedance analyzers is divided into either three- or five-part differential counts. The three-part differential count includes lymphocytes, monocytes and granulocytes. In the three-part differential count the instrument is not able to distinguish between neutrophils and eosinophils.[1] The granulocytes are the largest and are displayed in the right part of the histogram while lymphocytes are the smallest and are displayed in the left side of the histogram (Figure 6). Monocytes fall in between as mid-sized cells (even though they appear to be the largest cells viewed under the microscope). Based on the proportions of cell types present in the blood sample and the reagent system, the histogram generated by the computer contains one to several adjacent curves. Hidden within the curves are overlapping peaks that represent cell categories. The curves observed represent computer derived cell populations. The manufacturers use different lysing reagents based on cellular susceptibility to various reagents for the improvement of leukocyte differential count, a process known as chemical differentiation.[2] In

the five-part differential count the instrument gives a separate value for each type of cell (lymphocytes, monocytes, neutrophils, eosinophils and basophils). Lymphocytes, monocytes and neutrophils are displayed in one histogram; after exposure of WBC to lysing reagent in the chemical differentiation, eosinophils and basophils are displayed in a second separate histogram. The VetScan HM5 provides a five-part differential count of the leukocytes by combining impedance technology and chemical differentiation.

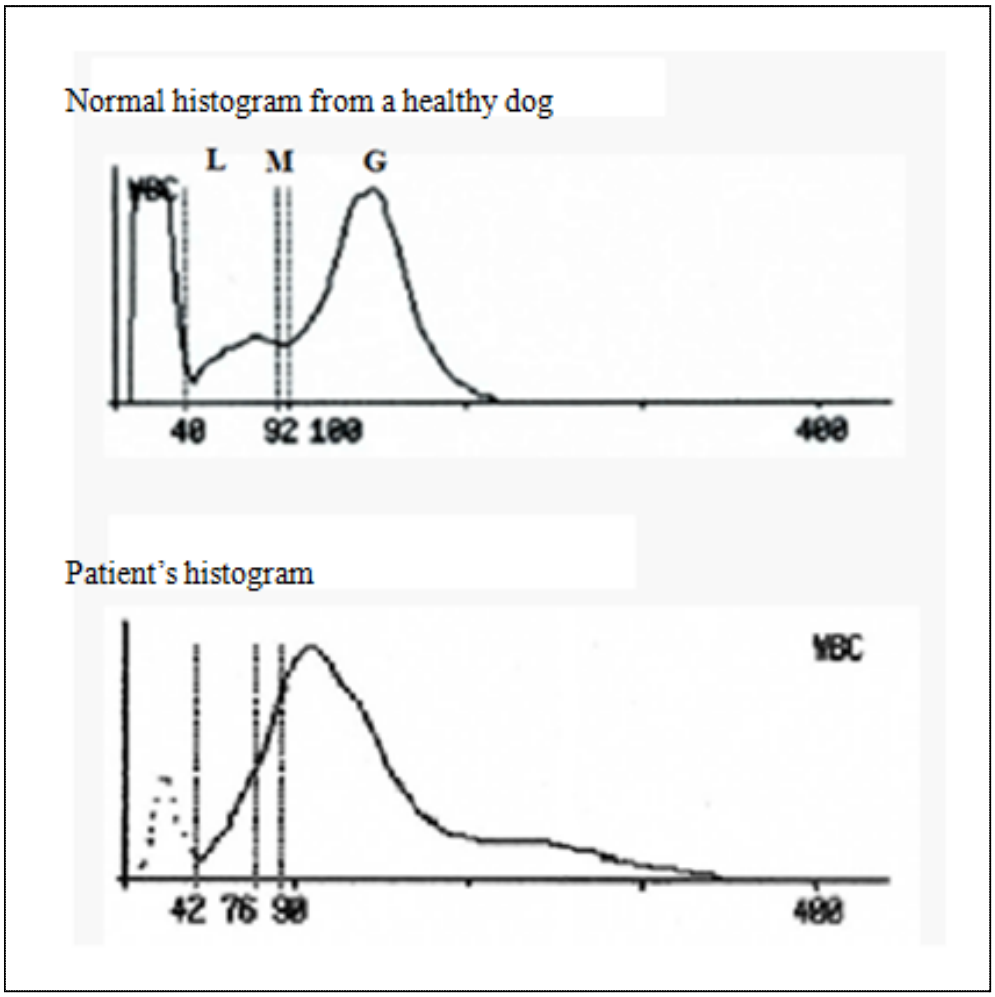
Figure 6: Example of cell distribution in a histogram from an impedance analyzer



Source: http://www.abaxis.com/veterinary/products/hm5.html

The VetScan HM5 shows on the CBC that the patient has an extreme leukocytosis and most of the WBC are neutrophils. These findings coincide with the histogram, which shows that there is mostly one cellular population distributed in the position where neutrophils (largest cells) are supposed to be. The LGLs are large cells (1.5-2x the size of a neutrophil) and they have been mistakenly identified by the impedance hematology analyzer as neutrophils. (Figure 7).

Figure 7: Comparison of the patient impedance WBC histogram with a normal WBC histogram



ADVIA 120 is a laser flow-cytometry based hematology analyzer[1]. In this type of analyzer a suspension of cells is broken into small droplets. Each droplet contains a single cell that passes through a laser beam. The cells absorb and scatter the light based on their size, nuclear characteristics and cytoplasmic granularity. These analyzers are equipped with light signal detectors that capture the light scattered by each cell and transforms it into electronic voltage pulses. A forward-angle scatter light detector captures the light diffracted around the cell and identifies the volume or size of the cell. A side-angle detector captures the light scattered at a 90° angle and identifies the internal complexity of the cell. [3] ADVIA 120 counts and differentiates leukocytes combining laser flow-cytometry with two different methods: peroxidase staining and basophil/lobularity identification. With the peroxidase staining method the erythrocytes are lysed and leukocytes are stained based on their content of peroxidase. Then the leukocytes are exposed to the laser and absorb and scatter the light based on their peroxidase activity. Lymphocytes and large unstained cells (LUCs) lack peroxidase activity and remain unstained. The information retrieved by the instrument regarding peroxidase content and cellular size are analyzed and different cellular populations are identified and displayed in a cytogram. The peroxidase content is displayed on the x-axis and the cellular size is displayed on the y-axis. These populations of cells are defined on a

sample-by-sample basis. In dogs the distinction between neutrophils and eosinophils is based on the peroxidase concentration, which is greater in neutrophils than eosinophils. With the basophil/lobularity method, RBCs are lysed and all leukocytes except for basophils are denudated of their cytoplasm using phtalic acid, surfactant and heat in another reaction chamber. In the BASO cytogram the nuclear configuration is displayed on the x-axis and the cellular size is plotted on the y-axis. Based on information from the two light detectors, the instrument divides the leukocytes into mononuclear (MN) and polymorphonuclear (PMN) cells. Each of the two methods provides a total WBC count and the instrument uses the information for an internal quality control.[4]

ADVIA 120 shows that the patient has a marked leukocytosis and was able to give a numeric value. The impedance analyzer correctly counted the total WBC, but failed to identify them. On the ADVIA 120 peroxidase cytogram the lymphocyte population is expanded toward the LUC region. LGLs are larger than normal lymphocytes and, as with all lymphocytes, lack peroxidase activity. ADVIA 120 most likely placed LGLs into the LUC population. Even if ADVIA 120 gives a more accurate estimate of the total WBC numbers and a more precise differential count, LGLs must be identified by a pathologist on a peripheral blood smear. The performance of today's hematology analyzers is markedly improved, but blood smears still need to be made and evaluated by an operator.

Large granular lymphocytes (LGL):

In dogs, different types of lymphomas and leukemias with LGL morphology have been described. Lymphomas with LGL morphology include the hepatosplenic lymphoma and the enteropathy associated T cell lymphoma with LGL morphology.[5,6] LGL leukemia in dogs most often arises in the spleen,[7,8] and could be acute or chronic with very different behavior and prognosis. In human medicine immunophenotypic analysis of LGL leukemias show that LGLs can arise from different cell lineage: T-cells (CD3+) or NK-cells (CD3-).[9] LGL leukemias originating from T-cell lineage are more frequent and less aggressive than LGL NK-cell lineage leukemias.[9] LGL leukemia arising from NK-cell lineage cannot be identified in veterinary medicine. Some canine LGL leukemias are presumed to be of NK origin because the cells stain negative for T-cell and B-cell surface markers and have been clinically very aggressive.[10]

Morphologically, LGLs are characterized as medium to large sized cells with round to oval eccentrically placed nuclei, with coarse chromatin and inconspicuous nucleoli, abundant cytoplasm and multiple paranuclear azurophilic granules.

No further investigations were performed on the patient's blood, and it was not possible to determine whether this was leukemia originating from the spleen or a leukemic phase of LGL lymphoma.

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