HumaCount 5L

| Case study book

Cat No. 164309

Human

Diagnostics Worldwide

REVISION LIST OF THE MANUAL

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CONTENTS

Preface

The objectives of this case study collection are to provide a better understanding of the haematology results obtained on the HumaCount 5L analyzer and a deeper insight into the correct usage of the analyzer's automated technology to include its strengths, limitations and any possible pitfalls. To do this, we have created a collection of clinically relevant cases and through their systematical evaluation we plan to demonstrate the correct and most efficient use of the clinical applications of the HumaCount 5L analyzer.

Introduction from our clinical evaluation site

The Department of Laboratory Medicine, Semmelweis University provides a wide and complex range of laboratory services for the University and to the whole region. In addition to routine clinical chemistry and laboratory medicine, the Department also performs specific tests in the field of endocrinology, immunology, microbiology, haemostasis and haematology.

The relationship with the clinical departments at the University which are acknowledged national centers of therapy and education, offers a unique opportunity to do outstanding research and development in diagnostics.

The characteristic cases presented in this book are representative for the patient population which is routinely assessed with the support of our Department. Patient heterogeneity and the variable patterns observed in their CBC parameters, well demonstrate the fascinating challenge that haematological investigations present to the clinicians and laboratory experts which without efficient and reliable instruments cannot be overcome.

The information that automated haematological systems provide differs significantly, depending on their principles and the technology applied. The most modern instruments with the biggest throughput and ability to measure novel parameters, however, are localized in large centers to which the patient should be referred. Therefore, a key step to establish a correct diagnosis is the referral. Any technical solution that improves the decision as to which patient in primary care should (or should not) be referred is welcome. We are convinced that this study testing the usefulness of a haematological automated system designed for primary care helps in, everyday practice, with the understanding and correct use of this important tool.

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General introduction to interpretation of HumaCount 5L results

The correct way to interpret the haematological results obtained on the HumaCount 5L analyzer is summarized in this section. To understand these, users need to have minimal but essential technical knowledge of the HumaCount 5L measurement technology. A short overview is given below. For a more detailed description of the operation and maintenance of the HumaCount 5L analyzer please refer to the user manual [REF] 164301.

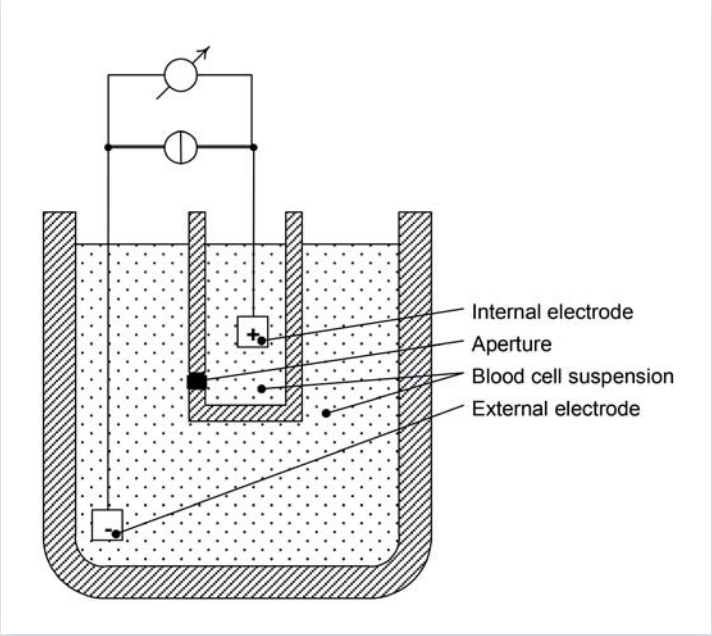
Measurement technology

The HumaCount 5L analyzer uses a combination of different measurement methods to provide results:

- Volumetric impedance (the Coulter method) is used to determine the cellular concentrations and volume distributions of leukocytes (WBC), erythrocytes (RBC), and platelets (PLT).
- Photometric measurement of light absorbance is used to determine hemoglobin (HGB) concentration.
- Optical measurement of light scattering and diffraction is used to determine 5-part leukocyte (LYM%, MON%, NEU%, EOS%, BAS %) differential parameters.

Volumetric Impedance Method

The volumetric impedance method (also known as the Coulter method) determines cellular concentrations and volume distributions of cells by detecting and measuring changes in electrical impedance when particles suspended in a conductive liquid pass through a small aperture. The method is "volumetric" because a small known volume of blood is precisely diluted with a conductive diluent and forced through the aperture at a fixed rate.



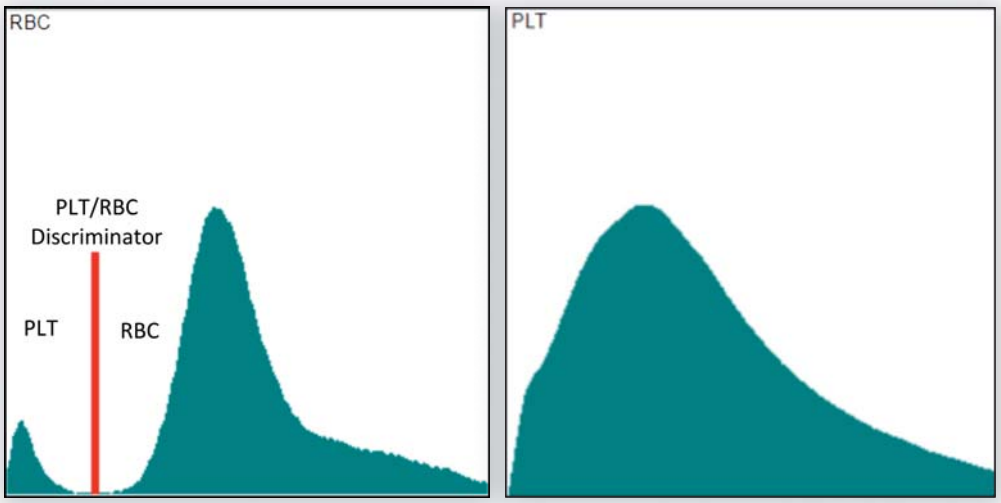
Volumetric Impedance Method

A constant direct current flows between the electrodes on both sides of the aperture. Each cell passing through the aperture causes a change in the electrical impedance of the conductive blood cell suspension. This change is detected by the HumaCount 5L electronics and converted to an electrical pulse. The quantity of pulses is equal to the number of particles. The intensity of each pulse is proportional to the volume of the particle. The volume distribution diagrams of the particles result in the WBC, RBC, and PLT histograms that are measured in femtoliters.

Electronic discrimination allows separation of erythrocytes (RBC) and platelets (PLT). A lytic reaction lyzes erythrocytes

to clearly measure leukocytes (WBC).

RBC and PLT impedance-based measurements are represented by histograms. These diagrams show the number of cells on the Y axis and the cell size on the X axis. Smaller cells are displayed on the left of the histogram, and larger cells are displayed on the right. The height of the histogram at a given size represents the number of cells with that size. The greater the histogram height indicates more cells.

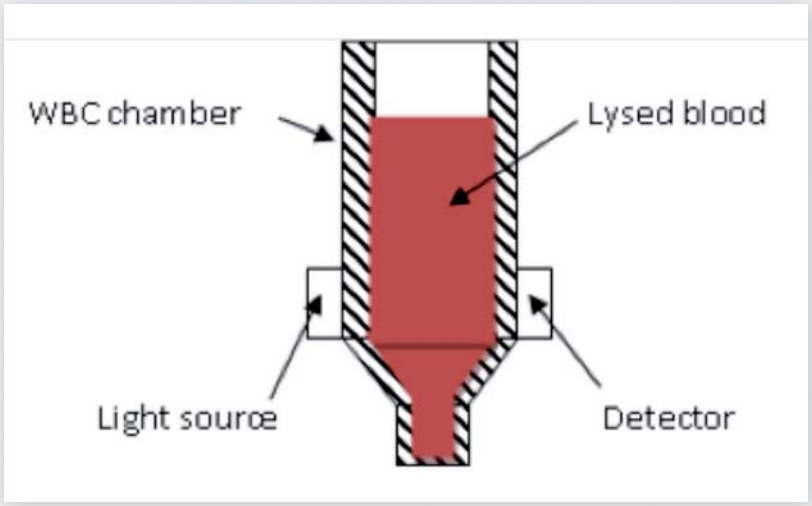


Result Screen Histograms

Discriminators (thresholds) are displayed with red colour. Platelets are located on the far left of the RBC histogram and to the left of the red discriminator. The PLT histogram is a magnified section of this region.

Photometric Light Absorbance Method

A lyzed blood sample dilution can be analyzed for hemoglobin (HGB) concentration based on its stable chromogen content. The reagent lyzes the red blood cells causing the release of cellular hemoglobin. The hemoglobin concentration is measured by making a photometric reading across the HumaCount 5L WBC chamber. The HGB measurement is calculated as the difference between a blank and a sample measurement with and without illumination thus compensating the effect of liquid refraction and incident light.

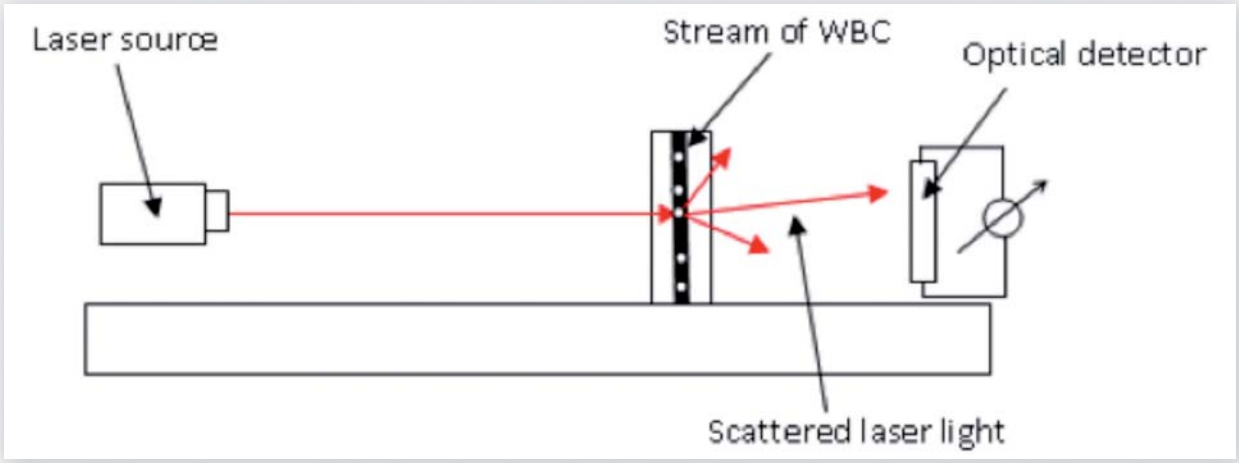


Photometric Light Absorbance Method

Optical Light Scatter and Diffraction Method

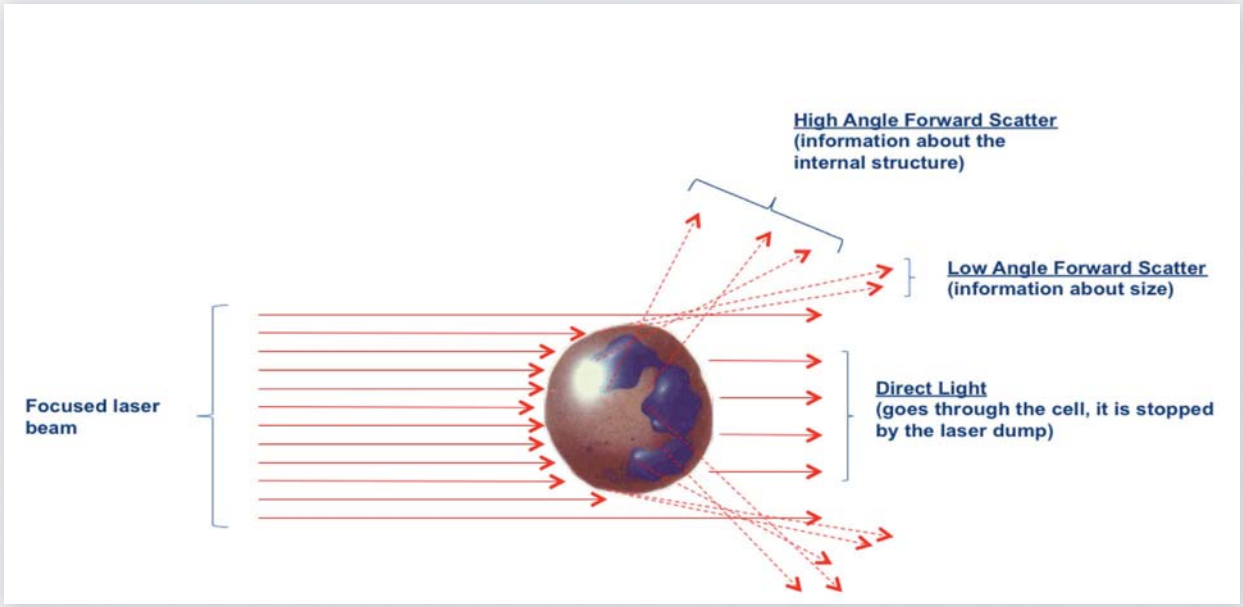
Optical measurement of light scattering and diffraction is used to determine the 5-part leukocyte (LYM%, MON%, NEU%, EOS%, BAS%) differential parameters. An optical measuring head contains a focused laser source that is used to illuminate a stream of leukocytes (WBC) suspended in an optically clear diluent moving through a flow cell.

The cells scatter light as they flow through the path of the laser beam. An optical detector senses changes in the intensity of the scattered laser light which are proportional to the cell volume and granularity of the cell's internal structure. The HumaCount 5L electronics convert these changes to electrical pulses which are gathered and stored for analysis. The five part population discrimination is based on analysis of the two dimensional volume and granularity distribution scatter diagram.



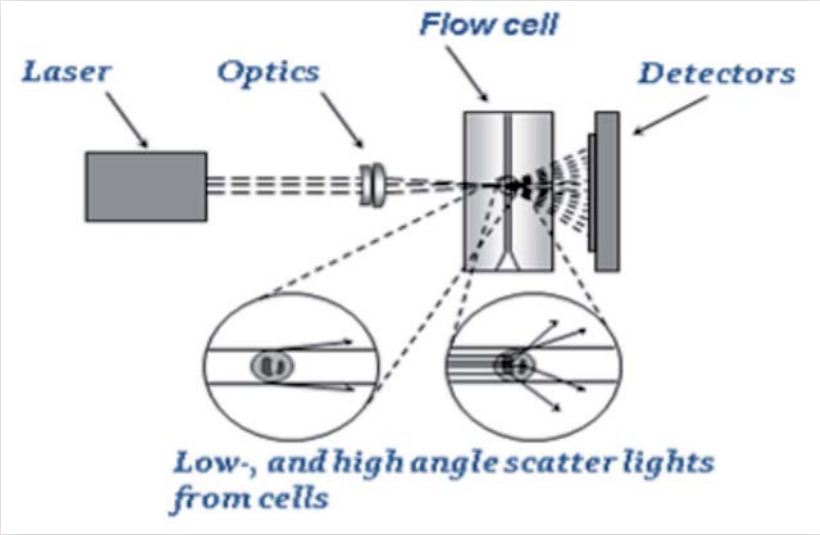
HumaCount 5L Optical Head Block Diagram

Cells with greater volume or size or with more granularity will tend to scatter greater amounts of light.
The intensity of scattered light is detected by an optical signal processing system.



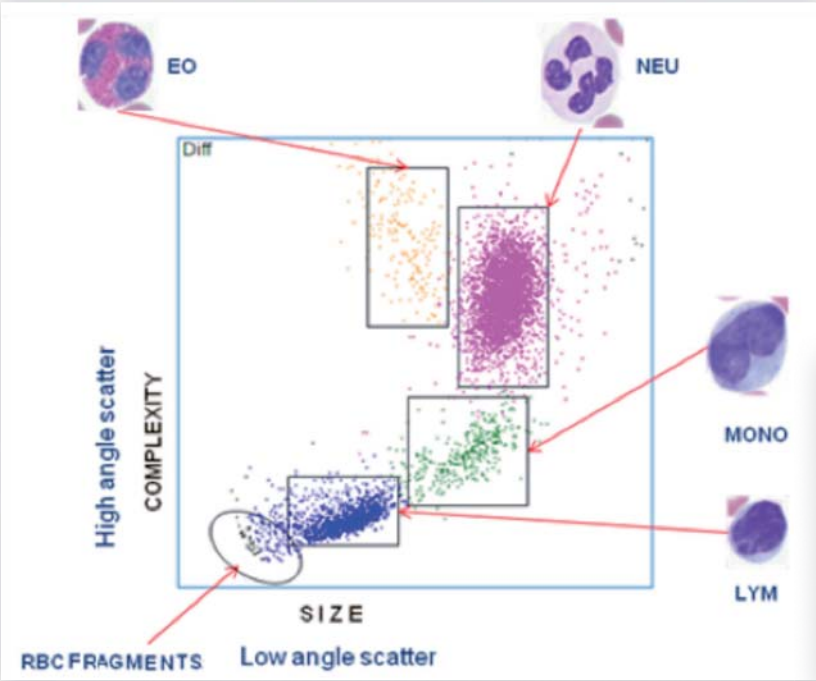
Cellural Light Scatter

External structure (and the size of the cell) causes lower angles of scatter, while the internal granularity or complexity causes higher angles of diffraction. Both low and high angles of light scatter are captured by optical sensors, providing the HumaCount 5L analyzer with two independent measurements for each cell crossing the path of the laser beam.

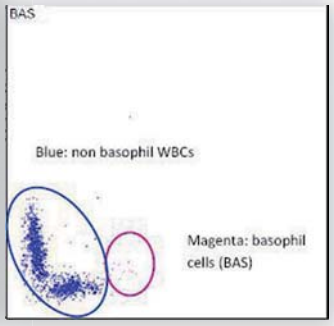


Optical Signal Processing System

The light scatter data is gathered and plotted as a two dimensional scatter diagram. Similar cells have similar scatter characteristics and tend to group together. This allows the analytical software to differentiate and identify the leukocyte populations and generate the 4-part differential (DIFF) and basophil (BAS) scatter diagrams.



4 DIFF Scattergram

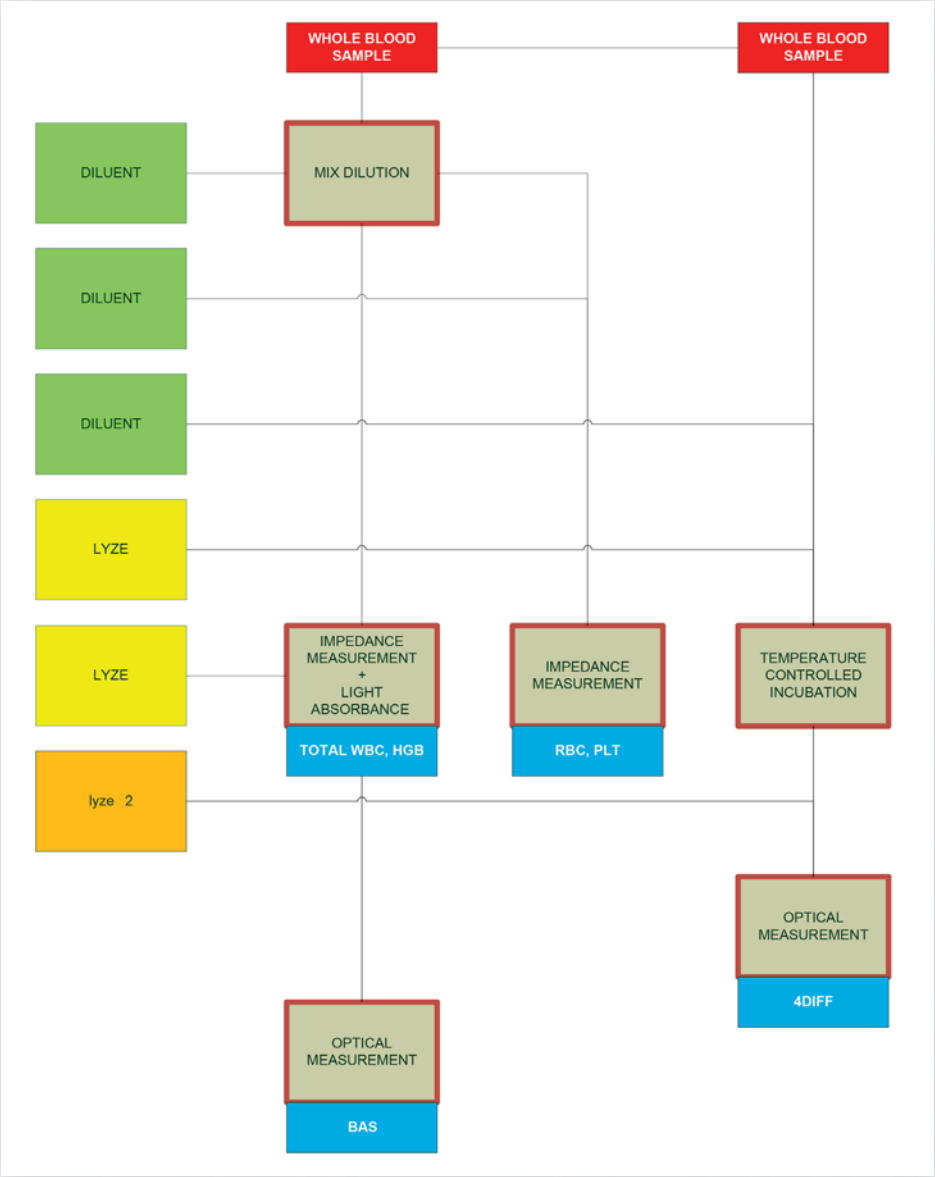


BAS Scattergram

In order to avoid artefacts, regular maintenance is needed and frequent measurement of dedicated haematological controls is required to ensure good analyzer performance. The process is described in HumaCount 5L user manual in details.

Measurement process

The HumaCount 5L measures RBC, PLT and WBC counts using different dilutions and a variety of methods. In the WBC dilution, RBCs are lyzed by a special reagent solution. The entire process is illustrated below.



HumaCount 5L Measurement Process

After presenting the sample tube and pressing the start button, the analyzer moves the sample rotor before taking the sample tube inside the analyzer. A sample needle pierces the tube cap (if present) and aspirates approximately 110 µL of primary blood sample. Blood sensor ensure that the primary blood sample is continuous and free of major bubbles before moving it to a shear valve. The shear valve separates the primary sample into 3 exact volumes of blood which are distributed to provide dilutions for the RBC, WBC, HGB and 5 part population measurements. Lysing reagents are used to eliminate RBCs in dilutions that are measured for WBCs.

RBC/PLT and WBC are measured by impedance technology when passed through 70 and 80 µm apertures respectively. Two portions of the sample are used to determine a five part subpopulation WBC differential via two independent measurements – a 4 part DIFF (neutrophil, lymphocyte, monocyte, and eosinophil) measurement and a separate BAS (basophil) measurement.

Sample results are displayed on the screen.

A step by step guide to the interpretation of results

To interpret the results, a general review of scattergrams and histograms is always needed and strongly recommended.

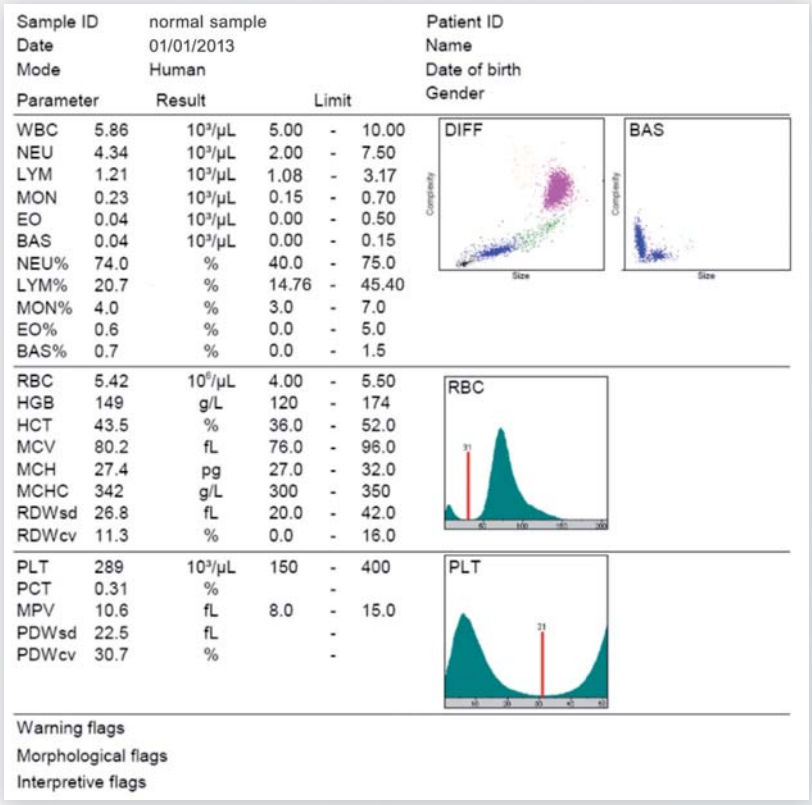
- In each WBC differential (DIFF) scattergram the cellular populations should be localised at their correct positions and be well separated from each other. Histograms should be well shaped, separated from each other and characteristic of normal RBC and PLT populations. This means that RBC's follow a normal distribution with a slight, asymmetric tail on the right end, due to the data processing algorithm. PLT's follow a Poisson distribution. (see illustration, 'Normal result').
- Then flags should be checked.

If abnormal scattergrams/histograms or any flag's are detected, an evaluation of blood smear is strongly suggested.

After making a general review the numerical results can be read and interpreted.

Please be aware like all automated haematology systems that the HumaCount 5L analyzer is intended for screening purposes only. If results are suggestive of haematological abnormalities, a microscopic evaluation of blood smear is always recommended.

Even in the absence of abnormalities, the results of automated cell counting may slightly differ from those obtained by the evaluation of microscopic blood smears. Please note that microscopic evaluation results have a higher statistical deviance as compared to automated methods. This is due to the fact that in a microscopic evaluation of a blood smear only around 100 cells are evaluated, whereas in automated counting methods they routinely analyze several thousands cells. This can lead to some discrepancy between numerical results of microscopic and automated methods especially in cell populations with lower numbers. (e.g. basophils, monocytes, eosinophils). However microscopic evaluation is always required in the event of the presence of abnormal samples.



Sample ID	normal sample				Patient ID	
Date	01/01/2013				Name	
Mode	Human				Date of birth	
					Gender	
Parameter	Result		Limit			
WBC	5.86	$10^3/\mu L$	5.00	-	10.00	
NEU	4.34	$10^3/\mu L$	2.00	-	7.50	
LYM	1.21	$10^3/\mu L$	1.08	-	3.17	
MON	0.23	$10^3/\mu L$	0.15	-	0.70	
EO	0.04	$10^3/\mu L$	0.00	-	0.50	
BAS	0.04	$10^3/\mu L$	0.00	-	0.15	
NEU%	74.0	%	40.0	-	75.0	
LYM%	20.7	%	14.76	-	45.40	
MON%	4.0	%	3.0	-	7.0	
EO%	0.6	%	0.0	-	5.0	
BAS%	0.7	%	0.0	-	1.5	
RBC	5.42	$10^6/\mu L$	4.00	-	5.50	
HGB	149	g/L	120	-	174	
HCT	43.5	%	36.0	-	52.0	
MCV	80.2	fL	76.0	-	96.0	
MCH	27.4	pg	27.0	-	32.0	
MCHC	342	g/L	300	-	350	
RDWsd	26.8	fL	20.0	-	42.0	
RDWcv	11.3	%	0.0	-	16.0	
PLT	289	$10^3/\mu L$	150	-	400	
PCT	0.31	%		-		
MPV	10.6	fL	8.0	-	15.0	
PDWsd	22.5	fL		-		
PDWcv	30.7	%		-		

Warning flags
Morphological flags
Interpretive flags

Printed result of a normal sample measured by HumaCount 5L

Limitations of HumaCount 5L technology

Even though the HumaCount 5L analyzer is high quality within its category and a very useful tool for automated haematological screening in small and medium sized clinical labs, users should realize there are some limitations which can be expected due to the technology utilized. However to help overcome these, there are some in-built flagging algorithms to indicate possible technical errors or the presence of immature and abnormal (atypical) cells which cannot be reliably distinguished on the HumaCount 5L analyzer. Due to their resistance to the lyzing procedure, nucleated red blood cells (NRBC) will be recognized as white blood cells. This may increase the total WBC count. Due to their similar size and structure NRBCs can interfere with the lymphocyte count. Furthermore because of their special response to the lyzing effect, such as atypical cells, blast cells, immature cells and activated lymphocytes may falsely increase the basophil and/or monocyte count. Activated lymphocytes (a variant form) and monocytes may produce a confluent population and as a result the analyzer monocyte count may be falsely elevated while conversely if lymphocyte activation is present (e.g. in case of some viral infections like mononucleosis) the lymphocyte count maybe falsely decreased. Please note that the analyzer doesn't provide any interpretive flags if a measured parameter is out of the linearity range, but a linearity flag " * " appears beside the numerical value of the parameter. If the count of a particular cell population is either strongly below or above the upper limit of the linearity range of the analyzer it may not give any numerical result for the population. However, cells will be clearly visible in the scattergram or histogram and the correct error flag will also be present. In cases where the count is above the linearity range, there is a high probability of coincidence occurring (i.e. more than one cell passing through the detector at the same time). In this event, a manual dilution of the sample with saline must be performed and re-tested to ensure the correct numerical results. Extremely high WBC counts can falsely increase the MCV of RBCs. Such a high WBC count can interfere with the RBC counting and in this case the real RBC count is somewhat lower than the displayed one. High cell counts or altered leukograms with abnormal cells are common findings in cases of haematological malignancies and careful interpretation of results is always essential. In general, blood smears of abnormal samples should be visually evaluated.

Reference ranges

Normal ranges may slightly vary among populations and may depend also on the technologies applied. It is recommended that you perform your own normal range study to establish normal ranges which are valid for the local population.

System Flags

The HumaCount 5L analyzer has a number of warning flags e.g. Linearity, High blank, General warning, Morphological, and Interpretive messages. The following tables describe these flags and their accompanying messages in detail. It also provides guidance on any actions that the operator can take to correct the problem described by the flag.

Normal range flags	Normal range flags are raised when a particular parameter is above or below the normal range, which is defined for the patient profile currently in effect.		
Flag	Meaning	Hierarchy	Colour Code
-	Parameter is below normal range	Lowest	Blue
--	Parameter is below half of the low limit of normal range	Low	Blue
+	Parameter is above the normal range	Lowest	Red
++	Parameter is more than double of the high limit of normal range	Low	Red
Linearity Range Flag	Linearity range flags are raised when a particular parameter is above or below the linearity range of the device.		
Flag	Meaning	Hierarchy	Colour Code
*	The related parameter is out of the linearity range	High	N/A
High blank flag	A high blank flag is raised when the blank measurement result of the particular parameter was higher than the blank limit.		
Flag	Meaning	Hierarchy	Colour Code
!	The blank value of the related primary parameter is high	Middle	N/A

Warning flags		Warning flags are raised when the analyzer detects a problem in the analysis process which may lead to incorrect or suspect results or in the parameters	
Flag	Meaning	Cause	Action
A	4 Diff Alarm	The 4 DIFF populations are not clearly distinguishable on the scatter diagram.	If other parameters predict a 'normal' sample then perform a cleaning procedure and repeat the measurement. If the 4 DIFF results reported then treat them as 'reduced reliability'. If not all the parameters are reported or the sample is predicted as pathological then perform a manual count on a stained smear.
B	WBC blank is high	WBC > WBC blank high limit	Check the cleanliness of the reagents and the HumaCount 5L. Perform an analyzer cleaning procedure. Repeat blank measurement. Treat WBC result as 'reduced reliability'.
b	RBC blank is high	RBC > RBC blank high limit	Check the cleanliness of the reagents and the HumaCount 5L. Perform an analyzer cleaning procedure. Repeat blank measurement. Treat RBC result as 'reduced reliability'.
C	WBC clogging	Drift in the probe voltage of the WBC capillary. The two main causes for drift are misconnection of reagents and the clogging of the capillary.	Check the reagent connections. Perform a cleaning procedure! See chapter: maintenance. Please repeat the measurement
c	RBC/PLT clogging	Perform a cleaning procedure! See chapter: maintenance.	Check the reagent connections. Perform a cleaning procedure! . Please repeat the measurement.
D	MON - NEU alert	Please repeat the measurement.	If other parameters indicate a 'normal' sample then perform a cleaning procedure and repeat the measurement. If MON, MON%, NEU, NEU% results are reported then treat them as 'reduced reliability'. If not all the parameters reported or the sample is predicted as pathological then perform a manual count on a stained smear.
E	EO - NEU alert	The EO and NEU populations are not clearly distinguishable on the scattergram.	If other parameters indicate a 'normal' sample then perform a cleaning procedure and repeat the measurement. If EO, EO%, NEU, NEU% results are reported then treat them as 'reduced reliability'. If not all the parameters reported or the sample is predicted as pathological then perform a manual count on a stained smear.
F	Differential blank high	More than 100 cells detected during the '4 Diff-blank' procedure.	Check the cleanliness of the reagents and the HumaCount 5L. Perform a flow cell cleaning of the HumaCount 5L. Repeat blank measurement. Treat 4 Diff results as 'reduced reliability'.
f	Basophil blank high	More than 100 cells detected during the 'Basophil-blank' procedure.	Check the cleanliness of the reagents and the HumaCount 5L. Perform a flow cell cleaning of the HumaCount 5L. Repeat blank measurement. Treat BAS result as 'reduced reliability'.
H	HGB blank high	Last accepted blank result: HGB ≥ 10 g/l	Check the cleanliness of the reagents and the HumaCount 5L. Perform an analyzer cleaning procedure. Repeat blank measurement. Treat HGB result as 'reduced reliability'.
I	PLT URI	The PLT-RBC gap is not clearly detectable on the PLT-RBC histogram: small MCV, fractured RBC-s, aggregated PLT-s (cold blood), side-effect of blood-transfusion.	Check the sample quality. Repeat the sample. If the problem persists then perform a manual count on a stained smear.

Warning flags		Warning flags are raised when the analyzer detects a problem in the analysis process which may lead to incorrect or suspect results or in the parameters	
Flag	Meaning	Cause	Action
M	Out of RBC linearity range	The RBC coincidence is over the limit: too high RBC count.	Check the sample homogeneity. Repeat the sample with manual pre-dilution.
m	Close to RBC linearity range	The RBC coincidence is close to the limit: too high RBC count.	Treat the RBC result as 'reduced reliability'. Check the sample homogeneity. Repeat the sample with manual pre-dilution.
O	High linearity range limit exceeded	If any of RBC,PLT or WBC results are higher than high linearity range limits.	Perform manual pre-dilution of sample and re-measure it.
o	Low linearity range limit exceeded	If any of RBC,PLT or WBC results are lower than Low linearity range limits.	None
p	PLT blank high	Last accepted blank result: PLT ≥ 15 * 10^3 cells/µl	Check the cleanliness of the reagents and the HumaCount 5L. Perform cleaning of the HumaCount 5L. Repeat blank measurement. Treat PLT result as 'reduced reliability'.
Q	MON - LYM alert	The MON and LYM populations are not clearly distinguishable on the scattergram.	If other parameters indicate a 'normal' sample then perform a cleaning procedure and repeat the measurement. If MON, MON%, LYM, LYM% results are reported then treat them as 'reduced reliability'. If not all the parameters reported or the sample is predicted as pathological then perform a manual count on a stained smear.
S	WBC measurement statistics warning	The distribution of the RBC detection changes in time. It points to clogging, non-homogeneous sample, cold sample, partial coagulation of the sample.	Perform an analyzer cleaning procedure. Check the homogeneity, the temperature and the coagulation of the sample. Repeat the sample.
s	RBC measurement statistics warning	The distribution of the RBC detection changes in time. It points to clogging, non-homogeneous sample, cold sample, partial coagulation of the sample.	Perform an analyzer cleaning procedure. Check the homogeneity, the temperature and the coagulation of the sample. Repeat the sample.
T	Blood detector error	Blood Detector malfunction (received signals are all zeros or not received signals)	Check Blood Detector (BD) and its connection to the LSDACQ card. Check sampling tube connection to BD.
u	Basophil high	The number of the BAS population is high or there are lyze-resistant cells in the sample.	Repeat the measurement or perform a manual count on a stained smear.
V	WBC vacuum warning	There is a (partial) clogging or leakage in the RBC part of measurement system. A faulty/ worn-out pump can also create vacuum errors.	Perform an analyzer cleaning procedure. Perform a self-test. If the vacuum problems persist then call for service.
v	RBC vacuum warning	There is a (partial) clogging or leakage in the RBC part of measurement system. A faulty/ worn-out pump can also create vacuum errors.	Perform an analyzer cleaning procedure. Perform a self-test. If the vacuum problems persist then call for service.
W	Sampling warning	Blood Detector detected insufficient sample volume.	Check the sample volume in sample tube. Check sampling process. Re-run sample.
X	Differential error	Insufficient cells detected during the 4 DIFF WBC differentiation.	Perform a flow cell cleaning procedure! Repeat the measurement. If the problem persists then ask for service.
x	Basophil error	Insufficient cells detected during the Basophil WBC differentiation.	If the total-WBC count is below 2 * 10^3 cells/µl then perform a manual count on a stained smear. Perform a flow cell cleaning procedure! Repeat the measurement. If the problem persists then ask for service.
Y	Differential percentage error	Algorithm error while distinguishing the WBC-s in the 4 DIFF procedure.	Repeat the measurement. If the problem persists on a given sample then perform a manual count on a stained smear.

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Warning flags		Warning flags are raised when the analyzer detects a problem in the analysis process which may lead to incorrect or suspect results or in the parameters	
Flag	Meaning	Cause	Action
y	Basophil percentage error	Algorithm error while distinguishing the WBC-s in the BAS procedure.	Repeat the measurement. If the problem persists on a given sample then perform a manual count on a stained smear.
Z	WBC noise high	During WBC counting there are too many small pulses (more than 10%) detected in the first 10 channels of the 255-channel full range. This may be caused by lyze resistant RBCs, electronic noise, contaminated WBC chamber or contaminated reagents.	Perform a clean process. Perform a hard clean process.

Morphological flag		Morphological flags are raised when the presence of morphologically abnormal cell types such as immature granulocytes are suspected.	
Flag	Meaning	Cause	Action
G	Immature granulocytes	The count in the region of (low - high angle scatter of HumaCount 5L) where the Immature granulocyte typically located is > 3% of the total WBC count.	Perform a manual count on a stained smear.
L	Atypical lymphocytes	The count in the region of (low - high angle scatter of HumaCount 5L) where the atypical lymphocytes are typically located is > 1% of the total WBC count.	Perform a manual count on a stained smear

Interpretive flag	Interpretive flags infer the presence of distributional abnormalities such as leukocytosis.
Flag	Meaning
Leukopenia	WBC --
Leukocytosis	WBC + or WBC ++
Neutropenia	NEU --
Neutrophilia	NEU ++
Lymphopenia	LYM --
Lymphocytosis	LYM ++
Monocytosis	MON ++
Eosinophilia	EOS ++
Basophilia	BAS ++
Anemia	RBC - or RBC --
Polycythemia	RBC + or RBC ++
Microcytic RBC	MCV - or MCV --
Macrocytic RBC	MCV + or MCV ++
Hypochromic	MCHC - or MCHC --
Hyperchromic	MCHC + or MCHC ++
Anisocytosis	RDW + or RDW ++
Thrombocytopenia	PLT --
Thrombocytosis	PLT + or PLT ++
Microcytic PLT	MPV - or MPV --
Macrocytic PLT	MPV + or MPV ++

Measurement related problems and remedies

Below are some typical problems related to the measurements. Please note that they are for information only. A more detailed description can be found in the HumaCount 5L user manual.

Small scattergram in the lower left corner

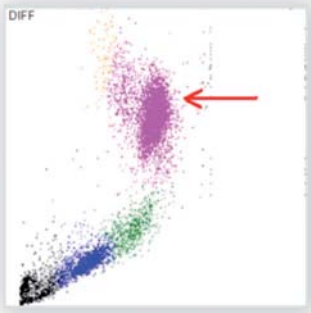
It basically indicates that a very low number of cells have been counted by the optical measurement system. The phenomenon is usually due to a system blockage, typically occurring in the temperature control unit or the flow cell.

Possible reasons:

- A blocked flow cell in the optical head
- A blocked sheath injection port

If this occurs, please contact service.

Scattergram shifted/bent left or right



The normally curved histogram bends to the left or to the right. Populations otherwise look acceptable, but the colour identification dividing the populations is halved.

Possible reasons:

- Optical amplifier LOW and HIGH angle factors were altered and are incorrect
- Malfunction in the optical amplifier
- The optical head has been replaced, but no calibration was made.

If this occurs, please contact service.

Scattergram smeared to upper right corner

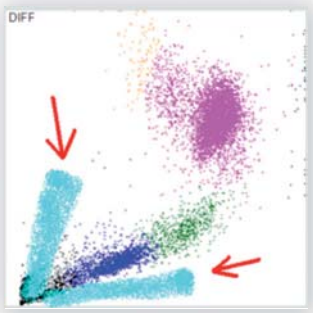
In this case, all cells generate extremely “intensive” signals in the optical amplifier. The system cannot compensate for the extreme high signals, and is unable to place them into the normal, expected intensity range. There is a possibility that the laser light path is not intersecting the sample stream
at the correct place.

Possible reasons:

- The laser alignment has been altered
- Noise in the laser (poor quality laser light)
- Optical head malfunction

If this occurs please contact service.

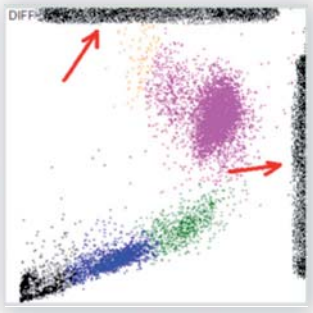
Triangular populations above/below normal scattergram area



As an example the population indicated in this diagram with light blue (cyan) appears on its own but it will not be coloured cyan on the screen. Instead it will be black or blue due to the system trying to interpret it as an irregular population.

If this occurs please contact service.

Thick or continuous lines on X or Y axis end



Two lines appear together or independently of one another. This basically indicates "unclassified", high intensity particles which cannot be represented in the "normal" analytical range where it would receive standard colour.

If this occurs please contact service.

Long, smeared population

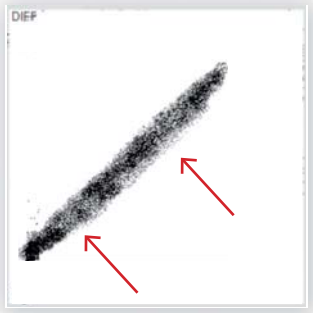
This image shows that neither the Thermal Control Unit, nor the reagents, nor the mixing have been able to do their required functions.

Possible reason:

- The two HC5L-Lyse and the HC5L-Diff reagents have been switched.

If trouble shooting of the above fails to resolve the issue, please contact service.

Thick group of cells shifted up and left

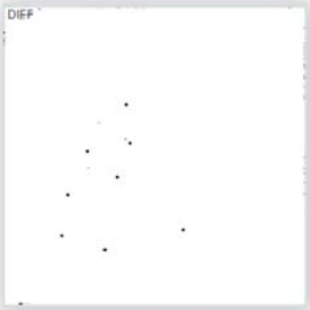


Possible reason:

- HC5L-Diluent reagent is connected to both HC5L-Lyse and HC5L-Diff inputs.

If troubleshooting of the above fails to resolve the issue, please contact service.

None or only very few cells on scattergram



What is displayed in the scattergram (and also on histograms) was actually measured by the optical system. However, the scattergram pattern suggests that either no cells or only a very few were seen, indicating that either no or only a few cells passed through the optical head.

Possible reasons:

- There was a sampling error – either insufficient amount of blood in the tube, or there was something obstructing the sample travel into the shear valve. Make sure you have enough sample and rerun sample.
- The loop of tube (below the shear valve) might be pinched or blocked. If blocked, no sample can be taken into the flow cell. Please contact service.
- A tube has become detached from the dilutor, or optical head, or sample path. Try flow cell cleaning procedure in maintenance menu.

If troubleshooting of the above fails to resolve the issue, please contact service.

Concentrated or collapsed scattergram

This image appears as a concentrated or collapsed group of cells. The cells appear to be unmodified, or unaffected by the chemical reactions that normally help in separating them.

Possible reason:

- Cells overlyzed due ambient temperature being too high.
  Try flow cell cleaning.

If troubleshooting of the above fails to resolve the issue, please contact service.

Smeared scattergram with concentrated center

There was a mechanical error or jam in the system during measurement or sampling and the SW previously stopped. During the re-start, the pneumatic functions injected whole blood or poorly diluted blood into the flow cell or onto the injector area.

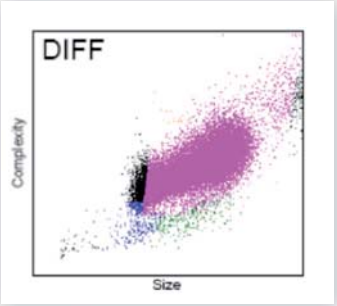
Start a Flow cell cleaning procedure (“Maintenance / Clean…”)

Fluctuating PLT background values

When the HumaCount 5L has a high daily workload and is running up to 300 samples a day (meaning 5 hours of continuous operation) it is possible to find this phenomenon.

Despite an in-built maintenance program, the system may require additional intensive cleaning periodically. If the Blank PLT values are increasing and decreasing unpredictably, please run a “Hard Cleaning” function.

Mirror artefact (or WBC ghosting)



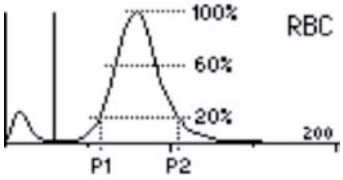
A doubled (or smeared along the X axis) WBC population can be seen on the 4 Diff scattergram. This phenomenon may be caused by disturbance of the stream (due to bubbles, dust, etc.).

- Rerun sample
- Run flow cell cleaning in maintenance menu and then rerun sample
- In case of no change, please, contact service

Specifications

Measured parameters

"cal" refers to calibration factors

White Blood Cells – WBC (cells/l, cells/µl)	Number of leukocytes WBC = WBCcal x (cells/l, cells/µl)
Red Blood Cells – RBC (cells/l, cells/µl)	Number of erythrocytes RBC = RBCcal x (cells/l, cells/µl)
Hemoglobin concentration – HGB (g/dl, g/l, mmol/l)	Measured photometrically at 540 nm; in each cycle blank measurement is performed on diluent HGB = HGBcal x (HGBmeasured – HGBblank)
Mean Corpuscular Volume – MCV (fl)	Average volume of individual erythrocytes derived from the RBC histogram.
Hematocrit – HCT (percentage, absolute)	Calculated from the RBC and MCV values. HCTpercentage = RBC x MCV x 100 HCTabsolute = RBC x MCV
Mean Corpuscular Hemoglobin – MCH (pg, fmol)	Average hemoglobin content of erythrocytes, calculated from RBC and HGB values. MCH = HGB / RBC
Mean Corpuscular Hemoglobin Concentration – MCHC (g/dl, g/l, mmol/l)	Calculated from the HGB and HCT values. MCHC = HGB / HCT absolute Unit of measurement is displayed according to the one chosen for HGB result (g/dl, g/l or moll/l)
Red Cell Distribution Width – RDW-SD (fl) Platelet Distribution Width – PDW-SD (fl) Red cell Distribution Width – RDW-CV (%) Platelet Distribution Width – PDW-CV (%)	The distribution width of the erythrocyte or platelet population derived from the histogram at 20% of peak  xDW-SD = RDWcal x (P2 - P1) (fl), xDW-CV = RDWcal x 0.56 x (P2 - P1) / (P2 + P1) by the factor of 0.56 CV is corrected to the 60% cut
Platelet – PLT (cells/l, cells/µl)	Number of thrombocytes (platelets) PLT = PLTcal x (cells/l, cells/µl)
Mean Platelet Volume – MPV (fl)	Average volume of individual platelets derived from the PLT histogram
Thrombocrit – PCT (percentage, absolute)	Calculated from the PLT and MPV values PCTpercentage = PLT x MPV x 100 PCTabsolute = PLT x MPV
White blood cell, 4 part differential: LYM (%) – lymphocytes MON (%) – monocytes and some eosinophils NEU (%) – neutrophil granulocytes EOS (%) – eosinophil granulocytes	Relative value are calculated from the optical 4 diff scatter populations
BAS (%) – basophil granulocytes	Relative value is calculated from the basophil optical scatter populations

Technical data

Main unit	Ref. 16430
Sample volume	Closed and Open-mode: 110 µl ; Small Sample Module 25µl
Sample type	Human whole blood with K3-EDTA anticoagulant
Tube Identification	By means of the front panel keyboard (enter ID) By means of the barcode labels (manual and/or auto-sampler)
Sampling method	Ceramic shear valve with 3 separated primary loops
Measured parameters	CBC in 5-part DIFF mode (24 parameters): WBC, LYM, MON, NEU, EOS, BAS, LYM%, MON%, NEU%, EOS%, BAS%, RBC, HCT, MCV, RDWsd, RDWcv HGB, MCH, MCHC, PLT, PCT, MPV, PDWsd, PDWcv
Throughput	60 tests/hour
Measurement method	Volumetric impedance change for WBC, RBC, PLT Photometry for HGB determination Light scattering 4-diff measurement: LYM, MON, NEU, EOS Light scattering basophil (BAS) measurement
Aperture diameter	WBC: 80 µm, RBC/PLT: 70 µm
HGB measurement	Light source: green LED with 540 nm wavelength Detector: light to frequency converter
Optical measurement	Light source: semiconductor laser diode with 650 nm wavelength and 7mW (Class IIIB laser device) Quartz flow cell with hydro-dynamic focusing Detector: fiber optic coupled PIN Si photodiodes Internal safety interlock
Auto-alignment system	Horizontal calibration of laser beam position. Fine calibration: with calibration material (Polystyrene micro-particle or Polystyrene microsphere, 5µm)
Reagents	HC5L-Diluent (20 liter) [REF] 16430/20 HC5L-Lyse CF (5 liter) [REF] 16430/30 HC5L-Lyse Diff (1 liter) [REF] 16430/40
Dilution ratios	WBC/BAS 1: 170 RBC/PLT 1: 21250 4 DIFF 1: 50
Sheath fluid	Diluent
Control material	CD Diff Control [REF] 3838
Quality Control	Levy-Jennings charts, QC database

Flagging	Pathological (diagnostic) flags Lab limits (normal ranges) Reagents alert (3 measurement pre-alert, online reagent replacement) Instrument alerts, internal container for reagents
Calibration	Manual and SW supported automatic mode
Languages available	English menu and support for other languages
Software upgrade	Via USB
Data storage capacity	100.000 records including flags, scatter- and histograms
Data processing	Intel ATOM N270 CPU
Data store	Windows® XP® Embedded
Display	800 x 600 colour graphic LCD, portrait layout
External printing	Via USB port, any Windows® XP® compatible printer
External keyboard	Via PS/2 or USB
Bar-Code reader	Optional Manual bar-code reader via USB, optional 2D-barcode reader via USB Built in Bar-Code reader in the Autosampler
Peripheral ports	USB (2.0) 4pc, Ethernet, PS/2
Power requirements	Power supply input: 100-240 VAC, 50/60 Hz, 400 VA
Operating temperature	15-30° C (59-86 °F);
Maximum relative humidity	80%

Performance data

Accuracy values and ranges

Accuracy	Experienced coefficient Against Reference (Cell-Dyn 3700)	Range
WBC	r = 1.00	0.5*10^3 / µl ≤ WBC ≤ 56.3*10^3/µl
RBC	r = 0.99	2.2*10^6 / µl ≤ RBC ≤ 6.3*10^6/µl
HGB	r = 0.99	68.8 g/l ≤ HGB ≤ 173 g/l
HCT	r = 0.99	19.5% ≤ HCT% ≤ 52.6%
PLT	r = 0.98	12.5 *10^3 / µl ≤ PLT ≤ 699*10^3/µl
NEU%	r = 0.99	5.8% ≤ NEU% ≤ 94.6%
LYM%	r = 0.99	2.5% ≤ LYM% ≤ 79.5%
MON%	r = 0.90	1.1% ≤ MON% ≤ 28.1%
EOS%	r = 0.95	0.0% ≤ EOS% ≤ 23.6%
BAS%	r = 0.55	0.1% ≤ BAS% ≤ 4.2%

N.B. the 5 part WBC is not reportable if the total WBC count is below 1*10^3/µl as the quantitation error becomes too great.

Linearity values and ranges

Linearity	Coefficient of determination	Range
WBC	r^2 ≥ 0.95	1*10^3 / µl ≤ WBC ≤ 100*10^3/µl
RBC	r^2 ≥ 0.95	0.4*10^6 / µl ≤ RBC ≤ 7.5*10^6/µl
HGB	r^2 ≥ 0.95	13 g/l ≤ HGB ≤ 227 g/l
PLT	r^2 ≥ 0.95	10*10^3 / µl ≤ PLT ≤ 873*10^3/µl

Carryover values and ranges

Carryover	High-to-low effect	Range
WBC	0.5% or less	LO ≤ 10*10^3/µl HI ≥ 75*10^3/µl
RBC	0.5% or less	LO ≤ 2.3*10^6/µl HI ≥ 7.5*10^6/µl
HGB	0.5% or less	LO ≤ 90 g/l HI ≥270 g/l
PLT	0.5% or less	LO ≤ 140*10^3 / µl HI ≥ 730*10^3 / µl

Reagent system

Diluent

HCL5L-Diluent:	Isotonic solution, used to dilute whole blood and quantitative and qualitative determination of RBC, WBC, PLT and HGB concentration

Lyse

HC5L-Lyse CF:	Reagent for stromatolysis of RBC and quantitative determination of WBC 5-part differentiation (LYM, MON, NEU, EOS, BAS) and HGB concentration measurement of human blood.

Diff

HC5L-Diff:	Quantitative determination of WBC, leukocyte 4-part differentiation (LYM, MON, NEU, EOS).

CHAPTER 1

Red blood cell abnormalities

Case 1 | Microcytic anemia, chronic gastrointestinal disease

History and clinical signs and symptoms

A 35 year old woman with known Crohn's disease presented at the outpatient clinic of the hospital with bloating and vomiting.

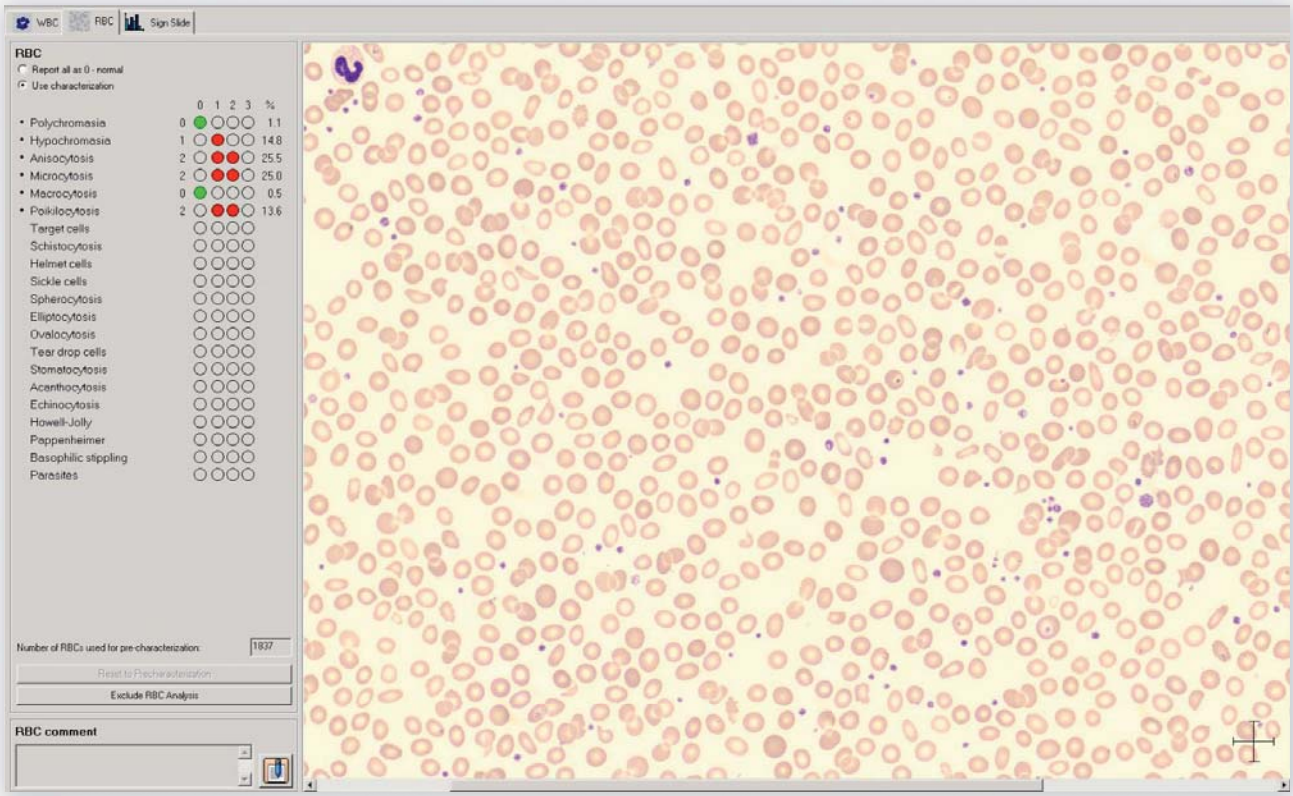
Hematologic findings

Sample ID			Patient 1				Patient ID	
Date			01/01/2013				Name	
Mode			Human				Date of birth	
Parameter			Result		Limit		Gender	
WBC	6.21		$10^3/\mu L$	5.00	-	10.00	DIFF	BAS
NEU	3.65		$10^3/\mu L$	2.00	-	7.50		
LYM	1.90		$10^3/\mu L$	1.08	-	3.17		
MON	0.51		$10^3/\mu L$	0.15	-	0.70		
EO	0.07		$10^3/\mu L$	0.00	-	0.50		
BAS	0.07		$10^3/\mu L$	0.00	-	0.15		
NEU%	58.8		%	40.0	-	75.0		
LYM%	30.6		%	14.76	-	45.40		
MON%	8.2	+	%	3.0	-	7.0		
EO%	1.2		%	0.0	-	5.0		
BAS%	1.2		%	0.0	-	1.5		
RBC	5.11		$10^6/\mu L$	4.00	-	5.50	RBC	
HGB	102	-	g/L	120	-	174		
HCT	37.9		%	36.0	-	52.0		
MCV	74.0	-	fL	76.0	-	96.0		
MCH	19.9	-	pg	27.0	-	32.0		
MCHC	269	-	g/L	300	-	350		
RDWsd	43.3	+	fL	20.0	-	42.0		
RDWcv	20.6	+	%	0.0	-	16.0		
PLT	363		$10^3/\mu L$	150	-	400	PLT	
PCT	0.45		%		-			
MPV	12.3		fL	8.0	-	15.0		
PDWsd	34.7		fL		-			
PDWcv	37.2		%		-			
Warning flags								
Morphological flags								
Interpretive flags			Microcytic RBC?, Hypochromic?, Anisocytosis?					

Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams appear normal. The neutrophil, monocyte, eosinophil and lymphocyte populations as well as the basophil population are well differentiated without any warning flags. The PLT histogram is normally shaped while the RBC histogram shows a left shift due to the presence of microcytic red blood cells and a smaller normal population (anisocytosis). The RBC and PLT populations are clearly differentiated. The WBC and RBC counts are within the normal range. However, the HGB, MCV, MCH and MCHC are lower than normal indicating smaller than normal red cells with decreased hemoglobin content (microcytosis, hypochromasia) as suggested by the interpretive flags. The RDWcv is significantly higher than normal and, therefore, indicates a large heterogeneity in the red cell population (anisocytosis).

Peripheral blood smear



The variations in red cell size and hemoglobin content are clearly visible.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	4	3.6
• Segmented neutrophil	61	55.0
• Eosinophil	1	0.9
• Basophil	1	0.9
• Lymphocyte	34	30.6
• Monocyte	10	9.0
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	111	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	25	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	7	-
• Artefact	2	-

Other laboratory findings

Other clinical laboratory results include slightly elevated CRP levels (12 mg/L [ref.range: 0-5 mg/L), normal iron levels (22.5 [6.6 – 26.0 µmol/L) with abnormal ferritin and serum transferrin receptor (STfr) values (9 [10 – 120] µg/L, and 5 [<3.5] mg/L respectively). Fecal occult blood was detected.
Electrolyte levels were within the normal range.

Radiological exams suggest multiple obstructions at the ileocecal region of the small intestine. A colonoscopy was not performed as the patient developed ileus (a contraindication of endoscopy).

Diagnosis

Microcytic anemia associated with occult intestinal bleeding, chronic gastrointestinal disease and ileus.

Disease course

The patient was transferred to the department of surgery. Following the surgical procedure a biological therapy was initiated. The patient's overall condition significantly improved along with the normalization of the RBC parameters.

Case 2 | Microcytic anemia due to chronic bleeding

History and clinical signs and symptoms

74 year old man contacted the physician due to symptoms of general weakness

Hematologic findings

Sample ID	Patient 2						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	3.58	-	$10^3/\mu L$	5.00	-	10.00	DIFF	BAS
NEU	2.07		$10^3/\mu L$	2.00	-	7.50		
LYM	0.90	-	$10^3/\mu L$	1.08	-	3.17		
MON	0.45		$10^3/\mu L$	0.15	-	0.70		
EO	0.14		$10^3/\mu L$	0.00	-	0.50		
BAS	0.01		$10^3/\mu L$	0.00	-	0.15		
NEU%	57.9		%	40.0	-	75.0		
LYM%	25.0		%	14.76	-	45.40		
MON%	12.7	+	%	3.0	-	7.0		
EO%	4.0		%	0.0	-	5.0		
BAS%	0.4		%	0.0	-	1.5		
RBC	3.60	-	$10^6/\mu L$	4.00	-	5.50	RBC	
HGB	78	-	g/L	120	-	174		
HCT	26.5	-	%	36.0	-	52.0		
MCV	73.5	-	fL	76.0	-	96.0		
MCH	21.8	-	pg	27.0	-	32.0		
MCHC	296	-	g/L	300	-	350		
RDWsd	43.6	+	fL	20.0	-	42.0		
RDWcv	20.3	+	%	0.0	-	16.0		
PLT	160		$10^3/\mu L$	150	-	400	PLT	
PCT	0.13		%		-			
MPV	8.2		fL	8.0	-	15.0		
PDWsd	25.6		fL		-			
PDWcv	38.0		%		-			
Warning flags								
Morphological flags								
Interpretive flags	Anemia?, Microcytic RBC?, Hypochromic?, Anisocytosis?							

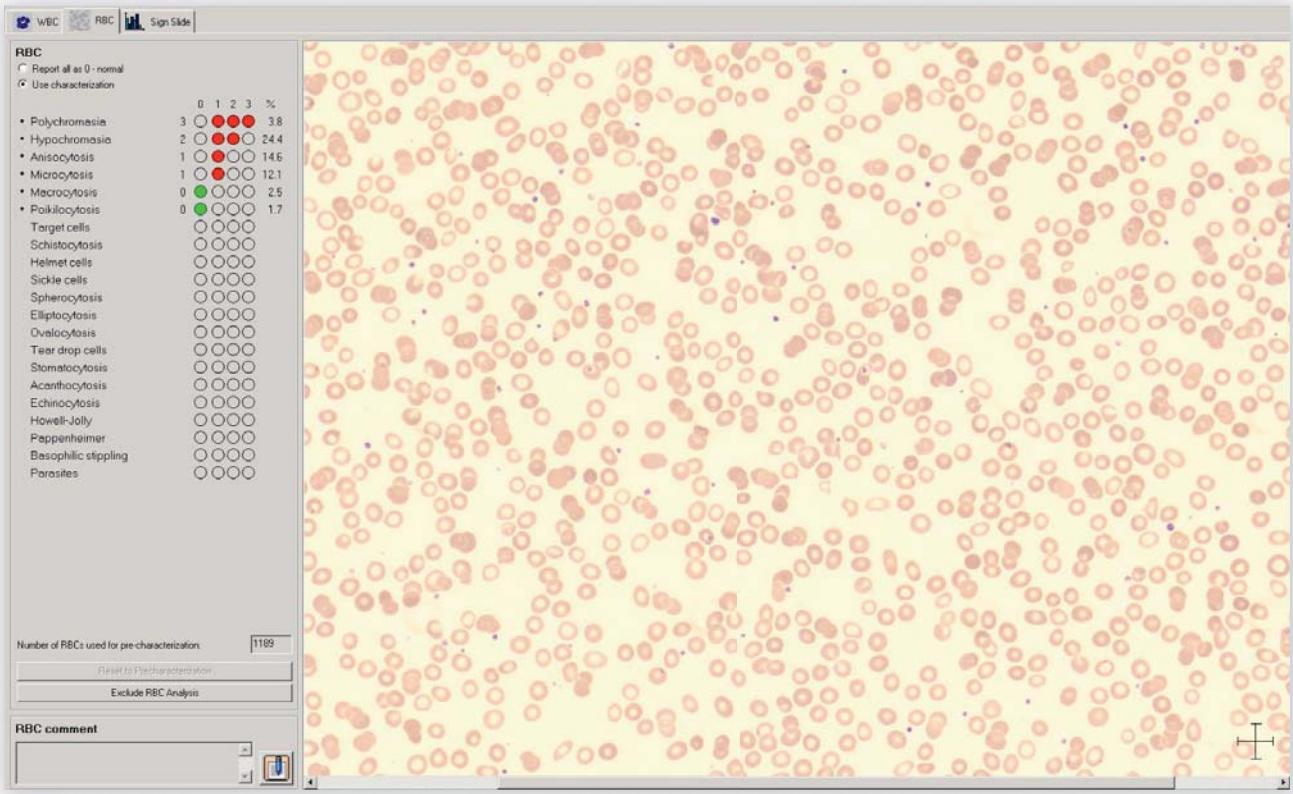
Interpretation

In the WBC DIFF and BAS scattergrams the neutrophil, monocyte, lymphocyte and eosinophil populations as well as the basophil population are well characterized without any warning flags. The WBC count indicates a mild leukopenia and relative monocytosis.

The RBC count is below the normal range. The low HGB indicates a severe anemia. Other red cell parameters (including HCT, MCV, MCH) are lower than normal indicating a smaller than normal red cell population with decreased hemoglobin content (microcytosis, hypochromasia). The RDWcv is significantly higher than normal supporting a large heterogeneity within the red cell population. (anisocytosis).

The RBC and PLT histograms are normally shaped and the RBC and PLT populations are clearly differentiated.

Peripheral blood smear



The microcytic hypochromic red cells are clearly visible on the peripheral blood smear.

WBC	Count	%
• Unidentified	1	0.9
• Band neutrophil	4	3.8
• Segmented neutrophil	66	61.1
• Eosinophil	5	4.6
• Basophil	1	0.9
• Lymphocyte	23	21.3
• Monocyte	11	10.2
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	1	0.9
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	4	-
• Thrombocyte aggregatio	2	-
Megakaryocyte	-	-
• Smudge cell	17	-
• Artefact	17	-

Other laboratory findings

Other clinical laboratory results include low iron levels (2.5 [6.6 – 26.0 of cancer).

Diagnosis

A microcytic anemia caused by an iron deficiency as a result of chronic bleeding from gastric cancer.

Disease course

The patient was transferred to the department of surgery. The surgical procedure was palliative.
Five units of packed red blood cells were transfused over the next few days and CBC slightly improved.
The patient expired two months later.

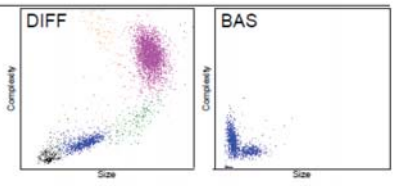
Case 3 | Megaloblastic anemia, vitamin B12 deficiency

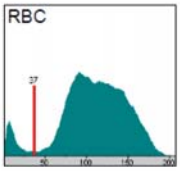
History and clinical signs and symptoms

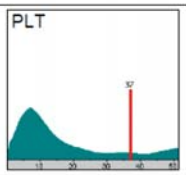
A 40 year old man contacted the physician due to symptoms of general weakness and unexplained weight loss (10 kg during the last four months).

Hematologic findings

Sample ID	Patient 3						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
							Gender	
Parameter	Result			Limit				
WBC	5.91		$10^3/\mu L$	5.00	-	10.00		
NEU	4.34		$10^3/\mu L$	2.00	-	7.50		
LYM	1.22		$10^3/\mu L$	1.08	-	3.17		
MON	0.17		$10^3/\mu L$	0.15	-	0.70		
EO	0.17		$10^3/\mu L$	0.00	-	0.50		
BAS	0.01		$10^3/\mu L$	0.00	-	0.15		
NEU%	73.5		%	40.0	-	75.0		
LYM%	20.7		%	14.76	-	45.40		
MON%	2.8	-	%	3.0	-	7.0		
EO%	2.8		%	0.0	-	5.0		
BAS%	0.2		%	0.0	-	1.5		
RBC	2.61	-	$10^6/\mu L$	4.00	-	5.50		
HGB	90	-	g/L	120	-	174		
HCT	30.0	-	%	36.0	-	52.0		
MCV	114.9	+	fL	76.0	-	96.0		
MCH	34.4	+	pg	27.0	-	32.0		
MCHC	299	-	g/L	300	-	350		
RDWsd	104.0	++	fL	20.0	-	42.0		
RDWcv	35.0	++	%	0.0	-	16.0		
PLT	132	-	$10^3/\mu L$	150	-	400		
PCT	0.18		%		-			
MPV	13.7		fL	8.0	-	15.0		
PDWsd	32.2		fL		-			
PDWcv	38.0		%		-			







Warning flags	
Morphological flags	
Interpretive flags	Anemia?, Macrocytic RBC?, Hypochromic?, Anisocytosis?

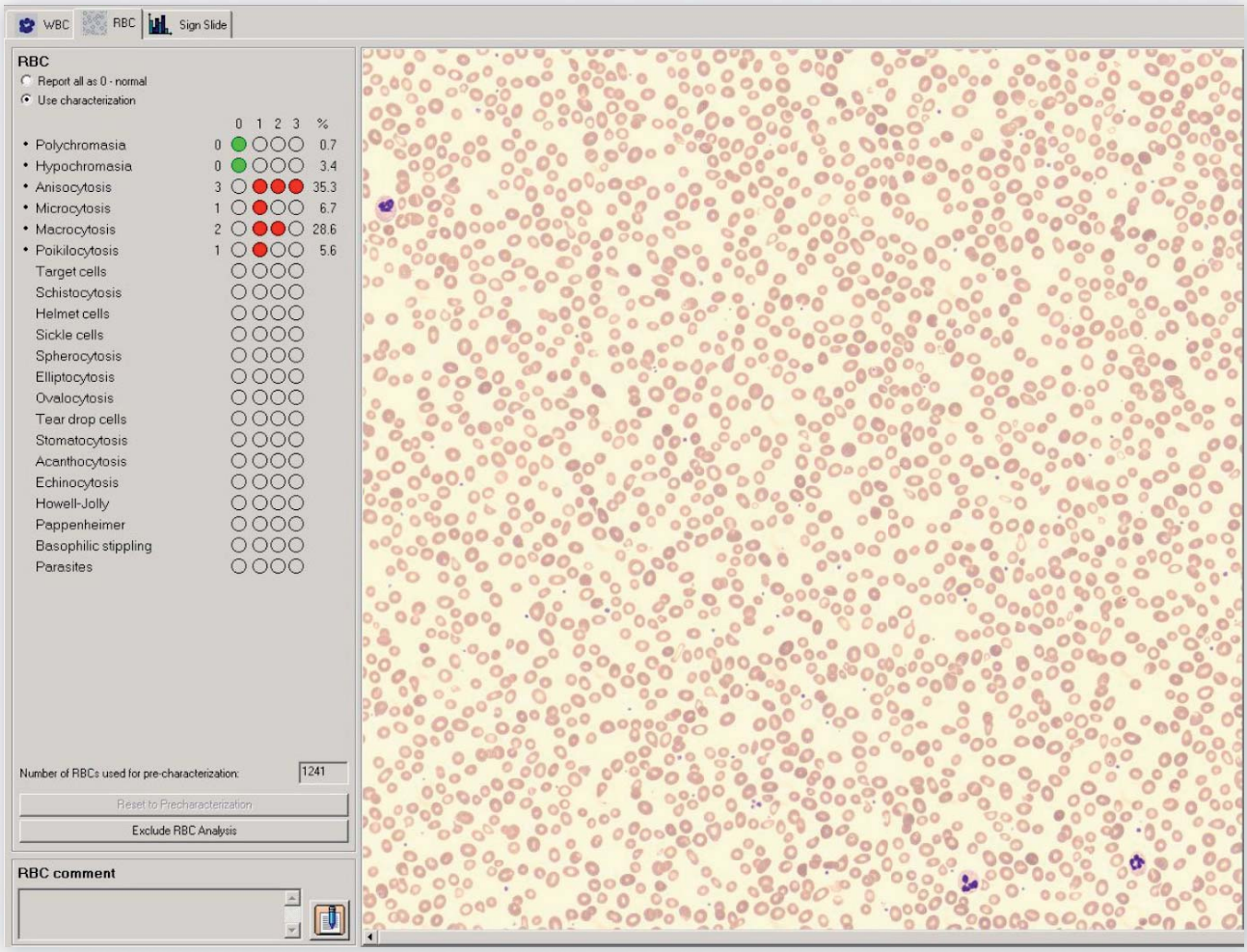
Interpretation

The WBC DIFF and BAS scattergrams appear normal. The WBC populations including neutrophil, monocyte, lymphocyte and eosinophil cells are clearly characterized without any warning flags. The WBC count is within the normal range.

The RBC histogram has an abnormal shape due to the presence of multiple RBC populations with different size characteristics. The red cell size distribution is widened (as it is also indicated by high RDWcv and the interpretative flag 'anisocytosis'). The RBC count is well below the normal range. The low HGB and HCT levels are in line with anemia. Of note, the red cell parameters MCV, MCH and MCHC indicate larger than normal red cells (see the interpretative flags 'macrocytic RBC' and 'hypochromic').

The PLT population is clearly differentiated from the RBCs. The morphological characteristics of the PLTs are normal.

Peripheral blood smear



The peripheral blood smear clearly indicates the presence of red cells of various sizes.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	74	69.2
• Eosinophil	1	0.9
• Basophil	-	-
• Lymphocyte	28	26.2
• Monocyte	3	2.8
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	1	0.9
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	107	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	6	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	3	-
• Artefact	5	-

Other laboratory findings

The levels of vitamin B12 are extremely low (30pg/mL [ref range: 197 – 866 pg/mL). Serological testing detected serum antibodies against parietal cells.

Folic acid levels and iron status parameters are normal. A colonoscopy excluded the presence of inflammatory bowel disease.

Diagnosis

A megaloblastic anemia due to vitamin B12 deficiency (pernicious anaemia with autoimmune background)

Disease course

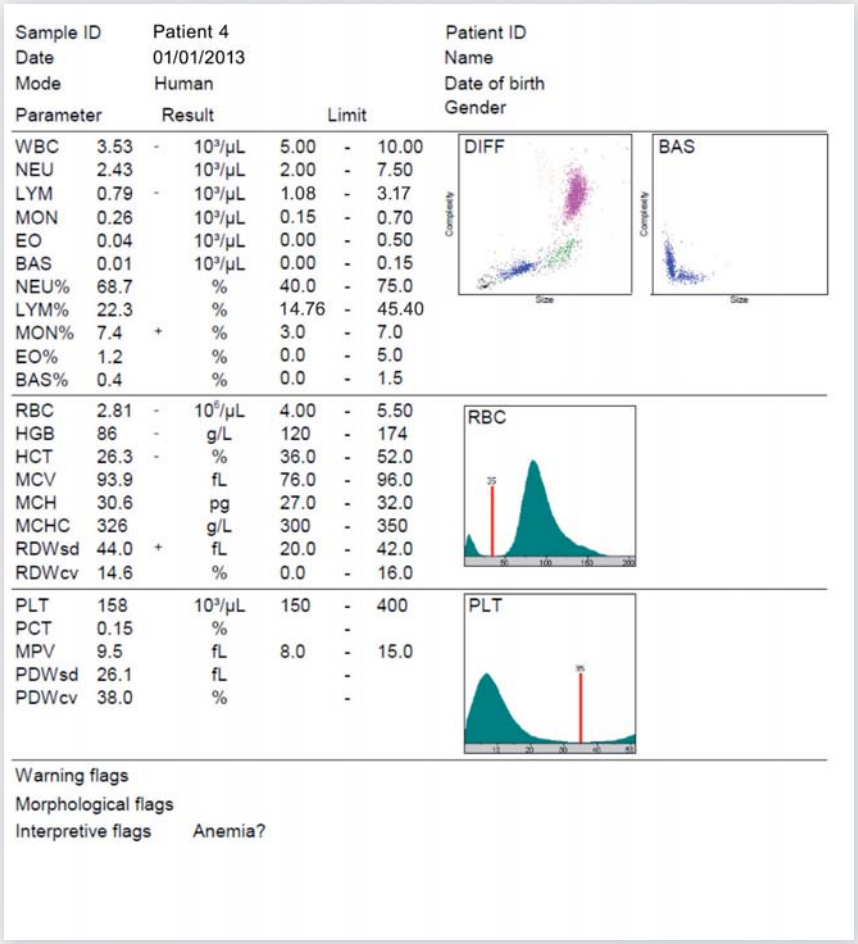
The red cell parameters responded to large doses of vitamin B12 administered intramuscularly.

Case 4 | Normocytic anemia due to chronic kidney disease

History and clinical signs and symptoms

A 68 year old woman on routine dialysis was tested as a regular assessment of her status.

Hematologic findings



Sample ID	Patient 4					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	3.53	-	$10^3/\mu L$	5.00	- 10.00		
NEU	2.43		$10^3/\mu L$	2.00	- 7.50		
LYM	0.79	-	$10^3/\mu L$	1.08	- 3.17		
MON	0.26		$10^3/\mu L$	0.15	- 0.70		
EO	0.04		$10^3/\mu L$	0.00	- 0.50		
BAS	0.01		$10^3/\mu L$	0.00	- 0.15		
NEU%	68.7		%	40.0	- 75.0		
LYM%	22.3		%	14.76	- 45.40		
MON%	7.4	+	%	3.0	- 7.0		
EO%	1.2		%	0.0	- 5.0		
BAS%	0.4		%	0.0	- 1.5		
RBC	2.81	-	$10^6/\mu L$	4.00	- 5.50		
HGB	86	-	g/L	120	- 174		
HCT	26.3	-	%	36.0	- 52.0		
MCV	93.9		fL	76.0	- 96.0		
MCH	30.6		pg	27.0	- 32.0		
MCHC	326		g/L	300	- 350		
RDWsd	44.0	+	fL	20.0	- 42.0		
RDWcv	14.6		%	0.0	- 16.0		
PLT	158		$10^3/\mu L$	150	- 400		
PCT	0.15		%		-		
MPV	9.5		fL	8.0	- 15.0		
PDWsd	26.1		fL		-		
PDWcv	38.0		%		-		
Warning flags							
Morphological flags							
Interpretive flags	Anemia?						

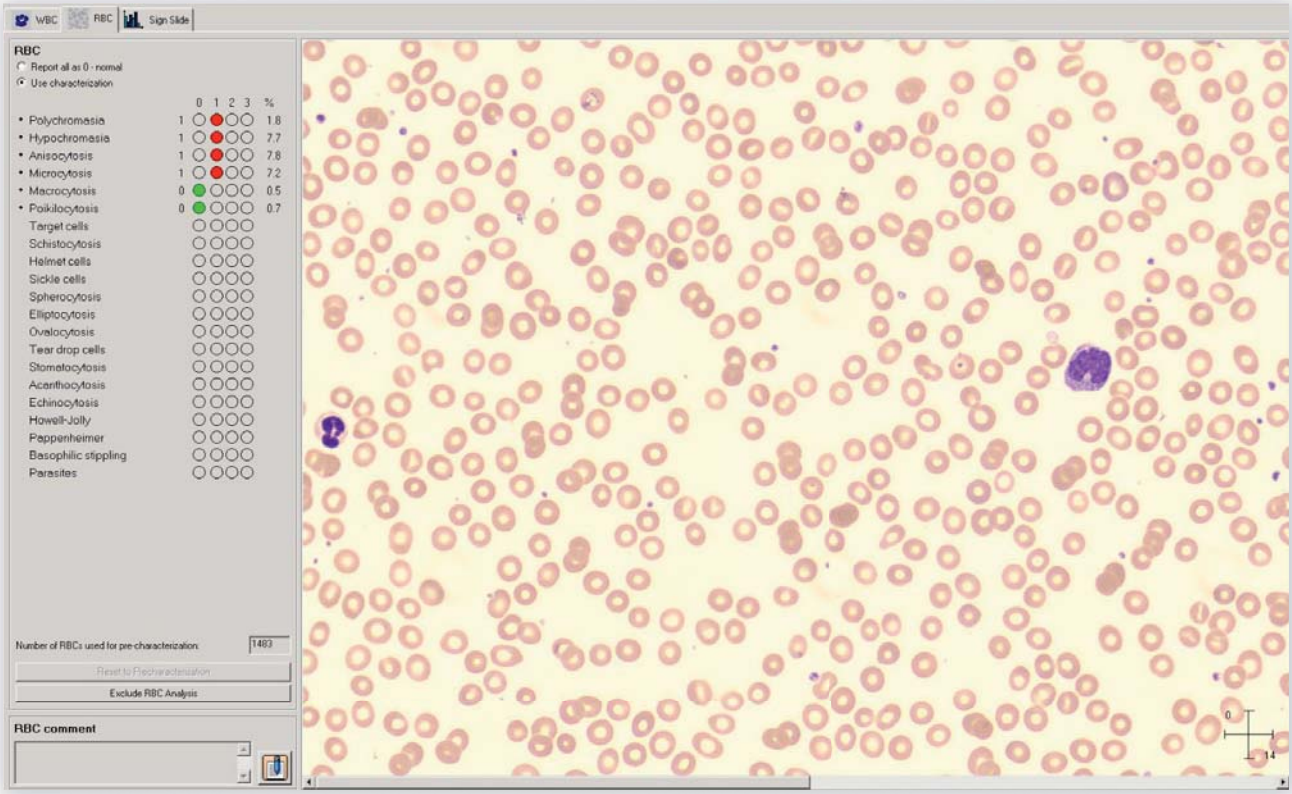
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined and the WBC subpopulations are well distinguished. The WBC count indicates a mild leukopenia and lymphopenia. Neutrophil, monocyte, lymphocyte and eosinophil populations as well as the basophil population are well characterized without any warning flags.

The RBC and PLT histograms are well defined and clearly separated. The RBC count is well below the normal range. The HGB and HCT are also lower than normal, while other red cell parameters are within the reference range indicating hypo-regenerative normocytic anemia.

The PLT count is normal with the PLT characteristics being un-remarkable.

Peripheral blood smear



The red cell characteristics appear normal.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	76	71.0
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	26	24.3
• Monocyte	5	4.7
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	107	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	-	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	7	-
• Artefact	8	-

Other laboratory findings

The reticulocyte count was 2.7%. No agglutination was observed. Other abnormal clinical laboratory results include an elevated blood urea nitrogen and creatinine levels and low GFR value (20.4 [2.5 – 8]mmol/L, 450 [44 – 80] µmol/L and 7.3 mL/min/1.73m2, respectively).

Diagnosis

Hypo-regenerative, normocytic anemia due to chronic kidney disease

Disease course

The initiated EPO therapy induced the formation of new red cells and the RBC count improved.

Case 5 | Macrocytic (megaloblastic) anemia, folic acid deficiency

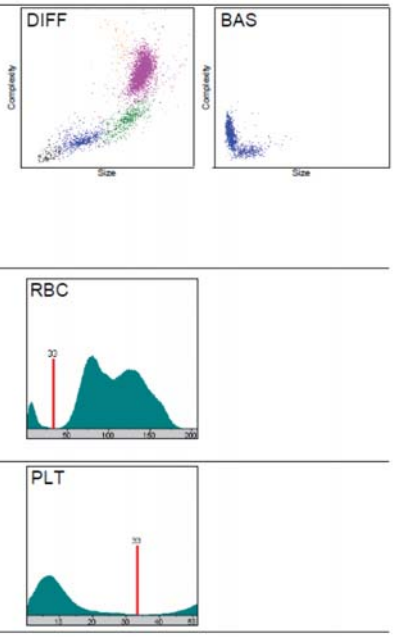
History and clinical signs and symptoms

A 38-year old adult unconscious man was admitted to the hospital with characteristic signs of encephalopathy. The clinical history indicated chronic drug and alcohol abuse. The patient has hepatosplenomegaly.

Hematologic findings

Sample ID Patient 5 Patient ID
Date 01/01/2013 Name
Mode Human Date of birth
Gender

Parameter	Result		Limit
WBC	5.22	$10^3/\mu L$	5.00 - 10.00
NEU	4.01	$10^3/\mu L$	2.00 - 7.50
LYM	0.61 -	$10^3/\mu L$	1.08 - 3.17
MON	0.48	$10^3/\mu L$	0.15 - 0.70
EO	0.09	$10^3/\mu L$	0.00 - 0.50
BAS	0.03	$10^3/\mu L$	0.00 - 0.15
NEU%	76.9 +	%	40.0 - 75.0
LYM%	11.7 -	%	14.76 - 45.40
MON%	9.1 +	%	3.0 - 7.0
EO%	1.7	%	0.0 - 5.0
BAS%	0.6	%	0.0 - 1.5
RBC	1.98 --	$10^6/\mu L$	4.00 - 5.50
HGB	68 -	g/L	120 - 174
HCT	21.5 -	%	36.0 - 52.0
MCV	108.8 +	fL	76.0 - 96.0
MCH	34.3 +	pg	27.0 - 32.0
MCHC	315	g/L	300 - 350
RDWsd	100.2 ++	fL	20.0 - 42.0
RDWcv	36.8 ++	%	0.0 - 16.0
PLT	73 --	$10^3/\mu L$	150 - 400
PCT	0.07	%	-
MPV	9.3	fL	8.0 - 15.0
PDWsd	29.1	fL	-
PDWcv	38.0	%	-



Warning flags
Morphological flags
Interpretive flags Anemia?, Macrocytic RBC?, Anisocytosis?, Thrombocytopenia?

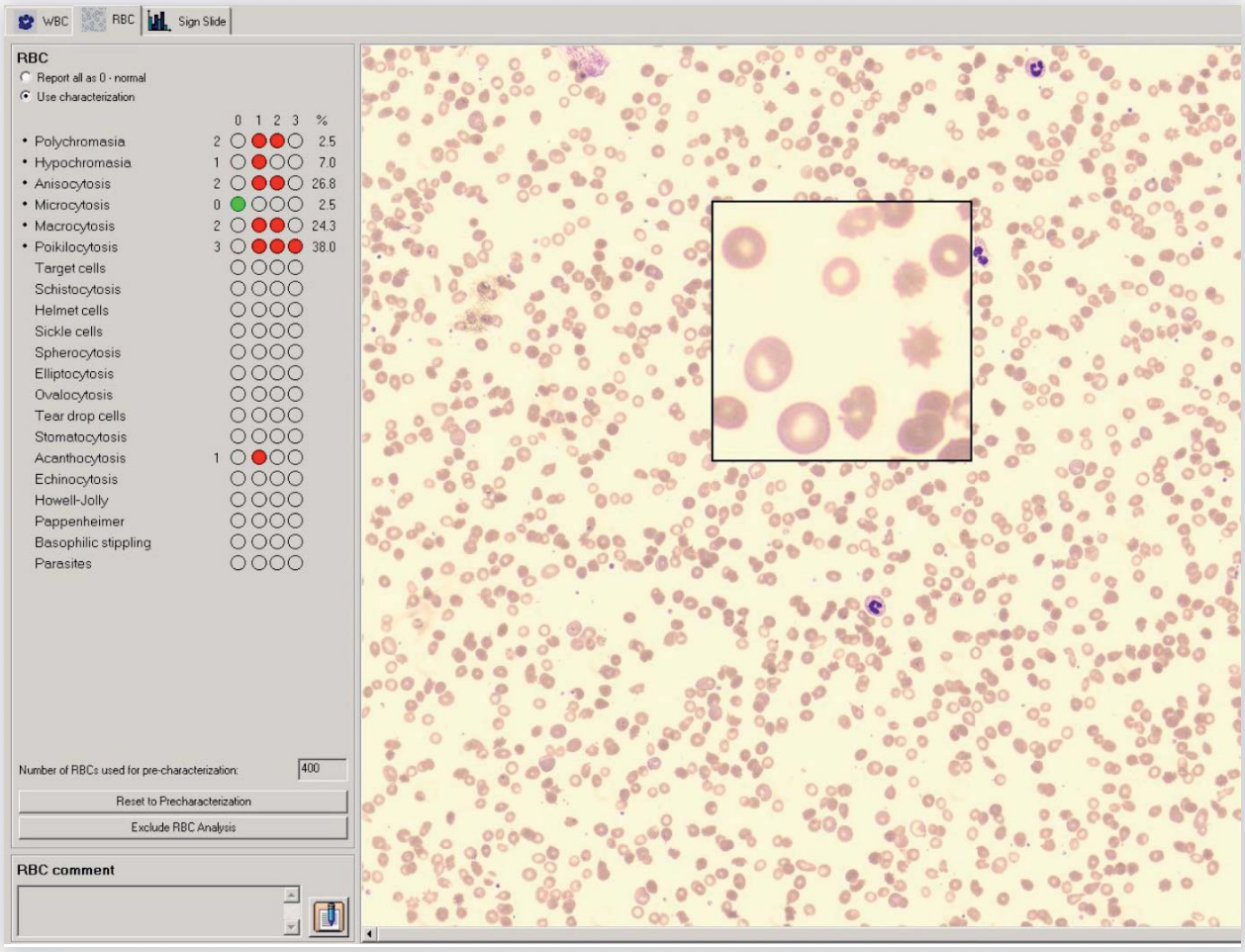
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The leukocyte subpopulations are well separated. The WBC count is normal. The lymphocyte count and percentage is decreased.

The RBC and PLT histograms are well separated. The population distribution of RBCs (i.e. RDWcv) is increased. There are two peaks within the RBC population indicating a large heterogeneity in the RBC size (see interpretative flag “anisocytosis”). The RBC count and HGB are markedly below the normal range (see interpretative flag “anemia”). The MCV is higher than the normal range (see interpretative flag “macrocytic RBC”).

The PLT population is normal in shape and distribution but PLT count is grossly decreased (see interpretative flag “thrombocytopenia”).

Peripheral blood smear



The RBCs present with abnormal characteristics such as macrocytosis, poikilocytosis, anisocytosis, polychromasia and hypochromasia. Some nucleated red cells (erythroblasts) are also present.The majority of the WBCs are mature neutrophil granulocytes.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	4	3.8 ✓
• Segmented neutrophil	75	70.8 ✓
• Eosinophil	2	1.9 ✓
• Basophil	1	0.9 ✓
• Lymphocyte	13	12.3 ✓
• Monocyte	10	9.4 ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	1	0.9 ✓
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	106	100

Non-WBC	Count	%
• Erythroblast (NRBC)	2	- ✓
• Giant thrombocyte	-	-
• Thrombocyte aggregatio	-	-
Megakaryocyte	-	-
• Smudge cell	10	- ✓
• Artefact	12	- ✓

Other laboratory findings

Abnormal findings included severe impairment of liver function (i.e. high levels of transaminases, LDH and ALP, significantly increased bilirubin levels and INR values and hyper-ammonemia (79 µmol/L [ref. range 14 – 55 µmol/L]). The folic acid levels were below the level of detection.

Diagnosis

Decompensated hepatic cirrhosis. The megaloblastic anemia was due to a dietary deficiency of folic acid.

Disease course

Intensive therapy was initiated. The patient was transfused with 8 units of packed RBCs to correct the anemia. The patient was also supplemented with vitamins. The RBC counts and HGB levels then normalized.

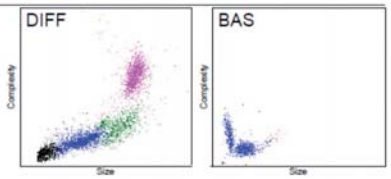
Case 6 | Hemolytic anemia, paroxysmal nocturnal hemoglobinuria

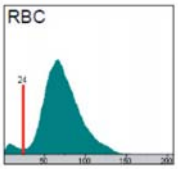
History and clinical signs and symptoms

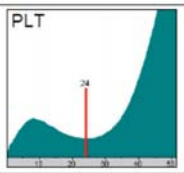
The diagnosis of paroxysmal nocturnal hemoglobinuria (PNH) was established 40 years ago. Since then a splenectomy was performed. The 51 year old man is periodically assessed. Blood was taken as a part of his routine check-ups. Currently he complains about fatigue and mild dyspnea. Occasionally he experiences a brownish discoloration of the urine.

Hematologic findings

Sample ID	Patient 6					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	5.48		$10^3/\mu L$	5.00	- 10.00		
NEU	1.86	-	$10^3/\mu L$	2.00	- 7.50		
LYM	2.44		$10^3/\mu L$	1.08	- 3.17		
MON	1.03	+	$10^3/\mu L$	0.15	- 0.70		
EO	0.04		$10^3/\mu L$	0.00	- 0.50		
BAS	0.11		$10^3/\mu L$	0.00	- 0.15		
NEU%	33.9	-	%	40.0	- 75.0		
LYM%	44.6		%	14.76	- 45.40		
MON%	18.8	++	%	3.0	- 7.0		
EO%	0.7		%	0.0	- 5.0		
BAS%	2.0	+	%	0.0	- 1.5		
RBC	3.26	-	$10^6/\mu L$	4.00	- 5.50		
HGB	70	-	g/L	120	- 174		
HCT	23.8	-	%	36.0	- 52.0		
MCV	73.1	-	fL	76.0	- 96.0		
MCH	21.6	-	pg	27.0	- 32.0		
MCHC	295	-	g/L	300	- 350		
RDWsd	49.9	+	fL	20.0	- 42.0		
RDWcv	23.3	+	%	0.0	- 16.0		
PLT	102	-	$10^3/\mu L$	150	- 400		
PCT	0.13		%		-		
MPV	12.8		fL	8.0	- 15.0		
PDWsd	32.4		fL		-		
PDWcv	37.8		%		-		
Warning flags	I						
Morphological flags							
Interpretive flags	Anemia?, Microcytic RBC?, Hypochromic?, Anisocytosis?						







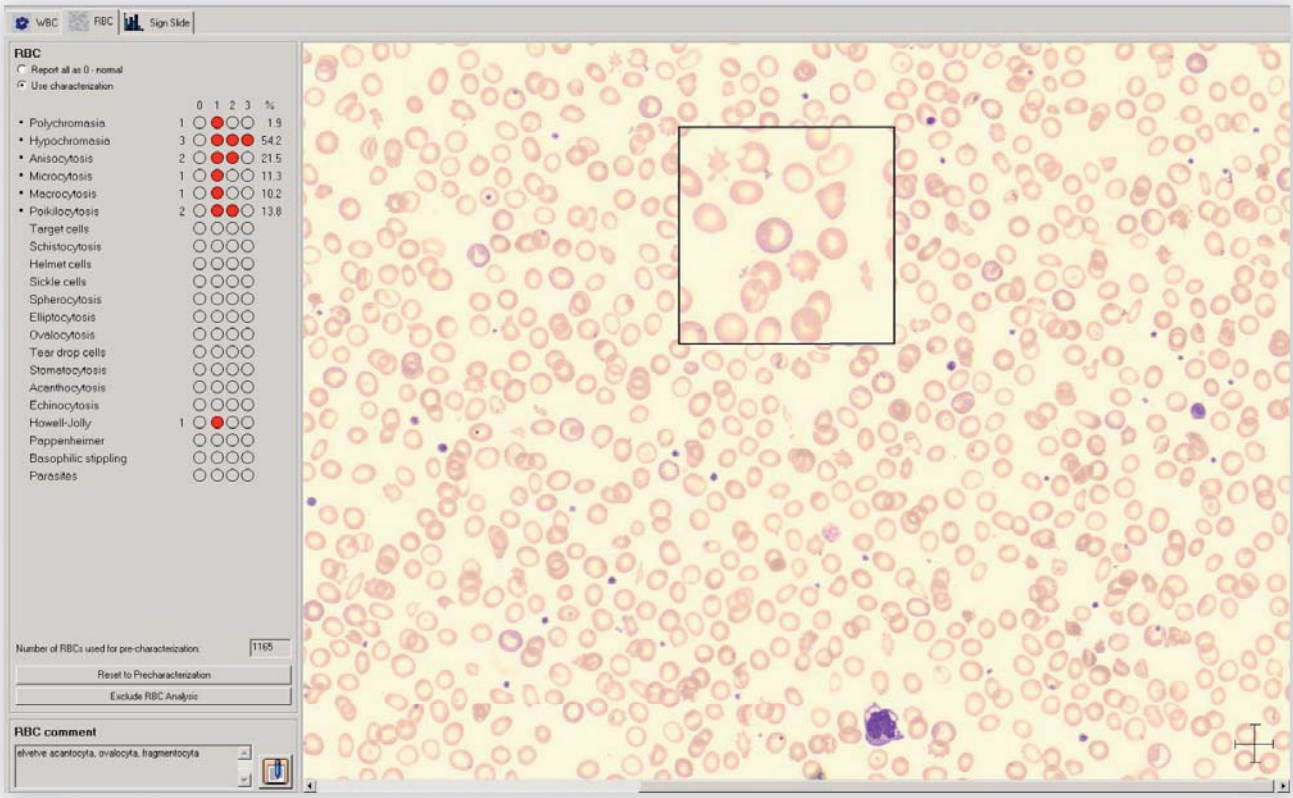
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC populations are well separated. The WBC count is within the normal range. Within the WBC differential the relative ratio of monocytes, lymphocytes and basophils are increased while the neutrophils are decreased. The absolute monocyte count is also elevated.

Evaluation of histograms reveals that the RBC and PLT populations are not clearly separated (see warning flag " I "). The RBC count and HGB levels along with MCV, MCH and MCHC parameters are lower than the normal. The distribution of red cells is widened (RDWcv elevated). The interpretive flags support the RBC morphological abnormalities of (interpretive flag "microcytic RBC", "anisocytosis" and "hypochromic"). The PLT count is also below the normal range.

An evaluation of the peripheral blood smear is recommended due to the warning flags.

Peripheral blood smear



The peripheral blood smear revealed mature leukocytes with an increase in monocyte prevalence.

The abnormalities of the red cells can be also clearly observed. The characteristics of red cells are consistent with those present in hemolytic anemia caused by cell membrane defects. Hypochromasia, anisocytosis, teardrop RBCs, acanthrocytes, ovulocytes, a few fragmentocytes, basophil stippling are all characteristic abnormalities.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	38	35.5 ✓
• Eosinophil	2	1.9
• Basophil	3	2.8
• Lymphocyte	46	43
• Monocyte	17	15.9
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	1	0.9
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	107	100

Non-WBC	Count	%
• Erythroblast (NRBC)	1	-
• Giant thrombocyte	96	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	10	-
• Artefact	6	-

Other laboratory findings

The reticulocyte count is 6% indicating a regenerative response. The serum total bilirubin level is increased about two fold (46 µmol/L [ref range: 0-21 µmol/L]), while the lactate dehydrogenase activity is increased about tenfold (4520 U/l [ref range: 230-460 U/L]) of the upper limit of normal reference range supporting the fact of hemolysis. There are no further abnormalities in other laboratory test results.

Diagnosis

Hemolytic anemia of unknown origin with thrombocytopenia.

Disease course

The initiation of specific biological therapy is considered.

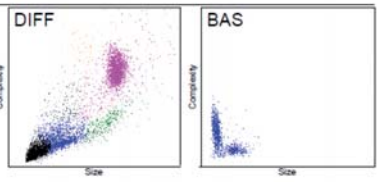
Case 7 | Hemolytic anemia caused by cold agglutinins

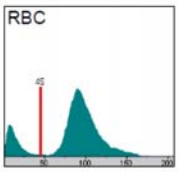
History and clinical signs and symptoms

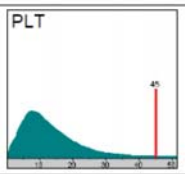
A 50 year old woman complained about acral cyanosis. When exposed to cold her fingers and toes become pale and painful.

Hematologic findings

Sample ID	Patient 7					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	5.13		$10^3/\mu L$	5.00	-	10.00	
NEU	2.83		$10^3/\mu L$	2.00	-	7.50	
LYM	1.96		$10^3/\mu L$	1.08	-	3.17	
MON	0.28		$10^3/\mu L$	0.15	-	0.70	
EO	0.06		$10^3/\mu L$	0.00	-	0.50	
BAS	0.00		$10^3/\mu L$	0.00	-	0.15	
NEU%	55.1		%	40.0	-	75.0	
LYM%	38.3		%	14.76	-	45.40	
MON%	5.4		%	3.0	-	7.0	
EO%	1.2		%	0.0	-	5.0	
BAS%	0.0		%	0.0	-	1.5	
RBC	0.81	--	$10^6/\mu L$	4.00	-	5.50	
HGB	104	-	g/L	120	-	174	
HCT	8.0	--	%	36.0	-	52.0	
MCV	98.6	+	fL	76.0	-	96.0	
MCH	128.0	++	pg	27.0	-	32.0	
MCHC	1298	++	g/L	300	-	350	
RDWsd	31.7		fL	20.0	-	42.0	
RDWcv	10.3		%	0.0	-	16.0	
PLT	225		$10^3/\mu L$	150	-	400	
PCT	0.29		%		-		
MPV	13.0		fL	8.0	-	15.0	
PDWsd	24.8		fL		-		
PDWcv	34.1		%		-		







Warning flags

Morphological flags

Interpretive flags Anemia?, Macrocytic RBC?, Hyperchromic?

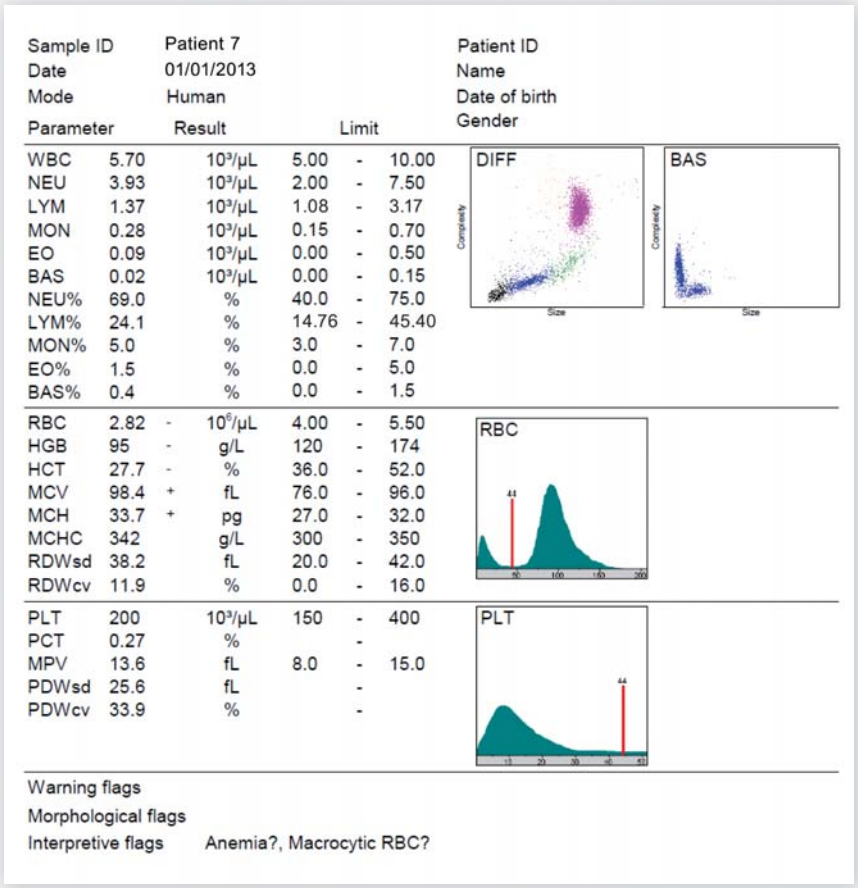
Interpretation

The WBC DIFF scattergram indicates well defined and clearly characterized WBC subpopulations only slightly dotted pattern can be recognized. The BAS scattergram is normal.

There is no warning or morphological flag. The RBC and PLT histograms have a normal characteristic and are well separated but the RBC count and HCT are extremely low while MCHC is grossly increased.

When the pre-analytical phase of measurement was controlled and the blood specimen was kept under body temperature (37 °C) for 15 minutes, the RBC counts increased compared to those measured previously. This case MCH and MCHC values also tended to normalize. The neutrophil count also increased because of releasing neutrophils from agglutinated clumps.

Repeated measurement after pre-warming (37 °C)

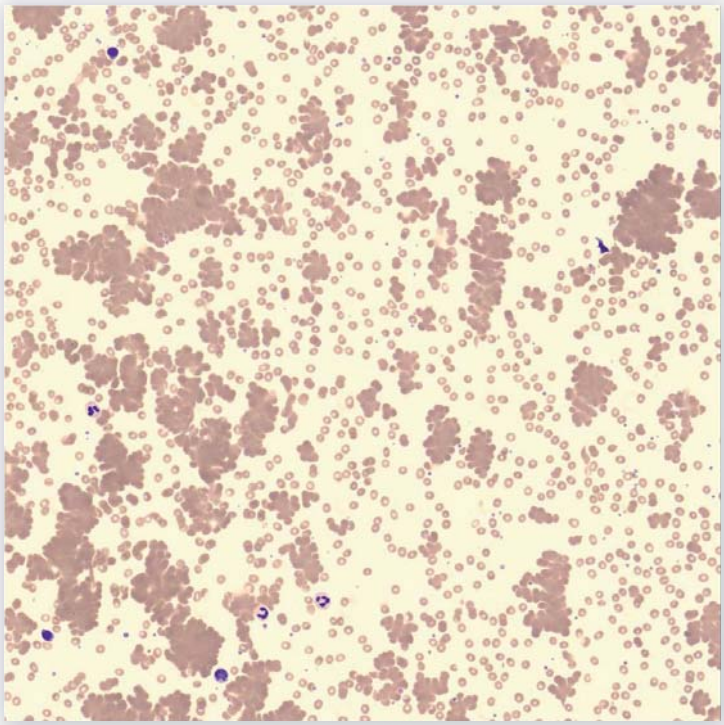


Sample ID	Patient 7					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	5.70		$10^3/\mu L$	5.00	-	10.00	
NEU	3.93		$10^3/\mu L$	2.00	-	7.50	
LYM	1.37		$10^3/\mu L$	1.08	-	3.17	
MON	0.28		$10^3/\mu L$	0.15	-	0.70	
EO	0.09		$10^3/\mu L$	0.00	-	0.50	
BAS	0.02		$10^3/\mu L$	0.00	-	0.15	
NEU%	69.0		%	40.0	-	75.0	
LYM%	24.1		%	14.76	-	45.40	
MON%	5.0		%	3.0	-	7.0	
EO%	1.5		%	0.0	-	5.0	
BAS%	0.4		%	0.0	-	1.5	
RBC	2.82	-	$10^6/\mu L$	4.00	-	5.50	
HGB	95	-	g/L	120	-	174	
HCT	27.7	-	%	36.0	-	52.0	
MCV	98.4	+	fL	76.0	-	96.0	
MCH	33.7	+	pg	27.0	-	32.0	
MCHC	342		g/L	300	-	350	
RDWsd	38.2		fL	20.0	-	42.0	
RDWcv	11.9		%	0.0	-	16.0	
PLT	200		$10^3/\mu L$	150	-	400	
PCT	0.27		%		-		
MPV	13.6		fL	8.0	-	15.0	
PDWsd	25.6		fL		-		
PDWcv	33.9		%		-		
Warning flags							
Morphological flags							
Interpretive flags	Anemia?, Macrocytic RBC?						

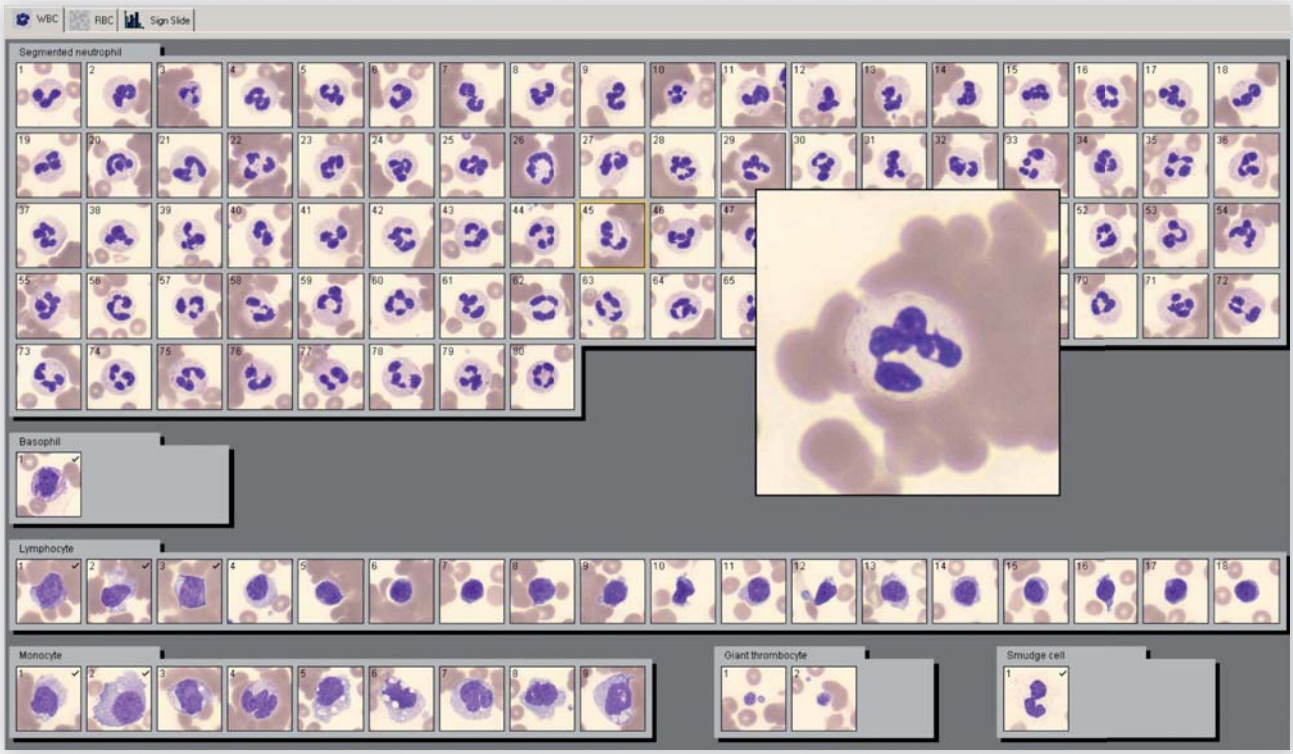
The RBC count, HGB, HCT and MCH values indicate a macrocytic hyper-chromic anemia.

Based on these findings the presence of cold agglutinins is reasonable to be postulated.

Peripheral blood smear



On the peripheral blood smear clumped RBC masses are clearly visible. Of note, this smear was done under routine conditions.



Agglutinated red blood cells capture neutrophils which causes a marked decrease in neutrophil count and an altered ratio of white blood cell populations at room temperature. (Lymphocytes and monocytes are also captured but more moderately.)

This effect decreases after pre-warming to 37 °C.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	80	74.1 ✓
• Eosinophil	-	-
• Basophil	1	0.9 ✓
• Lymphocyte	18	16.7 ✓
• **Monocyte**	9	8.3 ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	2	- ✓
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	1	- ✓
• Artefact	5	- ✓

Other laboratory findings

Other laboratory findings included marked impairment of hepatic function (associated with heavy drinking).

Diagnosis

Hemolytic anemia triggered by cold agglutinins

Disease course

The patient was strongly advised to wear protective gloves and socks when the weather is cold.

Avoidance of alcoholic beverages was also recommended. The patient's immunological assessment did not indicate any autoantibody responsible for hemolysis.

Case 8 | Erythrocytosis, polyglobulia, COPD

History and clinical signs and symptoms

A 55 year old man is a chain smoker. The diagnosis of chronic obstructive pulmonary disease (COPD) was established 3 years ago. The patient contacted his physician due to disabling dyspnea and fatigue.

Hematologic findings

Sample ID	Patient 8						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	18.15	+	$10^3/\mu L$	5.00	-	10.00	DIFF	BAS
NEU	16.23	++	$10^3/\mu L$	2.00	-	7.50		
LYM	1.43		$10^3/\mu L$	1.08	-	3.17		
MON	0.42		$10^3/\mu L$	0.15	-	0.70		
EO	0.02		$10^3/\mu L$	0.00	-	0.50		
BAS	0.05		$10^3/\mu L$	0.00	-	0.15		
NEU%	89.4	+	%	40.0	-	75.0		
LYM%	7.9	--	%	14.76	-	45.40		
MON%	2.3	-	%	3.0	-	7.0		
EO%	0.1		%	0.0	-	5.0		
BAS%	0.3		%	0.0	-	1.5		
RBC	6.66	+	$10^6/\mu L$	4.00	-	5.50	RBC	
HGB	189	+	g/L	120	-	174		
HCT	55.0	+	%	36.0	-	52.0		
MCV	82.6		fL	76.0	-	96.0		
MCH	28.4		pg	27.0	-	32.0		
MCHC	344		g/L	300	-	350		
RDWsd	34.8		fL	20.0	-	42.0		
RDWcv	13.7		%	0.0	-	16.0		
PLT	250		$10^3/\mu L$	150	-	400	PLT	
PCT	0.35		%		-			
MPV	13.8		fL	8.0	-	15.0		
PDWsd	32.2		fL		-			
PDWcv	37.1		%		-			
Warning flags								
Morphological flags	G							
Interpretive flags	Leukocytosis?, Neutrophilia?, Polycythemia?							

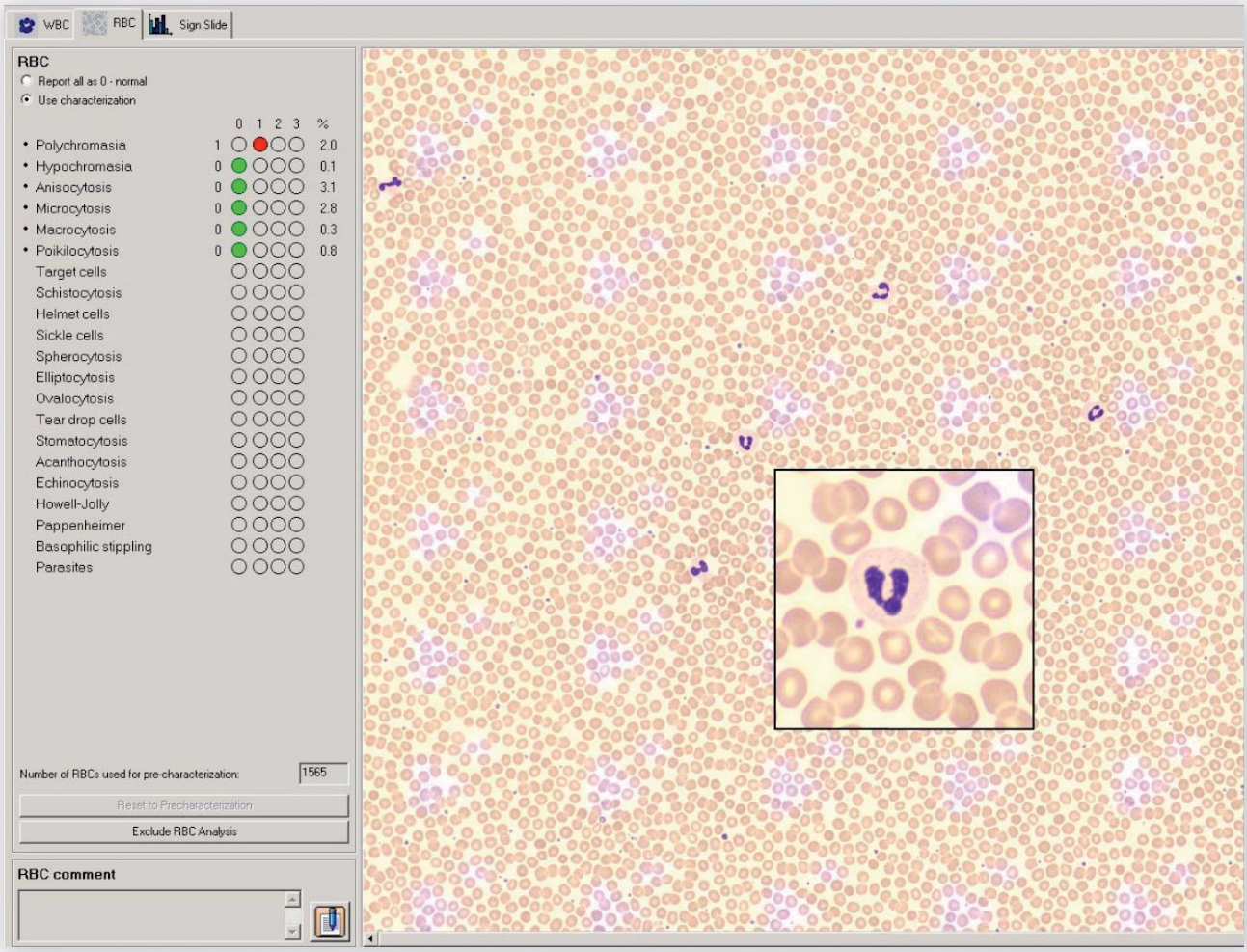
Interpretation

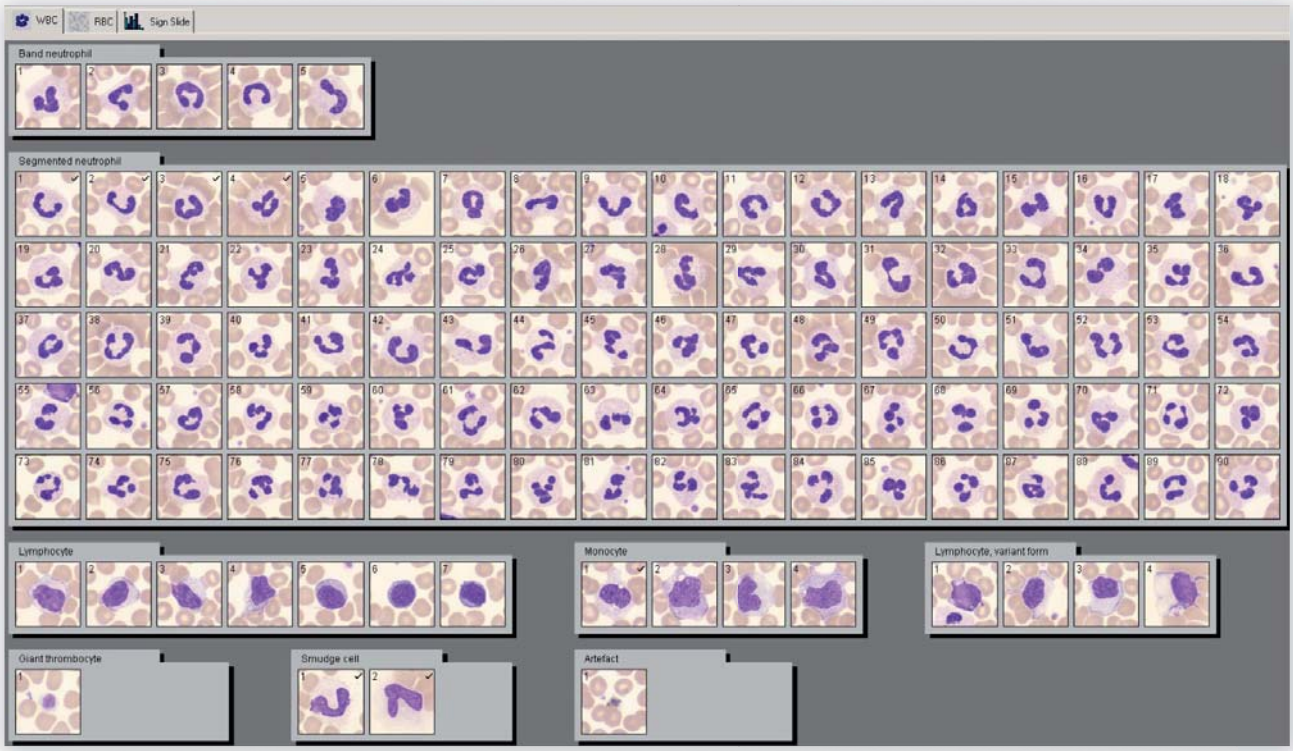
The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC subpopulations including neutrophil, monocyte, lymphocyte and eosinophil cells are distinguished. The WBC count is increased with the dominance of neutrophils (see interpretative flag “neutrophilia”). A Flag “G” indicates the possible presence of immature granulocytes thus evaluation of a peripheral blood smear is recommended.

The RBC and PLT populations are distinguished. Histograms are normally distributed. The RBC count is above the normal range. The HGB and HCT values are also significantly increased. The RBC size distribution is within the normal range.

The PLT count and morphological characteristics are within the normal range.

Peripheral blood smear





The peripheral blood smear is not remarkable.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	5	4.5
• Segmented neutrophil	90	81.8
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	7	6.4
• Monocyte	4	3.6
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	4	3.6
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	110	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	1	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	2	-
• Artefact	1	-

Other laboratory findings

Other physical findings are consistent with COPD. Inflammatory markers including CRP levels (25 mg/L [ref.range: 0- 5 mg/L) indicate inflammation. Based on X-ray a pulmonary infection was suspected.

Diagnosis

COPD, associated erythrocytosis and polyglobulia

Disease course

The patient was given wide-spectrum antibiotics, bronchodilators and, transient 100% oxygen. Soon the patient's overall condition improved.

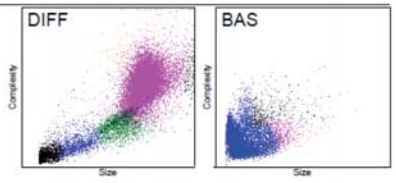
Case 9 | Polycythemia vera, nucleated red blood cells

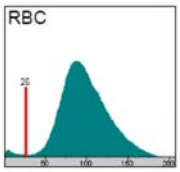
History and clinical signs and symptoms

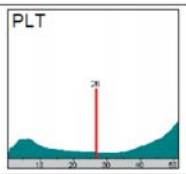
A 55 year old woman was diagnosed with polycythemia vera 5 years ago. This time the patient was admitted to the hospital as the transformation to leukemia was suspected. Clinical signs include dyspnea and edema in lower limbs. The abdominal ultrasonography revealed diffuse hepatosplenomegaly and enlarged lymph nodes.

Hematologic findings

Sample ID	Patient 9					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	104.9	*	$10^3/\mu L$	5.00	-	10.00	
NEU	85.56	*	$10^3/\mu L$	2.00	-	7.50	
LYM	8.08	*	$10^3/\mu L$	1.08	-	3.17	
MON	7.56	*	$10^3/\mu L$	0.15	-	0.70	
EO	0.42	*	$10^3/\mu L$	0.00	-	0.50	
BAS	3.36	*	$10^3/\mu L$	0.00	-	0.15	
NEU%	81.5	+	%	40.0	-	75.0	
LYM%	7.7	--	%	14.76	-	45.40	
MON%	7.2	+	%	3.0	-	7.0	
EO%	0.4		%	0.0	-	5.0	
BAS%	3.2	++	%	0.0	-	1.5	
RBC	3.48	-	$10^6/\mu L$	4.00	-	5.50	
HGB	105	-	g/L	120	-	174	
HCT	34.3	-	%	36.0	-	52.0	
MCV	98.6	+	fL	76.0	-	96.0	
MCH	30.2		pg	27.0	-	32.0	
MCHC	306		g/L	300	-	350	
RDWsd	77.3	+	fL	20.0	-	42.0	
RDWcv	---	**	%	0.0	-	16.0	
PLT	44	--	$10^3/\mu L$	150	-	400	
PCT	0.07		%		-		
MPV	15.6	+	fL	8.0	-	15.0	
PDWsd	59.1		fL		-		
PDWcv	38.0		%		-		







Warning flags	I
Morphological flags	GL
Interpretive flags	Anemia?, Macrocytic RBC?, Thrombocytopenia?, Macrocytic PLT?

Interpretation

WBC DIFF and BAS scattergrams are not defined.

WBC count is extremely elevated. WBC populations are confluent. Flag “G” warns the high prevalence of immature leukocytes. Flag “L” indicates the occurrence of atypical lymphocytes. Linearity flag “ * ” indicates that WBC counts are out of the linearity range.

Basophil cell count and prevalence are markedly increased.*

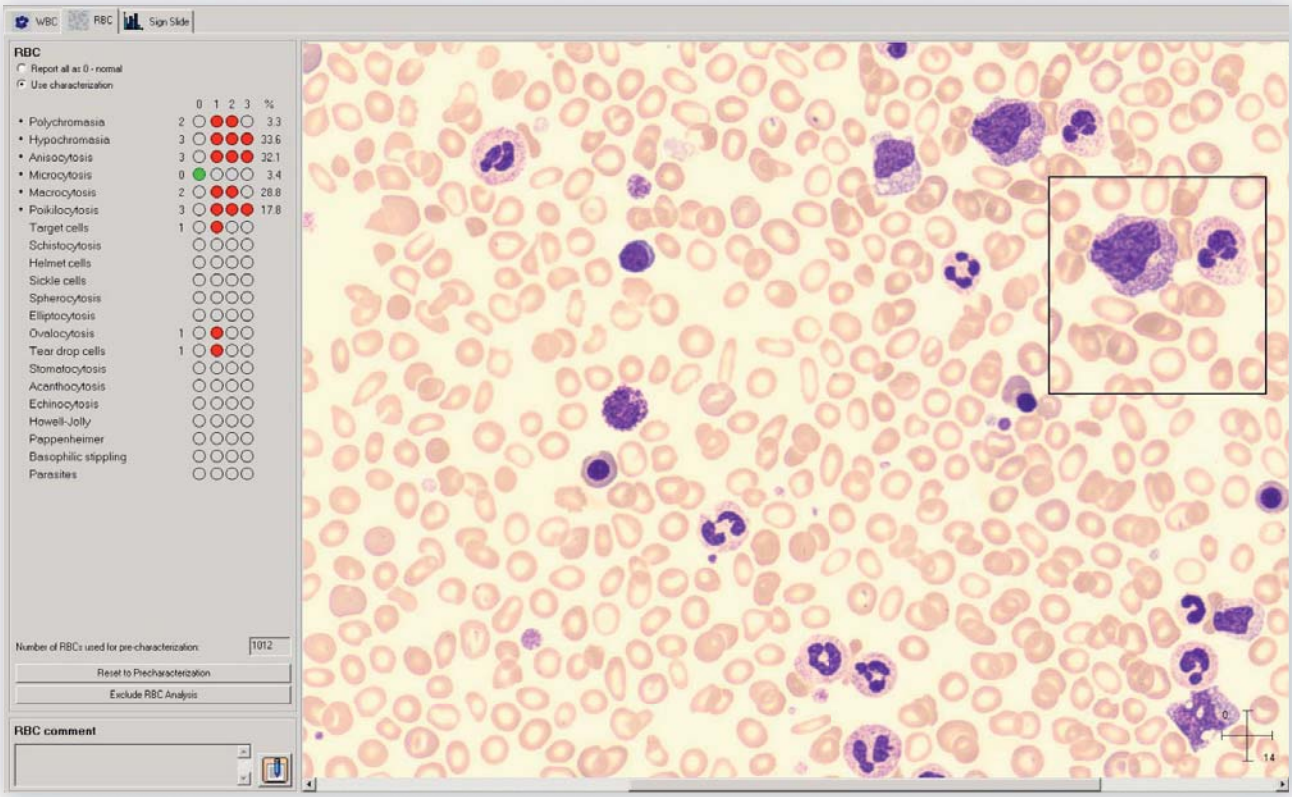
RBC and PLT histograms are not normal.

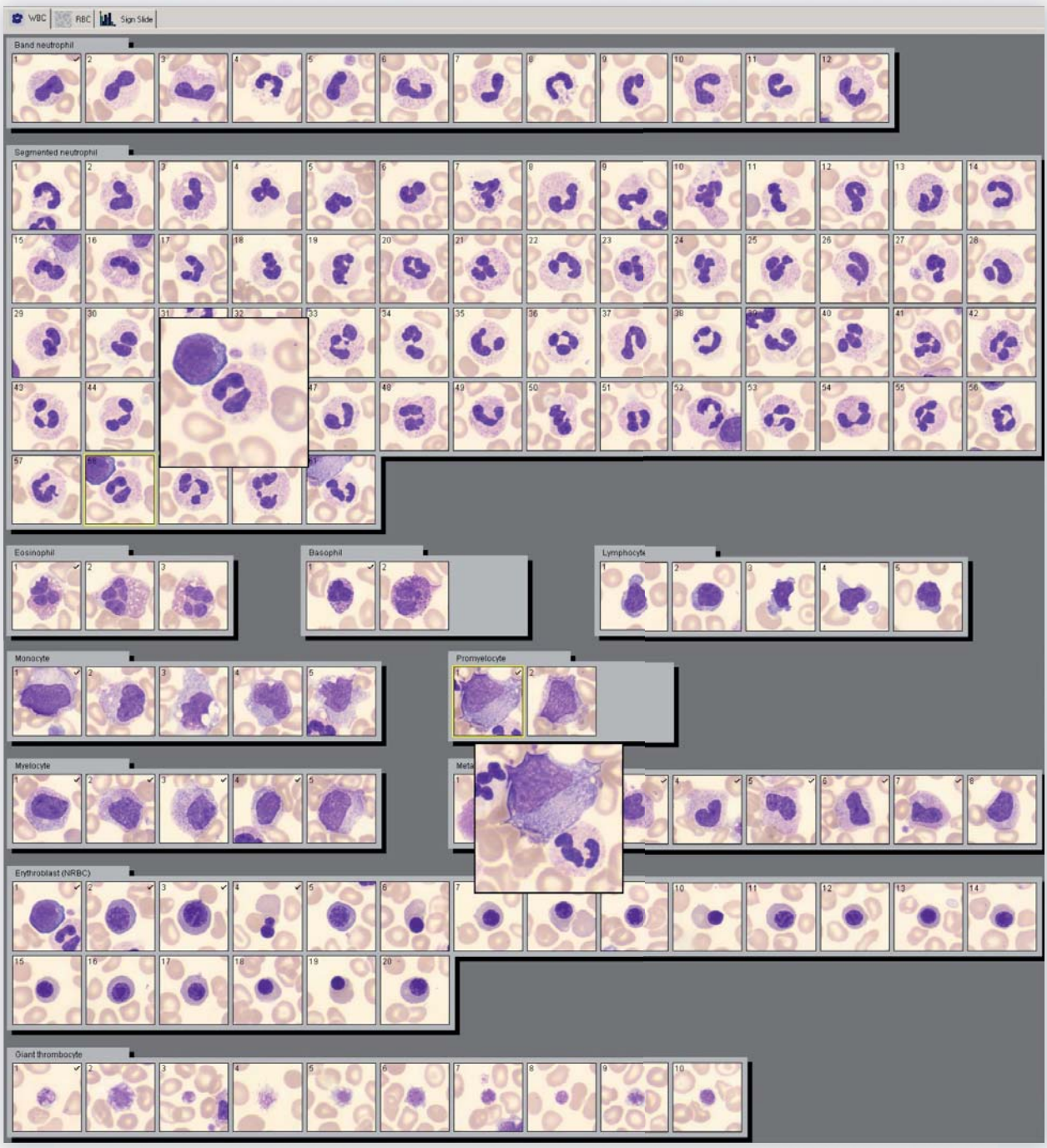
Flag “I” indicates that the PLT-RBC gap is not clearly detectable on the PLT-RBC histogram, suggesting the presence of aggregated or giant PLT-s.

RBC count, HGB and HCT values are below the lower limit of reference, MCV is increased indicating anemia (see flags “anemia”, “macrocytic RBC”). The RBC size distribution is very broad. RDWsd values are increased while RDWcv value is out of measurement range therefore not displayed. Both are indicative of anisocytosis. PLT count is very low, thrombocytopenia is present (see flag). High MPV is suggestive of macro-thrombocytes.

Due to morphological flags and the confluent leukocyte populations the visual assessment of a blood smear is strongly recommended.

Peripheral blood smear





In line with the diagnosis, different types of immature and atypical myeloid cells are present in peripheral blood. There is a high prevalence of normo-blasts (or nucleated red blood cells/NRBCs: 19%.) This finding supports the presence of extra-medullary erythropoiesis. Macro-thrombocytes are also present in the smear. There is a marked anisocytosis in the RBC population.

Of note, the prevalence of nucleated red blood cells is dramatically increased (about 19% of total WBC count). This falsely elevates leukocyte counts.* Therefore, the real WBC count in peripheral blood sample should be up to 81% of the measured one.

*NOTE: Nucleated red blood cells are resistant to lyzing procedure during the measurement. Therefore they are classified falsely to WBC population. This may increase the detected WBC count. In fact, WBC may be even lower than that provided.
Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system.

Due to their special response to lyzing effect abnormal, atypical and blast cells may falsely increase the basophil and monocyte counts.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	12	11.7
• Segmented neutrophil	61	59.2 ✓
• Eosinophil	3	2.9
• Basophil	2	1.9
• Lymphocyte	5	4.9
• Monocyte	5	4.9
• Promyelocyte	2	1.9
• Myelocyte	5	4.9
• Metamyelocyte	8	7.8
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	103	100

Non-WBC	Count	%
• Erythroblast (NRBC)	20	-
• Giant thrombocyte	10	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	9	-
• Artefact	2	-

Other laboratory findings

Other remarkable laboratory parameters include extremely high alkaline phosphatase and lactic dehydrogenase values (1500 U/L and 2320 U/L, respectively). This finding is in line with the basic diagnosis. Albumin levels are markedly decreased (20 g/L (ref. range: 35 – 50 g/L). Cytological evaluation (including flow-cytometry) of the bone marrow biopsy samples revealed a grade IV myelofibrosis with severely impaired hemopoietic activity but without any signs of malignant transformation.

Cytogenetic testing of peripheral blood verified the "JAK2 V617F" mutation.

Diagnosis

Polycythemia vera, macrocytic anemia, thrombocytopenia, chronic myelofibrosis with extra-medullary leukoerythropoiesis.

Disease course

Bone marrow biopsy did not reveal malignant transformation. Symptomatic therapy was applied.

CHAPTER 2

Platelet abnormalities

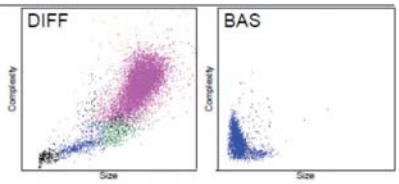
Case 10 | Thrombocytopenia, sepsis

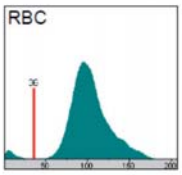
History and clinical signs and symptoms

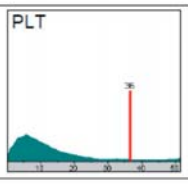
A 62 year old diabetic man was subjected to a left lower limb amputation. One day after the surgical intervention a general sickness and low-grade fever (38.3 °C) developed.

Hematologic findings

Sample ID	Patient 10						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	24.76	++	$10^3/\mu L$	5.00	-	10.00		
NEU	22.04	++	$10^3/\mu L$	2.00	-	7.50		
LYM	1.54		$10^3/\mu L$	1.08	-	3.17		
MON	1.06	+	$10^3/\mu L$	0.15	-	0.70		
EO	0.10		$10^3/\mu L$	0.00	-	0.50		
BAS	0.02		$10^3/\mu L$	0.00	-	0.15		
NEU%	89.0	+	%	40.0	-	75.0		
LYM%	6.2	--	%	14.76	-	45.40		
MON%	4.3		%	3.0	-	7.0		
EO%	0.4		%	0.0	-	5.0		
BAS%	0.1		%	0.0	-	1.5		
RBC	2.95	-	$10^6/\mu L$	4.00	-	5.50		
HGB	95	-	g/L	120	-	174		
HCT	30.7	-	%	36.0	-	52.0		
MCV	103.8	+	fL	76.0	-	96.0		
MCH	32.2	+	pg	27.0	-	32.0		
MCHC	310		g/L	300	-	350		
RDWsd	56.8	+	fL	20.0	-	42.0		
RDWcv	14.9		%	0.0	-	16.0		
PLT	76	-	$10^3/\mu L$	150	-	400		
PCT	0.10		%		-			
MPV	13.2		fL	8.0	-	15.0		
PDWsd	35.1		fL		-			
PDWcv	38.0		%		-			







Warning flags	
Morphological flags	G
Interpretive flags	Leukocytosis?, Neutrophilia?, Anemia?, Macrocytic RBC?

Interpretation

The WBC count is elevated with a dominant neutrophil population. A Flag “G” warns of the possible presence of immature neutrophils. The cell subpopulations are not clearly characterized (see DIFF scattergram), hence, the evaluation of a peripheral blood smear is strongly recommended even in absence of a warning flag related to the WBC population separation.

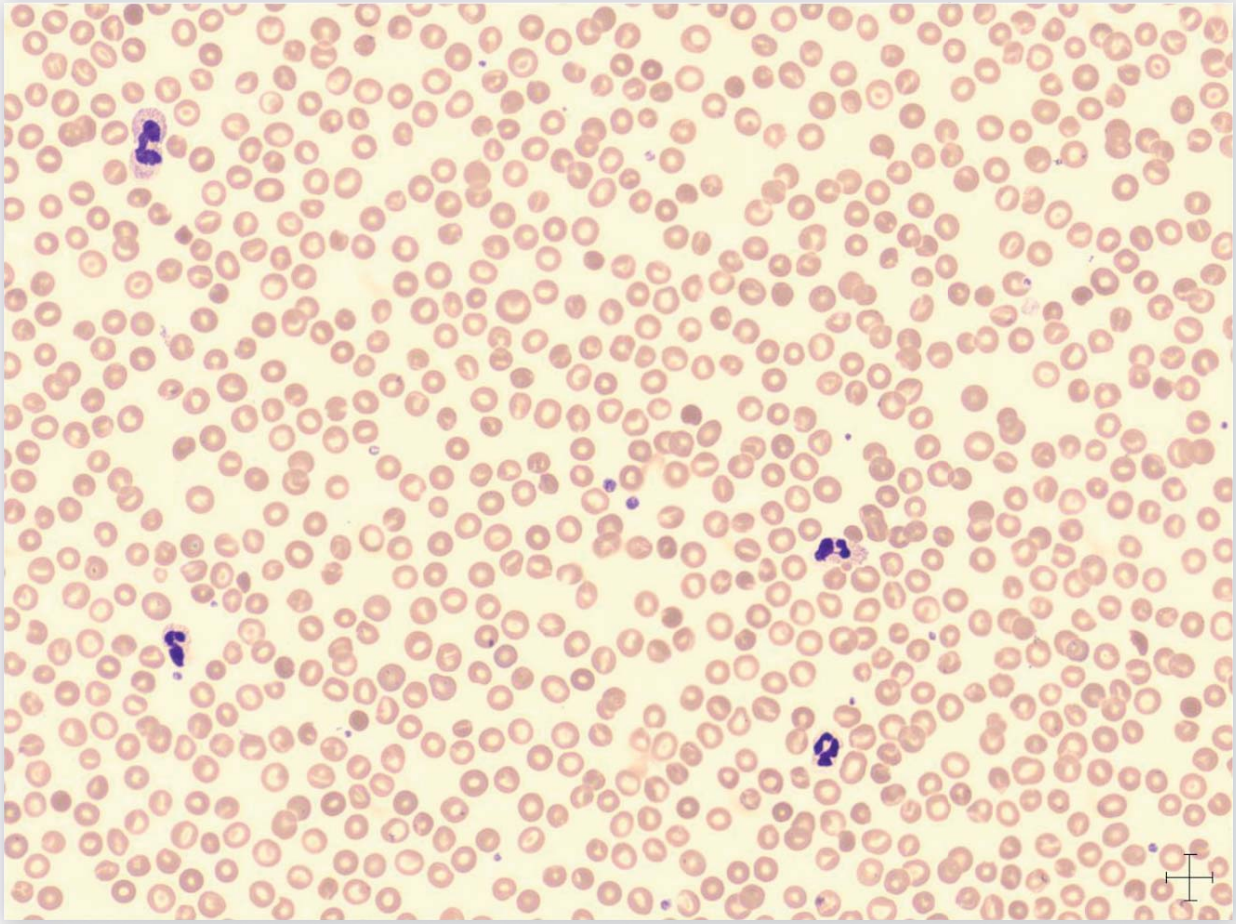
The RBC and PLT histograms are clearly separated.

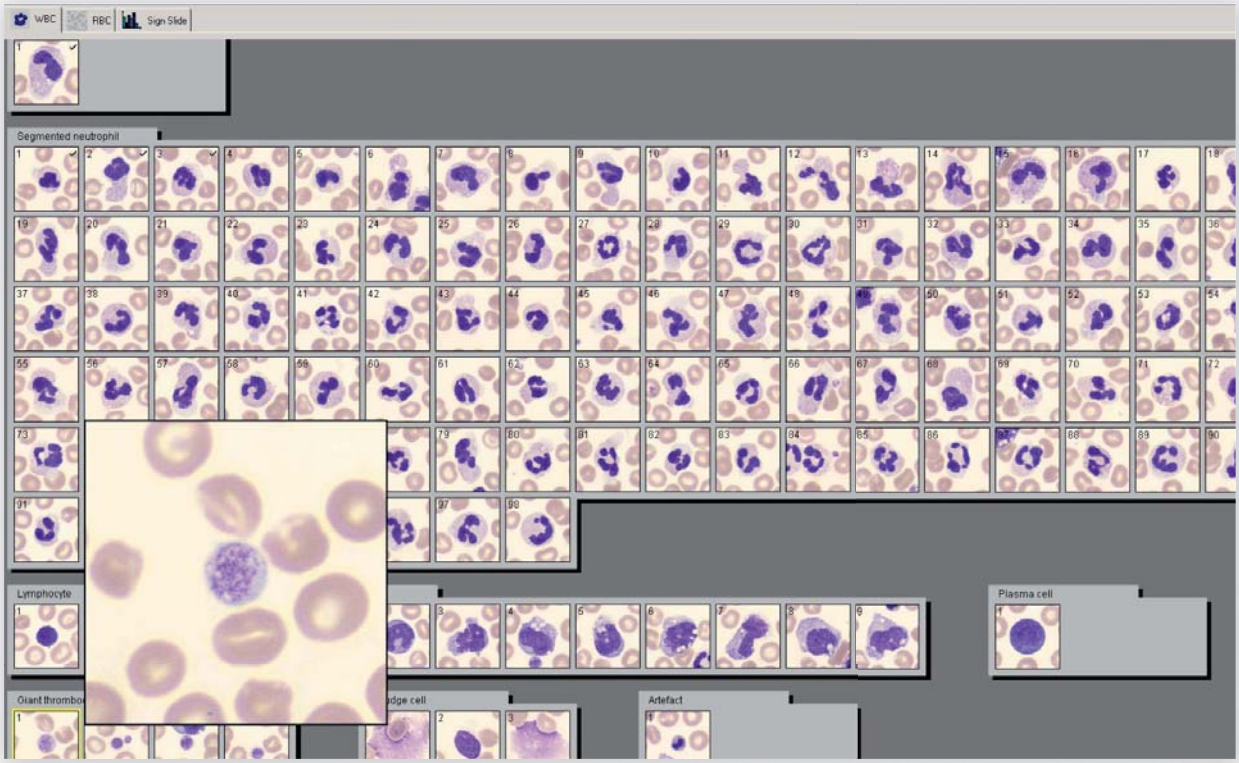
The RBC count is below the normal range indicating severe anemia (see interpretative flag “anemia”). The low HGB and HCT are present without major abnormalities in the RBC characteristics however the increased RDWsd together with a slightly elevated MCV is indicative of possible anisocytosis.

The PLT count is markedly below the normal reference range.

Please note, that interpretive flag “thrombocytopenia” only appears if PLT count is less than half of the low limit of normal range (normal range flag “--”).

Peripheral blood smear





The high prevalence of neutrophils with the presence of some immature leukocytes is consistent with a bacterial infection. Apart from a mild anisocytosis the RBC morphological characteristics in general are normal, indicating the reactive nature of the anemia. The presence of macro-thrombocytes may be a sign of increased bone marrow activity.

WBC	Count	%	
• Unidentified	0	0	■
• Band neutrophil	1	0.9	■
• Segmented neutrophil	98	87.5	■
• Eosinophil	-	-	
• Basophil	-	-	
• Lymphocyte	9	8	■
• Monocyte	4	3.6	■
• Promyelocyte	-	-	
• Myelocyte	-	-	
• Metamyelocyte	-	-	
Immature eosinophil	-	-	
Immature basophil	-	-	
Promonocyte	-	-	
Prolymphocyte	-	-	
• Blast (no lineage spec)	-	-	
• Lymphocyte, variant form	-	-	
• Plasma cell	1	0.9	
Large granular lymphocyte	-	-	
Hairy cell	-	-	
Sezary cell	-	-	
Other	-	-	
Total	112	100	

Non-WBC	Count	%	
• Erythroblast (NRBC)	-	-	
• Giant thrombocyte	4	-	■
• Thrombocyte aggregation	-	-	
Megakaryocyte	-	-	
• Smudge cell	3	-	■
• Artefact	1	-	

The microscopic evaluation of the peripheral blood smear does not confirm the presense of immature granulocytes indicated by the flag “G”. This is an example that automated flagging algorithms,-despite their clear importance in rapid screenings-are not always reliable.

Other findings

Other abnormal clinical laboratory results include high CRP and pro-calcitonin levels (82 [<5] mg/L and 4 [<0.5]ng/mL). The INR value is 2.8 [0.9-1.15]. Liver and renal function tests (including transaminases, alkaline phosphatase, lactic dehydrogenase, blood urea nitrogen and creatinine levels) are suggestive of the development of multi-organ failure. A blood culture was not obtained (the patient was already given antibiotics). The patient then developed bruises.

Diagnosis

Sepsis with severe anemia and thrombocytopenia.

Disease course

Platelet concentrates and packed RBCs were transfused. Two days after the initiation of antibiotic therapy the patient's general status dramatically improved. Within ten days the CBC count tended to return to the normal range and the other biochemical parameters also normalized.

Case 11 | Thrombocytopenia of unknown origin

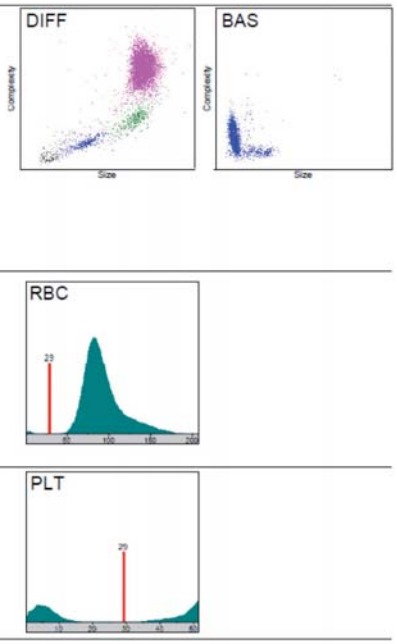
History and clinical signs and symptoms

30 year old man contacted the doctor due to severe pain and bleeding blisters in his mouth.

Hematologic findings

Sample ID Patient 11 Patient ID
Date 01/01/2013 Name
Mode Human Date of birth
Gender

Parameter	Result			Limit		
WBC	10.39	+	$10^3/\mu L$	5.00	-	10.00
NEU	9.41	+	$10^3/\mu L$	2.00	-	7.50
LYM	0.56	·	$10^3/\mu L$	1.08	-	3.17
MON	0.41		$10^3/\mu L$	0.15	-	0.70
EO	0.01		$10^3/\mu L$	0.00	-	0.50
BAS	0.00		$10^3/\mu L$	0.00	-	0.15
NEU%	90.6	+	%	40.0	-	75.0
LYM%	5.4	--	%	14.76	-	45.40
MON%	3.9		%	3.0	-	7.0
EO%	0.1		%	0.0	-	5.0
BAS%	0.0		%	0.0	-	1.5
RBC	4.70		$10^6/\mu L$	4.00	-	5.50
HGB	150		g/L	120	-	174
HCT	43.8		%	36.0	-	52.0
MCV	93.1		fL	76.0	-	96.0
MCH	31.8		pg	27.0	-	32.0
MCHC	341		g/L	300	-	350
RDWsd	35.8		fL	20.0	-	42.0
RDWcv	13.9		%	0.0	-	16.0
PLT	11	--	$10^3/\mu L$	150	-	400
PCT	0.00		%		-	
MPV	---	**	fL	8.0	-	15.0
PDWsd	0.0		fL		-	
PDWcv	38.2		%		-	



Warning flags
Morphological flags
Interpretive flags Thrombocytopenia?

Interpretation

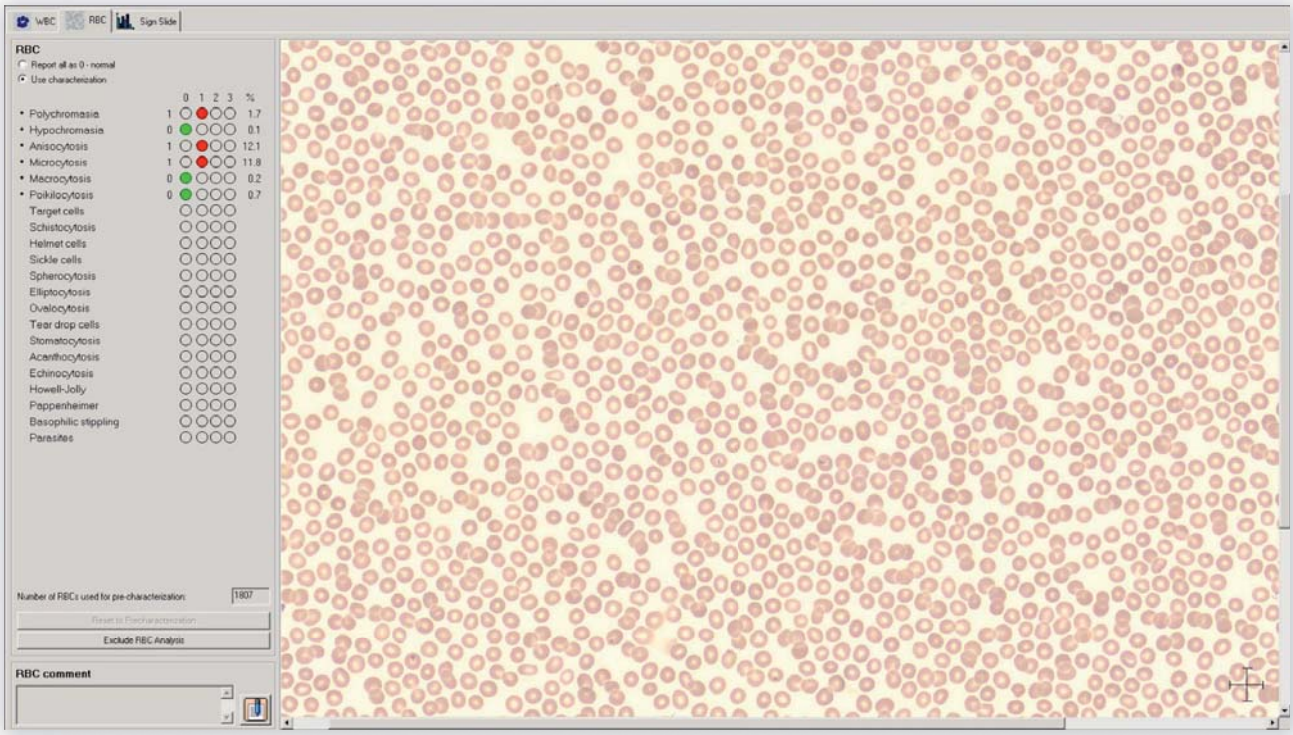
The WBC differential (DIFF) and basophil (BAS) scattergrams are well defined. The WBC count is slightly elevated. The major leukocyte subpopulations are well characterized. The overwhelming majority of the WBCs are neutrophils. There is also a marked absolute and relative lymphopenia and a mild neutrophilia.

The RBC and PLT histograms are well separated (with negligible PLT peak). The shape and size distribution of the RBC histogram is normal. The HGB and RBC morphology is also within the normal range.

The platelets are markedly decreased (see interpretative flag “thrombocytopenia”).

The hematologic findings are characteristic for severe thrombocytopenia.

Peripheral blood smear



No platelets are visible in the peripheral blood smear.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	98	89.9
• Eosinophil	1	0.9
• Basophil	1	0.9
• Lymphocyte	5	4.6
• Monocyte	4	3.7
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	109	100

Non-WBC	Count	%
• Erythroblast (NRBC)	1	-
• Giant thrombocyte	96	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	10	-
• Artefact	6	-

Other laboratory findings

Other laboratory results were normal. Serological tests to detect a wide range of pathogens and autoimmune disorders did not provide any explanation for the thrombocytopenia.

Diagnosis

Thrombocytopenia of unknown origin

Disease course

Several regimes, including the administration of immune modulatory agents and steroids, failed to increase the PLT count. Finally the patient responded to a course of biological therapy.

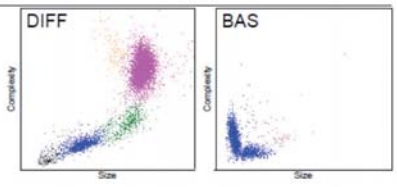
Case 12 | Transient thrombocytosis

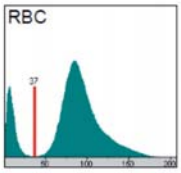
History and clinical signs and symptoms

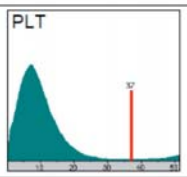
A 60 year old man was subjected to bladder stoma surgery 3 weeks ago. Postoperatively a systemic infection developed that was effectively treated with antibiotics. A blood sample was taken to monitor the patient's status.

Hematologic findings

Sample ID	Patient 12					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	12.02	+	$10^3/\mu L$	5.00	- 10.00		
NEU	8.87	+	$10^3/\mu L$	2.00	- 7.50		
LYM	2.08		$10^3/\mu L$	1.08	- 3.17		
MON	0.66		$10^3/\mu L$	0.15	- 0.70		
EO	0.22		$10^3/\mu L$	0.00	- 0.50		
BAS	0.19	+	$10^3/\mu L$	0.00	- 0.15		
NEU%	73.8		%	40.0	- 75.0		
LYM%	17.3		%	14.76	- 45.40		
MON%	5.5		%	3.0	- 7.0		
EO%	1.8		%	0.0	- 5.0		
BAS%	1.6	+	%	0.0	- 1.5		
RBC	4.14		$10^6/\mu L$	4.00	- 5.50		
HGB	125		g/L	120	- 174		
HCT	39.2		%	36.0	- 52.0		
MCV	94.6		fL	76.0	- 96.0		
MCH	30.2		pg	27.0	- 32.0		
MCHC	320		g/L	300	- 350		
RDWsd	47.2	+	fL	20.0	- 42.0		
RDWcv	16.0		%	0.0	- 16.0		
PLT	726	+	$10^3/\mu L$	150	- 400		
PCT	0.63		%		-		
MPV	8.7		fL	8.0	- 15.0		
PDWsd	24.4		fL		-		
PDWcv	37.5		%		-		







Warning flags

Morphological flags

Interpretive flags Leukocytosis?, Thrombocytosis?

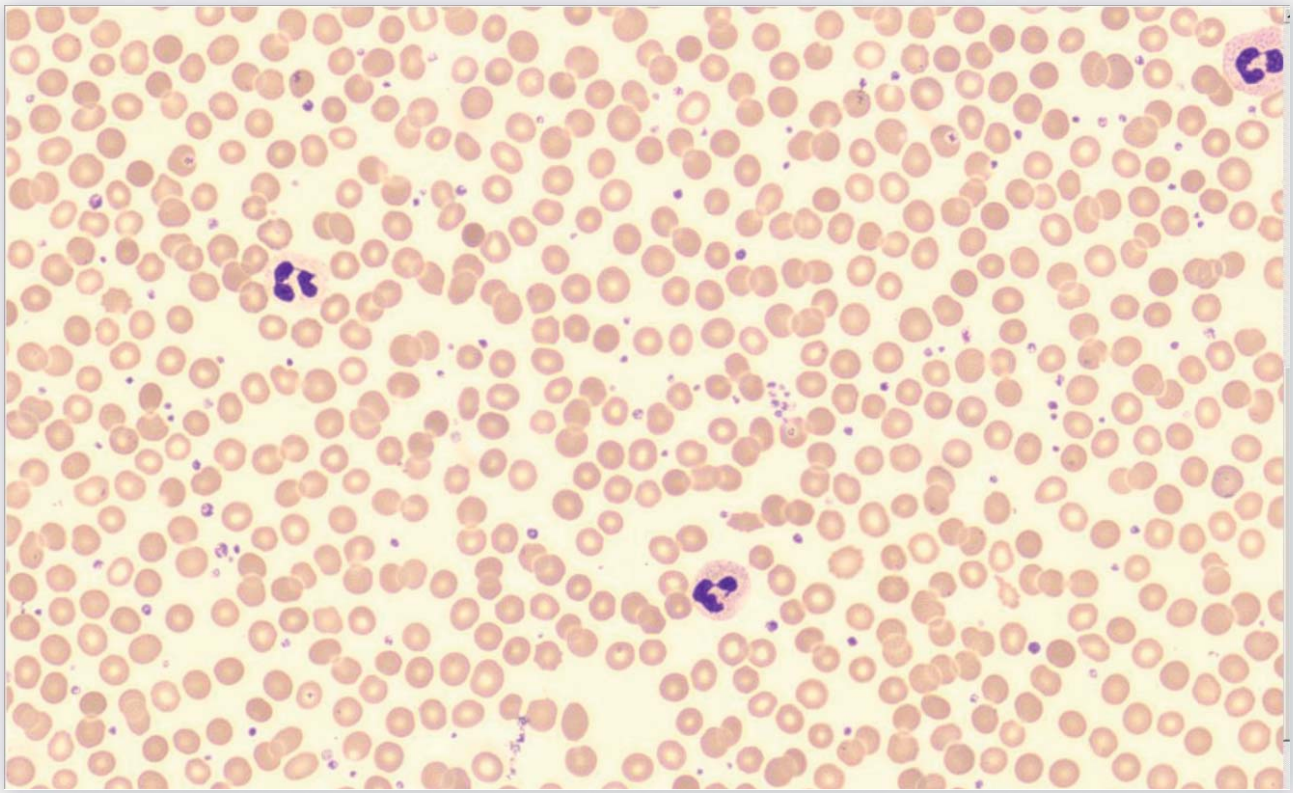
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC subpopulations including monocytes, neutrophils, lymphocytes and eosinophils are well defined. The WBC count is slightly elevated (see interpretative flag "leukocytosis"). The BAS count and percentage is slightly increased (without clinical relevance).

The PLT and RBC histograms are clearly separated. The red cell parameters are within the normal range. The increased RDWsd has no clinical relevance. The markedly elevated peak left of the RBC histogram is consistent with the high PLT count. The PLT count is markedly higher than the upper limit of normal (see interpretative flag "thrombocytosis").

As there weren't any warning or morphological flags, the assessment of a peripheral blood smear was not expected to provide any additional useful information.

Peripheral blood smear



The peripheral blood smear clearly indicates the increased prevalence of platelets.

WBC	Count	%
• Unidentified	1	0.9
• Band neutrophil	3	2.7
• Segmented neutrophil	74	67.3 ✓
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	22	20 ✓
• Monocyte	7	6.3
• Promyelocyte	-	-
• Myelocyte	1	0.9
• Metamyelocyte	1	0.9
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	1	0.9
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	110	100

Non-WBC	Count	%
• Erythroblast (NRBC)	1	-
• Giant thrombocyte	96	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	10	-
• Artefact	6	-

Other laboratory findings

Other routine clinical laboratory results are unremarkable. CRP levels are normal (3.5 mg/L (ref value: <5 mg/L).

Diagnosis

Transient thrombocytosis

Disease course

According to his current physical and laboratory status (including a repeated complete blood count) the patient has fully recovered. The thrombocytosis is considered as an coincidental finding.

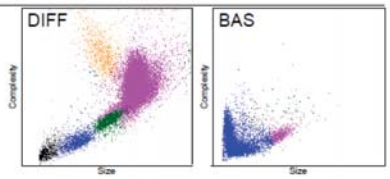
Case 13 | Extreme thrombocythemia in chronic myeloid leukemia (need for manual sample dilution)

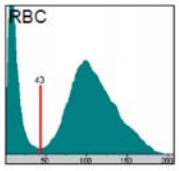
History and clinical signs and symptoms

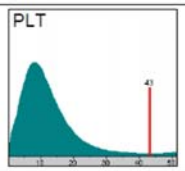
A 45 year old woman was diagnosed with chronic myeloid leukemia (CML) 1 year ago. The patient is regularly treated with chemotherapeutic regime as protocol.

Hematologic findings

Sample ID	Patient 13					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	33.20	++	$10^3/\mu L$	5.00	-	10.00	
NEU	23.97	++	$10^3/\mu L$	2.00	-	7.50	
LYM	2.46		$10^3/\mu L$	1.08	-	3.17	
MON	2.29	++	$10^3/\mu L$	0.15	-	0.70	
EO	1.53	++	$10^3/\mu L$	0.00	-	0.50	
BAS	2.95	++	$10^3/\mu L$	0.00	-	0.15	
NEU%	72.2		%	40.0	-	75.0	
LYM%	7.4	-	%	14.76	-	45.40	
MON%	6.9		%	3.0	-	7.0	
EO%	4.6		%	0.0	-	5.0	
BAS%	8.9	++	%	0.0	-	1.5	
RBC	3.49	-	$10^6/\mu L$	4.00	-	5.50	
HGB	107	-	g/L	120	-	174	
HCT	38.0		%	36.0	-	52.0	
MCV	108.6	+	fL	76.0	-	96.0	
MCH	30.5		pg	27.0	-	32.0	
MCHC	281	-	g/L	300	-	350	
RDWsd	83.1	+	fL	20.0	-	42.0	
RDWcv	---	**	%	0.0	-	16.0	
PLT	---	**	$10^3/\mu L$	150	-	400	
PCT	---	**	%		-		
MPV	---	**	fL	8.0	-	15.0	
PDWsd	---	**	fL		-		
PDWcv	---	**	%		-		







Warning flags	O
Morphological flags	G
Interpretive flags	Leukocytosis?, Neutrophilia?, Monocytosis?, Eosinophilia?, Basophilia?, Anemia?, Macrocytic RBC?, Hypochromic?

Interpretation

The WBC DIFF scattergram is abnormal. The WBC DIFF scattergram indicates a confluent population with neutrophils as dominant cell population. The WBC count and the count of each granulocyte subpopulations including basophils are dramatically increased (see interpretive flags “leukocytosis, neutrophilia, monocytosis, eosinophilia, basophilia”). However due to the abnormal WBC differential (DIFF) scattergram and the possible presence of immature granulocytes (flag “G”) abnormal cells are suspected. *

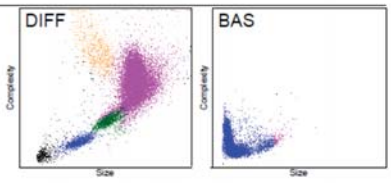
The RBC count is just below the normal range. The red cell (RBC) population is heterogeneous and the RBC histogram is widened, RDWsd and MCV are increased, HGB and RBC are decreased indicating macrocytic hypochromic anemia and anisocytosis (see interpretive flags). RDWcv is value is out of the upper limit of linearity range therefore not visible (see flag “ ** ”). Of note, the marked peak left of the RBC histogram suggests a significantly increased PLT count. The PLT histogram is normal in shape and RBC and PLT histograms are well separated.

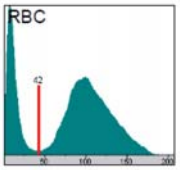
The absence of a definitive PLT count and warning flag 'O' and flag "**" in the presence of the well-defined population of thrombocytes indicates a PLT count above the upper limit of the linearity range of detection. Therefore, measurement should be repeated from a 2-fold, saline-diluted sample. Indeed, PLT count is around 1800 x 10^3/µL.

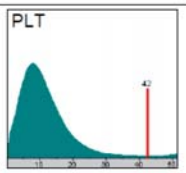
Result from the 2-fold diluted sample (with saline)

Sample ID Patient 13 Patient ID
Date 01/01/2013 Name
Mode Human Date of birth
Gender

Parameter	Result			Limit		
WBC	12.34	+	10^3/µL	5.00	-	10.00
NEU	9.63	+	10^3/µL	2.00	-	7.50
LYM	0.86	-	10^3/µL	1.08	-	3.17
MON	0.93	+	10^3/µL	0.15	-	0.70
EO	0.58	+	10^3/µL	0.00	-	0.50
BAS	0.35	++	10^3/µL	0.00	-	0.15
NEU%	78.0	+	%	40.0	-	75.0
LYM%	7.0	-	%	14.76	-	45.40
MON%	7.5	+	%	3.0	-	7.0
EO%	4.7		%	0.0	-	5.0
BAS%	2.8	+	%	0.0	-	1.5
RBC	1.81	--	10^6/µL	4.00	-	5.50
HGB	52	--	g/L	120	-	174
HCT	19.2	-	%	36.0	-	52.0
MCV	106.2	+	fL	76.0	-	96.0
MCH	28.5		pg	27.0	-	32.0
MCHC	269	-	g/L	300	-	350
RDWsd	76.5	+	fL	20.0	-	42.0
RDWcv	---	**	%	0.0	-	16.0
PLT	905	++	10^3/µL	150	-	400
PCT	0.89		%		-	
MPV	9.8		fL	8.0	-	15.0
PDWsd	20.0		fL		-	
PDWcv	33.8		%		-	





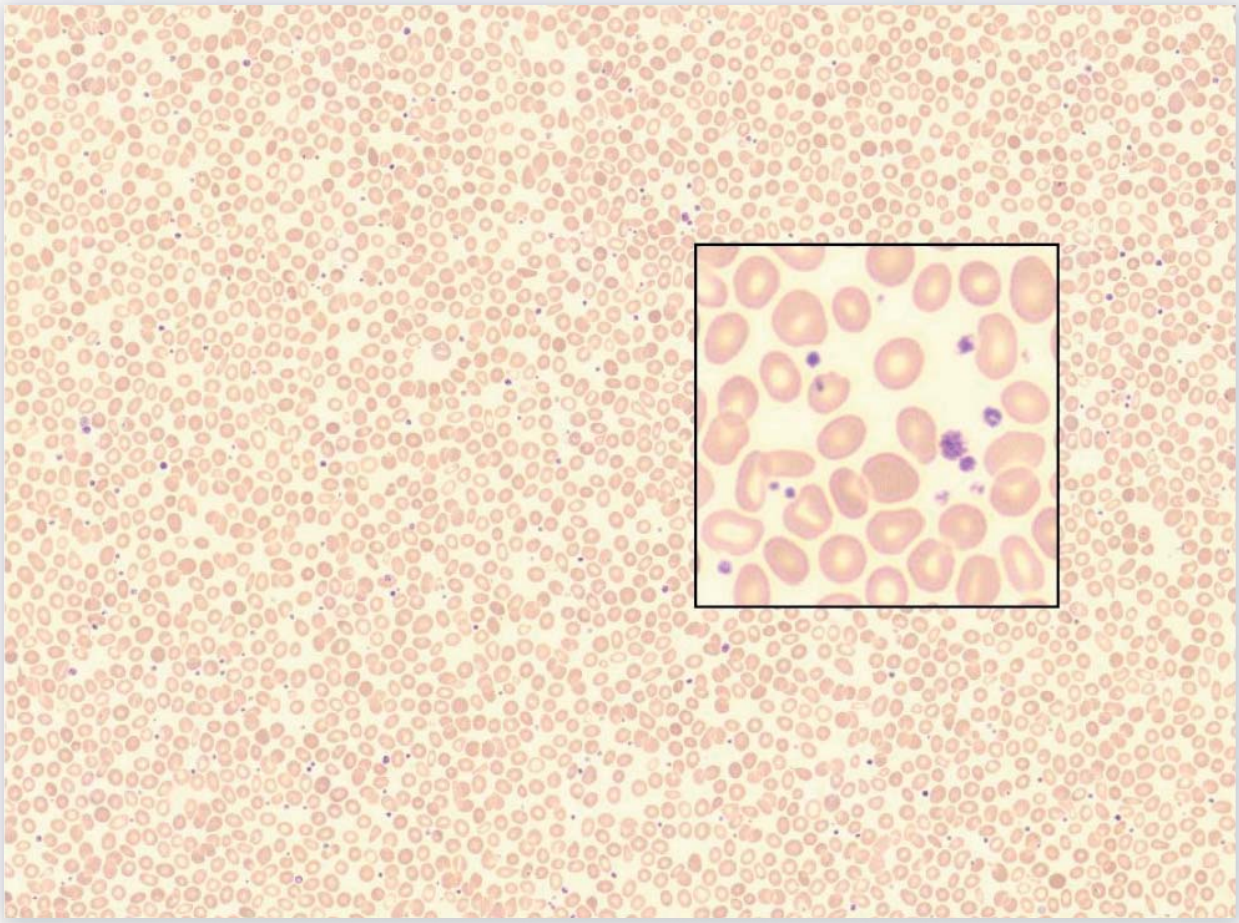


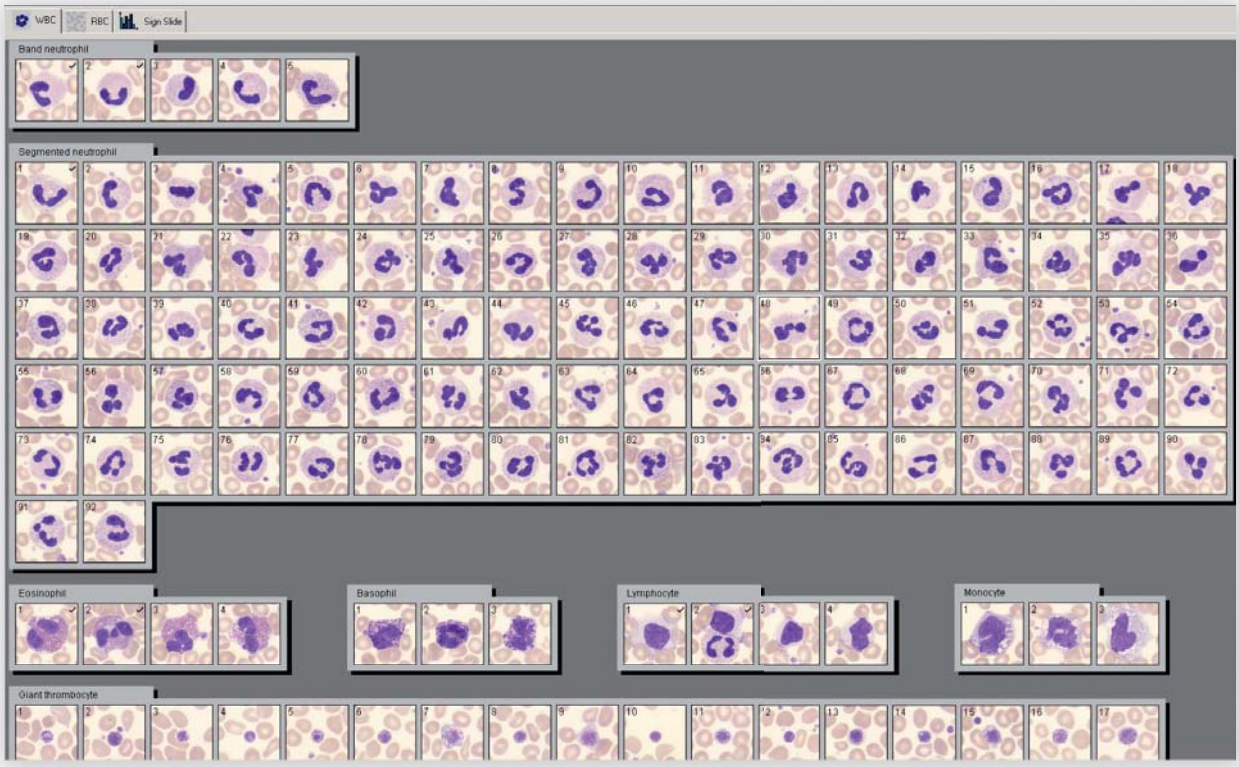
Warning flags
Morphological flags G
Interpretive flags Leukocytosis?, Basophilia?, Anemia?, Macrocytic RBC?, Hypochromic?, Thrombocytosis?

Please note that WBC and RBC results should be evaluated from the undiluted sample in this case.

The assessment of a peripheral blood smear is essential because of abnormal WBC DIFF scattergram and flag "G" indicating the possible presence of immature granulocytes.

Peripheral blood smear





The most remarkable finding in the peripheral blood smear is the presence of giant thrombocytes with thrombocythemia. As the patient is in remission, no other striking abnormality is visible. There is no monocytosis and no marked basophilia in the smear.*

*Due to their special response to lyzing effect abnormal and immature cells may falsely increase the analyzers monocyte, lymphocyte and basophil counts which is shown here if compared analyzer's results with those obtained by the microscopic evaluation of the smear.

Because of confluent WBC populations analyzer's neutrophil percentage significantly differs from that of the microscopic evaluation.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	5	4.5 ■ ✓
• Segmented neutrophil	92	82.9 ■ ✓
• Eosinophil	4	3.6 ■ ✓
• Basophil	3	2.7 ■ ✓
• Lymphocyte	4	3.6 ■ ✓
• Monocyte	3	2.7 ■ ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	111	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	17	- ■ ✓
• Thrombocyte aggregation	1	- ■ ✓
Megakaryocyte	-	-
• Smudge cell	1	- ■ ✓
• Artefact	1	- ■ ✓

Other laboratory findings

Other laboratory findings are not remarkable

Diagnosis

Chronic myeloid leukemia currently in remission with thrombocythemia

Disease course

The patient is registered for stem cell transplantation waiting list. A HLA-identical bone marrow donor is searched for.

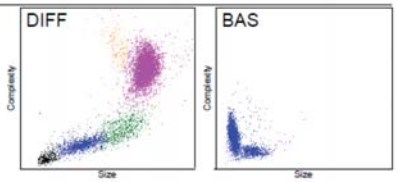
Case 14 | Thrombocytosis, anemia

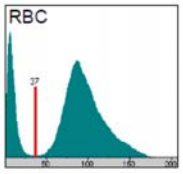
History and clinical signs and symptoms

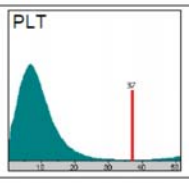
A 35 year old man was diagnosed with schizophrenia 10 years ago. He is also a known drug user.
He suffered multiple traumas in an accident.

Hematologic findings

Sample ID	Patient 14					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	13.08	+	$10^3/\mu L$	5.00	- 10.00		
NEU	9.78	+	$10^3/\mu L$	2.00	- 7.50		
LYM	2.04		$10^3/\mu L$	1.08	- 3.17		
MON	0.86	+	$10^3/\mu L$	0.15	- 0.70		
EO	0.31		$10^3/\mu L$	0.00	- 0.50		
BAS	0.08		$10^3/\mu L$	0.00	- 0.15		
NEU%	74.8		%	40.0	- 75.0		
LYM%	15.6		%	14.76	- 45.40		
MON%	6.6		%	3.0	- 7.0		
EO%	2.4		%	0.0	- 5.0		
BAS%	0.6		%	0.0	- 1.5		
RBC	3.48	-	$10^6/\mu L$	4.00	- 5.50		
HGB	100	-	g/L	120	- 174		
HCT	33.7	-	%	36.0	- 52.0		
MCV	96.9	+	fL	76.0	- 96.0		
MCH	28.7		pg	27.0	- 32.0		
MCHC	296	-	g/L	300	- 350		
RDWsd	55.2	+	fL	20.0	- 42.0		
RDWcv	17.4	+	%	0.0	- 16.0		
PLT	897	++	$10^3/\mu L$	150	- 400		
PCT	0.75		%		-		
MPV	8.3		fL	8.0	- 15.0		
PDWsd	25.1		fL		-		
PDWcv	38.0		%		-		







Warning flags

Morphological flags

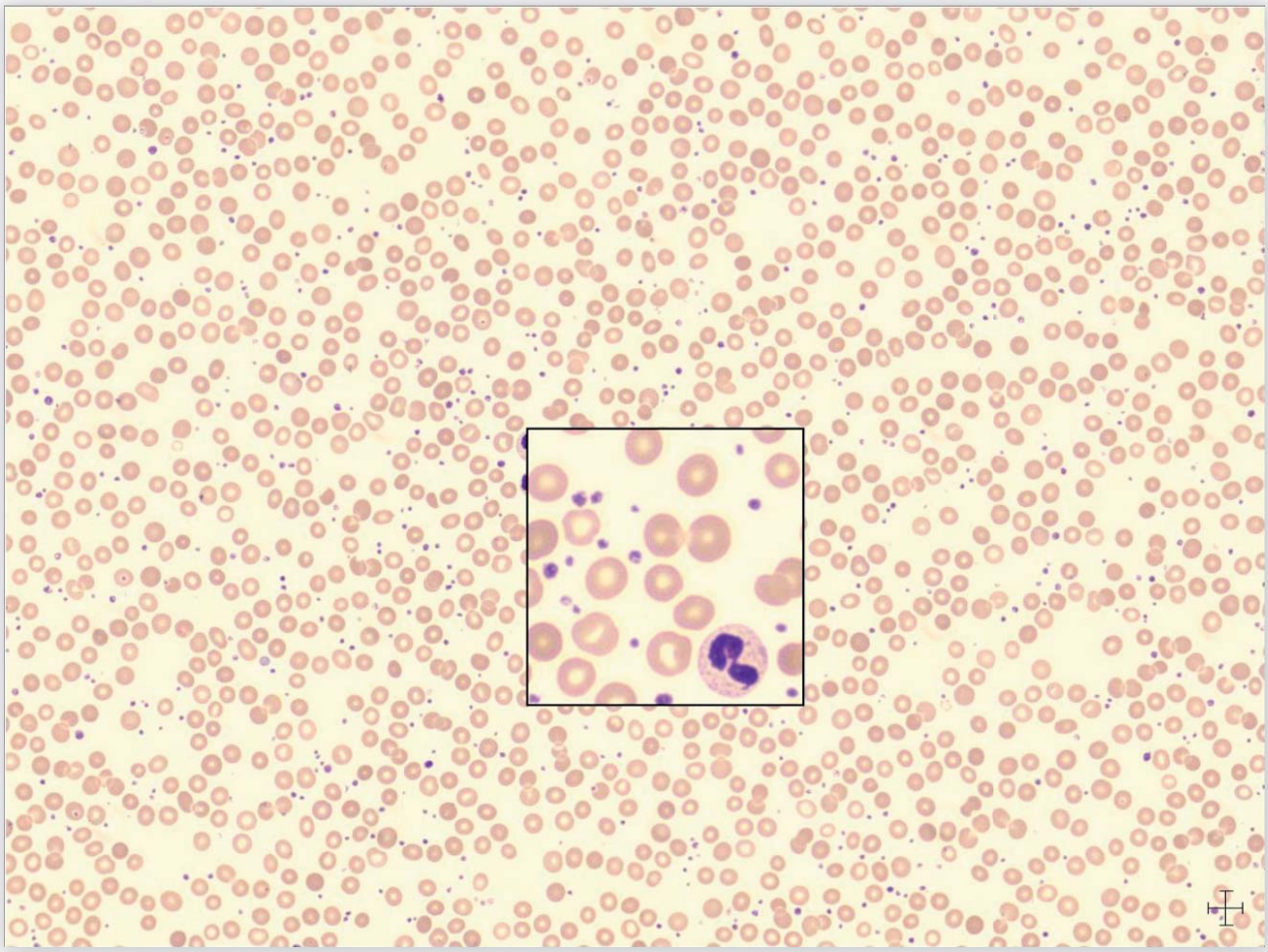
Interpretive flags: Leukocytosis?, Anemia?, Macrocytic RBC?, Hypochromic?, Anisocytosis?, Thrombocytosis?

Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC including monocyte, neutrophil, eosinophil and lymphocyte populations are well characterized. The WBC count is slightly increased (see interpretative flag “leukocytosis”).

The PLT and RBC histograms are readily distinguishable. The decreased RBC, HGB, HCT and MCHC with increased MCV and RDW parameters are indicative of mild a macrocytic, hypochromic anemia and anisocytosis (see interpretive flags). The markedly elevated peak left of the RBC histogram is consistent with the high PLT count. The PLT count is markedly higher than the upper limit of the normal range.

Peripheral blood smear



The peripheral blood smear clearly indicates the increased prevalence of platelets.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	80	74.1
• Eosinophil	7	6.5
• Basophil	-	-
• Lymphocyte	12	11.1
• Monocyte	9	8.3
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	4	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	11	-
• Artefact	4	-

Other laboratory findings

Other routine clinical laboratory results are consistent with systemic cell damage (with increased lactic dehydrogenase and creatine kinase values) and impaired hepatic function (high transaminase activities). CRP levels were elevated (45 mg/L [ref value: <5 mg/L]).

Diagnosis

Transient thrombocytosis due to unknown origin with a macrocytic hypochromic anemia.

Disease course

Multiple surgical procedures were performed to stabilize the patient's status. Efforts were made to maintain drug abstinence. Antipsychotic therapy was adjusted.

CHAPTER 3 | WBC ABNORMALITIES

3.1 Neutrophilia

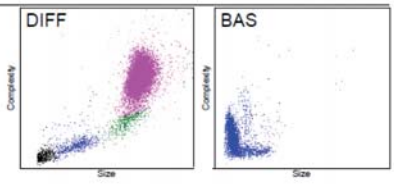
Case 15 | Infection (probable bacterial)

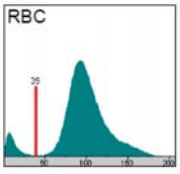
History and clinical signs and symptoms

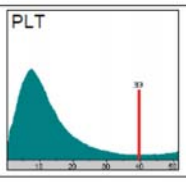
The 75 year old female patient with cancer presented at the hospital with symptoms of general sickness and unproductive cough.

Hematologic findings

Sample ID	Patient 15						Patient ID
Date	01/01/2013						Name
Mode	Human						Date of birth
Parameter	Result			Limit			Gender
WBC	28.00	++	$10^3/\mu L$	5.00	-	10.00	
NEU	26.18	++	$10^3/\mu L$	2.00	-	7.50	
LYM	1.23		$10^3/\mu L$	1.08	-	3.17	
MON	0.45		$10^3/\mu L$	0.15	-	0.70	
EO	0.06		$10^3/\mu L$	0.00	-	0.50	
BAS	0.08		$10^3/\mu L$	0.00	-	0.15	
NEU%	93.5	+	%	40.0	-	75.0	
LYM%	4.4	--	%	14.76	-	45.40	
MON%	1.6	-	%	3.0	-	7.0	
EO%	0.2		%	0.0	-	5.0	
BAS%	0.3		%	0.0	-	1.5	
RBC	3.62	-	$10^6/\mu L$	4.00	-	5.50	
HGB	115	-	g/L	120	-	174	
HCT	36.8		%	36.0	-	52.0	
MCV	101.8	+	fL	76.0	-	96.0	
MCH	31.8		pg	27.0	-	32.0	
MCHC	312		g/L	300	-	350	
RDWsd	46.6	+	fL	20.0	-	42.0	
RDWcv	14.3		%	0.0	-	16.0	
PLT	240		$10^3/\mu L$	150	-	400	
PCT	0.31		%		-		
MPV	12.9		fL	8.0	-	15.0	
PDWsd	30.7		fL		-		
PDWcv	37.6		%		-		







Warning flags	D
Morphological flags	
Interpretive flags	Leukocytosis?, Neutrophilia?, Anemia?, Macrocytic RBC?

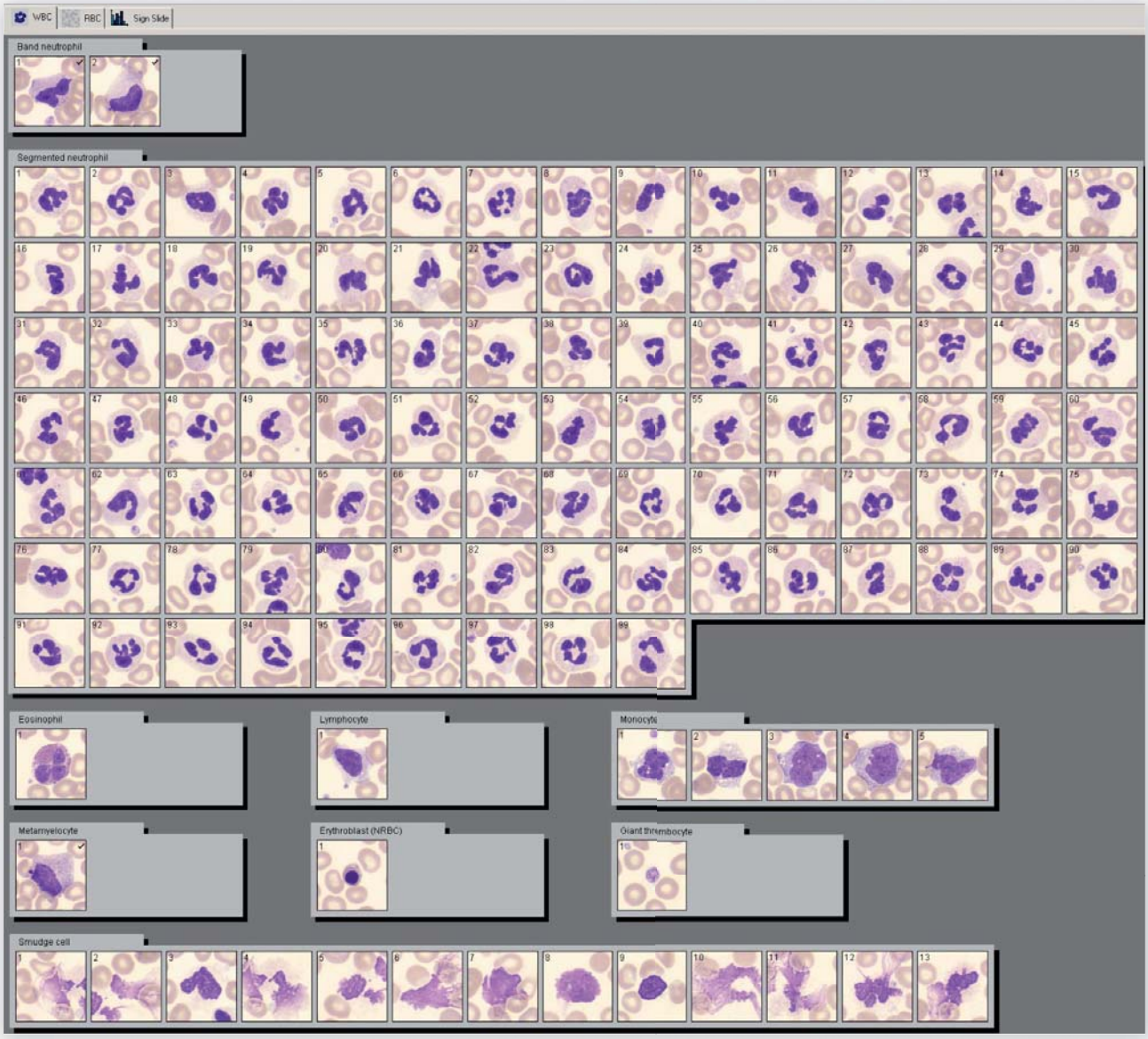
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are generally a normal pattern. The WBC DIFF scattergram presents a dominant neutrophil population among the leukocytes. However, a “D” flag indicates that there is some overlap between the population of less defined WBCs (monocytes) and the neutrophils. Leukocytosis is indicated by the elevated WBC count.

The PLT and RBC populations are well separated, histogram’s shapes are normal. An increased MCV, RDWsd, decreased HGB and RBC values show a mild macrocytic anemia.

Due to the warning “D” flag, the assessment of a peripheral blood smear is recommended.

Peripheral blood smear



There are some immature neutrophils; however, the overwhelming majority of the neutrophils are well segmented and present the morphological characteristics of mature cells including segmentation.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	80	74.1
• Eosinophil	7	6.5
• Basophil	-	-
• Lymphocyte	12	11.1
• Monocyte	9	8.3
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	4	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	11	-
• Artefact	4	-

Other laboratory findings

Other abnormal clinical laboratory results include elevated CRP levels (165 mg/L [ref.range: 0 -5 mg/L) and lower than normal iron levels (3.3 [6.6 – 26.0 µmol/L). Urinalysis excluded renal and urinary tract infection. A blood culture was not obtained as antibiotics were already administered. The findings of the physical exam and chest X-ray are characteristic for lobar pneumonia.

Diagnosis

Systemic sepsis and a mild macrocytic, normochromic anemia

Disease course

In spite of the extensive antimicrobial therapy, the patient's status deteriorated. The patient was transferred to the Intensive Care Unit, but she expired three days later.

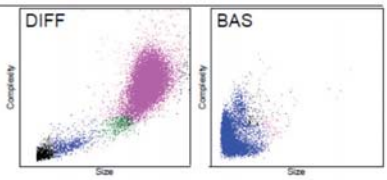
Case 16 | Severe neutrophilia caused by metastases in the bone marrow

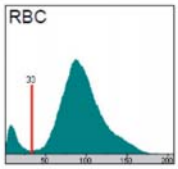
History and clinical signs and symptoms

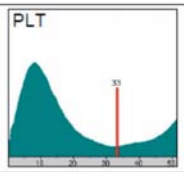
An 85 year old woman was admitted to the Department of Medicine with a terminal stage of lung cancer with multiple metastases.

Hematologic findings

Sample ID	Patient 16					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	68.54	++	$10^3/\mu L$	5.00	-	10.00	
NEU	64.91	++	$10^3/\mu L$	2.00	-	7.50	
LYM	2.40		$10^3/\mu L$	1.08	-	3.17	
MON	0.82	+	$10^3/\mu L$	0.15	-	0.70	
EO	0.07		$10^3/\mu L$	0.00	-	0.50	
BAS	0.34	++	$10^3/\mu L$	0.00	-	0.15	
NEU%	94.7	+	%	40.0	-	75.0	
LYM%	3.5	--	%	14.76	-	45.40	
MON%	1.2	--	%	3.0	-	7.0	
EO%	0.1		%	0.0	-	5.0	
BAS%	0.5		%	0.0	-	1.5	
RBC	5.00		$10^6/\mu L$	4.00	-	5.50	
HGB	151		g/L	120	-	174	
HCT	47.8		%	36.0	-	52.0	
MCV	95.7		fL	76.0	-	96.0	
MCH	30.3		pg	27.0	-	32.0	
MCHC	316		g/L	300	-	350	
RDWsd	63.3	+	fL	20.0	-	42.0	
RDWcv	20.0	+	%	0.0	-	16.0	
PLT	357		$10^3/\mu L$	150	-	400	
PCT	0.47		%		-		
MPV	13.1		fL	8.0	-	15.0	
PDWsd	32.8		fL		-		
PDWcv	37.2		%		-		







Warning flags	D
Morphological flags	G
Interpretive flags	Leukocytosis?, Neutrophilia?, Basophilia?, Anisocytosis?

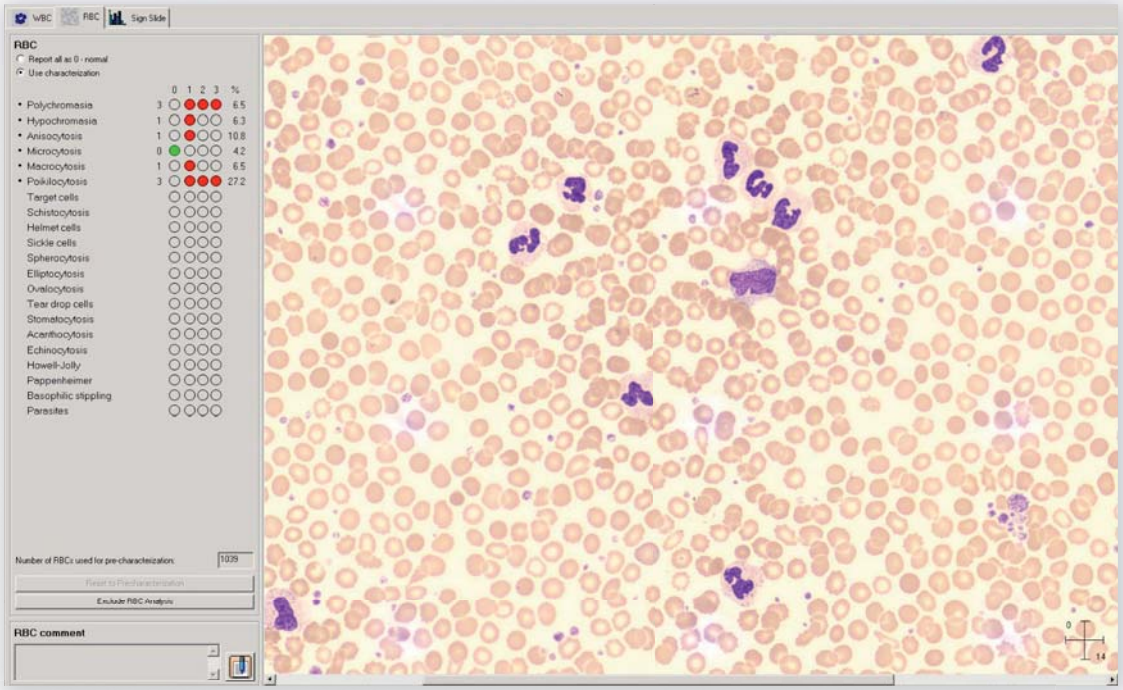
Interpretation

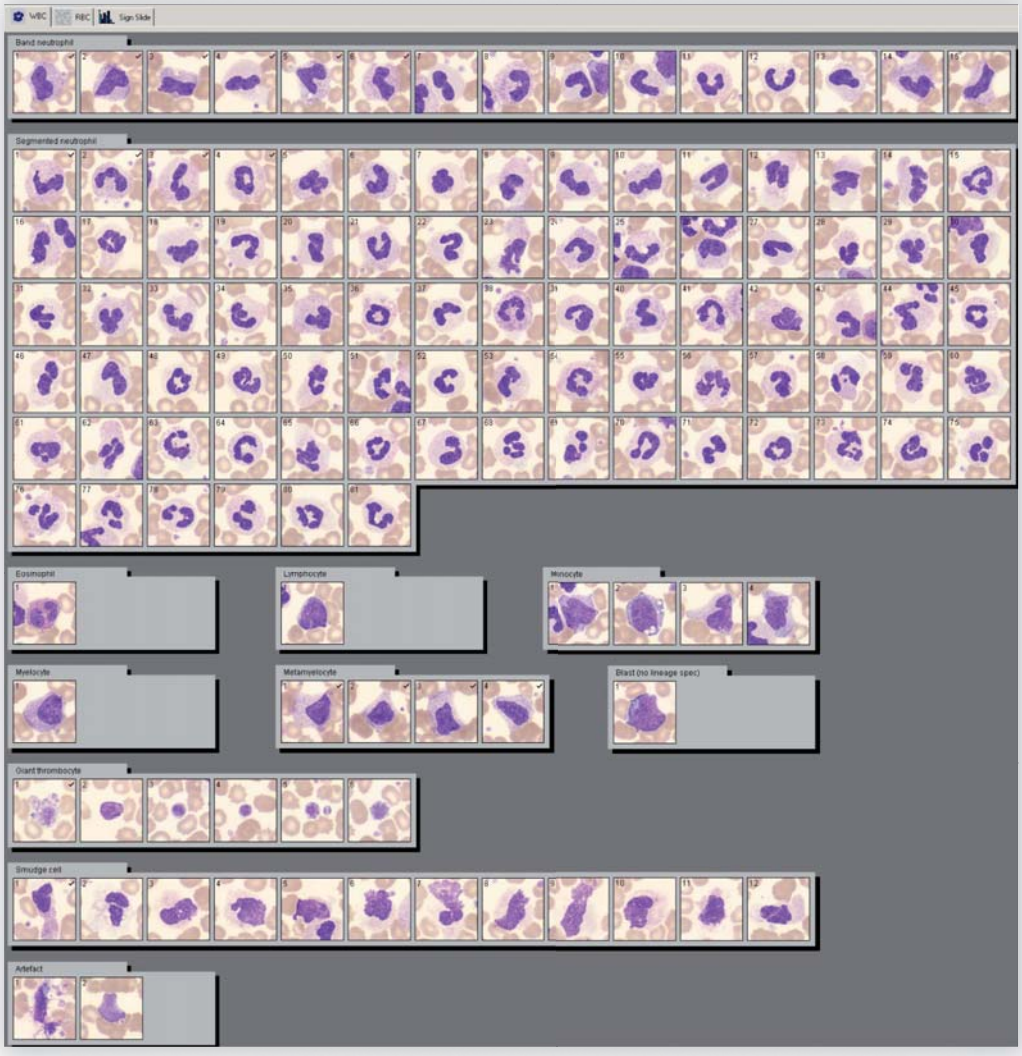
The WBC differential (DIFF) and basophil (BAS) scattergrams are not clearly differentiated. The monocyte and neutrophil populations are not clearly distinguishable (see warning flag “D”). The WBC count is markedly increased with a dominant neutrophil population (see interpretative flags “leukocytosis” and “neutrophilia”). The prevalence of immature cells is probably significant (see morphological flag “G”).

The RBC and PLT histograms are clearly separated and the red cell and platelet morphological parameters are within the normal range except for increased RDW values. The elevated RDW together with the abnormal shape of RBC histogram (widened right shoulder) can be explained by the presence of RBC agglutination.

Due to the warning and morphological flags, the assessment of a peripheral blood smear is strongly recommended.

Peripheral blood smear





The dominance of segmented neutrophils is clear. There are also immature granulocytes (band neutrophil cells) and different types of immature mononuclear cells.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	15	13.9
• Segmented neutrophil	81	75.0
• Eosinophil	1	0.9
• Basophil	-	-
• Lymphocyte	1	0.9
• Monocyte	4	3.7
• Promyelocyte	-	-
• Myelocyte	1	0.9
• Metamyelocyte	4	3.7
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	1	0.9
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	6	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	12	-
• Artefact	2	-

Other laboratory findings

Other abnormal clinical laboratory results included high CRP levels (150 mg/L (ref value: <5 mg/L), high liver enzyme values and low albumin levels. Kidney function was also severely impaired.

Diagnosis

Systemic cancer with bone marrow metastases.

Disease course

The patient's general status rapidly deteriorated. The patient expired a few days later probably due to complications of systemic cancer.

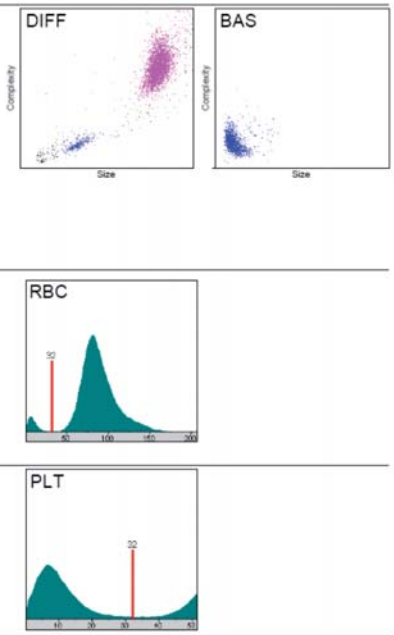
Case 17 | Sepsis with severe anemia

History and clinical signs and symptoms

Two days after cholecystectomy a 50 year old man experienced periodic chills and fever (39.7°C).

Hematologic findings

Sample ID			Patient 17				Patient ID
Date			01/01/2013				Name
Mode			Human				Date of birth
Parameter	Result			Limit			Gender
WBC	5.41		$10^3/\mu L$	5.00	-	10.00	
NEU	5.02		$10^3/\mu L$	2.00	-	7.50	
LYM	0.35	--	$10^3/\mu L$	1.08	-	3.17	
MON	0.02	--	$10^3/\mu L$	0.15	-	0.70	
EO	0.00		$10^3/\mu L$	0.00	-	0.50	
BAS	0.03		$10^3/\mu L$	0.00	-	0.15	
NEU%	92.7	+	%	40.0	-	75.0	
LYM%	6.4	--	%	14.76	-	45.40	
MON%	0.3	--	%	3.0	-	7.0	
EO%	0.0		%	0.0	-	5.0	
BAS%	0.6		%	0.0	-	1.5	
RBC	2.85	-	$10^6/\mu L$	4.00	-	5.50	
HGB	76	-	g/L	120	-	174	
HCT	25.5	-	%	36.0	-	52.0	
MCV	89.4		fL	76.0	-	96.0	
MCH	26.8	-	pg	27.0	-	32.0	
MCHC	300		g/L	300	-	350	
RDWsd	37.7		fL	20.0	-	42.0	
RDWcv	14.7		%	0.0	-	16.0	
PLT	112	-	$10^3/\mu L$	150	-	400	
PCT	0.10		%		-		
MPV	8.9		fL	8.0	-	15.0	
PDWsd	25.7		fL		-		
PDWcv	38.0		%		-		
Warning flags							
Morphological flags							
Interpretive flags	Lymphopenia?, Anemia?						



Interpretation

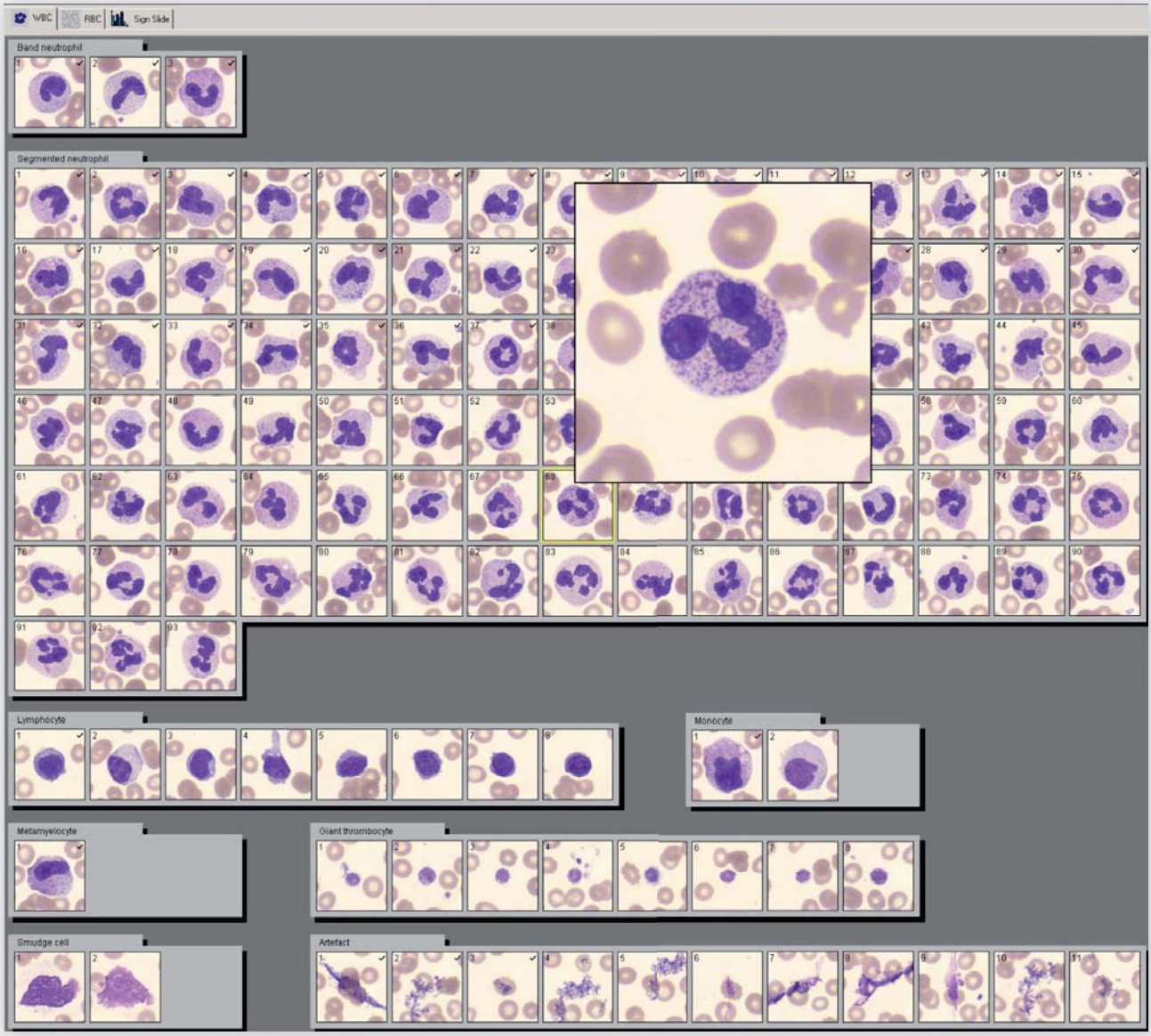
The WBC DIFF and BAS scattergrams are clearly defined. The WBC differential populations are well characterized. The WBC count is within the normal reference range. The overwhelming majority of WBCs are neutrophils. There is also an absolute and relative lymphopenia.

The RBC and PLT populations are clearly separated and the histograms have a normal shape. Both RBC and PLT counts are below the normal range. A low HGB and HCT are present without major abnormalities in the RBC characteristics.

The hematologic findings are characteristic for severe bacterial infection or sepsis (please, note that sepsis often occurs without an elevated WBC count). Blood loss due to a recent surgery and extensive intravenous fluid therapy may contribute to the low RBC count.

Because of abnormal numerical results the evaluation of a peripheral blood smear is strongly recommended.

Peripheral blood smear



The segmented neutrophils present the characteristic features of activation. Toxic granulation can be observed in cell's cytoplasm.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	3	2.8
• Segmented neutrophil	93	86.1
• Eosinophil	-	-
• Basophil	1	0.9
• Lymphocyte	8	7.4
• Monocyte	2	1.9
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	1	0.9
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	9	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	2	-
• Artefact	11	-

Other laboratory findings

Other abnormal clinical laboratory results include high CRP and pro-calcitonin levels (170 [<5] mg/L and 16 [<0.5]ng/mL). A blood culture identified the presence of E.coli.

Diagnosis

Septicemia due to Bacterial infection with severe anemia

Disease course

Targeted antibiotic treatment resolved the sepsis. In a few days the temperature normalized and the CBC count tended to return to the normal range.

Case 18 | Leukocytosis, neutrophilia, reactive thrombocytosis, pneumonia

History and clinical signs and symptoms

Three days following a Cesarean section a 35 year old woman experienced chills and high fever (39.3 °C). Her general status quickly deteriorated. Physical exam revealed tenderness of the uterus.

Hematologic findings

Sample ID	Patient 18						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	24.46	++	$10^3/\mu L$	5.00	-	10.00	DIFF	BAS
NEU	19.13	++	$10^3/\mu L$	2.00	-	7.50		
LYM	2.67		$10^3/\mu L$	1.08	-	3.17		
MON	1.91	+	$10^3/\mu L$	0.15	-	0.70		
EO	0.46		$10^3/\mu L$	0.00	-	0.50		
BAS	0.29	+	$10^3/\mu L$	0.00	-	0.15		
NEU%	78.2	+	%	40.0	-	75.0		
LYM%	10.9	-	%	14.76	-	45.40		
MON%	7.8		%	3.0	-	7.0		
EO%	1.9		%	0.0	-	5.0		
BAS%	1.2		%	0.0	-	1.5		
RBC	4.08		$10^6/\mu L$	4.00	-	5.50	RBC	
HGB	137		g/L	120	-	174		
HCT	40.6		%	36.0	-	52.0		
MCV	99.7	+	fL	76.0	-	96.0		
MCH	33.5	+	pg	27.0	-	32.0		
MCHC	336		g/L	300	-	350		
RDWsd	47.2		fL	20.0	-	42.0		
RDWcv	14.9		%	0.0	-	16.0		
PLT	593	+	$10^3/\mu L$	150	-	400	PLT	
PCT	0.49		%		-			
MPV	8.2		fL	8.0	-	15.0		
PDWsd	22.3		fL		-			
PDWcv	38.1		%		-			
Warning flags								
Morphological flags	G							
Interpretive flags	Leukocytosis?, Neutrophilia?, Macrocytic RBC?, Thrombocytosis?							

Interpretation

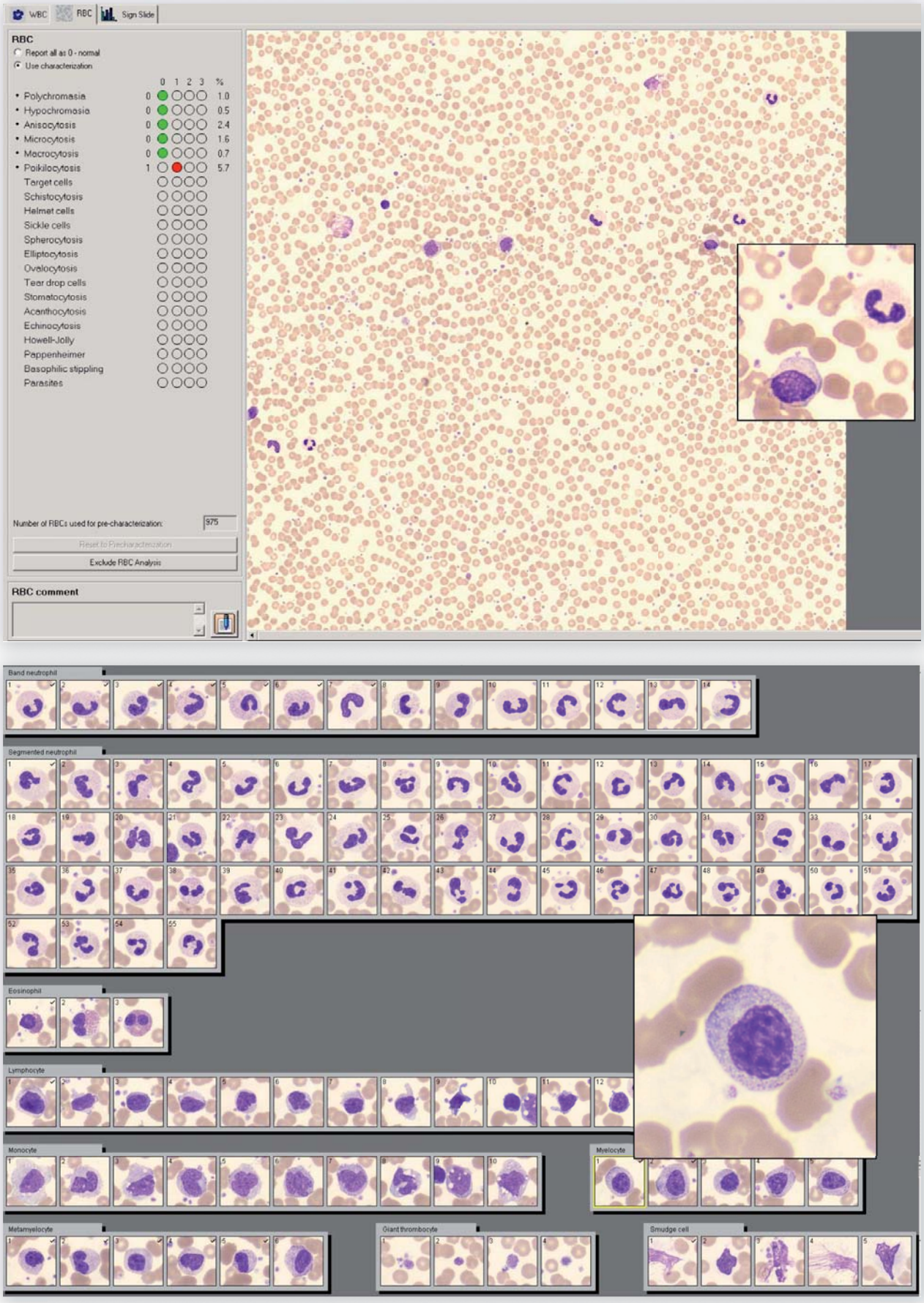
The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The leukocyte subpopulations are well separated. The WBC count exceeds markedly the upper limit of normal (see interpretative flag “leukocytosis”). The overwhelming majority of the WBCs are neutrophils (see interpretative flag “neutrophilia”). Also, except for the elevated monocyte count, the absolute number of the other leukocyte subpopulations is within the normal range. The morphological flag “G” suggests some immature granulocytes.

The RBC and PLT populations are clearly separated. The RBC and PLT histograms size distribution are normal. The red cell count is within the normal range. The red cell, MCV however, is somewhat elevated, see interpretative flag “macrocytic RBC”); however in this case this finding is of no clinical relevance.

The PLT count exceeds the upper limit of the normal range (see interpretative flag ‘thrombocytosis’).

Due to the well characterized blood cell populations and the absence of warning flags no additional information from the peripheral blood smear is anticipated.

Peripheral blood smear



The peripheral blood smear is consistent with the results of the automated CBC count.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	14	13.0
• Segmented neutrophil	55	50.9 ✓
• Eosinophil	3	2.8
• Basophil	-	-
• Lymphocyte	15	13.9
• Monocyte	10	9.3
• Promyelocyte	-	-
• Myelocyte	5	4.6
• Metamyelocyte	6	5.6
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	4	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	5	-
• Artefact	4	-

Other laboratory findings

The CRP level exceeded 20-fold the upper limit of the normal range (102 mg/L [ref range: <5 mg/L). The chest X-ray revealed diffuse opacity over the lungs. No other abnormal clinical laboratory results were detected. No blood culture was obtained.

Diagnosis

Pneumonia

Disease course

The patient's symptoms dramatically improved after one week of antibiotic therapy.

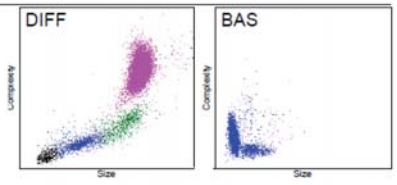
Case 19 | Leukocytosis, neutrophilia, pneumonia

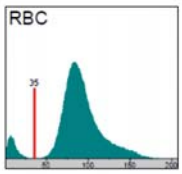
History and clinical signs and symptoms

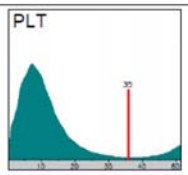
A 50 year old woman contacted the physician due to a high fever (38.8 °C), and cough. Her physical exam revealed rales and crackles over the right lung upon examination. She also complained about dyspnea and fatigue.

Hematologic findings

Sample ID	Patient 19					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	21.91	+	$10^3/\mu L$	5.00	- 10.00		
NEU	18.21	++	$10^3/\mu L$	2.00	- 7.50		
LYM	2.32		$10^3/\mu L$	1.08	- 3.17		
MON	1.23	+	$10^3/\mu L$	0.15	- 0.70		
EO	0.02		$10^3/\mu L$	0.00	- 0.50		
BAS	0.13		$10^3/\mu L$	0.00	- 0.15		
NEU%	83.1	+	%	40.0	- 75.0		
LYM%	10.6	-	%	14.76	- 45.40		
MON%	5.6		%	3.0	- 7.0		
EO%	0.1		%	0.0	- 5.0		
BAS%	0.6		%	0.0	- 1.5		
RBC	4.42		$10^6/\mu L$	4.00	- 5.50		
HGB	139		g/L	120	- 174		
HCT	41.3		%	36.0	- 52.0		
MCV	93.4		fL	76.0	- 96.0		
MCH	31.5		pg	27.0	- 32.0		
MCHC	337		g/L	300	- 350		
RDWsd	39.0		fL	20.0	- 42.0		
RDWcv	14.5		%	0.0	- 16.0		
PLT	268		$10^3/\mu L$	150	- 400		
PCT	0.22		%		-		
MPV	8.1		fL	8.0	- 15.0		
PDWsd	23.2		fL		-		
PDWcv	38.2		%		-		







Warning flags

Morphological flags

Interpretive flags Leukocytosis?, Neutrophilia?

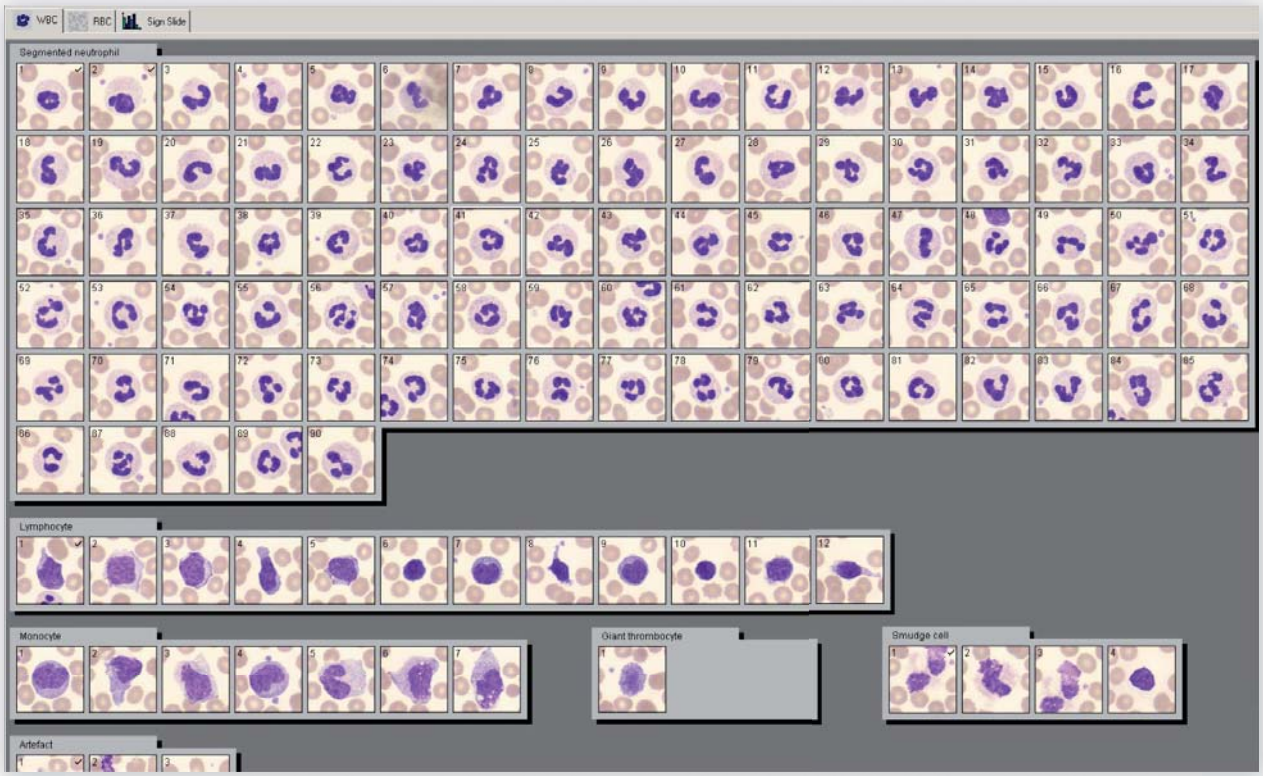
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are well defined. The WBC sub-populations are well separated. The WBC count exceeds markedly the upper limit of the normal range (see interpretative flag "leukocytosis"). The overwhelming majority of the WBCs are neutrophils (see interpretative flag "neutrophilia"). The absolute number of the other leukocyte subpopulations are within the normal range.

The RBC and PLT histograms are clearly distinguished. The shape and size distribution of RBC and PLT histograms are normal. The red cell and platelet morphology is within the normal range.

Due to the well characterized blood cell populations and the absence of warning flags no additional information from the peripheral blood smear is anticipated.

Peripheral blood smear



The peripheral blood smear is consistent with the results of automated CBC measurement.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	90	82.6
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	12	11.0
• Monocyte	7	6.4
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	109	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	1	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	4	-
• Artefact	3	-

Other laboratory findings

CRP levels exceeded 20-fold the upper limit of normal (102 mg/L [ref range: <5 mg/L). Chest X-ray revealed diffuse opacity over the right lung. Other laboratory markers were normal.

Diagnosis

Pneumonia with neutrophilia

Disease course

The patient's symptoms dramatically improved after a week of antibiotic course.

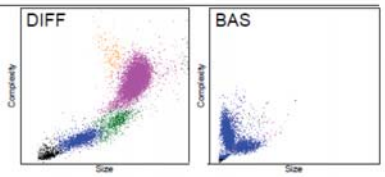
Case 20 | Neonatal infection

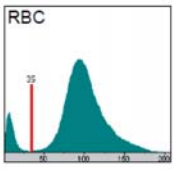
History and clinical signs and symptoms

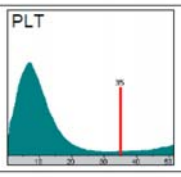
Following a vaginal birth, a term male neonate presented with the early signs of disturbed post-natal adaptation during his first post-natal day. Early onset of sepsis was suspected.

Hematologic findings

Sample ID	Patient 20					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	22.95	++	$10^3/\mu L$	5.00	- 10.00		
NEU	16.43	++	$10^3/\mu L$	2.00	- 7.50		
LYM	4.73	+	$10^3/\mu L$	1.08	- 3.17		
MON	1.29	+	$10^3/\mu L$	0.15	- 0.70		
EO	0.44		$10^3/\mu L$	0.00	- 0.50		
BAS	0.07		$10^3/\mu L$	0.00	- 0.15		
NEU%	71.6		%	40.0	- 75.0		
LYM%	20.6		%	14.76	- 45.40		
MON%	5.6		%	3.0	- 7.0		
EO%	1.9		%	0.0	- 5.0		
BAS%	0.3		%	0.0	- 1.5		
RBC	4.32		$10^6/\mu L$	4.00	- 5.50		
HGB	147		g/L	120	- 174		
HCT	44.0		%	36.0	- 52.0		
MCV	102.0	+	fL	76.0	- 96.0		
MCH	34.0	+	pg	27.0	- 32.0		
MCHC	334		g/L	300	- 350		
RDWsd	54.3	+	fL	20.0	- 42.0		
RDWcv	16.1	+	%	0.0	- 16.0		
PLT	353		$10^3/\mu L$	150	- 400		
PCT	0.30		%		-		
MPV	8.5		fL	8.0	- 15.0		
PDWsd	24.2		fL		-		
PDWcv	38.0		%		-		







Warning flags	
Morphological flags	G
Interpretive flags	Leukocytosis?, Neutrophilia?, Macrocytic RBC?, Anisocytosis?

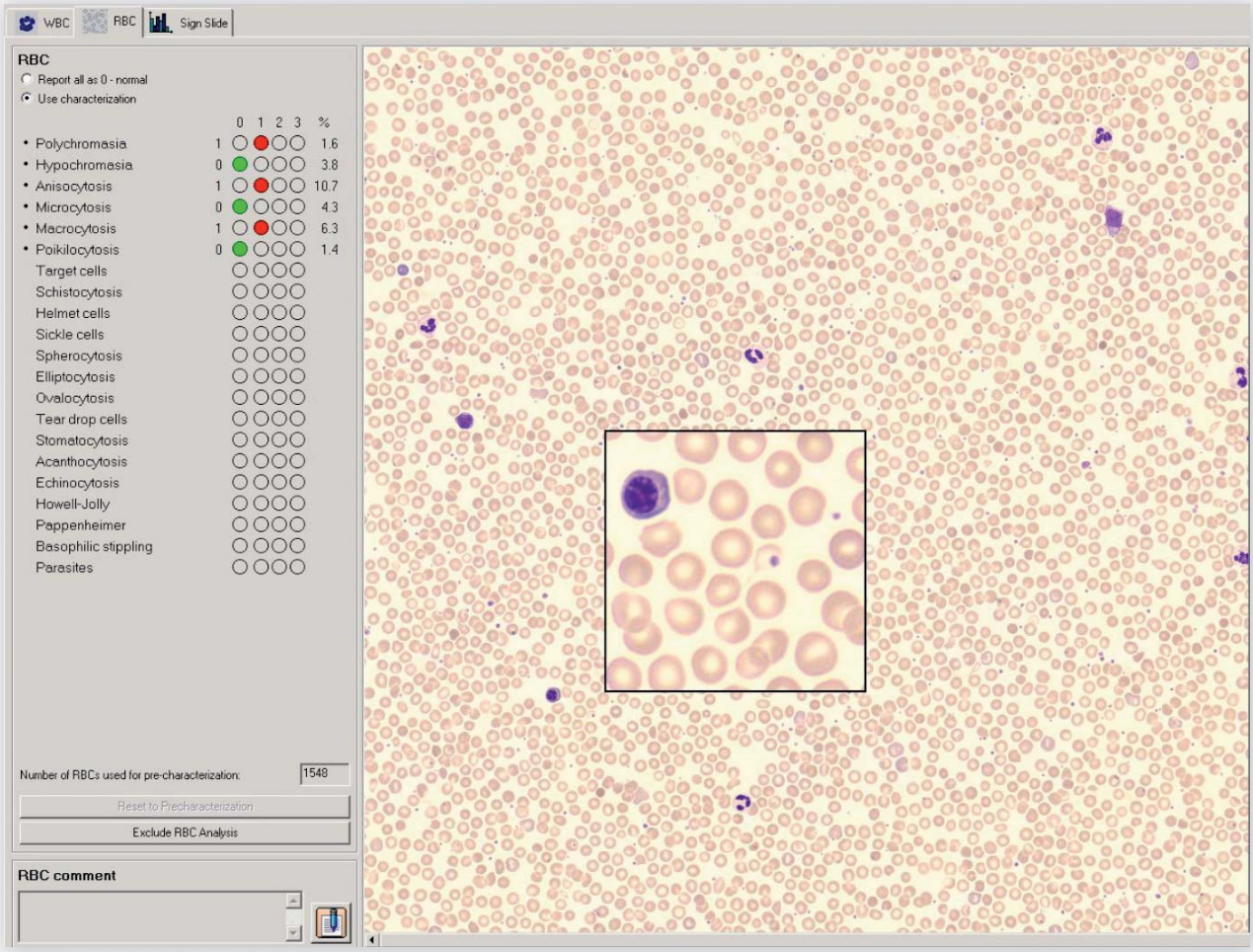
Interpretation

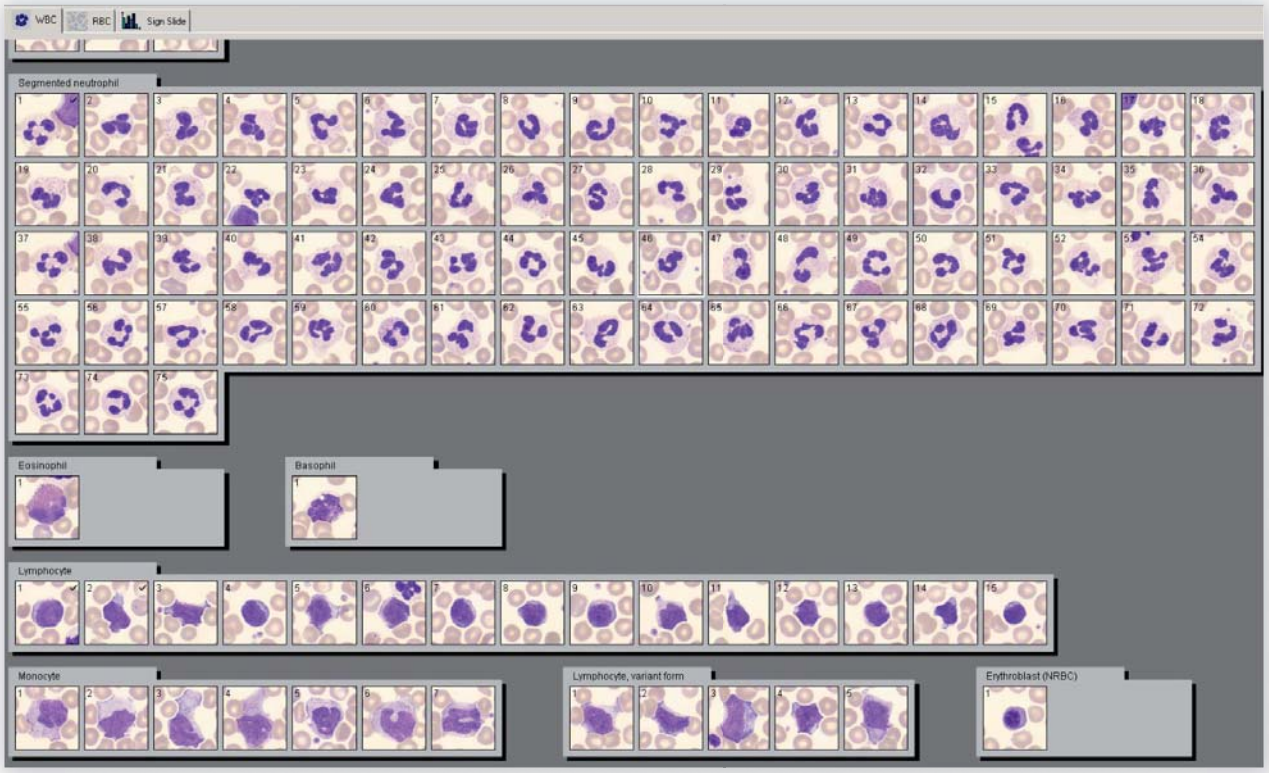
The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC count is increased with a dominant neutrophil population (see interpretative flags “leukocytosis” and “neutrophilia”). A flag „G” indicates that some immature cells may be present. The monocyte count is increased. Elevated lymphocyte count is a physiologic finding in neonates.

The RBC and PLT populations are clearly separated. The RBC and PLT histograms are normal in shape and distribution. Increased RDW values indicate anisocytosis (see flag „anisocytosis”). The MCV and MCH values are slightly more elevated than the indicated adult reference range but are appropriate for a term neonate (see interpretative flag “macrocytic RBC”).

Due to the elevated WBC count and the morphological Flag „G” the assessment of a peripheral blood smear is recommended.

Peripheral blood smear

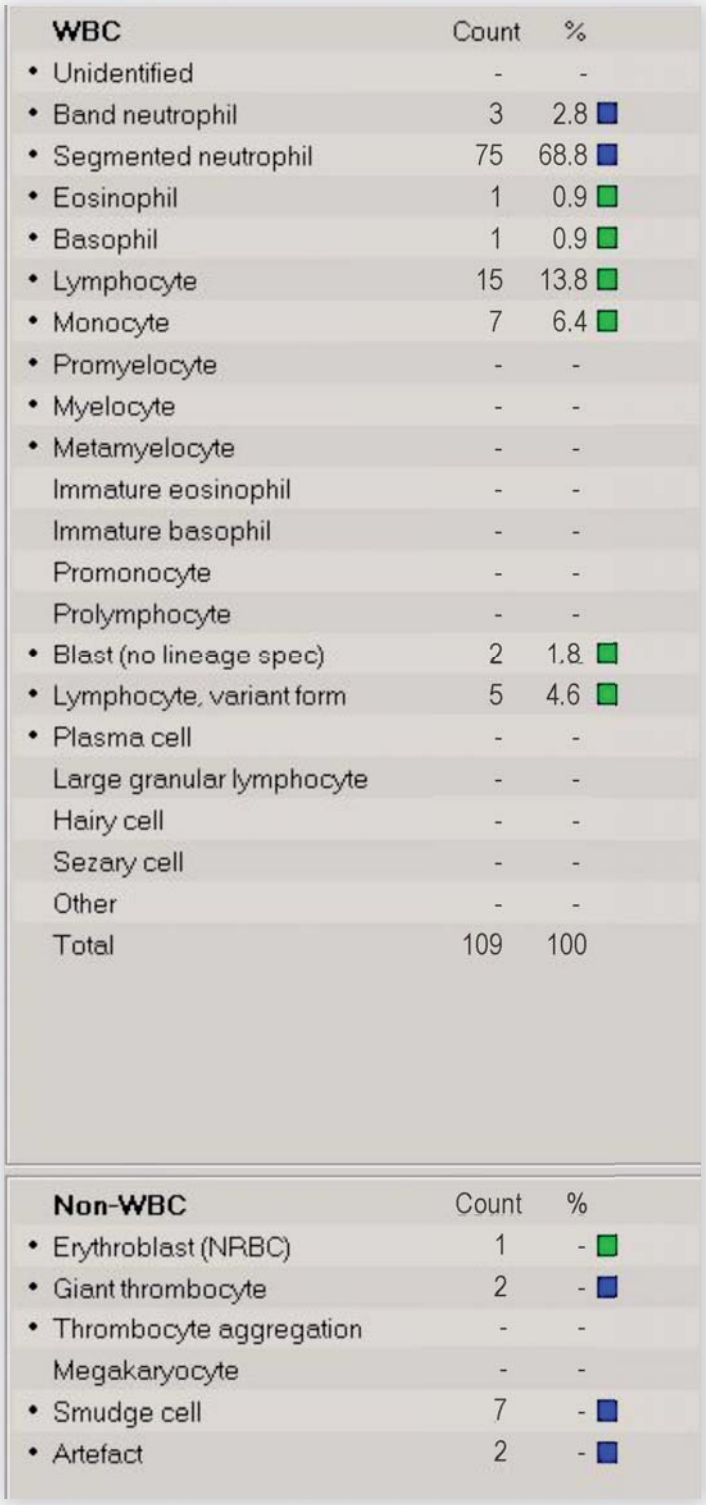




There are some immature neutrophils, however, the overwhelming majority of neutrophils are well segmented and correspond to a mature neutrophil population.

A few nucleated red cells were also observed.*

*Note: During measurement, nucleated red blood cells are resistant to the lyzing procedure and are therefore, falsely classified to the WBC population resulting in an elevated count. Like other similar systems, the HumaCount 5L is unable to reliability distinguish blasts, immature and abnormal cells, however as it gives a "G" morphological flag it indicates that a microscopic evaluation of smear should be performed. Otherwise, independent of the presence of flags the microscopic evaluation of the smear is always strongly recommended if any abnormal results (e.g. neutrophilia) can be seen.



WBC	Count	%
• Unidentified	-	-
• Band neutrophil	3	2.8
• Segmented neutrophil	75	68.8
• Eosinophil	1	0.9
• Basophil	1	0.9
• Lymphocyte	15	13.8
• Monocyte	7	6.4
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	2	1.8
• Lymphocyte, variant form	5	4.6
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	109	100

Non-WBC	Count	%
• Erythroblast (NRBC)	1	-
• Giant thrombocyte	2	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	7	-
• Artefact	2	-

Other laboratory findings

Acute phase reactants did not support a systemic infection. CRP and pro-calcitonin levels were almost normal, 6 mg/L and 0.7 ng/L, respectively, however, the sensitivity of these parameters in a neonate is low. Maternal vaginal culture indicated the vaginal colonization of Group B Streptococcus bacteria.

Diagnosis

Early detection of a systemic neonatal infection

Disease course

The infant was immediately transferred to the intensive care unit. Broad-spectrum antibiotic therapy was initiated. The infant's status stabilized and he was discharged a few days later.

Chapter 3 | WBC ABNORMALITIES

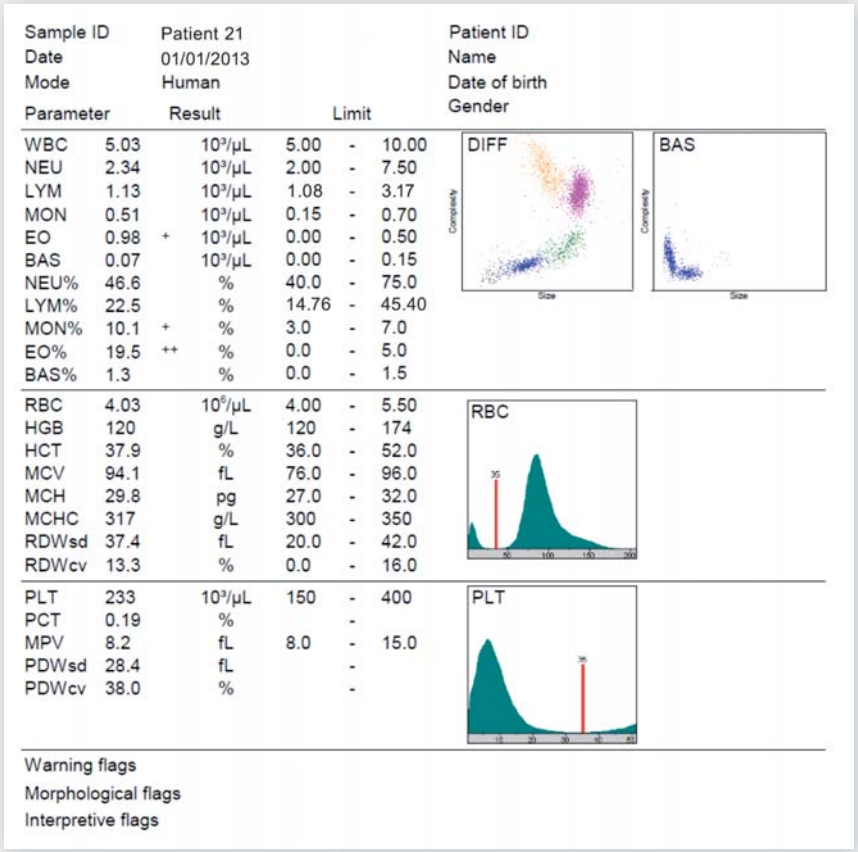
3.2 Eosinophilia

Case 21 | Eosinophilia, carcinoid syndrome

History and clinical signs and symptoms

A 65 year old woman was subjected to surgery in order to remove multiple metastases of a gastrointestinal tumor.

Hematologic findings



Sample ID	Patient 21					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	5.03		$10^3/\mu L$	5.00	-	10.00	
NEU	2.34		$10^3/\mu L$	2.00	-	7.50	
LYM	1.13		$10^3/\mu L$	1.08	-	3.17	
MON	0.51		$10^3/\mu L$	0.15	-	0.70	
EO	0.98	+	$10^3/\mu L$	0.00	-	0.50	
BAS	0.07		$10^3/\mu L$	0.00	-	0.15	
NEU%	46.6		%	40.0	-	75.0	
LYM%	22.5		%	14.76	-	45.40	
MON%	10.1	+	%	3.0	-	7.0	
EO%	19.5	++	%	0.0	-	5.0	
BAS%	1.3		%	0.0	-	1.5	
RBC	4.03		$10^6/\mu L$	4.00	-	5.50	
HGB	120		g/L	120	-	174	
HCT	37.9		%	36.0	-	52.0	
MCV	94.1		fL	76.0	-	96.0	
MCH	29.8		pg	27.0	-	32.0	
MCHC	317		g/L	300	-	350	
RDWsd	37.4		fL	20.0	-	42.0	
RDWcv	13.3		%	0.0	-	16.0	
PLT	233		$10^3/\mu L$	150	-	400	
PCT	0.19		%		-		
MPV	8.2		fL	8.0	-	15.0	
PDWsd	28.4		fL		-		
PDWcv	38.0		%		-		

Warning flags

Morphological flags

Interpretive flags

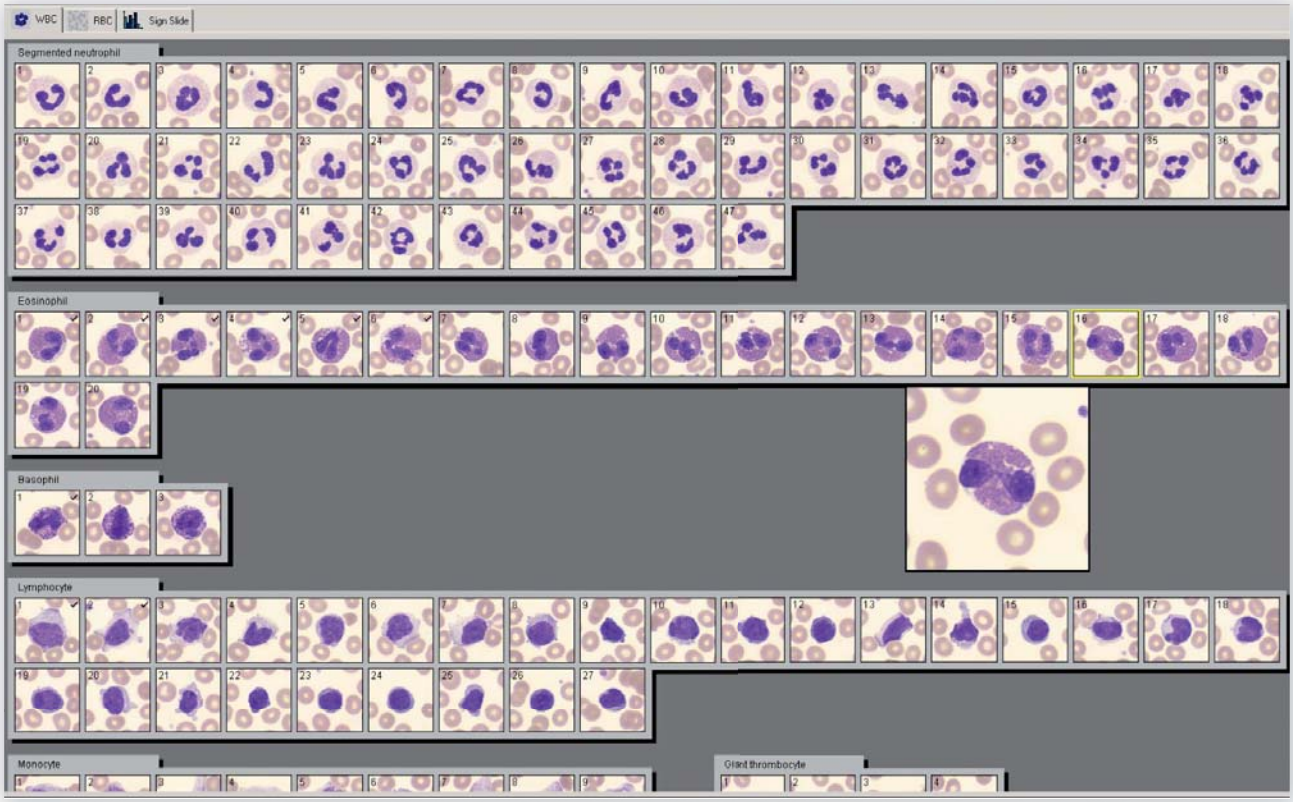
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC count is within the normal range. However, within the WBC population the eosinophils are markedly increased. Please note, that interpretive flag “eosinophilia” only appears if EO count is more than double of the high limit of normal range (normal range flag “++”). The different subpopulations of neutrophils, lymphocytes, monocytes and eosinophils (NEU, LYM, MON and EO, respectively) are well characterized.

The RBC and PLT populations are well separated. The morphological characteristics are not unusual.

Due to the absence of warning and morphological flags the visual assessment of a peripheral blood smear probably does not provide any additional information.

Peripheral blood smear



The peripheral blood smear validates the highly prevalent eosinophils.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	47	44.3 ✓
• Eosinophil	20	18.9
• Basophil	3	2.8
• Lymphocyte	27	25.5
• Monocyte	9	8.5
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	106	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	4	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	7	-
• Artefact	9	-

Other laboratory findings

Serum levels of chromogranin A, the marker of carcinoid tumors markedly exceeded the upper limit of normal.

Diagnosis

Eosinophilia in carcinoid syndrome

Disease course

Chromogranin A levels dropped dramatically after the surgery. One month later the eosinophil count was in the normal range.

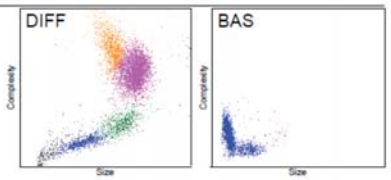
Case 22 | Eosinophilia, asthma

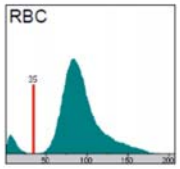
History and clinical signs and symptoms

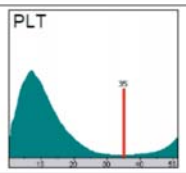
A 38 year old man with known asthma contacted his family physician due to severe dyspnea and wheezing.

Hematologic findings

Sample ID	Patient 22					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	9.10		$10^3/\mu L$	5.00	-	10.00	
NEU	5.21		$10^3/\mu L$	2.00	-	7.50	
LYM	1.13		$10^3/\mu L$	1.08	-	3.17	
MON	0.72	+	$10^3/\mu L$	0.15	-	0.70	
EO	1.92	++	$10^3/\mu L$	0.00	-	0.50	
BAS	0.12		$10^3/\mu L$	0.00	-	0.15	
NEU%	57.3		%	40.0	-	75.0	
LYM%	12.4	-	%	14.76	-	45.40	
MON%	7.9	+	%	3.0	-	7.0	
EO%	21.1	++	%	0.0	-	5.0	
BAS%	1.3		%	0.0	-	1.5	
RBC	4.14		$10^6/\mu L$	4.00	-	5.50	
HGB	123		g/L	120	-	174	
HCT	38.9		%	36.0	-	52.0	
MCV	93.9		fL	76.0	-	96.0	
MCH	29.7		pg	27.0	-	32.0	
MCHC	316		g/L	300	-	350	
RDWsd	44.1	+	fL	20.0	-	42.0	
RDWcv	15.6		%	0.0	-	16.0	
PLT	210		$10^3/\mu L$	150	-	400	
PCT	0.20		%		-		
MPV	9.6		fL	8.0	-	15.0	
PDWsd	28.2		fL		-		
PDWcv	38.0		%		-		







Warning flags

Morphological flags

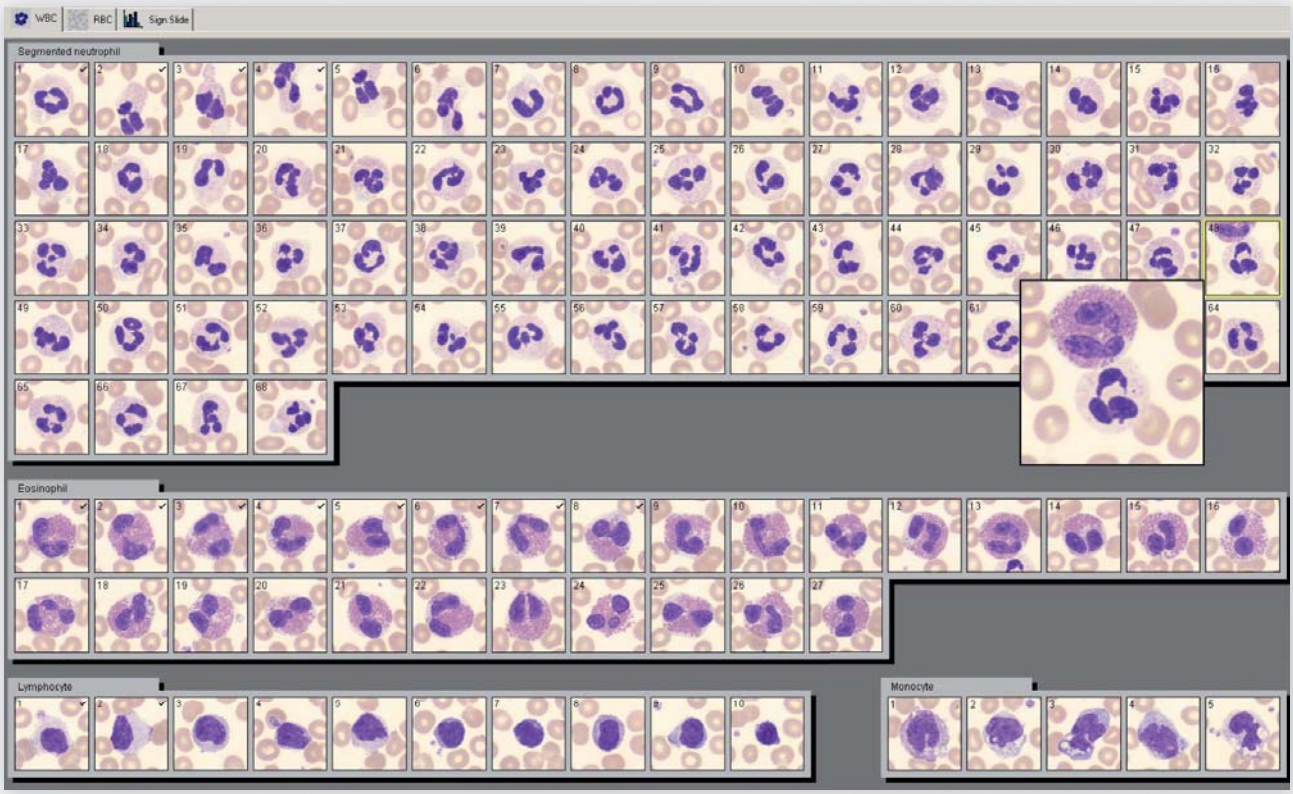
Interpretive flags Eosinophilia?

Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The WBC count is normal. The different subpopulations of neutrophils, lymphocytes, monocytes and eosinophils are well defined. However, within the WBC population the prevalence of eosinophils is markedly elevated (see also interpretative flag “eosinophilia”). The slightly elevated monocyte number and percentage are of no clinical relevance.

The PLT and RBC histograms are clearly separated and their shapes are normal.

Peripheral blood smear



The substantially higher than normal prevalence of eosinophils is the finding on this blood smear.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	68	61.8 ✓
• Eosinophil	27	24.5
• Basophil	-	-
• Lymphocyte	10	9.1
• Monocyte	5	4.5
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	110	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	10	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	7	-
• Artefact	2	-

Other laboratory findings

Other routine clinical chemistry results are not abnormal. Physical findings and lung function are characteristic for moderate asthma.

Diagnosis

Eosinophilia associated with allergic asthma.

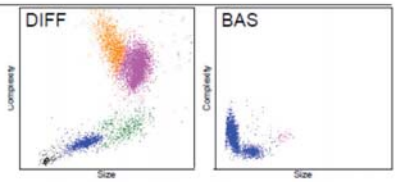
Case 23 | Eosinophilia, helminthiasis

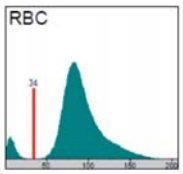
History and clinical signs and symptoms

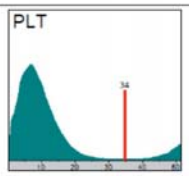
A 20 year old man was routinely assessed at a local health care provider as a part of his job application. Blood was taken on this occasion. No unusual physical signs or symptoms were detected.

Hematologic findings

Sample ID	Patient 23					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	9.79		$10^3/\mu L$	5.00	- 10.00		
NEU	4.31		$10^3/\mu L$	2.00	- 7.50		
LYM	1.56		$10^3/\mu L$	1.08	- 3.17		
MON	0.48		$10^3/\mu L$	0.15	- 0.70		
EO	3.28	++	$10^3/\mu L$	0.00	- 0.50		
BAS	0.17	+	$10^3/\mu L$	0.00	- 0.15		
NEU%	44.0		%	40.0	- 75.0		
LYM%	15.9		%	14.76	- 45.40		
MON%	4.9		%	3.0	- 7.0		
EO%	33.5	++	%	0.0	- 5.0		
BAS%	1.7	+	%	0.0	- 1.5		
RBC	3.89		$10^6/\mu L$	4.00	- 5.50		
HGB	125		g/L	120	- 174		
HCT	36.3	-	%	36.0	- 52.0		
MCV	93.3		fL	76.0	- 96.0		
MCH	32.2	+	pg	27.0	- 32.0		
MCHC	345		g/L	300	- 350		
RDWsd	41.0		fL	20.0	- 42.0		
RDWcv	14.9		%	0.0	- 16.0		
PLT	217		$10^3/\mu L$	150	- 400		
PCT	0.14		%		-		
MPV	6.3	-	fL	8.0	- 15.0		
PDWsd	21.2		fL		-		
PDWcv	38.2		%		-		







Warning flags

Morphological flags

Interpretive flags Eosinophilia?, Microcytic PLT?

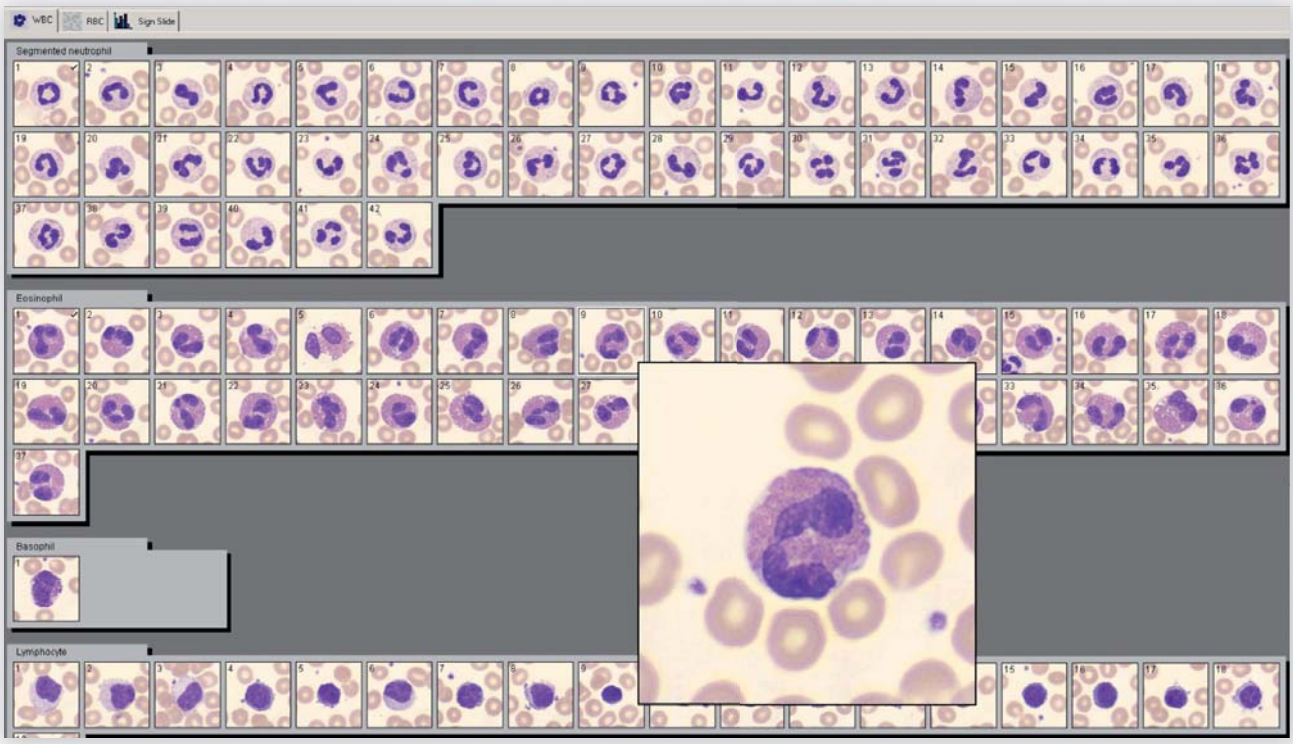
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. The blood cell populations are well characterized. The WBC count was within the normal reference range. The percentage and absolute number of eosinophils was markedly elevated (see interpretive flag “eosinophilia”). Additionally there is a slight elevation in absolute number and percentage of basophils.

The RBC and PLT histograms are well separated. The shape and size distribution of RBC and PLT histograms are normal. The RBC count and morphologic parameters are also within the normal range.

The PLT count is normal. Decreased MPV value (see interpretive flag “microcytic PLT”) has no clinical relevancy.

Peripheral blood smear



The high prevalence of eosinophils in the peripheral blood smear was observed.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	42	40.4 ✓
• Eosinophil	37	35.6 ✓
• Basophil	1	1 ✓
• Lymphocyte	19	18.3 ✓
• Monocyte	5	4.8 ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	104	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	2	- ✓
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	10	-
• Artefact	4	-

Other laboratory findings

Serological tests suggested intestinal helminthiasis.

Diagnosis

Helminthiasis-induced eosinophilia

Disease course

The marked eosinophilia returned to the normal after a course of anti-helminth therapy.

Chapter 3 | WBC ABNORMALTIES

3.3 Lymphocytosis

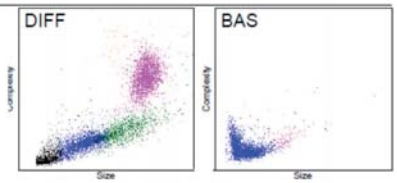
Case 24 | Mononucleosis with lymphocytosis

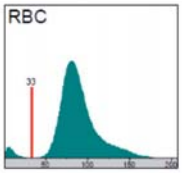
History and clinical signs and symptoms

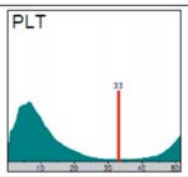
An adolescent girl presented at the outpatient clinic with swollen glands. She complained about increased pharyngeal pain that intensified when she swallowed. Other remarkable signs and symptoms included general fatigue, low grade fever [38.2 °C], swollen glands around the neck and extensive salivation.

Hematologic findings

Sample ID	Patient 24					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	11.05	+	$10^3/\mu L$	5.00	-	10.00	
NEU	4.21		$10^3/\mu L$	2.00	-	7.50	
LYM	4.39	+	$10^3/\mu L$	1.08	-	3.17	
MON	1.90	+	$10^3/\mu L$	0.15	-	0.70	
EO	0.06		$10^3/\mu L$	0.00	-	0.50	
BAS	0.50	++	$10^3/\mu L$	0.00	-	0.15	
NEU%	38.1		%	40.0	-	75.0	
LYM%	39.7		%	14.76	-	45.40	
MON%	17.2	+	%	3.0	-	7.0	
EO%	0.5		%	0.0	-	5.0	
BAS%	4.5	++	%	0.0	-	1.5	
RBC	4.24		$10^6/\mu L$	4.00	-	5.50	
HGB	133		g/L	120	-	174	
HCT	38.3		%	36.0	-	52.0	
MCV	90.4		fL	76.0	-	96.0	
MCH	31.4		pg	27.0	-	32.0	
MCHC	347		g/L	300	-	350	
RDWsd	35.9		fL	20.0	-	42.0	
RDWcv	14.4		%	0.0	-	16.0	
PLT	124	-	$10^3/\mu L$	150	-	400	
PCT	0.08		%		-		
MPV	6.2	-	fL	8.0	-	15.0	
PDWsd	23.1		fL		-		
PDWcv	38.2		%		-		







Warning flags	
Morphological flags	G
Interpretive flags	Basophilia?, Microcytic PLT?

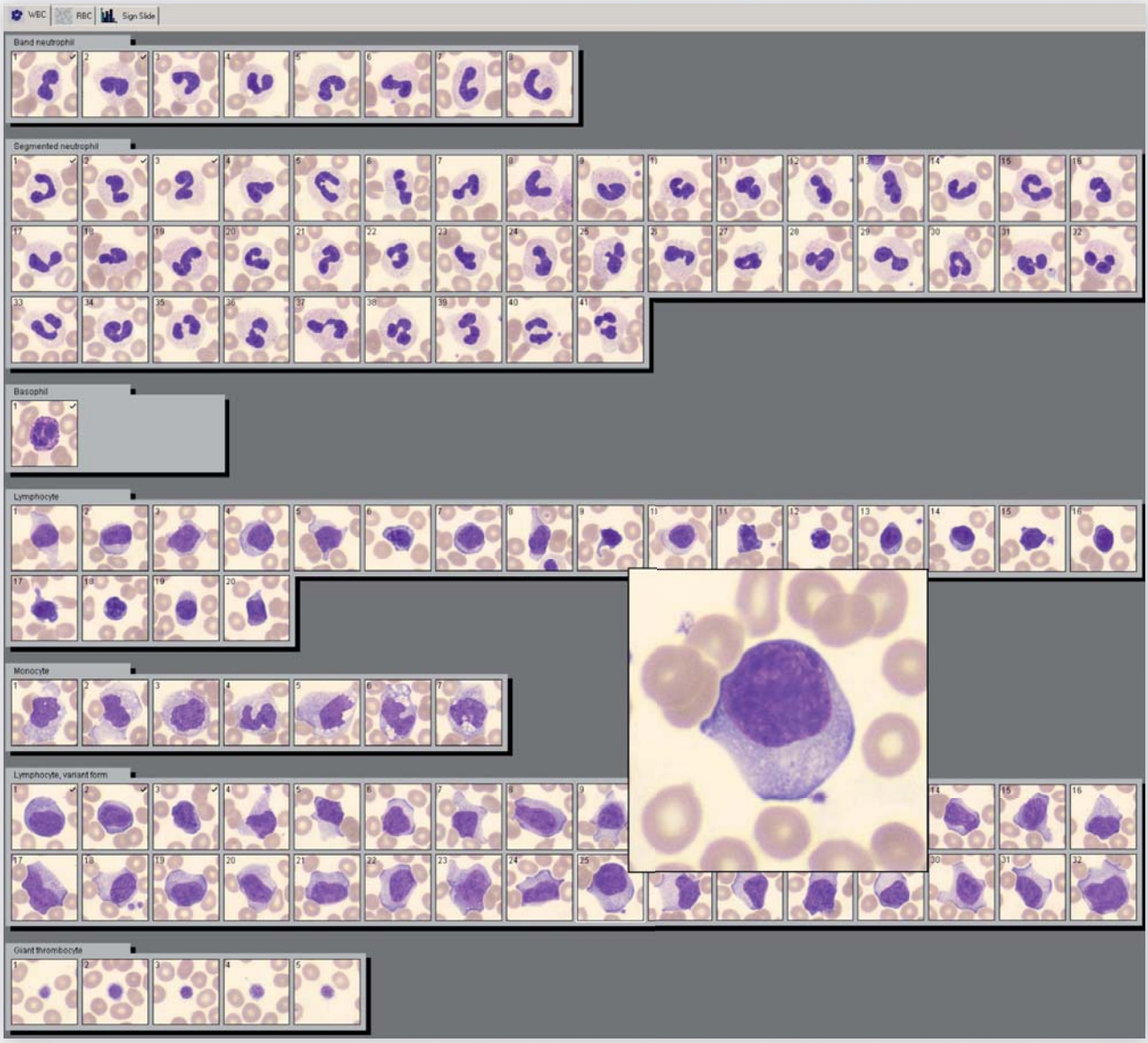
Interpretation

Despite the absence of any warning flags, the WBC differential (DIFF) scattergram is not clearly defined. Lymphocyte and monocyte populations are confluent. The WBC count is slightly above the upper limit of the normal range. A flag „G" indicates that immature cells may be present. The basophil (BAS) scattergram indicates a large number of leukocytes.¬ The lymphocyte, monocyte and basophil counts and percentages are above the upper limit of normal range.

The RBC and PLT histograms are well separated and are normal in shape and distribution. The RBC and PLT counts are within the normal range. Decreased MPV value (see interpretive flag "microcytic PLT") is not clinically relevant here.

Due to the morphologic Flag „G" and the marked increase of basophil cells the assessment of a peripheral blood smear is recommended.

Peripheral blood smear



The percentage of large mononuclear cells presenting the characteristics of monocytes and variant lymphocytes were 6,4% and 29,4% respectively. There is no marked basophilia detected in the peripheral blood smear.*

* Due to their specific response to lyze reagent immature cells and activated lymphocytes may result in a falsely increased basophil count. Activated lymphocytes (named as variant forms when evaluated in a microscopic smear) have a similar response to that of monocytes to the effect of lyze reagent. This means they are mistakenly counted as monocytes resulting in a falsely elevated monocyte count with a decreased lymphocyte count. The analyzer registers a warning flag "G" indicating to the operator that they should conduct a microscopic smear.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	8	7.3
• Segmented neutrophil	41	37.6 ✓
• Eosinophil	-	-
• Basophil	1	0.9
• Lymphocyte	20	18.3
• Monocyte	7	6.4
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	32	29.4
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	109	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	5	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	43	-
• Artefact	9	-

Other laboratory findings

Abnormal findings included elevated LDH values (655 U/L [reference range: 230 – 460 U/L); CRP was 61.4 mg/L [<5 mg/L]. Serological tests verified the infection with Epstein-Barr virus.

Diagnosis

Pfeiffer's disease (Infectious mononucleosis)

Disease course

After 4 weeks on symptomatic therapy the patient's general condition improved. A full recovery required several months.

Case 25 | Leukocytosis and lymphocytosis caused by infectious mononucleosis

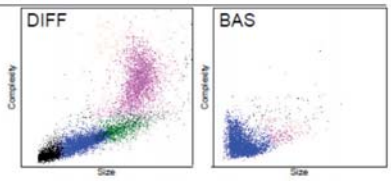
History and clinical signs and symptoms

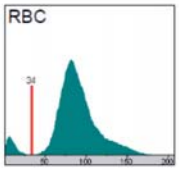
A young man age 22 years presented at the outpatient clinic with fever and general malaise.
He complained about diffuse pain in his neck. His tonsils were swollen. Other remarkable signs and symptoms included fever [38.9 °C] and fatigue.

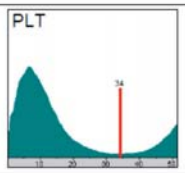
Hematologic findings

Sample ID Patient 25 — Patient ID
Date 01/01/2013 — Name
Mode Human — Date of birth
Gender

Parameter	Result			Limit		
WBC	20.93	++	$10^3/\mu L$	5.00	-	10.00
NEU	5.30		$10^3/\mu L$	2.00	-	7.50
LYM	11.51	++	$10^3/\mu L$	1.08	-	3.17
MON	3.01	++	$10^3/\mu L$	0.15	-	0.70
EO	0.08		$10^3/\mu L$	0.00	-	0.50
BAS	1.03	++	$10^3/\mu L$	0.00	-	0.15
NEU%	25.3	-	%	40.0	-	75.0
LYM%	55.0	+	%	14.76	-	45.40
MON%	14.4	++	%	3.0	-	7.0
EO%	0.4		%	0.0	-	5.0
BAS%	4.9	++	%	0.0	-	1.5
RBC	4.42		$10^6/\mu L$	4.00	-	5.50
HGB	133		g/L	120	-	174
HCT	40.4		%	36.0	-	52.0
MCV	91.4		fL	76.0	-	96.0
MCH	30.0		pg	27.0	-	32.0
MCHC	328		g/L	300	-	350
RDWsd	41.0		fL	20.0	-	42.0
RDWcv	15.4		%	0.0	-	16.0
PLT	224		$10^3/\mu L$	150	-	400
PCT	0.20		%		-	
MPV	8.7		fL	8.0	-	15.0
PDWsd	27.3		fL		-	
PDWcv	38.0		%		-	







Warning flags
Morphological flags G
Interpretive flags Leukocytosis?, Monocytosis?, Lymphocytosis?, Basophilia?

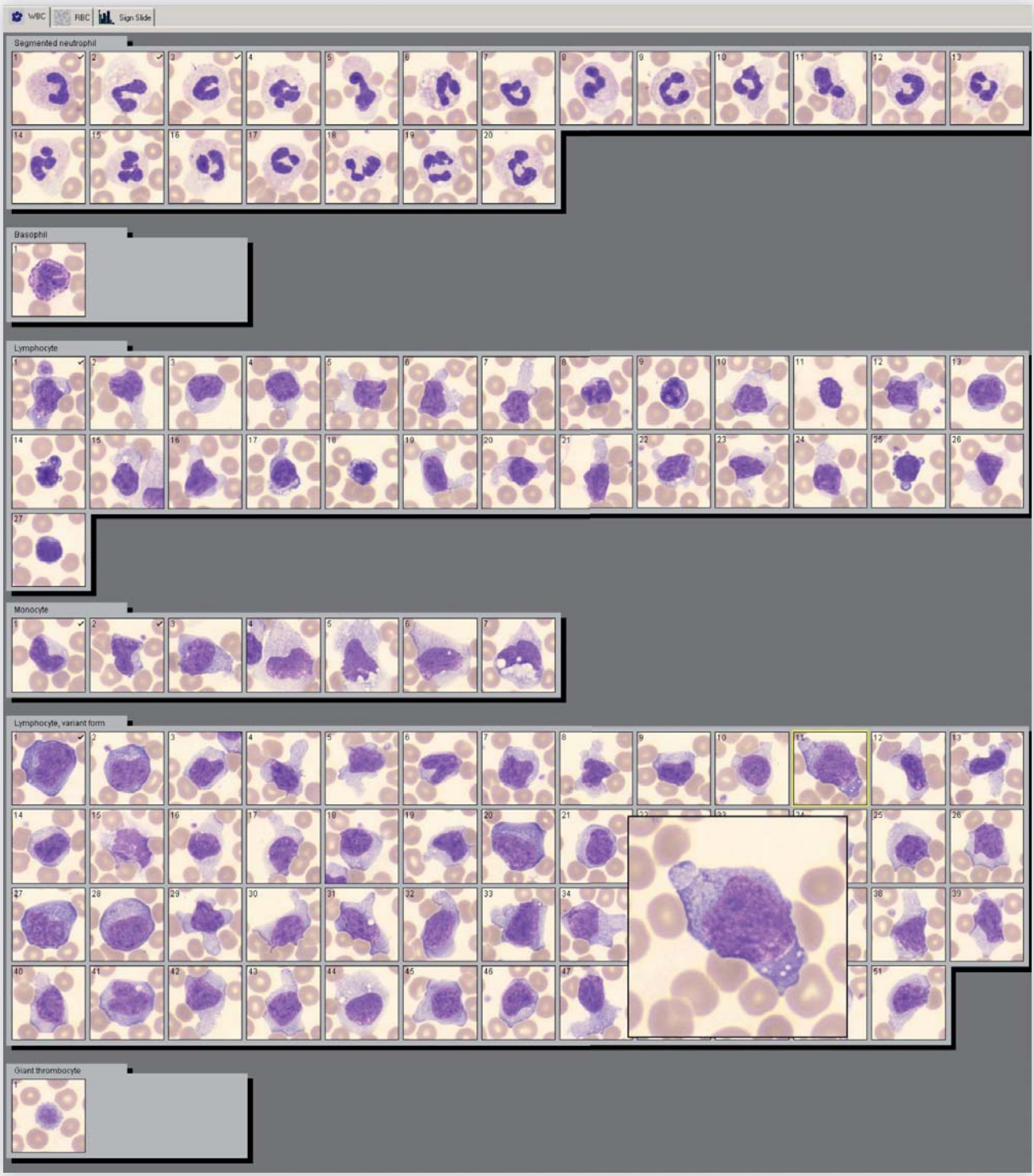
Interpretation

The WBC DIFF and BAS scattergrams are not clearly defined. The leukocyte populations are confluent. The WBC count is about twice the upper limit of normal. Morphological Flag "G" indicates the presence of immature cells. A high number and percentage of basophil (undetermined) cells were also detected.

The RBC and PLT histograms are well separated and normal in shape and distribution. The RBC and PLT counts are within the normal range.

Due to the presence of the morphological and interpretive flags and the marked increase of basophil cells* the assessment of peripheral blood smear is highly recommended.

Peripheral blood smear



A large number of monocytes along with variant and activated lymphocytes are observed. No blasts or any other abnormal cells raise the suspicion that a malignancy is present.

* Due to their special response to lyzing effect atypical cells, blast cells, immature cells and activated lymphocytes may falsely increase the basophil count. Activated lymphocytes (variant form) and monocytes form a confluent population and therefore the analyzer monocyte count is falsely elevated while lymphocyte count is falsely decreased. This can be seen if results are compared to that of the manual smear.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	20	18.9 ✓
• Eosinophil	-	-
• Basophil	1	0.9
• Lymphocyte	27	25.5
• Monocyte	7	6.6
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	51	48.1
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	106	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	1	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	14	-
• Artefact	8	-

Other laboratory findings

Remarkable findings included high alkaline phosphatase, transaminase, lactic dehydrogenase values and bilirubin levels (exceeding 8–15 times the upper limit of normal). Serological tests indicated acute infection with Epstein-Barr virus.

Diagnosis

Pfeiffer's disease (Infectious mononucleosis)

Disease course

The patient was discharged home. Symptomatic therapy was recommended until full recovery.

Chapter 3 | WBC ABNORMALTIES

3.4 Basophilia

Case 26 | Basophilia, polycythemia rubra vera

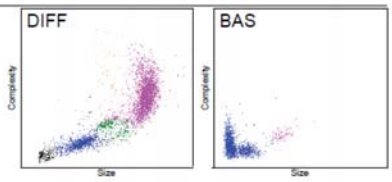
History and clinical signs and symptoms

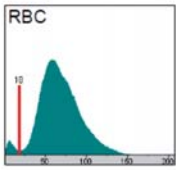
Three years ago an abnormally high RBC was measured when the 63 year old man was participating in a screening. At repeated measurements, high PLT and WBC counts (1.5 and 3x upper limit of normal (ULN), respectively) were detected. The diagnosis of myelo-proliferative disease was established and supported by molecular biology testing (i.e. JAK2 mutation).

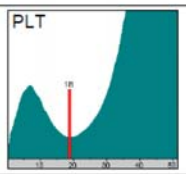
Hydroxycarbamide therapy was initiated; then PLT, WBC and RBC counts tended to normalize. The patient reported nasal bleeding at several occasions. The patient was scheduled for a control visit.

Hematologic findings

Sample ID	Patient 26					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	6.08		$10^3/\mu L$	5.00	- 10.00		
NEU	3.73		$10^3/\mu L$	2.00	- 7.50		
LYM	1.45		$10^3/\mu L$	1.08	- 3.17		
MON	0.47		$10^3/\mu L$	0.15	- 0.70		
EO	0.07		$10^3/\mu L$	0.00	- 0.50		
BAS	0.36	++	$10^3/\mu L$	0.00	- 0.15		
NEU%	61.3		%	40.0	- 75.0		
LYM%	23.9		%	14.76	- 45.40		
MON%	7.7	+	%	3.0	- 7.0		
EO%	1.1		%	0.0	- 5.0		
BAS%	6.0	++	%	0.0	- 1.5		
RBC	7.88	+	$10^6/\mu L$	4.00	- 5.50		
HGB	165		g/L	120	- 174		
HCT	54.8	+	%	36.0	- 52.0		
MCV	69.5	-	fL	76.0	- 96.0		
MCH	21.0	-	pg	27.0	- 32.0		
MCHC	302		g/L	300	- 350		
RDWsd	55.7	+	fL	20.0	- 42.0		
RDWcv	25.4	+	%	0.0	- 16.0		
PLT	207		$10^3/\mu L$	150	- 400		
PCT	0.18		%		-		
MPV	8.6		fL	8.0	- 15.0		
PDWsd	27.7		fL		-		
PDWcv	35.2		%		-		
Warning flags	D						
Morphological flags	G						
Interpretive flags	Basophilia?, Polycythemia?, Microcytic RBC?, Anisocytosis?						







Interpretation

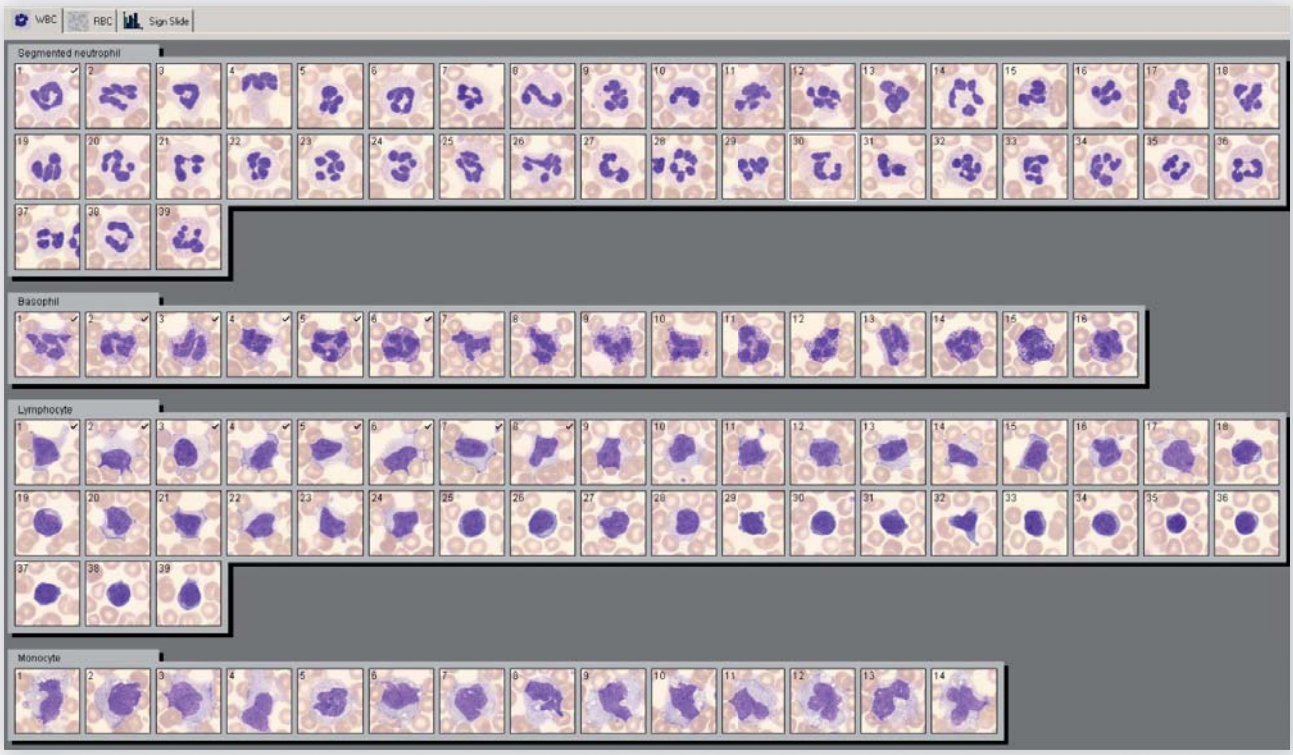
The WBC DIFF scattergram is not normal, monocyte and neutrophil populations are not clearly distinguishable (warning flag „D"). The WBC count is within the normal range. The basophil count is markedly increased.

The RBC and PLT histograms have a normal shape but they are not clearly separated. RBC count is elevated. The flag "anisocytosis" indicates a heterogeneity in RBC size. This heterogeneity is further supported by the shape and widening of RBC histogram (reflected by a high RDW value).

The PLT count is within the normal range.

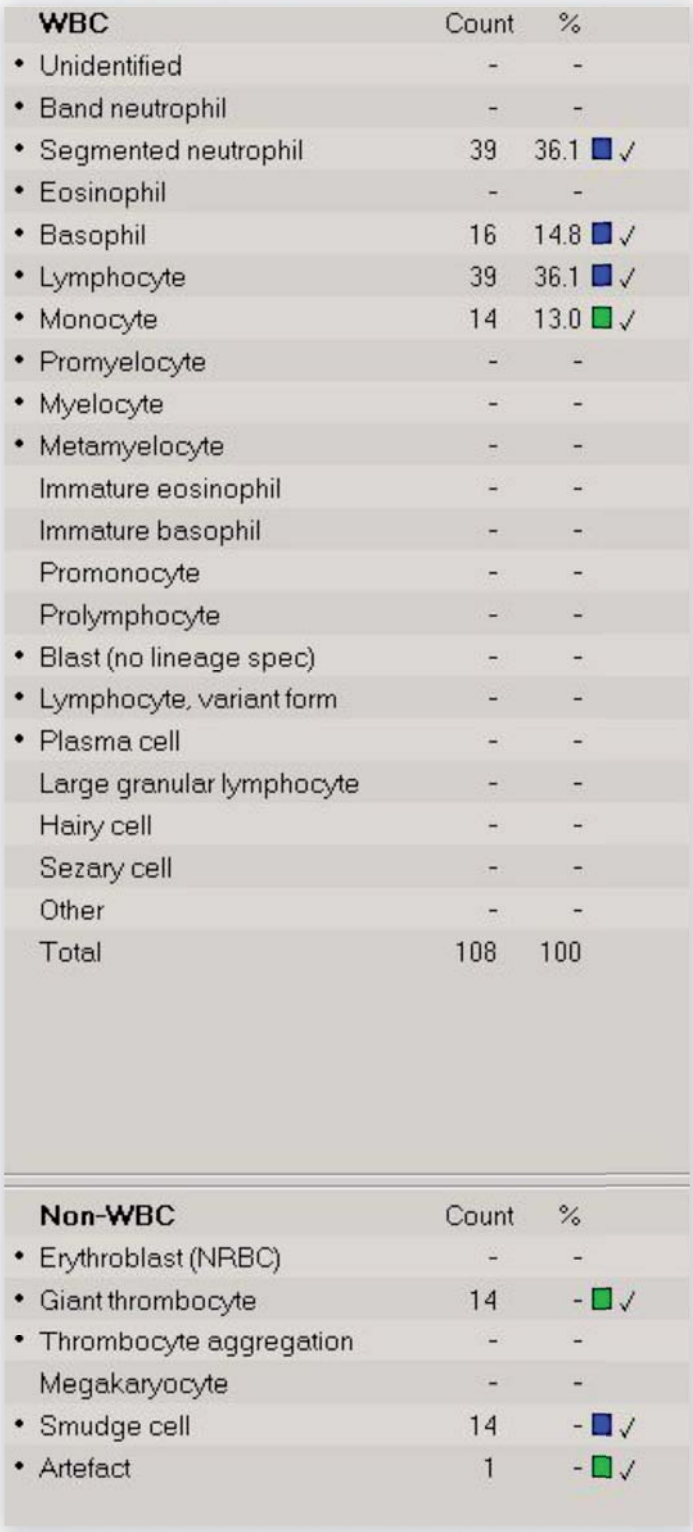
The assessment of a peripheral blood smear is highly recommended due to the abnormal WBC DIFF scattergram with warning flag "D" and morphological flag "G" (possible presence of immature granulocytes).

Peripheral blood smear



The dominant alteration in the peripheral blood smear is the marked presence of basophil cells (14.8%). The other WBC subpopulations present include neutrophil cells (36.1%), lymphocytes (36.1%) and monocytes (13.0%). The high prevalence of basophil cells is a characteristic feature of the disease.

Because of confluent WBC populations (probably due to the altered cellular structures in the disease), the analyzer's result significantly differs from that of the microscopic evaluation.



WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	39	36.1 ✓
• Eosinophil	-	-
• Basophil	16	14.8 ✓
• Lymphocyte	39	36.1 ✓
• Monocyte	14	13.0 ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	14	- ✓
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	14	- ✓
• Artefact	1	- ✓

Other laboratory findings

Other laboratory findings include LDH and GGT activity over 3-fold and 1.5-fold of upper limit of normal, respectively.

Diagnosis

Polycythemia rubra vera with marked basophilia

Disease course

The patient responded to maintenance therapy with hydroxycarbamide.
The patient's current status is stable; his next visit was scheduled one month later.

Chapter 3 | WBC ABNORMALITIES

3.5 Miscellaneous cases

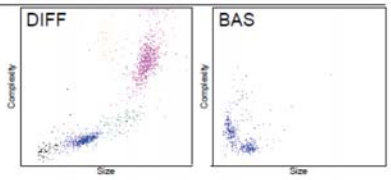
Case 27 | Severe leukopenia

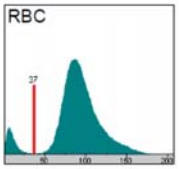
History and clinical signs and symptoms

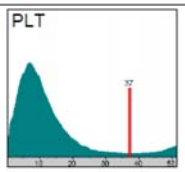
A 45 year old woman with Hodgkin's disease was treated with chemotherapy. The patient was regularly sampled to monitor her status.

Hematologic findings

Sample ID	Patient 27						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	1.69	--	$10^3/\mu L$	5.00	-	10.00		
NEU	0.89	--	$10^3/\mu L$	2.00	-	7.50		
LYM	0.62	-	$10^3/\mu L$	1.08	-	3.17		
MON	0.09	-	$10^3/\mu L$	0.15	-	0.70		
EO	0.06		$10^3/\mu L$	0.00	-	0.50		
BAS	0.03		$10^3/\mu L$	0.00	-	0.15		
NEU%	52.7		%	40.0	-	75.0		
LYM%	36.8		%	14.76	-	45.40		
MON%	5.4		%	3.0	-	7.0		
EO%	3.4		%	0.0	-	5.0		
BAS%	1.7	+	%	0.0	-	1.5		
RBC	4.13		$10^6/\mu L$	4.00	-	5.50		
HGB	120		g/L	120	-	174		
HCT	39.3		%	36.0	-	52.0		
MCV	95.3		fL	76.0	-	96.0		
MCH	29.1		pg	27.0	-	32.0		
MCHC	305		g/L	300	-	350		
RDWsd	44.6	+	fL	20.0	-	42.0		
RDWcv	14.9		%	0.0	-	16.0		
PLT	233		$10^3/\mu L$	150	-	400		
PCT	0.24		%		-			
MPV	10.4		fL	8.0	-	15.0		
PDWsd	27.4		fL		-			
PDWcv	38.0		%		-			
Warning flags								
Morphological flags								
Interpretive flags	Leukopenia?, Neutropenia?, Lymphopenia?							







Interpretation

The WBC DIFF and BAS scattergrams are clearly defined. The WBC DIFF subpopulations are well characterized. Of note, the WBC counts including neutrophil and lymphocyte counts were well below the lower limit of the normal reference range (see also interpretative flags "leukopenia", „neutropenia" and "lymphopenia").

The RBC and PLT histograms are clearly separated and have a normal shape and size. The HGB, RBC and PLT counts are also within the normal range.

Peripheral blood smear

The number of leukocytes in the peripheral blood smear were extremely low; the observed leukocytes are apparently normal.

WBC	Count	%
• Unidentified	1	0.9
• Band neutrophil	3	2.7
• Segmented neutrophil	59	53.6 ✓
• Eosinophil	2	1.8
• Basophil	-	-
• Lymphocyte	39	35.5
• Monocyte	4	3.6
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	2	1.8
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	110	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	14	-
• Thrombocyte aggregation	1	-
Megakaryocyte	-	-
• Smudge cell	3	-
• Artefact	7	-

Other laboratory findings

Other laboratory findings including inflammatory markers such as acute phase reactants were not remarkable.

Diagnosis

Chemotherapy induced leukopenia.

Disease course

Chemotherapy was suspended. Leukocyte counts increased within 10 days.

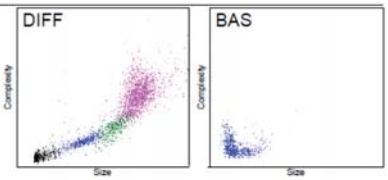
Case 28 | Myelodysplasia

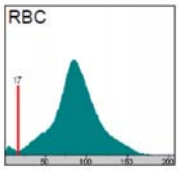
History and clinical signs and symptoms

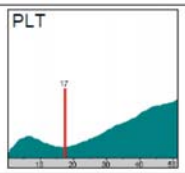
A 58 year old man with known myelodysplasia (diagnosed 3 years before) presented at the outpatient clinic due to fatigue and general weakness.

Hematologic findings

Sample ID	Patient 28					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	3.24	-	$10^3/\mu L$	5.00	- 10.00		
NEU	2.04		$10^3/\mu L$	2.00	- 7.50		
LYM	0.74	-	$10^3/\mu L$	1.08	- 3.17		
MON	0.45		$10^3/\mu L$	0.15	- 0.70		
EO	0.00		$10^3/\mu L$	0.00	- 0.50		
BAS	0.01		$10^3/\mu L$	0.00	- 0.15		
NEU%	63.0		%	40.0	- 75.0		
LYM%	22.8		%	14.76	- 45.40		
MON%	13.8	+	%	3.0	- 7.0		
EO%	0.0		%	0.0	- 5.0		
BAS%	0.4		%	0.0	- 1.5		
RBC	2.48	-	$10^6/\mu L$	4.00	- 5.50		
HGB	62	-	g/L	120	- 174		
HCT	21.8	-	%	36.0	- 52.0		
MCV	88.0		fL	76.0	- 96.0		
MCH	25.2	-	pg	27.0	- 32.0		
MCHC	286	-	g/L	300	- 350		
RDWsd	72.6	+	fL	20.0	- 42.0		
RDWcv	21.8	+	%	0.0	- 16.0		
PLT	36	--	$10^3/\mu L$	150	- 400		
PCT	0.02		%		-		
MPV	6.0	-	fL	8.0	- 15.0		
PDWsd	23.7		fL		-		
PDWcv	38.0		%		-		







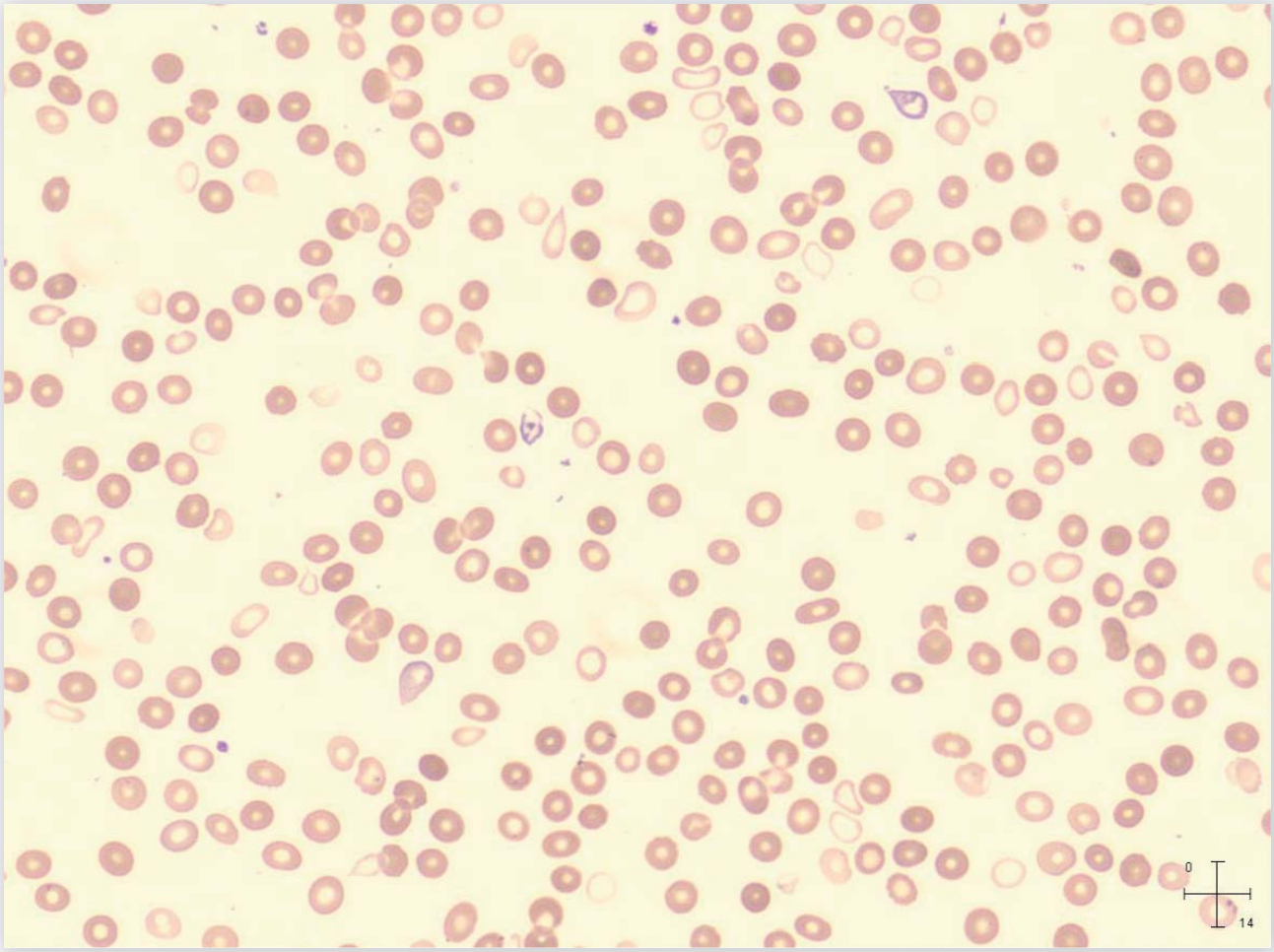
Warning flags	ID
Morphological flags	G
Interpretive flags	Anemia?, Hypochromic?, Anisocytosis?, Thrombocytopenia?, Microcytic PLT?

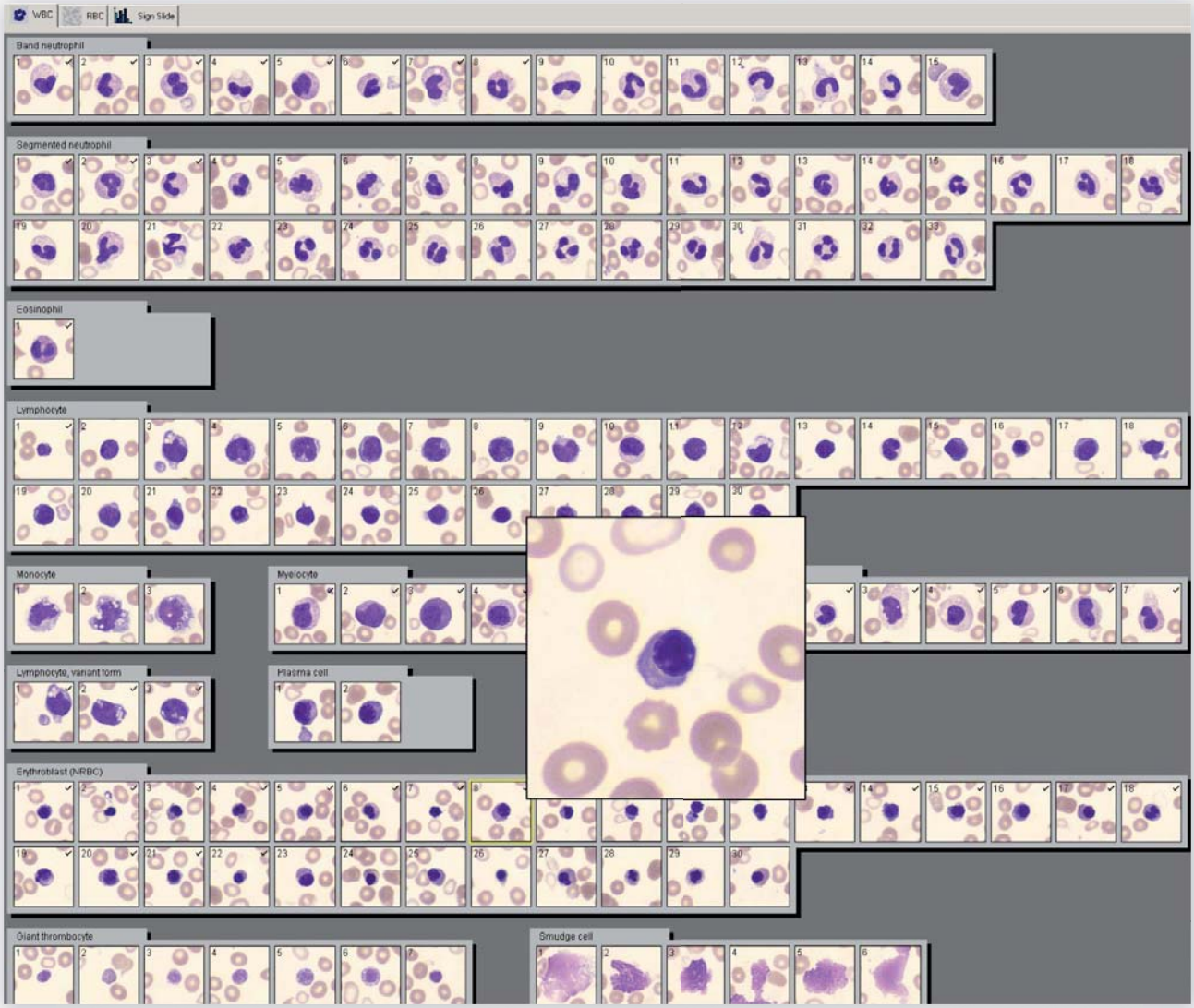
Interpretation

The WBC differential (DIFF) and basophil (BAS) scattergrams are clearly defined. However, the neutrophils and monocytes populations are not distinguished clearly on WBC differential scattergram (warning Flag "D").The WBC count indicates a mild leukopenia with lymphopenia and relative monocytosis (see interpretive flags). Flag "G" suggests immature granulocytes.

The RBC and PLT histograms are abnormally distributed. The Flag "I" warns that the RBC and PLT populations are not clearly separated. The confluent population (see on PLT histogram) suggests red blood cell fragmentation. Broadening of RBC histogram and increased RDW values are indicative of a heterogeneous size distribution of RBC's (see flag "anisocytosis"). Numerical results indicate a normocytic hypochromic anemia (decreased HGB, RBC, MCH, HCT) and severe thrombocytopenia. Due to several warning flags, overlapping populations on the WBC scattergram and histograms a peripheral blood smear should be microscopically evaluated.

Peripheral blood smear





The peripheral blood smear clearly indicates leukopenia. Within the small population of leukocytes the prevalence of immature leukocytes in different stages of maturity is strikingly high. There are also remarkable abnormalities in the RBC population, including hypochromasia, anisocytosis, teardrop erythrocytes and fragmentocytes. Erythroblasts (nucleated erythrocytes/NRBCs) were also prevalent.*

* NOTE: Nucleated red blood cells (NRBCs) are resistant to the lyzing procedure during the measurement. Therefore they are classified falsely into the WBC population. This may increase the detected WBC count. In fact, the WBC may be even lower than that provided.

Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	15	15.0
• Segmented neutrophil	33	33.0 ✓
• Eosinophil	1	1.0
• Basophil	-	-
• Lymphocyte	30	30.0
• Monocyte	3	3.0
• Promyelocyte	-	-
• Myelocyte	6	6.0
• Metamyelocyte	7	7.0
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	3	3.0
• Plasma cell	2	2.0
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	100	100

Non-WBC	Count	%
• Erythroblast (NRBC)	30	-
• Giant thrombocyte	7	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	6	-
• Artefact	4	-

NOTE: Monocytosis as indicated on the A5 result is not confirmed by the microscopic evaluation of the smear. The false monocyte result of the A5 system is due to the presence of immature cells (warning flag "G") leading to improper separation of the neutrophils and monocytes as indicated by warning flag "D". This also illustrates that automated results are for rapid screening purposes only. The importance of microscopic evaluation of blood smears in case of abnormal results, especially in case of warning flags is high.

Other laboratory findings

Clinical laboratory results (renal and hepatic function, cardiac status) are not remarkable.

Diagnosis

Myelodysplasia with severe hypochromic, normocytic anemia and thrombocythopenia.

Disease course

The patient was transfused and soon his status improved significantly.

CHAPTER 4

Hematologic malignancies

Case 29 | Chronic lymphoid leukemia (CLL) with anemia

History and clinical signs and symptoms

A 75 year old man was diagnosed with CLL five years before. He presented at the clinic for regular assessment.

Hematologic findings

Sample ID	Patient 29						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	41.60	++	$10^3/\mu L$	5.00	-	10.00	DIFF	BAS
NEU	1.21	-	$10^3/\mu L$	2.00	-	7.50		
LYM	27.79	++	$10^3/\mu L$	1.08	-	3.17		
MON	11.15	++	$10^3/\mu L$	0.15	-	0.70		
EO	0.33		$10^3/\mu L$	0.00	-	0.50		
BAS	1.12	++	$10^3/\mu L$	0.00	-	0.15		
NEU%	2.9	--	%	40.0	-	75.0		
LYM%	66.8	+	%	14.76	-	45.40		
MON%	26.8	++	%	3.0	-	7.0		
EO%	0.8		%	0.0	-	5.0		
BAS%	2.7	+	%	0.0	-	1.5		
RBC	2.50	-	$10^6/\mu L$	4.00	-	5.50	RBC	
HGB	80	-	g/L	120	-	174		
HCT	23.8	-	%	36.0	-	52.0		
MCV	95.5		fL	76.0	-	96.0		
MCH	32.1	+	pg	27.0	-	32.0		
MCHC	336		g/L	300	-	350		
RDWsd	61.4	+	fL	20.0	-	42.0		
RDWcv	18.9	+	%	0.0	-	16.0		
PLT	226		$10^3/\mu L$	150	-	400	PLT	
PCT	0.22		%		-			
MPV	9.6		fL	8.0	-	15.0		
PDWsd	26.6		fL		-			
PDWcv	37.7		%		-			
Warning flags	D							
Morphological flags	GL							
Interpretive flags	Leukocytosis?, Monocytosis?, Lymphocytosis?, Basophilia?, Anemia?, Anisocytosis?							

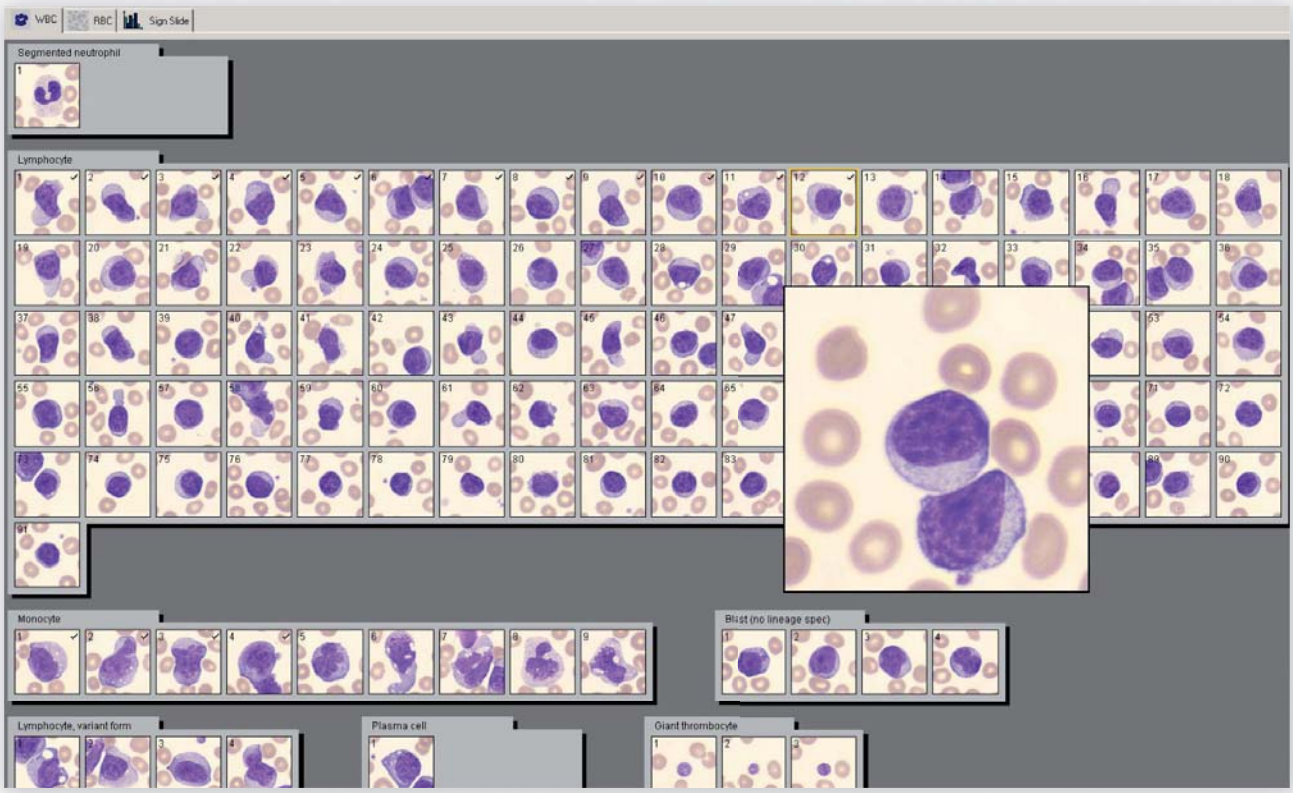
Interpretation

The WBC DIFF scattergram is abnormal. The WBC numeric count indicates a leukocytosis with a characteristic dominance of lymphocytes and other less characterized cells (classified by the instrument as monocytes). The Flag "D" indicates that the WBC populations are not separated sufficiently. Morphological flags "G" and "L" indicate immature granulocytes and atypical lymphocytes respectively. The microscopic evaluation of the peripheral blood smear is a must.

The RBC histogram is abnormally distributed (broadened) while the PLT histogram is normal. Severe anemia is present as indicated by the low RBC, HGB and HCT values. Other red cell parameters (including MCV, MCH, MCHC) are within the normal reference range except for an increased RDW which indicates anisocytosis (may be caused by regenerative response).

The PLT count is within the normal range. The RBC and PLT histograms are clearly separated.

Peripheral blood smear



The predominance of mononuclear cells among leukocytes is clear. The majority of the leukocytes are lymphocytes; however, abnormal cells such as blast and variant lymphocytes do also occur.

WBC	Count	%	
• Unidentified	-	-	
• Band neutrophil	-	-	
• Segmented neutrophil	1	0.9	✓
• Eosinophil	-	-	
• Basophil	-	-	
• Lymphocyte	91	82.7	✓
• Monocyte	9	8.2	✓
• Promyelocyte	-	-	
• Myelocyte	-	-	
• Metamyelocyte	-	-	
Immature eosinophil	-	-	
Immature basophil	-	-	
Promonocyte	-	-	
Prolymphocyte	-	-	
• Blast (no lineage spec)	4	3.6	✓
• Lymphocyte, variant form	4	3.6	✓
• Plasma cell	1	0.9	✓
Large granular lymphocyte	-	-	
Hairy cell	-	-	
Sezary cell	-	-	
Other	-	-	
Total	110	100	

Non-WBC	Count	%	
• Erythroblast (NRBC)	-	-	
• Giant thrombocyte	3	-	✓
• Thrombocyte aggregation	-	-	
Megakaryocyte	-	-	
• Smudge cell	11	-	✓
• Artefact	1	-	

NOTE: Monocytosis and basophilia as indicated on the A5 result are not confirmed by the microscopic evaluation of the smear. The false results of the A5 system are due to the presence of immature and atypical cells causing improper separation of WBC's as indicated by the warning flags "GL" and "D". This also illustrates that automated results are for rapid screening purposes only. The importance of microscopic evaluation of peripheral blood smears in the case of abnormal results, especially in presence of warning flags is high.

Other laboratory findings

No remarkable findings.

Diagnosis

Chronic lymphocytic leukemia and related anemia

Disease course

Six units of packed RBCs were given to improve the anemia. A chemotherapeutic regime was modified in order to avoid the hematologic toxicity

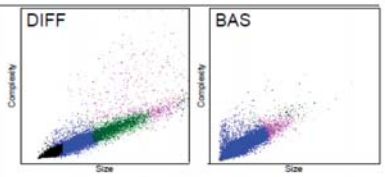
Case 30 | Chronic lymphoid leukemia with high WBC count (need for manual sample dilution)

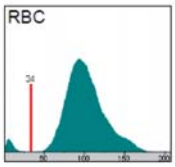
History and clinical signs and symptoms

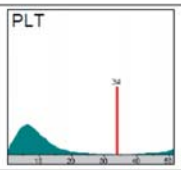
A 77 year old man was diagnosed with indolent B-cell lymphoma 5 years before. Since the diagnosis the patient was subjected to different therapeutic regimes, but white blood cell counts did not respond. Currently the patient is not given chemotherapy. He returned to check his CBC. There are no specific complaints. Physical signs and symptoms include fatigue, mild hepatomegaly.

Hematologic findings

Sample ID	Patient 30					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	---	**	$10^3/\mu L$	5.00	-	10.00	
NEU	---	**	$10^3/\mu L$	2.00	-	7.50	
LYM	---	**	$10^3/\mu L$	1.08	-	3.17	
MON	---	**	$10^3/\mu L$	0.15	-	0.70	
EO	---	**	$10^3/\mu L$	0.00	-	0.50	
BAS	---	**	$10^3/\mu L$	0.00	-	0.15	
NEU%	---	**	%	40.0	-	75.0	
LYM%	---	**	%	14.76	-	45.40	
MON%	---	**	%	3.0	-	7.0	
EO%	---	**	%	0.0	-	5.0	
BAS%	---	**	%	0.0	-	1.5	
RBC	3.11	-	$10^6/\mu L$	4.00	-	5.50	
HGB	110	-	g/L	120	-	174	
HCT	31.7	-	%	36.0	-	52.0	
MCV	102.0	+	fL	76.0	-	96.0	
MCH	35.4	+	pg	27.0	-	32.0	
MCHC	347		g/L	300	-	350	
RDWsd	55.6	+	fL	20.0	-	42.0	
RDWcv	15.6		%	0.0	-	16.0	
PLT	94	-	$10^3/\mu L$	150	-	400	
PCT	0.11		%		-		
MPV	11.4		fL	8.0	-	15.0	
PDWsd	28.5		fL		-		
PDWcv	35.3		%		-		
Warning flags	DO						
Morphological flags	G						
Interpretive flags	Anemia?, Macrocytic RBC?						







Interpretation

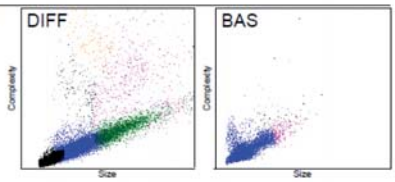
The RBC and PLT histograms are characteristic and well separated. The RBC population is homogenous, but is low in size. The low RBC count indicates an anemia (flagged).
The elevated RDWsd indicates a heterogeneity in RBC size distribution. The mean cell hemoglobin (MCH) is above the upper level of normal in the majority of RBCs. The anemia is flagged as macrocytic.

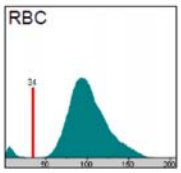
The flag 'thrombocytopenia' warns that the platelet count (PLT) is low which is in line with the numerical count.

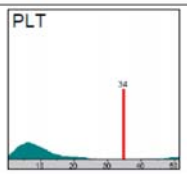
The instrument has not provided a WBC count however the DIFF and BAS scattergrams clearly show a WBC population with abnormal characteristic. The WBC DIFF scattergram indicates a confluent population (Warning flag 'D'); the Warning flag 'O' and linearity flag " ** " are also present and indicates that the WBC count exceeds the upper limit of the linearity range. Therefore, the sample was 2-fold diluted and re-measured to obtain numerical WBC results. Please, note that WBC counts should be multiplied by a factor of 2 and the real total WBC count should be around 160 G/L.

Sample diluted 2-fold with saline:

Sample ID	Patient 30					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	84.08	++	$10^3/\mu L$	5.00	-	10.00	
NEU	0.67	--	$10^3/\mu L$	2.00	-	7.50	
LYM	72.48	++	$10^3/\mu L$	1.08	-	3.17	
MON	8.83	++	$10^3/\mu L$	0.15	-	0.70	
EO	0.67	+	$10^3/\mu L$	0.00	-	0.50	
BAS	1.43	++	$10^3/\mu L$	0.00	-	0.15	
NEU%	0.8	--	%	40.0	-	75.0	
LYM%	86.2	++	%	14.76	-	45.40	
MON%	10.5	+	%	3.0	-	7.0	
EO%	0.8		%	0.0	-	5.0	
BAS%	1.7	+	%	0.0	-	1.5	
RBC	1.60	--	$10^6/\mu L$	4.00	-	5.50	
HGB	56	--	g/L	120	-	174	
HCT	16.2	--	%	36.0	-	52.0	
MCV	101.4	+	fL	76.0	-	96.0	
MCH	34.9	+	pg	27.0	-	32.0	
MCHC	345		g/L	300	-	350	
RDWsd	55.3	+	fL	20.0	-	42.0	
RDWcv	15.4		%	0.0	-	16.0	
PLT	53	--	$10^3/\mu L$	150	-	400	
PCT	0.06		%		-		
MPV	11.1		fL	8.0	-	15.0	
PDWsd	30.0		fL		-		
PDWcv	35.4		%		-		







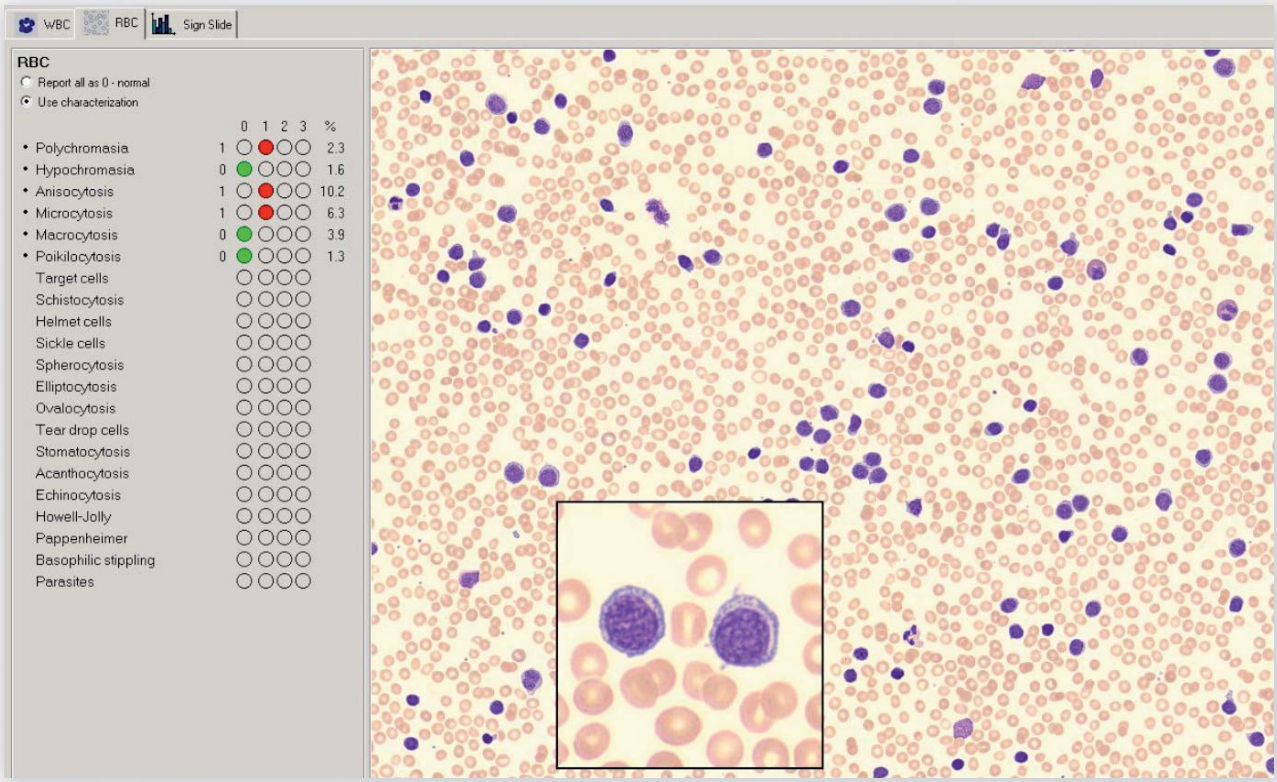
Warning flags	
Morphological flags	G
Interpretive flags	Leukocytosis?, Neutropenia?, Monocytosis?, Lymphocytosis?, Basophilia?, Anemia?, Macrocytic RBC?, Thrombocytopenia?

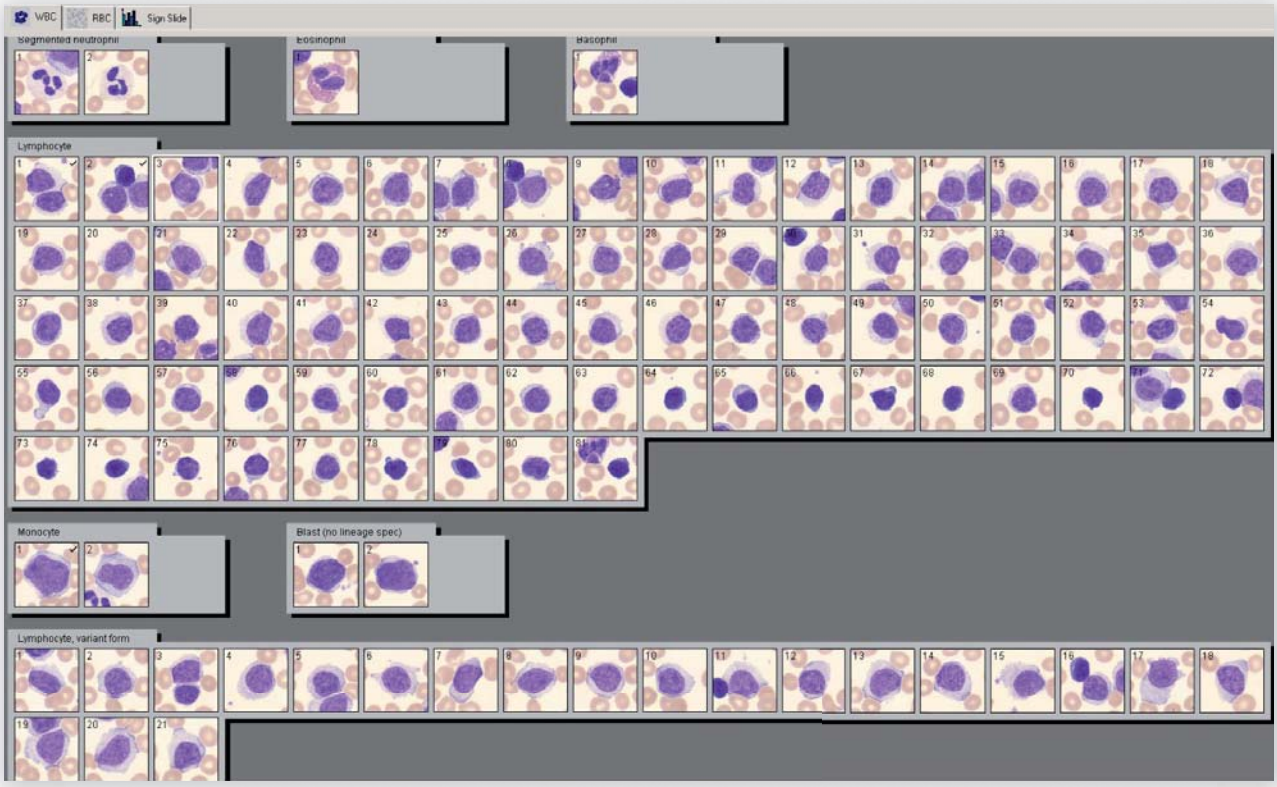
According to the diluted sample the WBC count is extremely high (over 160 G/L). The overwhelming majority of WBCs are lymphocytes in line with the diagnosis of chronic lymphoid leukemia. The high proportion of basophil cells (BAS) and monocytes (MON) along with the confluent population on the DIFF scattergram suggests that abnormal mononuclear cells are highly prevalent.*
(The morphological flag "G" is not relevant here.)

* Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system.

The assessment of a peripheral blood smear is essential.

Peripheral blood smear





The peripheral blood smear is characterized by the dominant WBC form, i.e. lymphocytes corresponds to the clinical diagnosis. RBCs are low in number and contain various amount of HGB.

Macrocytosis is not confirmed by the microscopic analysis. Falsely elevated MCV value is caused by the extremely high WBC count which interferes with the RBC counting. The real RBC count may be somewhat lower than the displayed one.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	2	1.8 ✓
• Eosinophil	1	0.9
• Basophil	1	0.9
• Lymphocyte	81	73.6
• Monocyte	2	1.8
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	2	1.8
• Lymphocyte, variant form	21	19.1
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	110	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	-	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	9	-
• Artefact	1	-

NOTE: Monocytosis and basophilia as indicated on the A5 result are not confirmed by the microscopic evaluation of the smear. The false results of the A5 system are due to the presence of highly abnormal lymphocytes causing improper separation of WBC populations as indicated by the confluent WBC scattergram. This also illustrates that automated results are for rapid screening purposes only. The microscopic evaluation of peripheral blood smears in the case of abnormal WBC scattergrams or results with or without the presence of any warning or morphological flags is essential.

Other laboratory findings

Other laboratory findings are not remarkable.

Diagnosis

Chronic lymphoid leukemia

Disease course

The RBC count is low but this case does not justify transfusion yet. Erythropoietin was administered to trigger erythropoiesis.

The patient's status is stable; his next visit was scheduled one month later.

Case 31 | Chronic lymphoid leukemia (CLL)

History and clinical signs and symptoms

A 45 year old man was diagnosed with indolent chronic lymphoid leukemia 6 years before. Quite recently night sweating and lymphadenopathy in the cervical region has developed. The patient returned to his scheduled control visit. Physical signs and symptoms include fatigue, the spleen is enlarged.

Hematologic findings

Sample ID	Patient 31						Patient ID	
Date	01/01/2013						Name	
Mode	Human						Date of birth	
Parameter	Result			Limit			Gender	
WBC	118.4	*	$10^3/\mu L$	5.00	-	10.00	DIFF	BAS
NEU	4.50	*	$10^3/\mu L$	2.00	-	7.50		
LYM	93.43	*	$10^3/\mu L$	1.08	-	3.17		
MON	11.60	*	$10^3/\mu L$	0.15	-	0.70		
EO	0.47	*	$10^3/\mu L$	0.00	-	0.50		
BAS	8.41	*	$10^3/\mu L$	0.00	-	0.15		
NEU%	3.8	--	%	40.0	-	75.0		
LYM%	78.9	+	%	14.76	-	45.40		
MON%	9.8	+	%	3.0	-	7.0		
EO%	0.4		%	0.0	-	5.0		
BAS%	7.1	++	%	0.0	-	1.5		
RBC	5.28		$10^6/\mu L$	4.00	-	5.50	RBC	
HGB	135		g/L	120	-	174		
HCT	43.5		%	36.0	-	52.0		
MCV	82.4		fL	76.0	-	96.0		
MCH	25.6	-	pg	27.0	-	32.0		
MCHC	310		g/L	300	-	350		
RDWsd	30.4		fL	20.0	-	42.0		
RDWcv	11.9		%	0.0	-	16.0		
PLT	199		$10^3/\mu L$	150	-	400	PLT	
PCT	0.29		%		-			
MPV	14.4		fL	8.0	-	15.0		
PDWsd	26.0		fL		-			
PDWcv	29.8		%		-			
Warning flags	D							
Morphological flags	GL							
Interpretive flags								

Interpretation

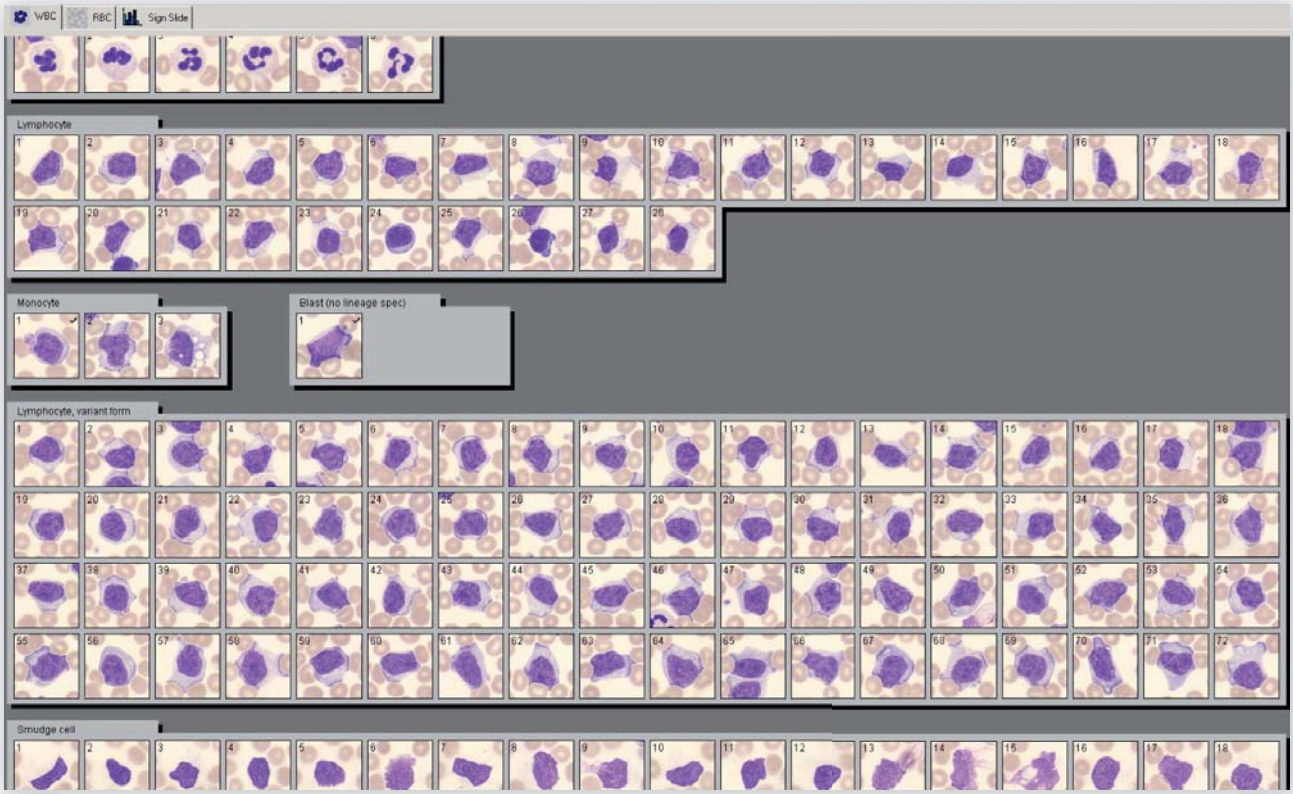
The WBC DIFF scattergram is abnormal, lymphocytes and monocytes are not separated. Thus the instrument was unable to differentiate a significant portion of the WBC population (see warning flag "D"). Based on the WBC DIFF and BAS scattergrams the presence of abnormal cells is highly suspected. Beside abnormal scattergram characteristics the WBC count is very high and percentile distrubutions' are pathologic. Linearity flag " * " indicates that WBC counts are out of the linearity range. Morphological flags 'G' and 'L' are indicative of immature granulocytes and atypical lymphocytes.*

*Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system. Due to their altered cytoplasmic and membrane structure they can mimic other cells often falsely elevating basophil and monocyte counts can be seen in this example when comparing results to those of a microscopic evaluation.

Based on the histograms, the red cell (RBC) and platelet (PLT) populations are clearly defined and well separated. The RBC parameters are without any pathologic alterations. The PLT count is slightly below the lower normal limit

The assessment of a peripheral blood smear is essential due to the high WBC count, undifferentiated WBC populations and warning flags.

Peripheral blood smear



Peripheral blood smear characterized by variant lymphocytes corresponds to the clinical diagnosis.
The relative prevalence of the WBCs was as follows: neutrophil cells (5.5%), lymphocytes (25.5%), monocytes (2.7%), variant (enlarged) lymphocytes (65.5%) and blasts (0.9%) and smudge cells.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	6	5.5 ✓
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	28	25.5 ✓
• Monocyte	3	2.7 ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	1	0.9 ✓
• Lymphocyte, variant form	72	65.5 ✓
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	110	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	-	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	22	- ✓
• Artefact	-	-

NOTE: Monocytosis and basophilia as indicated on the A5 result are not confirmed by the microscopic evaluation of the smear. The false results of the A5 system are due to the presence of highly abnormal lymphocytes causing improper separation of WBC populations as indicated by the WBC scattergram and the warning flags “G”. This also illustrates that automated results are for rapid screening purposes only. The microscopic evaluation of peripheral blood smears in the case of abnormal WBC scattergrams or results with or without the presence of any warning or morphological flags is essential.

Other laboratory findings

Other laboratory findings are not remarkable.

Diagnosis

Chronic lymphoid leukemia

Disease course

Physical signs and symptoms include fatigue, the spleen is enlarged.

The patient was reluctant to chemotherapy. His next visit was scheduled one month later.

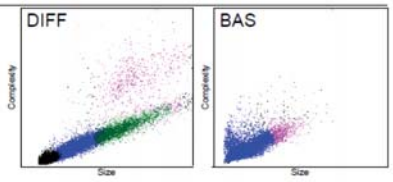
Case 32 | Chronic B-cell leukemia with high WBC count (need for manual sample dilution)

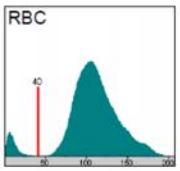
History and clinical signs and symptoms

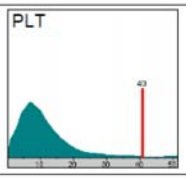
A 55 year old woman was diagnosed with chronic B-cell (B-CLL) 2 years ago. The patient is regularly treated with standard chemotherapeutic regime. Now she returned for the next course of therapy. Lymphadenopathy developed in the retroperitoneal region. A mild hepatosplenomegaly was also present.

Hematologic findings

Sample ID			Patient 32				Patient ID	
Date			01/01/2013				Name	
Mode			Human				Date of birth	
Parameter			Result		Limit		Gender	
WBC	---	**	$10^3/\mu L$	5.00	-	10.00		
NEU	---	**	$10^3/\mu L$	2.00	-	7.50		
LYM	---	**	$10^3/\mu L$	1.08	-	3.17		
MON	---	**	$10^3/\mu L$	0.15	-	0.70		
EO	---	**	$10^3/\mu L$	0.00	-	0.50		
BAS	---	**	$10^3/\mu L$	0.00	-	0.15		
NEU%	---	**	%	40.0	-	75.0		
LYM%	---	**	%	14.76	-	45.40		
MON%	---	**	%	3.0	-	7.0		
EO%	---	**	%	0.0	-	5.0		
BAS%	---	**	%	0.0	-	1.5		
RBC	3.10	-	$10^6/\mu L$	4.00	-	5.50		
HGB	104	-	g/L	120	-	174		
HCT	34.9	-	%	36.0	-	52.0		
MCV	112.5	+	fL	76.0	-	96.0		
MCH	33.6	+	pg	27.0	-	32.0		
MCHC	299	-	g/L	300	-	350		
RDWsd	61.0	+	fL	20.0	-	42.0		
RDWcv	15.5		%	0.0	-	16.0		
PLT	189		$10^3/\mu L$	150	-	400		
PCT	0.24		%		-			
MPV	12.9		fL	8.0	-	15.0		
PDWsd	29.4		fL		-			
PDWcv	35.3		%		-			







Warning flags	O
Morphological flags	G
Interpretive flags	Anemia?, Macrocytic RBC?, Hypochromic?

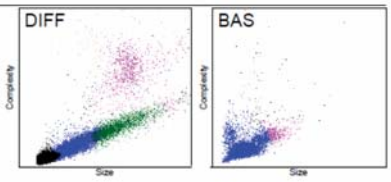
Interpretation

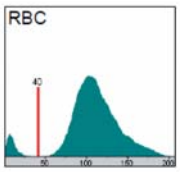
The RBC and PLT histograms are normal in shape and well separated. The red cell (RBC) population is low in size. The low RBC count indicates anemia (flagged).

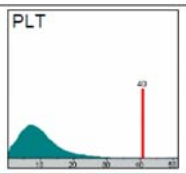
The elevated RDWsd indicates the heterogeneity in RBC size (anisocytosis). The mean cell hemoglobin concentration (MCHC) is below the lower level of normal in the majority of RBCs. The anemia is flagged as macrocytic and hypochromic.

The instrument was unable to provide a WBC count but the DIFF and BAS sctattergrams clearly show the presence of cells. The WBC DIFF scattergram indicates a confluent population; the Warning Flag "O" is also present indicating that cell count is over the upper limit of linearity range. Therefore, the sample was diluted 2-fold in saline and re-measured. Please, note that the WBC count should be multiplied by a factor of 2 and the real numerical counts should be around 2 fold of the displayed values.

Sample ID		Patient 32					Patient ID	
Date		01/01/2013					Name	
Mode		Human					Date of birth	
							Gender	
Parameter	Result			Limit				
WBC	82.10	++	$10^3/\mu L$	5.00	-	10.00		
NEU	2.22		$10^3/\mu L$	2.00	-	7.50		
LYM	66.67	++	$10^3/\mu L$	1.08	-	3.17		
MON	10.67	++	$10^3/\mu L$	0.15	-	0.70		
EO	0.16		$10^3/\mu L$	0.00	-	0.50		
BAS	2.38	++	$10^3/\mu L$	0.00	-	0.15		
NEU%	2.7	--	%	40.0	-	75.0		
LYM%	81.2	+	%	14.76	-	45.40		
MON%	13.0	+	%	3.0	-	7.0		
EO%	0.2		%	0.0	-	5.0		
BAS%	2.9	+	%	0.0	-	1.5		
RBC	1.57	--	$10^6/\mu L$	4.00	-	5.50		
HGB	52	--	g/L	120	-	174		
HCT	17.8	--	%	36.0	-	52.0		
MCV	112.8	+	fL	76.0	-	96.0		
MCH	33.3	+	pg	27.0	-	32.0		
MCHC	295	-	g/L	300	-	350		
RDWsd	61.2	+	fL	20.0	-	42.0		
RDWcv	15.5		%	0.0	-	16.0		
PLT	104	-	$10^3/\mu L$	150	-	400		
PCT	0.11		%		-			
MPV	10.8		fL	8.0	-	15.0		
PDWsd	27.6		fL		-			
PDWcv	35.4		%		-			







Warning flags

Morphological flags G

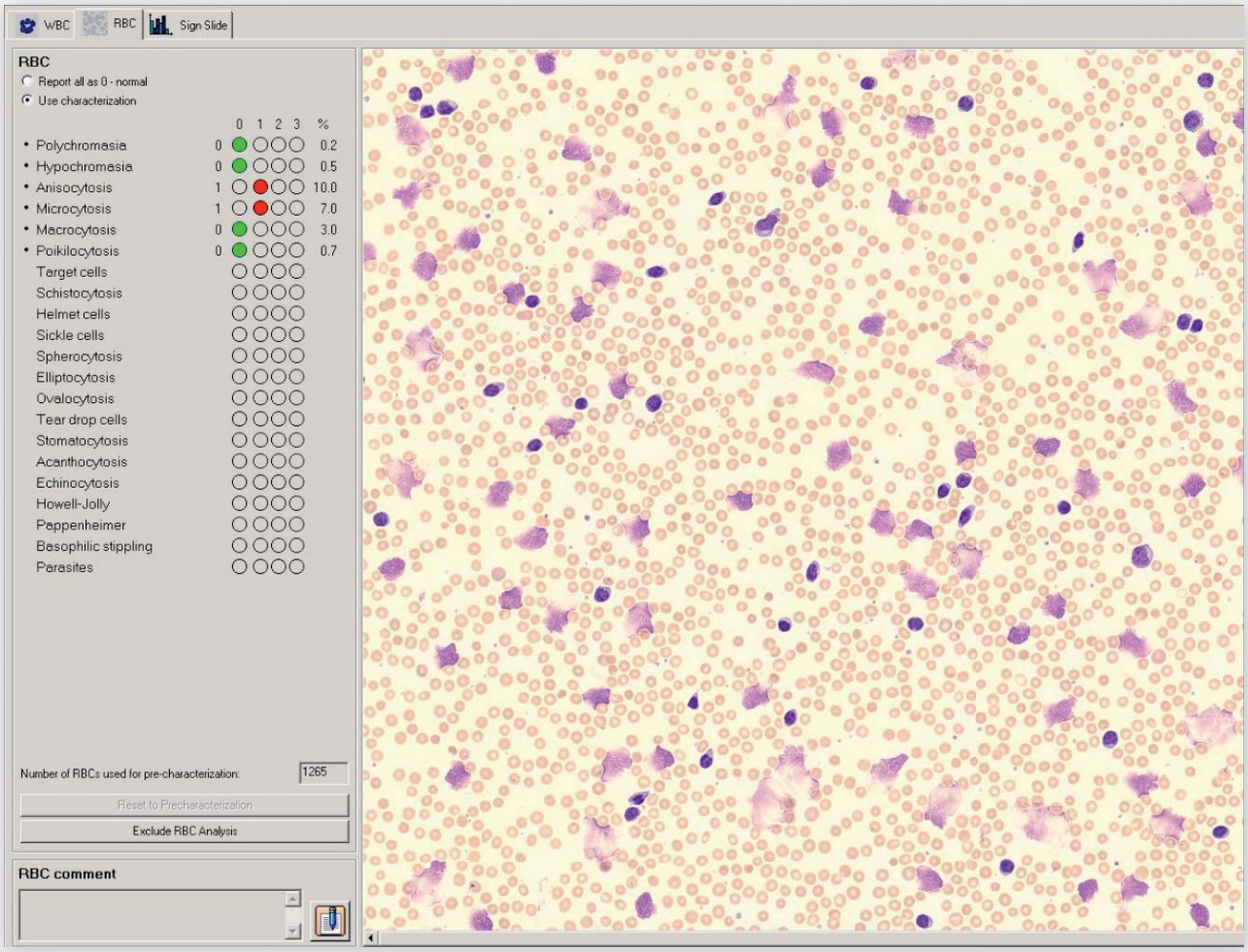
Interpretive flags Leukocytosis?, Monocytosis?, Lymphocytosis?, Basophilia?, Anemia?, Macrocytic RBC?, Hypochromic?

According to the diluted sample the WBC count is extremely high (about 160 G/L). The overwhelming majority of the WBCs are lymphocytes in line with the diagnosis of B-CCL. The high proportion of basophil cells (BAS) and monocytes (MON) along with confluent population on scattergram suggests that abnormal cells are highly prevalent.* Flag "G" indicates immature granulocytes.

The assessment of peripheral blood smear is essential.

*Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system. Due to their special response to lyzing effect atypical and blast cells may falsely increase the basophil or monocyte counts which is shown in the result of microscopic evaluation of the smear.

Peripheral blood smear



Macrocytosis is not confirmed by the microscopic analysis. Falsely elevated MCV value is caused by the extremely high WBC count which interferes with the RBC counting. The real RBC count may be somewhat lower than the displayed one.

The Peripheral blood smear characterized by the highly abundant lymphocytes (including atypical forms) and remnants of abnormally fragile lymphocytes ('smudge cells'). The peripheral blood smear corresponds to the clinical diagnosis and indicates the progression of disease.

WBC	Count	%
• Unidentified	-	-
• **Band neutrophil**	-	-
• Segmented neutrophil	5	4.6 ✓
• Eosinophil	-	-
• Basophil	-	-
• Lymphocyte	88	81.5
• Monocyte	2	1.9
• Promyelocyte	-	-
• Myelocyte	1	0.9
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	-	-
• Lymphocyte, variant form	12	11.1
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	1	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	152	-
• Artefact	-	-

NOTE: Monocytosis and basophilia as indicated on the A5 result are not confirmed by the microscopic evaluation of the smear. The false results of the A5 system are due to the presence of highly abnormal lymphocytes causing improper separation of WBC populations as indicated by the WBC scattergram and the warning flags "G". This also illustrates that automated results are for rapid screening purposes only. The microscopic evaluation of peripheral blood smears in the case of abnormal WBC scattergrams or results with or without the presence of any warning or morphological flags is essential.

Other laboratory findings

Other laboratory finidings are not remarkable.

Diagnosis

Chronic Lymphocytic (B-Cell) leukemia

Disease course

The WBC count did not respond to standard chemotherapeutic regime; the patient had to be switched on another one.

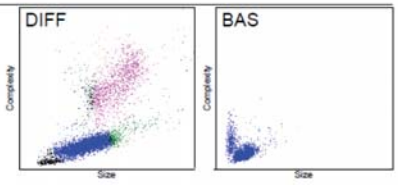
Case 33 | Hairy cell leukemia

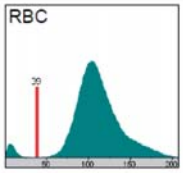
History and clinical signs and symptoms

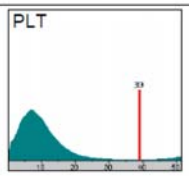
Last year the 55 year old man suffered a complicated deep vein thrombosis of the right leg. Splenomegaly was detected by physical exam. The routine CBC revealed abnormalities in WBC subpopulations; peripheral smear indicated the presence of abnormal cells. The patient was referred to the outpatient clinic of hematology.

Hematologic findings

Sample ID		Patient 33					Patient ID	
Date		01/01/2013					Name	
Mode		Human					Date of birth	
							Gender	
Parameter	Result			Limit				
WBC	13.21	+	$10^3/\mu L$	5.00	-	10.00		
NEU	1.78	-	$10^3/\mu L$	2.00	-	7.50		
LYM	10.91	++	$10^3/\mu L$	1.08	-	3.17		
MON	0.40		$10^3/\mu L$	0.15	-	0.70		
EO	0.07		$10^3/\mu L$	0.00	-	0.50		
BAS	0.05		$10^3/\mu L$	0.00	-	0.15		
NEU%	13.5	--	%	40.0	-	75.0		
LYM%	82.6	+	%	14.76	-	45.40		
MON%	3.0		%	3.0	-	7.0		
EO%	0.5		%	0.0	-	5.0		
BAS%	0.4		%	0.0	-	1.5		
RBC	4.49		$10^6/\mu L$	4.00	-	5.50		
HGB	130		g/L	120	-	174		
HCT	50.7		%	36.0	-	52.0		
MCV	112.8	+	fL	76.0	-	96.0		
MCH	29.0		pg	27.0	-	32.0		
MCHC	257	-	g/L	300	-	350		
RDWsd	52.4	+	fL	20.0	-	42.0		
RDWcv	13.8		%	0.0	-	16.0		
PLT	149	-	$10^3/\mu L$	150	-	400		
PCT	0.15		%		-			
MPV	10.2		fL	8.0	-	15.0		
PDWsd	27.3		fL		-			
PDWcv	35.4		%		-			
Warning flags			Q					
Morphological flags								
Interpretive flags			Leukocytosis?, Lymphocytosis?, Macrocytic RBC?, Hypochromic?					







Interpretation

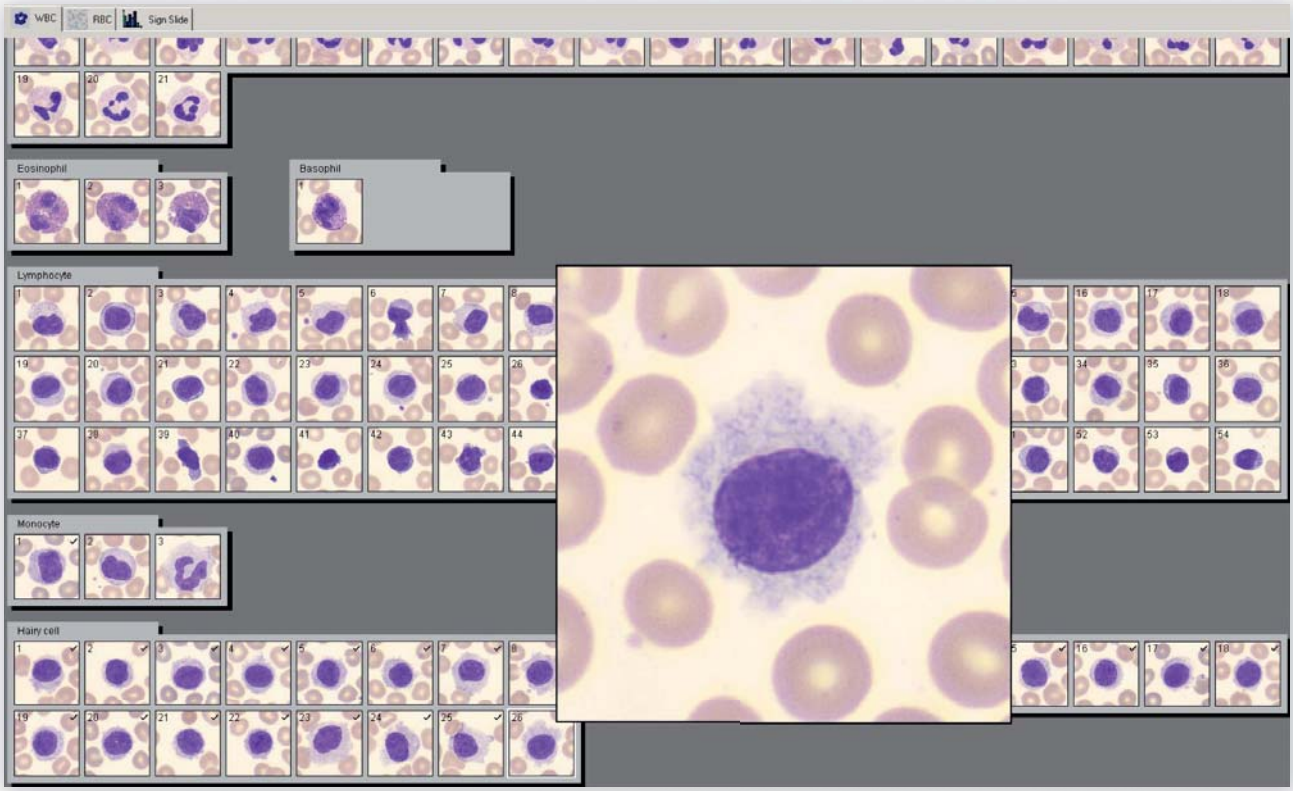
The WBC DIFF scattergram is abnormal, the monocyte-lymphocyte subpopulations are not clearly defined (see warning flag "Q"). The WBC count is just above the normal. Marked neutropenia and lymphocytosis is present. The RBC and PLT histograms are well separated and normal in characteristic.

The red cell (RBC) population is homogenous, the cells are somewhat larger (elevated MCV and decreased MCHC).

The Platelet count (PLT) is just below the normal.

Because of abnormal DIFF scattergram and numerical findings, a microscopic evaluation of the blood smear was recommended.

Peripheral blood smear



The peripheral blood smear is characterized by lymphocytes (74.1%). However, 24.1% of lymphocytes are abnormal with a phenotype of 'hairy cell'. .

WBC	Count	%	
• Unidentified	-	-	
• Band neutrophil	-	-	
• Segmented neutrophil	21	19.4	✓
• Eosinophil	3	2.8	✓
• Basophil	1	0.9	✓
• Lymphocyte	54	50.0	✓
• Monocyte	3	2.8	✓
• Promyelocyte	-	-	
• Myelocyte	-	-	
• Metamyelocyte	-	-	
Immature eosinophil	-	-	
Immature basophil	-	-	
Promonocyte	-	-	
Prolymphocyte	-	-	
• Blast (no lineage spec)	-	-	
• Lymphocyte, variant form	-	-	
• Plasma cell	-	-	
Large granular lymphocyte	-	-	
Hairy cell	26	24.1	✓
Sezary cell	-	-	
Other	-	-	
Total	108	100	

Non-WBC	Count	%	
• Erythroblast (NRBC)	-	-	
• Giant thrombocyte	1	-	✓
• Thrombocyte aggregation	-	-	
Megakaryocyte	-	-	
• Smudge cell	14	-	✓
• Artefact	11	-	

NOTE: Differences between analyzer's result and microscopic evaluation are due to the presence of highly abnormal lymphocytes causing improper separation of WBC populations as indicated by the WBC scattergram and the warning flag "Q". This also illustrates that automated results are for rapid screening purposes only. The microscopic evaluation of peripheral blood smears in the case of abnormal WBC scattergrams or results with or without the presence of any warning or morphological flags is essential.

Other laboratory findings

BRAF V600E mutation was demonstrated. The diagnosis of hairy cell leukemia was established.

Diagnosis

Hairy cell leukemia

Disease course

The therapeutic protocol was designed. The patient's next visit was scheduled.

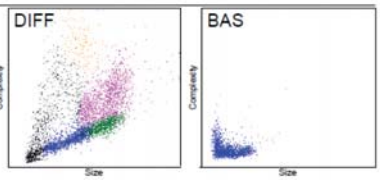
Case 34 | Acute myelo-monocytic leukemia (AMML)

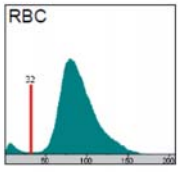
History and clinical signs and symptoms

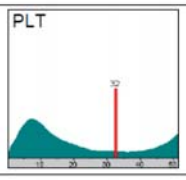
A 65 year old woman was diagnosed with acute myelo-monocytic leukemia (AMML) 3 months before. AMML was suspected based on leukocytosis with highly prevalent atypical myeloid cells in the peripheral blood. The exact diagnosis was established with the appropriate flow cytometric and cytogenetic methods. The patient returned to the next course of her chemotherapy regime. There was a marked susceptibility for bruising. Physical signs and symptoms were not specific.

Hematologic findings

Sample ID	Patient 34					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	8.45		$10^3/\mu L$	5.00	- 10.00		
NEU	---	**	$10^3/\mu L$	2.00	- 7.50		
LYM	---	**	$10^3/\mu L$	1.08	- 3.17		
MON	---	**	$10^3/\mu L$	0.15	- 0.70		
EO	---	**	$10^3/\mu L$	0.00	- 0.50		
BAS	0.04		$10^3/\mu L$	0.00	- 0.15		
NEU%	---	**	%	40.0	- 75.0		
LYM%	---	**	%	14.76	- 45.40		
MON%	---	**	%	3.0	- 7.0		
EO%	---	**	%	0.0	- 5.0		
BAS%	0.5		%	0.0	- 1.5		
RBC	3.51	-	$10^6/\mu L$	4.00	- 5.50		
HGB	97	-	g/L	120	- 174		
HCT	31.7	-	%	36.0	- 52.0		
MCV	90.4		fL	76.0	- 96.0		
MCH	27.6		pg	27.0	- 32.0		
MCHC	305		g/L	300	- 350		
RDWsd	39.9		fL	20.0	- 42.0		
RDWcv	13.7		%	0.0	- 16.0		
PLT	89	-	$10^3/\mu L$	150	- 400		
PCT	0.10		%		-		
MPV	10.8		fL	8.0	- 15.0		
PDWsd	27.0		fL		-		
PDWcv	30.6		%		-		
Warning flags	AD						
Morphological flags	G						
Interpretive flags	Anemia?						







Interpretation

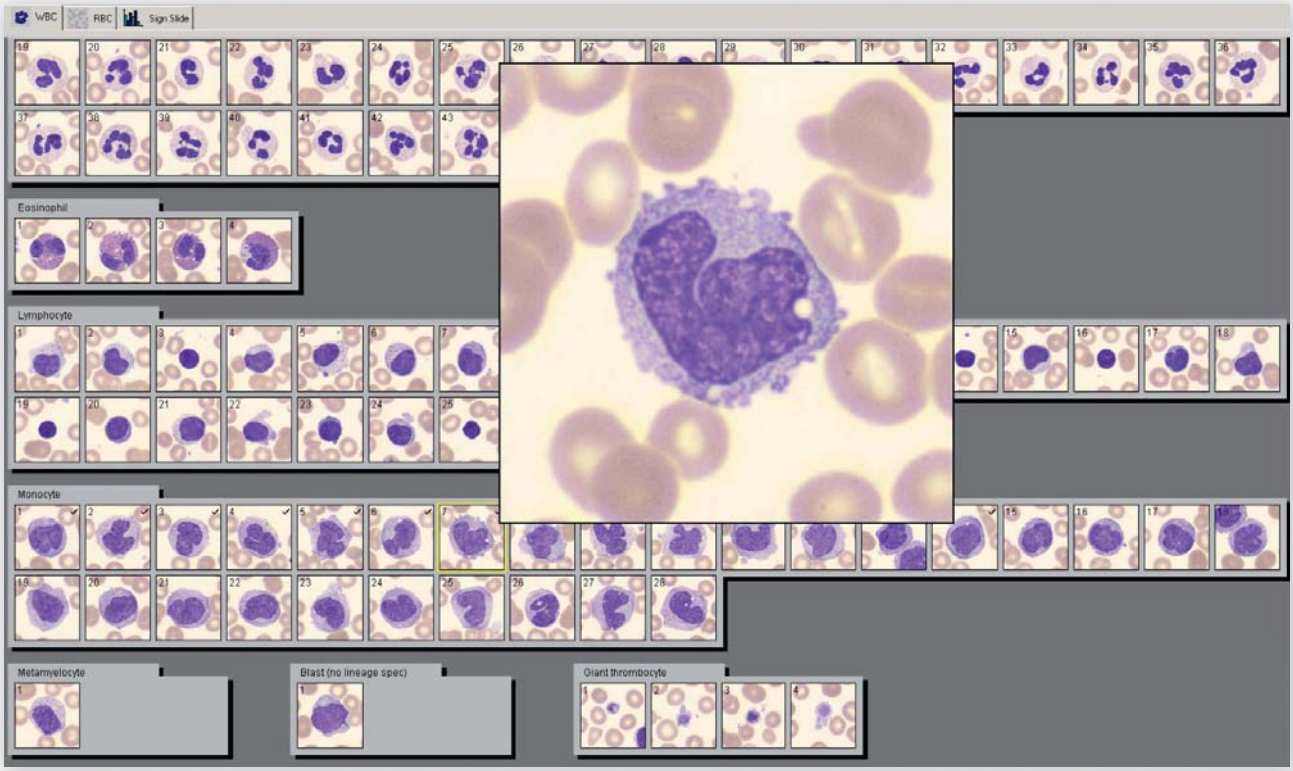
The instrument provided just a normal WBC count without any differential counts. The WBC differential (DIFF) scattergram is abnormal and indicates a confluent population; a peripheral blood smear should be analyzed. Based on the WBC scattergram there is a large number of (probably morphologically abnormal) cells that the instrument was not able to identify (see warning flags 'A' and 'D').*
Morphological flag 'G' indicates immature granulocytes.

* Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system.

The RBC and PLT histograms shape are normal and well separated. The RBC count and HGB and HCT levels are decreased indicating anemia (see flag). The red cell (RBC) population is homogenous; the cells' size and hemoglobin content are within the normal range.

Due to warning flags AD and the absence of differential WBC count the assessment of a peripheral blood smear is essential.

Peripheral blood smear



The peripheral blood smear is characterized by the highly abundant mononuclear cells; these resemble an immature form of monocytes (i.e. pro-monocytes). The peripheral blood smear corresponds to the clinical diagnosis'.

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	-	-
• Segmented neutrophil	44	40.7 ✓
• Eosinophil	4	3.7 ✓
• Basophil	-	-
• Lymphocyte	30	27.8 ✓
• Monocyte	28	25.9 ✓
• Promyelocyte	-	-
• Myelocyte	-	-
• Metamyelocyte	1	0.9 ✓
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	1	0.9 ✓
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	108	100

Non-WBC	Count	%
• Erythroblast (NRBC)	-	-
• Giant thrombocyte	4	- ✓
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	13	- ✓
• Artefact	5	- ✓

Other laboratory findings

Other laboratory findings are not remarkable.

Diagnosis

Acute myelo-monocytic leukemia

Disease course

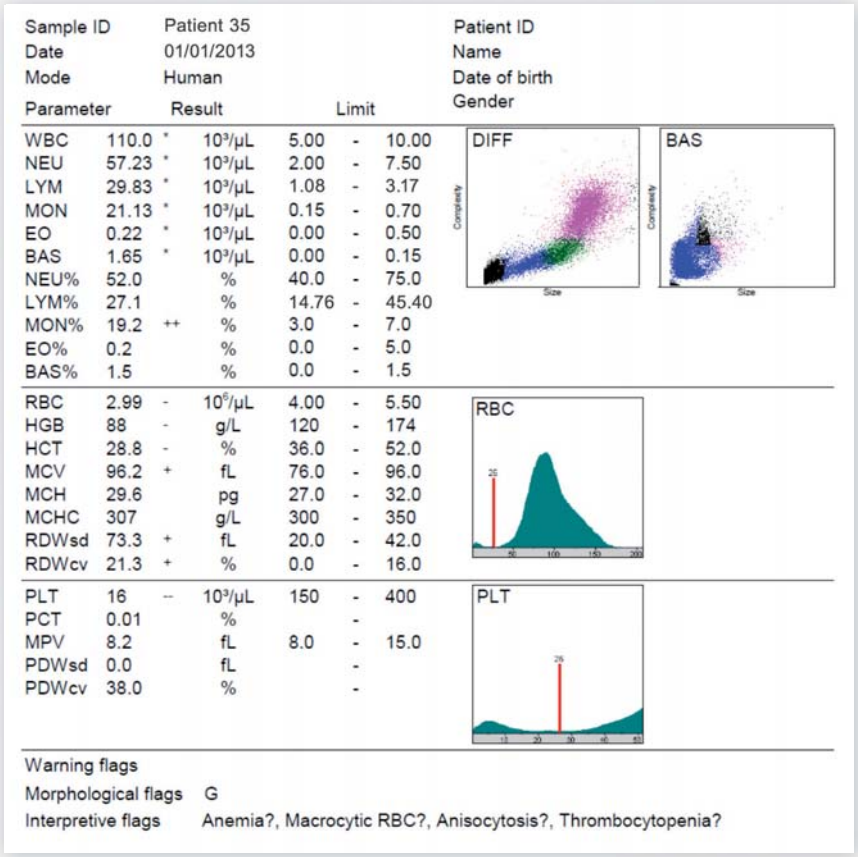
The chemotherapeutic cycles were continued.

Case 35 | Acute myeloid leukemia (AML), monocytosis, nucleated red blood cells (NRBCs)

History and clinical signs and symptoms

A 60 year old woman was diagnosed with acute myeloid leukemia three months ago. She was admitted to the hospital to a palliative chemotherapy treatment.

Hematologic findings



Sample ID	Patient 35					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
						Gender	
Parameter	Result			Limit			
WBC	110.0	*	$10^3/\mu L$	5.00	-	10.00	
NEU	57.23	*	$10^3/\mu L$	2.00	-	7.50	
LYM	29.83	*	$10^3/\mu L$	1.08	-	3.17	
MON	21.13	*	$10^3/\mu L$	0.15	-	0.70	
EO	0.22	*	$10^3/\mu L$	0.00	-	0.50	
BAS	1.65	*	$10^3/\mu L$	0.00	-	0.15	
NEU%	52.0		%	40.0	-	75.0	
LYM%	27.1		%	14.76	-	45.40	
MON%	19.2	++	%	3.0	-	7.0	
EO%	0.2		%	0.0	-	5.0	
BAS%	1.5		%	0.0	-	1.5	
RBC	2.99	-	$10^6/\mu L$	4.00	-	5.50	
HGB	88	-	g/L	120	-	174	
HCT	28.8	-	%	36.0	-	52.0	
MCV	96.2	+	fL	76.0	-	96.0	
MCH	29.6		pg	27.0	-	32.0	
MCHC	307		g/L	300	-	350	
RDWsd	73.3	+	fL	20.0	-	42.0	
RDWcv	21.3	+	%	0.0	-	16.0	
PLT	16	--	$10^3/\mu L$	150	-	400	
PCT	0.01		%		-		
MPV	8.2		fL	8.0	-	15.0	
PDWsd	0.0		fL		-		
PDWcv	38.0		%		-		
Warning flags							
Morphological flags	G						
Interpretive flags	Anemia?, Macrocytic RBC?, Anisocytosis?, Thrombocytopenia?						

Interpretation

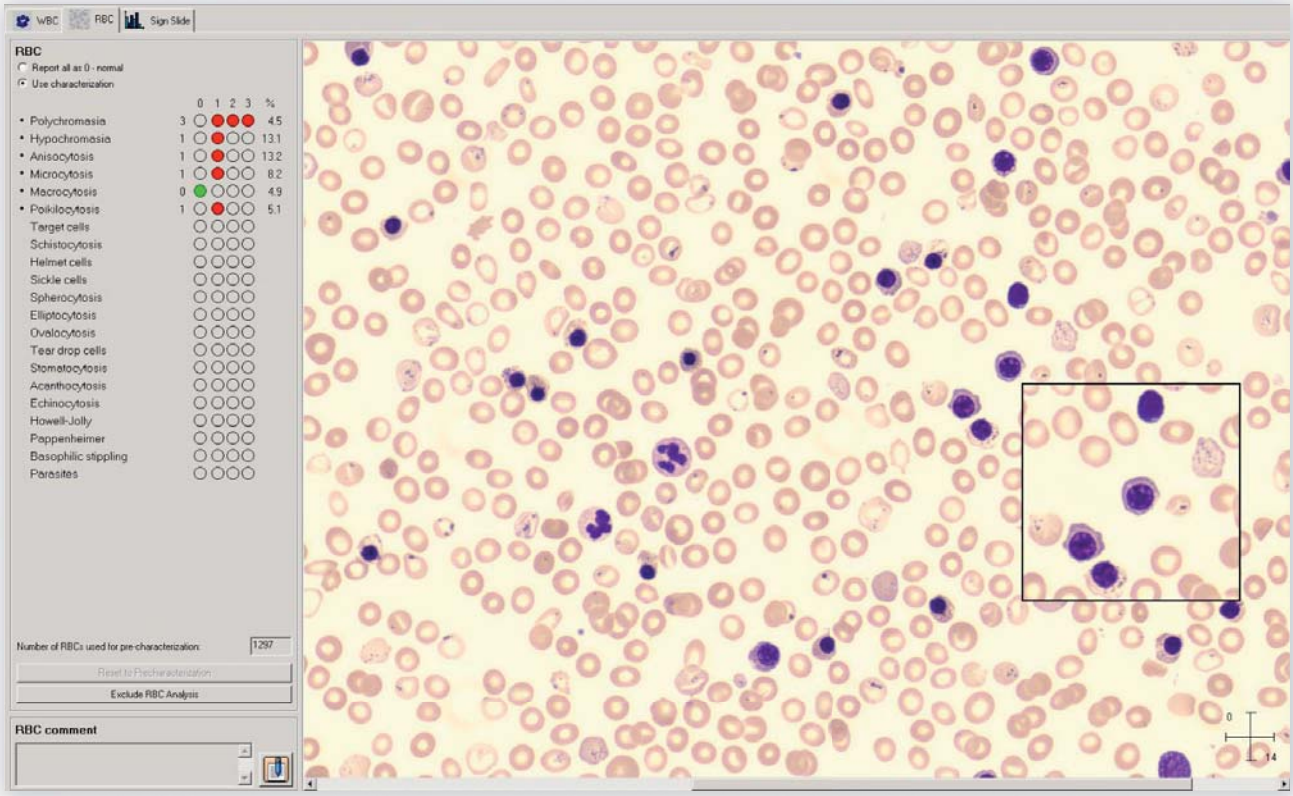
The WBC differential (DIFF) and basophil (BAS) scattergrams are not well defined. The WBC count is markedly elevated. (The flag " * " indicates that the measured parameter is out of the linearity range.) The WBC populations are confluent; the populations of neutrophils and monocytes are overlapping. (Flag " G " warns of the high percentage of immature leukocytes.) The basophil cell count and percentage are markedly increased.

The RBC count is significantly lower than normal (see interpretative flag "anemia"). The HGB and HCT values are consistent with the low RBC count. The RBC parameters indicate an elevated MCV. The RBC histogram is abnormally distributed. These findings are indicative of macrocytosis and anisocytosis (see flags).

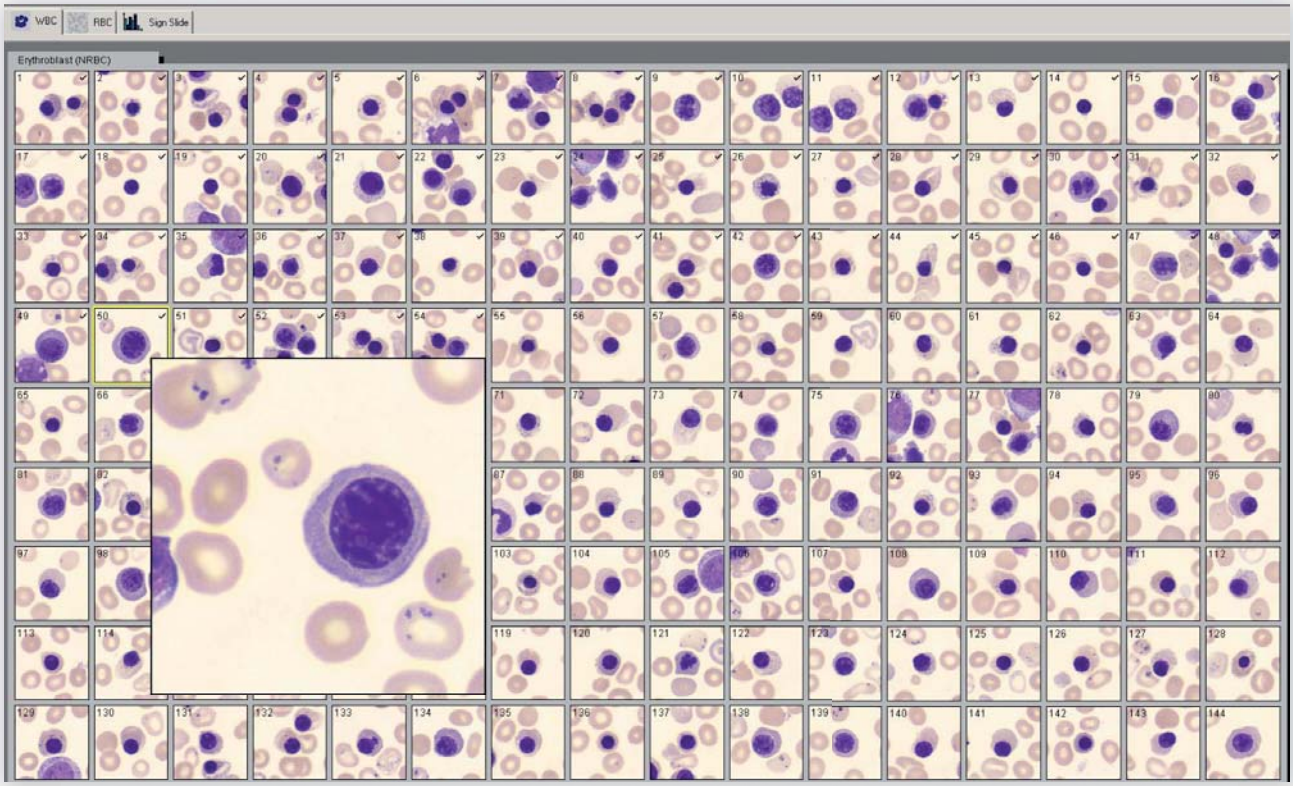
In spite of low RBC count, the PLT peak is not increased relative to RBC peak in the histogram. In fact, PLTs are absent.

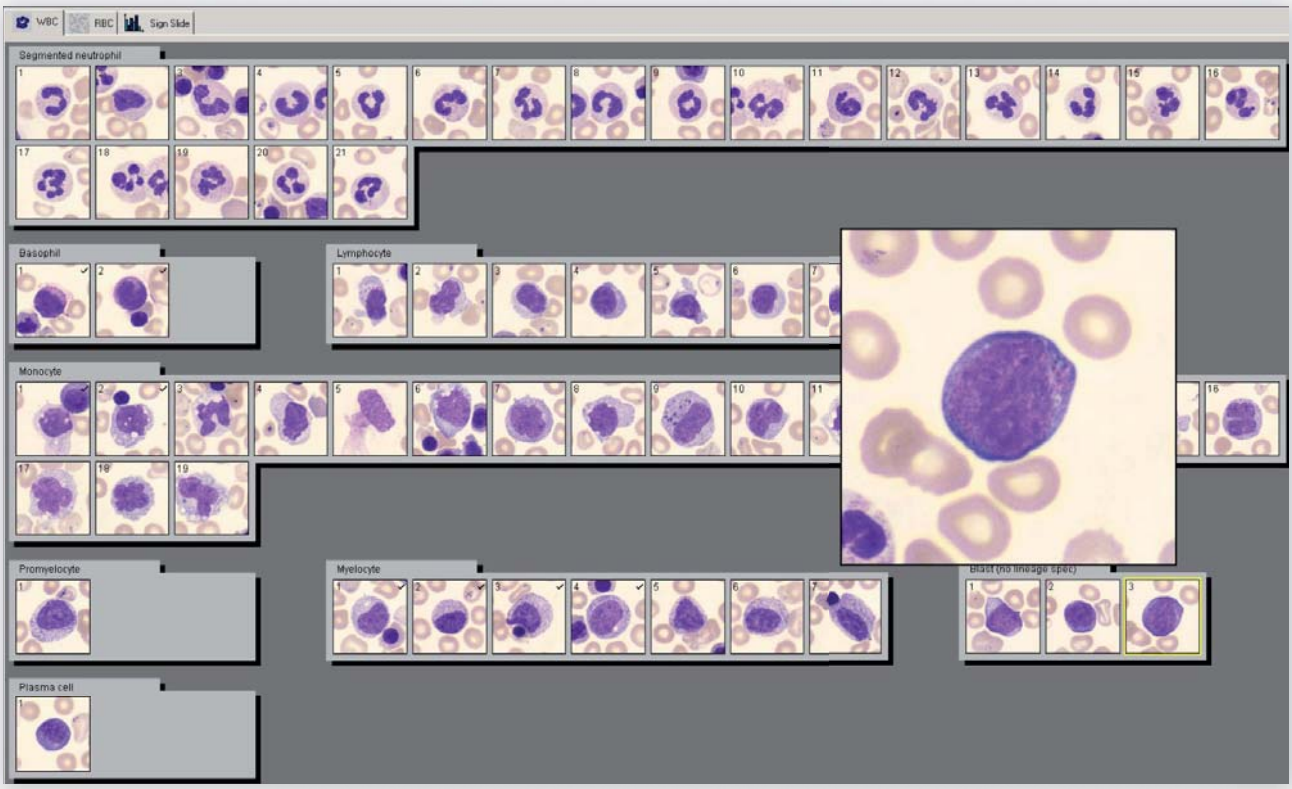
Due to the morphological flags and the confluent leukocyte populations, the visual assessment of a peripheral blood smear is strongly recommended.

Peripheral blood smear



Macrocytosis is not confirmed by the microscopic analysis. Falsely elevated MCV value is caused by the extremely high WBC count which interferes with the RBC counting. The real RBC count may be somewhat lower than the displayed one.





In line with the diagnosis of acute myeloid leukemia, immature myeloid cells such as myelocytes and pro-myelocytes are present. The percentage of monocytes and basophils is also increased (24.4% and 2.6% respectively). The prevalence of erythroblasts is dramatically increased (about 22% of total WBC count). This may lead to a falsely elevated WBC count. Therefore, the real WBC count in the peripheral blood sample should be up to 78% of the originally measured WBC.*

WBC	Count	%
• Unidentified	8	10.3
• Band neutrophil	7	9
• Segmented neutrophil	21	26.9 ✓
• Eosinophil	-	-
• Basophil	2	2.6
• Lymphocyte	9	11.6
• Monocyte	19	24.4
• Promyelocyte	1	1.3
• Myelocyte	7	9
• Metamyelocyte	-	-
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	3	3.8
• Lymphocyte, variant form	-	-
• Plasma cell	1	1.3
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	109	100

Non-WBC	Count	%
• Erythroblast (NRBC)	199	-
• Giant thrombocyte	-	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	11	-
• Artefact	24	-

* NOTE: Nucleated red blood cells are resistant to the lyzing procedure during the measurement. Therefore they are classified falsely into the WBC population. This may increase the detected WBC count. In fact, WBC may be even lower than that provided. Because of their similar size and structure NRBCs are counted mainly to the lymphocyte population. Thus analyzer's lymphocyte count is falsely elevated .

Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system.

This also illustrates that automated results are for rapid screening purposes only. The importance of microscopic evaluation of peripheral blood smears in case of abnormal results, especially in case of warning flags is high.

Other laboratory findings

Currently there is no other remarkable laboratory or physical findings.

Diagnosis

Acute myeloid leukemia in its acute phase with severe anemia and thrombocytopenia

Disease course

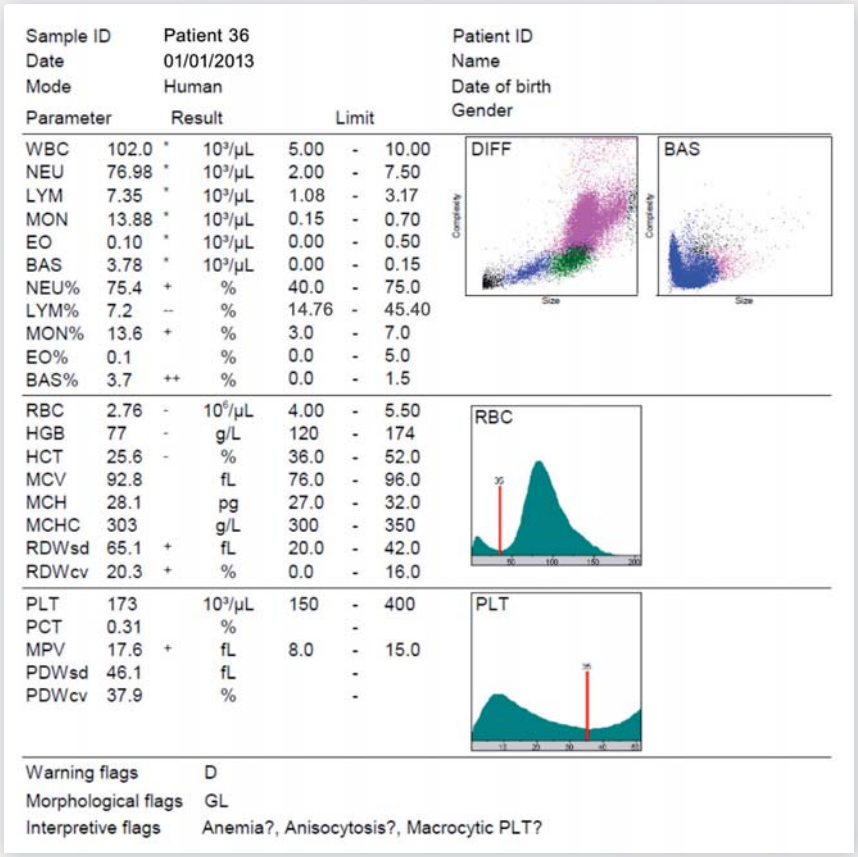
Anemia and thrombocytopenia were treated by the transfusion of packed RBCs and PLT concentrates.
The scheduled course of chemotherapy was continued.

Case 36 | Chronic myelo-monocytic leukemia (CMML) with monocytosis

History and clinical signs and symptoms

A 70 year old woman was diagnosed with chronic myelo-monocytic leukemia 6 months ago and was remitted following the appropriate treatment. Now the patient returned for a scheduled assessment.

Hematologic findings



Sample ID	Patient 36					Patient ID
Date	01/01/2013					Name
Mode	Human					Date of birth
Parameter	Result			Limit		Gender
WBC	102.0	*	$10^3/\mu L$	5.00	- 10.00	
NEU	76.98	*	$10^3/\mu L$	2.00	- 7.50	
LYM	7.35	*	$10^3/\mu L$	1.08	- 3.17	
MON	13.88	*	$10^3/\mu L$	0.15	- 0.70	
EO	0.10	*	$10^3/\mu L$	0.00	- 0.50	
BAS	3.78	*	$10^3/\mu L$	0.00	- 0.15	
NEU%	75.4	+	%	40.0	- 75.0	
LYM%	7.2	--	%	14.76	- 45.40	
MON%	13.6	+	%	3.0	- 7.0	
EO%	0.1		%	0.0	- 5.0	
BAS%	3.7	++	%	0.0	- 1.5	
RBC	2.76	-	$10^6/\mu L$	4.00	- 5.50	
HGB	77	-	g/L	120	- 174	
HCT	25.6	-	%	36.0	- 52.0	
MCV	92.8		fL	76.0	- 96.0	
MCH	28.1		pg	27.0	- 32.0	
MCHC	303		g/L	300	- 350	
RDWsd	65.1	+	fL	20.0	- 42.0	
RDWcv	20.3	+	%	0.0	- 16.0	
PLT	173		$10^3/\mu L$	150	- 400	
PCT	0.31		%		-	
MPV	17.6	+	fL	8.0	- 15.0	
PDWsd	46.1		fL		-	
PDWcv	37.9		%		-	

Warning flags	D
Morphological flags	GL
Interpretive flags	Anemia?, Anisocytosis?, Macrocytic PLT?

Interpretation

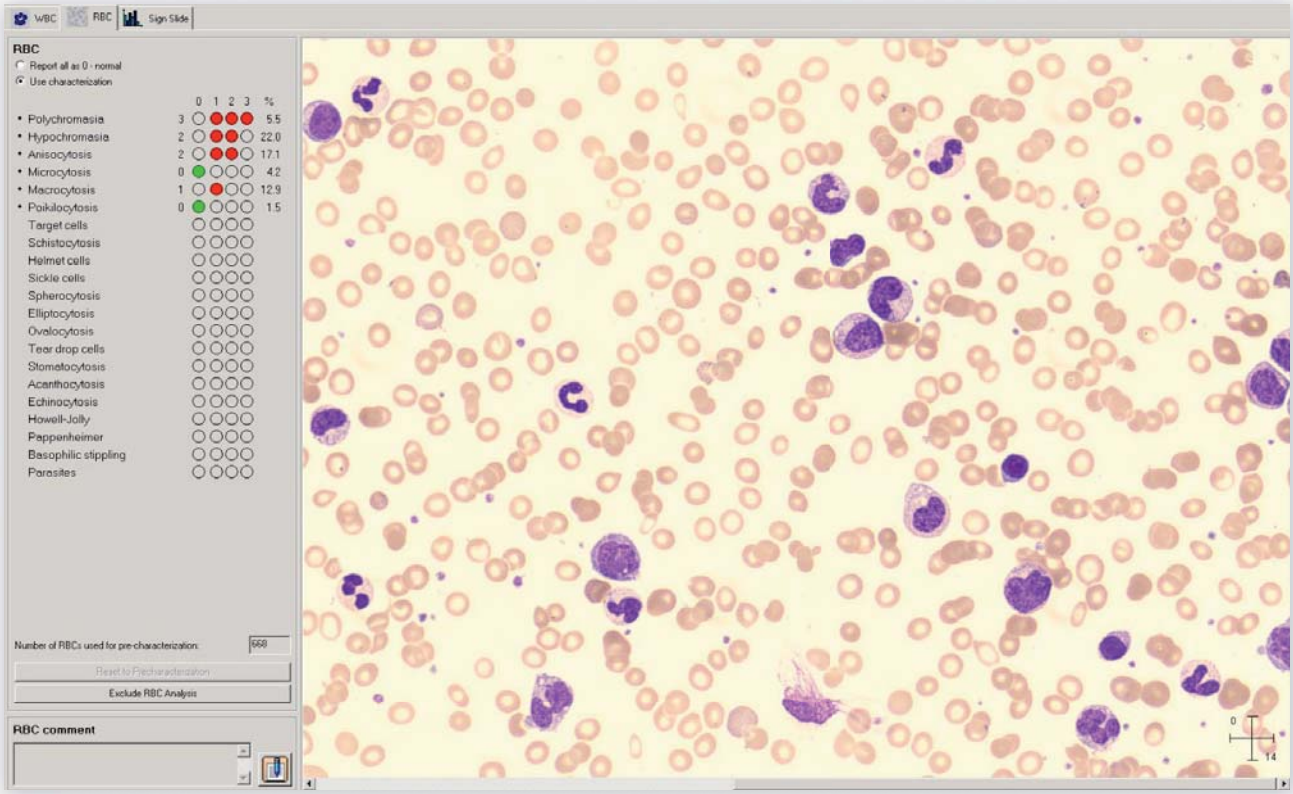
The WBC differential (DIFF) and basophil (BAS) populations are not clearly defined. The WBC count is markedly elevated. (The flag "*" indicates that the measured parameter is out of the linearity range.)
The WBC populations are confluent. The WBC populations of neutrophils and monocytes are overlapping. (Flag "D" warns that the MON and NEU populations are not clearly distinguishable on the scattergram.)
Flag "G" and "L" suggests the presence of immature neutrophils and atypical lymphocytes, respectively.
The basophil cell count and percentage are markedly increased.

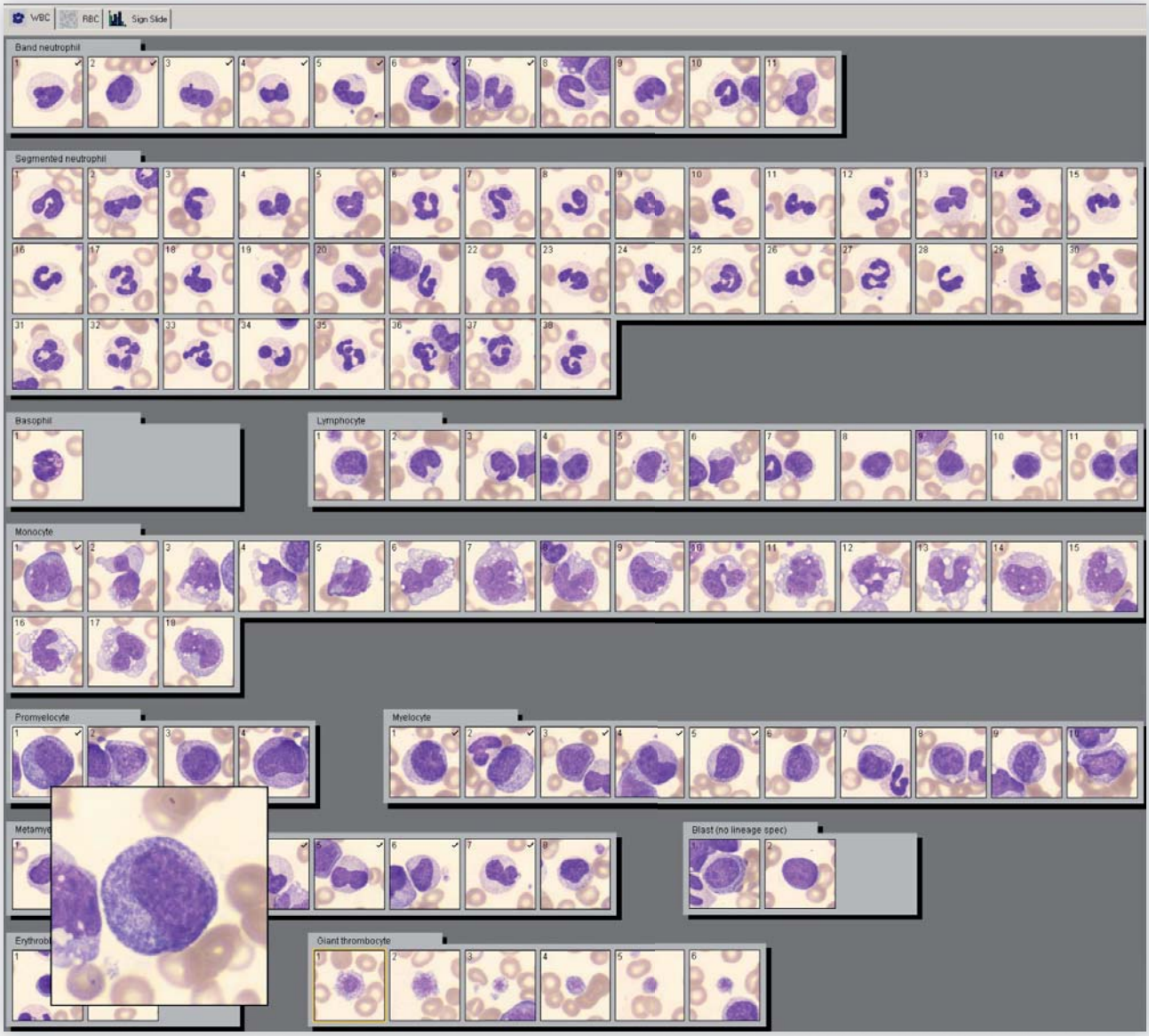
The RBC and PLT histograms are clearly separated. The RBC count is lower than normal. The HGB and HCT values are also low and support a severe anemia (see also the interpretative flag "anemia"). The RBC population is heterogeneous (see RDWcv and the interpretative flag "anisocytosis").

The PLT count is within the normal reference range. However, the PLT volume (MPV) is higher than normal and raises the presence of macrocytic PLTs.

Due to the abnormal scattergrams, the presence of warning and morphological flags, the visual assessment of a peripheral blood smear is strongly recommended.

Peripheral blood smear





In line with the diagnosis of chronic myeloid leukemia, the atypical myeloid cells including blasts, pro-myelocytes, myelocytes, metamyelocytes and band forms of neutrophils are present. A few normoblast (erythroblast/NRBCs)* can also be observed. Macro-thrombocytes are also present in the peripheral blood smear. (Basophil percentage is 1% while lymphocytes percentage is 10.7% according to the manual count).

WBC	Count	%
• Unidentified	-	-
• Band neutrophil	11	10.7
• Segmented neutrophil	38	36.9 ✓
• Eosinophil	-	-
• Basophil	1	1.0
• Lymphocyte	11	10.7
• Monocyte	18	17.5
• Promyelocyte	4	3.9
• Myelocyte	10	9.7
• **Metamyelocyte**	8	7.8
Immature eosinophil	-	-
Immature basophil	-	-
Promonocyte	-	-
Prolymphocyte	-	-
• Blast (no lineage spec)	2	1.9
• Lymphocyte, variant form	-	-
• Plasma cell	-	-
Large granular lymphocyte	-	-
Hairy cell	-	-
Sezary cell	-	-
Other	-	-
Total	103	100

Non-WBC	Count	%
• Erythroblast (NRBC)	2	-
• Giant thrombocyte	6	-
• Thrombocyte aggregation	-	-
Megakaryocyte	-	-
• Smudge cell	6	-
• Artefact	6	-

* NOTE: Nucleated red blood cells are resistant to the lyzing procedure during the measurement. Therefore they are classified falsely into the WBC population. This may increase the detected WBC count. In fact, WBC may be even lower than that provided. Because of their similar size and structure NRBCs are counted mainly to the lymphocyte population. Thus analyzer's lymphocyte count is falsely elevated .

Blasts, immature and abnormal cells cannot be distinguished reliably with HumaCount 5L system.

This also illustrates that automated results are for rapid screening purposes only. The importance of microscopic evaluation of peripheral blood smears in case of abnormal results, especially in case of warning flags is high.

Other laboratory findings

Currently there is no other remarkable laboratory or physical findings.

Diagnosis

Anemia due to a chronic myelo-monocytic leukemia relapsed

Disease course

In order to correct the anemia the patient was given an RBC transfusion. The next course of chemotherapy was scheduled.

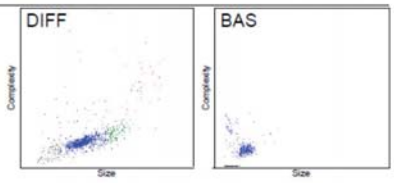
Case 37 | Hairy cell leukemia, pancytopenia

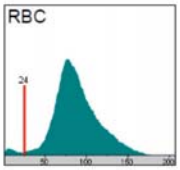
History and clinical signs and symptoms

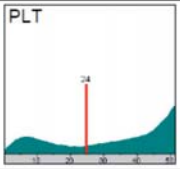
A 38 year old man was diagnosed with hairy cell leukemia 6 months earlier was currently being treated with a course of specific monoclonal antibody. A blood sample was taken as a part of his regular monitoring. Bruises and signs of sub-mucosal bleeding were also present.

Hematologic findings

Sample ID	Patient 37					Patient ID	
Date	01/01/2013					Name	
Mode	Human					Date of birth	
Parameter	Result			Limit		Gender	
WBC	1.42	--	$10^3/\mu L$	5.00	- 10.00		
NEU	0.06	--	$10^3/\mu L$	2.00	- 7.50		
LYM	1.24		$10^3/\mu L$	1.08	- 3.17		
MON	0.11	-	$10^3/\mu L$	0.15	- 0.70		
EO	0.01		$10^3/\mu L$	0.00	- 0.50		
BAS	0.00		$10^3/\mu L$	0.00	- 0.15		
NEU%	4.0	--	%	40.0	- 75.0		
LYM%	87.1	+	%	14.76	- 45.40		
MON%	8.0	+	%	3.0	- 7.0		
EO%	0.6		%	0.0	- 5.0		
BAS%	0.3		%	0.0	- 1.5		
RBC	2.49	-	$10^6/\mu L$	4.00	- 5.50		
HGB	72	-	g/L	120	- 174		
HCT	22.3	-	%	36.0	- 52.0		
MCV	89.3		fL	76.0	- 96.0		
MCH	28.7		pg	27.0	- 32.0		
MCHC	321		g/L	300	- 350		
RDWsd	63.9	+	fL	20.0	- 42.0		
RDWcv	19.1	+	%	0.0	- 16.0		
PLT	38	--	$10^3/\mu L$	150	- 400		
PCT	0.04		%		-		
MPV	10.6		fL	8.0	- 15.0		
PDWsd	33.7		fL		-		
PDWcv	38.0		%		-		







Warning flags	IQ
Morphological flags	G
Interpretive flags	Leukopenia?, Neutropenia?, Anemia?, Anisocytosis?, Thrombocytopenia?

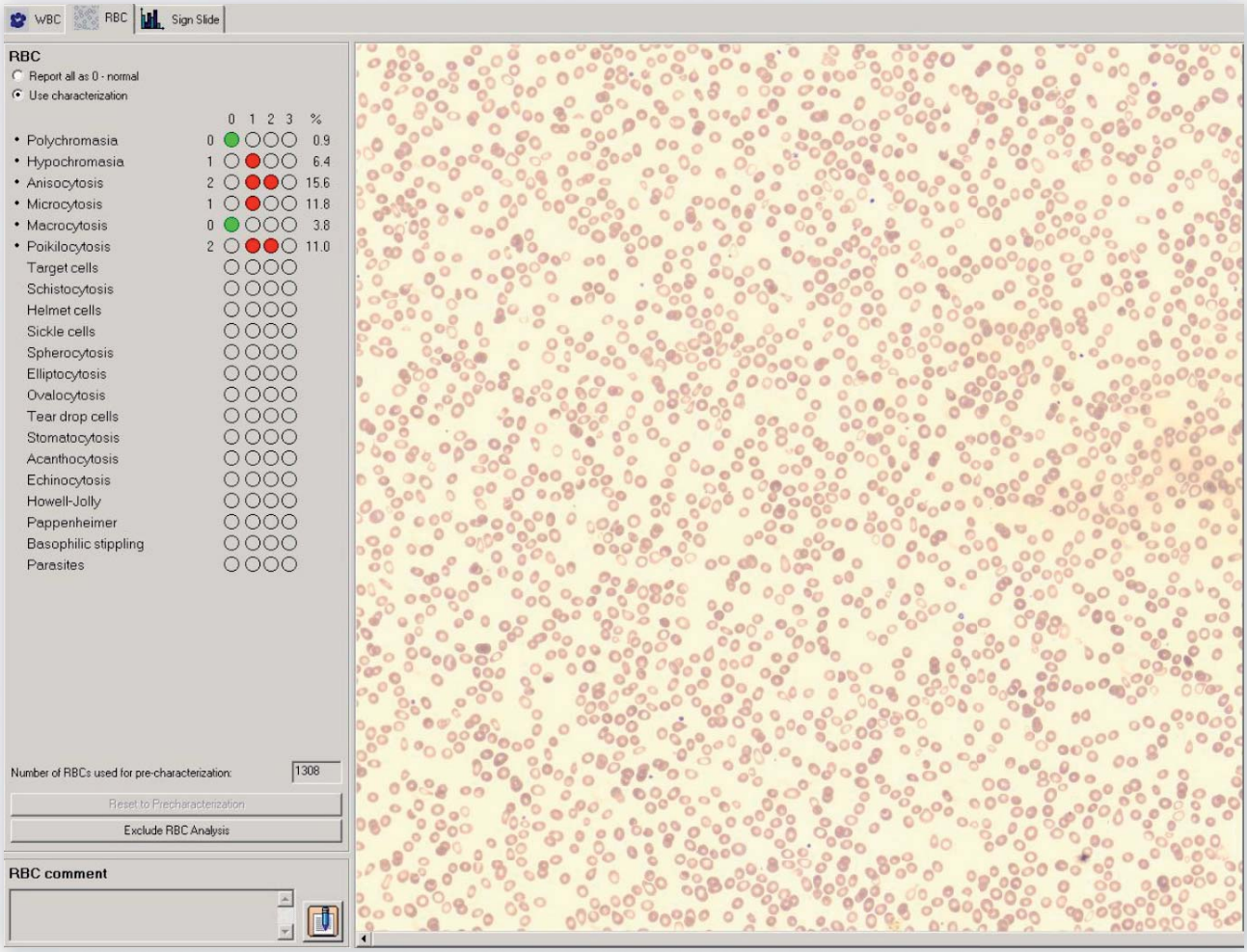
Interpretation

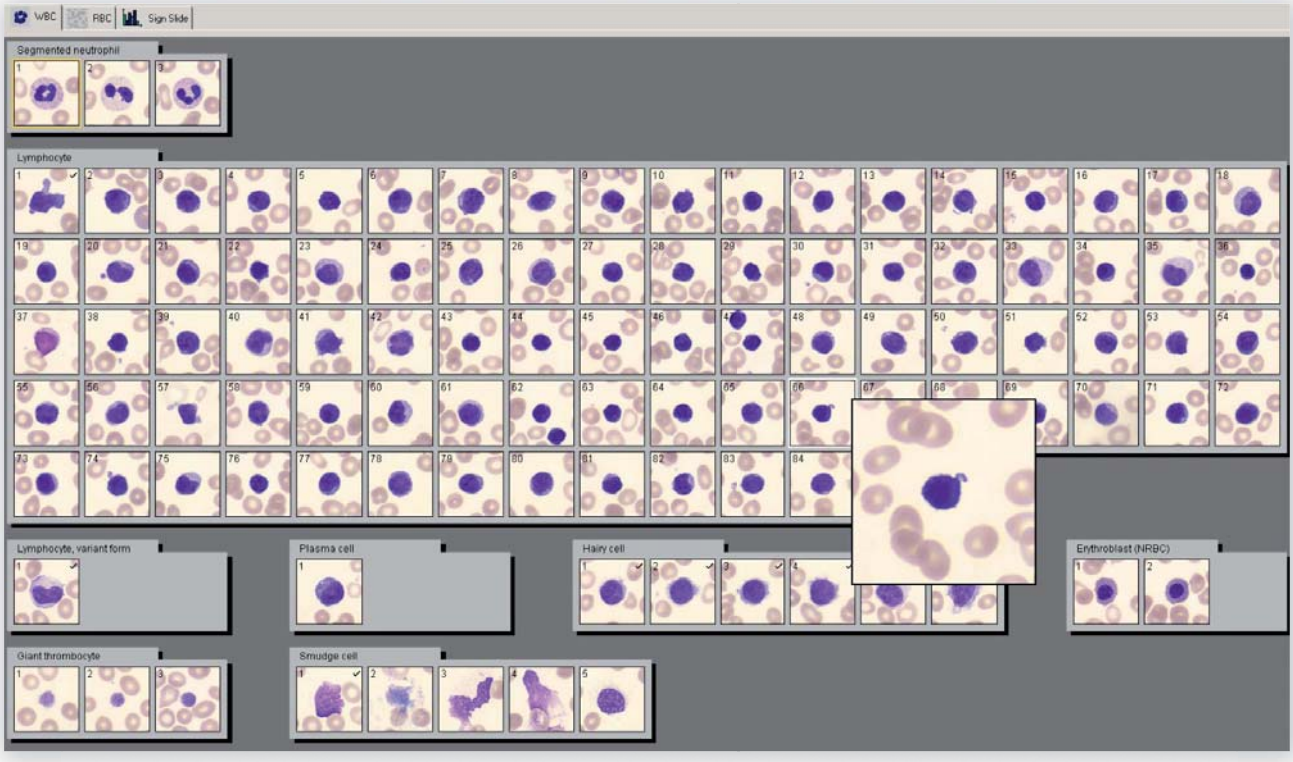
The WBC differential (DIFF) and basophil (BAS) scattergrams are well separated and defined. The WBC count, particularly the neutrophil count is extremely low (see interpretative flag “leukopenia” and “neutropenia”). The morphological flag “ G ” suggests the presence of immature granulocytes. The number of other leukocyte subpopulations is also below the lower limit of normal range. The monocyte and lymphocyte populations are confluent (warning flag “Q”).*

The RBC and PLT populations are not clearly separated (see warning flag “ I ”). The RBC histogram is abnormally distributed. Both the red cell and platelet counts are also low (see interpretative flag “anisocytosis”, “anemia” and “thrombocytopenia”).

Due to the warning and morphological flags the evaluation of peripheral blood smear is strongly recommended.

Peripheral blood smear





The peripheral blood smear supports the markedly reduced presence of neutrophil cells and the dominance of lymphoid cells in peripheral blood. The cells specific for this disease (i.e. the 'hairy cells') were also detected.*

The morphological assessment of the red cells indicate anisocytosis, poikilocytosis and hypochromasia.

WBC	Count	%	
• Unidentified	11	10.2	✓
• Band neutrophil	-	-	
• Segmented neutrophil	3	2.8	✓
• Eosinophil	-	-	✓
• Basophil	-	-	✓
• Lymphocyte	86	79.6	✓
• Monocyte	-	-	
• Promyelocyte	-	-	
• Myelocyte	-	-	
• Metamyelocyte	-	-	
Immature eosinophil	-	-	
Immature basophil	-	-	
Promonocyte	-	-	
Prolymphocyte	-	-	
• Blast (no lineage spec)	-	-	
• Lymphocyte, variant form	1	0.9	
• Plasma cell	1	0.9	
Large granular lymphocyte	-	-	
Hairy cell	6	5.6	
Sezary cell	-	-	
Other	-	-	
Total	108	100	

Non-WBC	Count	%	
• Erythroblast (NRBC)	2	-	✓
• Giant thrombocyte	3	-	✓
• Thrombocyte aggregation	-	-	
Megakaryocyte	-	-	
• Smudge cell	5	-	
• Artefact	110	-	

* Atypical lymphocytes (e.g. hairy cells) and monocytes form a confluent population and therefore analyzers monocyte count is falsely elevated while lymphocyte count is falsely decreased as can be seen if analyzer's results are compared to that of the microscopic evaluation.

Other laboratory findings

There were no other remarkable laboratory findings. Inflammatory markers were within the normal range.

Diagnosis

Pancytopenia in a patient with hairy cell leukemia

Disease course

Packed RBCs and platelet concentrates were transfused in order to correct the anemia and thrombocytopenia. The next cycle of chemotherapy was delayed.

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