Celltac

Over 30 years of experience with In Vitro Diagnostics

Celltac Clinical data book

Hematology 5 part diff scattergram interpretation

Reviewed by

Mitsuru Murata, MD, PhD

Department of Laboratory Medicine,
School of Medicine, Keio University

Fighting Disease with Electronics

NIHON KOHDEN

Celltac WBC 5 part differential methodology

Principle of flow cytometry

Nihon Kohden has developed flow cytometry technology using a laser diode for its Celltac series. In the optical system of the Celltac 5-part differential analyzers, three kinds of cell information are obtained:

● Size ● Complexity ● Granularity

Three measurements are taken simultaneously for each cell as it passes through the flow cell in the optical path (See Figure 1).

Three light detectors around the flow cell measure three parameters:

- FSS – Forward small angle scattered light
- FLS – Forward large angle scattered light
- SDS – Side angle scattered light

The size of each cell is determined from FSS, its complexity from FLS, and its granularity from SDS.

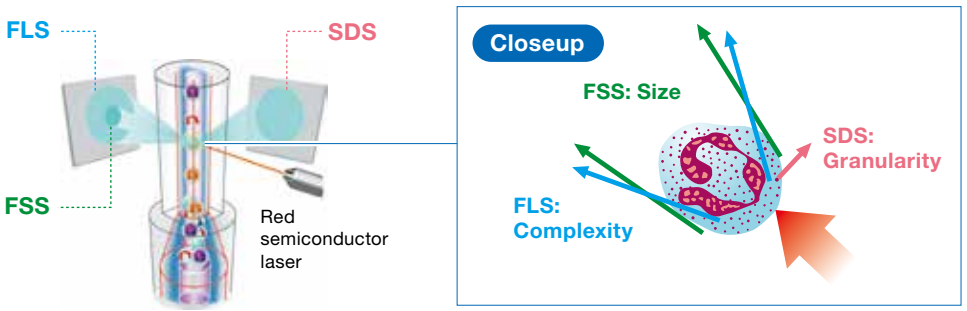


Figure 1. Internal structure of the flow cell unit

Preserving cells close to their in vivo state for measurement accuracy

A key feature of the Celltac WBC differential is that it provides accurate morphological information about the cells by preserving them in almost their original state within the circulation. Celltac 5-part differential analyzers do not alter the blood cells by staining, shrinkage or differential lysis which can distort the measurement results.

Single-channel measurement reduces cost

Celltac 5-part differential analyzers measure each cell under identical conditions. This eliminates measurement errors that can occur with multiple channel methods. This detection method also minimizes reagent consumption and therefore reduces cost.

Scattergram interpretation

Three parameters for the cells (FSS, FLS, and SDS) are plotted in a three dimensional matrix. This leads to better cluster separation than conventional two-dimensional methods (See Figure 2).

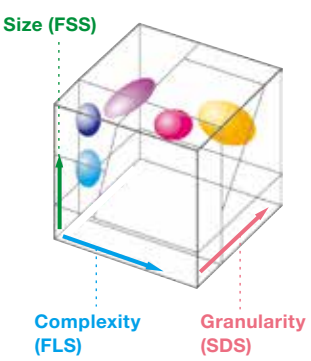
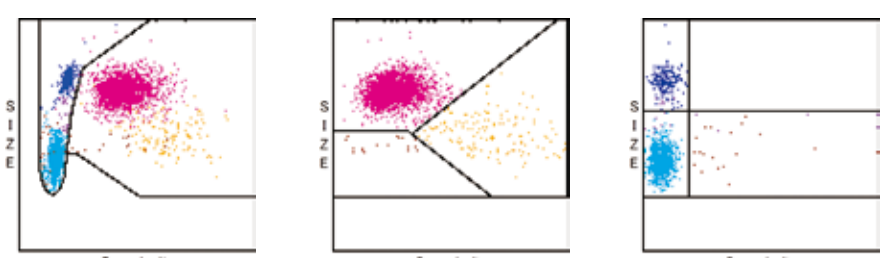


Figure 2. 3 dimensional matrix

The scattergrams below are known as:

1. **S-C (MAIN)** : size-complexity distribution
2. **S-G (NE/EO)** : size-granularity distribution
3. **S-G (LY/MO/BA)** : size-granularity distribution

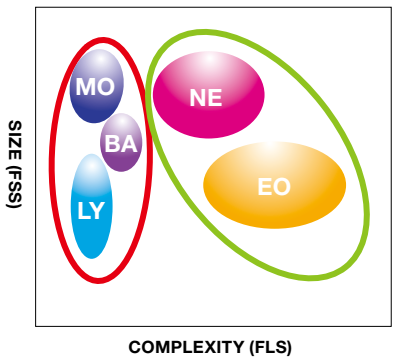


1 S-C (MAIN) 2 S-G (NE/EO) 3 S-G (LY/MO/BA)

Scattergram usefulness

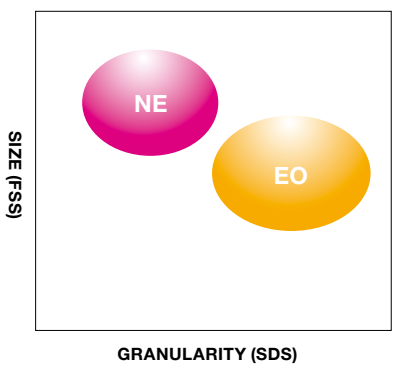
The three types of cellular information obtained with Celltac's flow cytometry method (Size, Complexity, and Granularity) correlate well with visual microscopic film examination. This allows the smooth integration of Celltac 5-part differential analyzers into the routine hematology laboratory. The operator may check for morphological abnormalities and predict the characteristics by examining the scattergram.

1 S-C (MAIN) scattergram



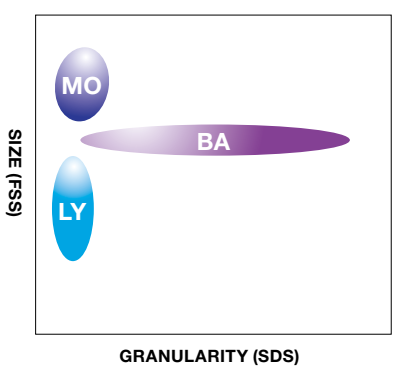
In the S-C (MAIN) scattergram, the leukocyte populations are divided into a mononuclear cell + BA area for those with lower FLS intensity (◯) and NE + EO area for those with higher FLS intensity (◯). Lymphocytes (LY) and monocytes (MO) are plotted in the mononuclear cell area. The basophilic population appears in the same area. But complexity is slightly higher than MO and LY subpopulations due to water-solubility of the basophilic granules. In the NE + EO area, neutrophils (NE) and eosinophils (EO) are plotted.

2 S-G (NE/EO) scattergram



The S-G (NE/EO) scattergram shows the NE/EO area which is on the right side of the S-C (MAIN) scattergram. The difference is that the horizontal axis uses granularity to separate NE and EO subpopulations.

3 S-G (LY/MO/BA) scattergram



The S-G (LY/MO/BA) scattergram shows the mononuclear cell + BA area which is on the left side of the S-C (MAIN) scattergram. Here, the horizontal axis uses granularity to separate LY, MO, and BA subpopulations.

POINTS

Size = FSS – Forward small angle scattered light
Complexity = FLS – Forward large angle scattered light
Granularity = SDS – Side angle scattered light

LY Lymphocyte
MO Monocyte
NE Neutrophil
EO Eosinophil
BA Basophil

Flags and Messages

Celltac 5-part differential analyzers provide various several flags and messages to help you to identify abnormal conditions and analyze the results.

Flags suggesting a numeric abnormality

The following flags appear with the result when a numerical value is outside the normal range. Take appropriate action according to your laboratory policy.

	Flag	Appears when (default values)
WBC	Leukocytosis	WBC > 18 x 10^3/µL
	Leukopenia	WBC < 2.5 x 10^3/µL
	Neutrophilia	NE > 11 x 10^3/µL
	Neutropenia	NE < 1.0 x 10^3/µL
	Lymphocytosis	LY > 4.0 x 10^3/µL
	Lymphopenia	LY < 0.8 x 10^3/µL
	Monocytosis	MO > 1.0 x 10^3/µL
	Eosinophilia	EO > 0.7 x 10^3/µL
	Basophilia	BA > 0.2 x 10^3/µL
RBC	Erythrocytosis	RBC > 6.5 x 10^6/µL
	Anemia	HGB < 10.0 g/dL
	Anisocytosis	RDW > 20.0%
	Microcytosis	MCV < 70.0 fL
	Macrocytosis	MCV > 110 fL
	Hypochromia	MCHC < 29.0 g/dL
	Abnormal MCHC	MCHC < 28.0 g/dL MCHC > 38.0 g/dL
PLT	Thrombocytosis	PLT > 600 x 10^3/µL
	Thrombocytopenia	PLT < 60 x 10^3/µL

You can individually set all the flagging limits (other than MCHC). For details of how to change flagging limits, please refer to the Operator's manual.

Flags suggesting a morphological abnormality

The following flags appear on the result of an abnormal distribution pattern in the scattergram or histogram and suggest a morphological abnormality.

Flag	Suspected morphological abnormality
Blasts	Blast cells
Immature Gr	Immature granulocytes
Left shift	Left shift
Atypical Ly	Atypical lymphocytes
Plt Clumps	Platelet clumps

Other messages

The following flags can also appear where there is an abnormal distribution pattern in the scattergram or histogram.

Flag	Appears when
Ly-Mo Interference (LMI)	LY and MO subpopulations are not clearly separated on the S-C (MAIN) scattergram
Ne-Eo Interference (NEI)	NE and EO subpopulations are not clearly separated on the S-G (NE/EO) scattergram
Small Nucleated Cell (SNC)	Gap between WBC impedance and optical count values exceeds the threshold
Poor Hemolyzation	RBC ghosts interfere with WBC histogram development
PLT-RBC Interference	PLT and RBC histogram plots are not clearly separated

Symbols

The following symbols are displayed next to the numeric result for the relevant parameter and are caused by data abnormality or a mechanical error. Take appropriate action by considering the relevant factors in each situation.

Symbol	Appears when	Countermeasure
*	Interfering factor or abnormal cells suspected	Microscopic examination of blood film
C	PLT clumps suspected	Microscopic examination of blood film
!	Poor hemolyzation or abnormal MCHC value	Microscopic examination of blood film
?	Mechanical error suspected	Confirm the diluent temperature and clean the flow paths
H	Numeric result higher than the set normal range	Review according to your laboratory protocol
L	Numeric result lower than the set normal range	Review according to your laboratory protocol

[CAUTION]

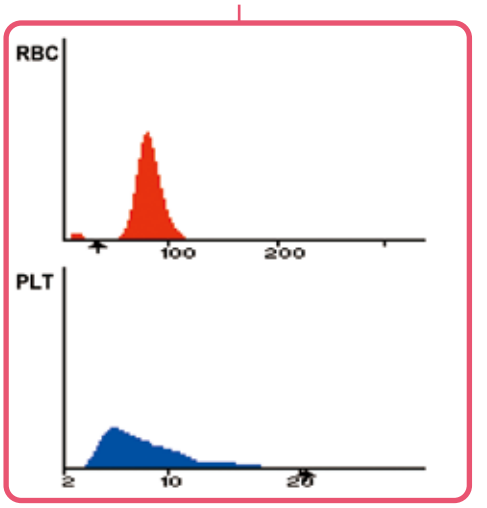
1. These flags are intended only to identify possibly abnormalities and assist in determining if further investigation is required. These flags by themselves cannot be used for judging cell abnormalities or disease diagnosis.
2. When a flag appears and "*" symbol is displayed beside the result it suggests the result should be treated with caution. In this case, check the result by an alternative method.

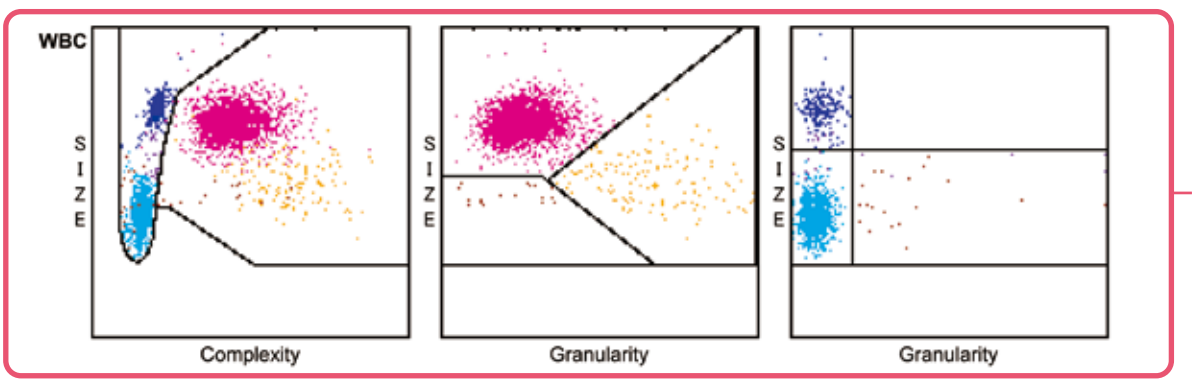
Sample Case (Normal)

Information from Celltac

Numeric data | Flags | Histograms*

WBC	4.8	[10³/μL]	
NE	3.1	66.1	[%]
LY	1.2	24.0	[%]
MO	0.3	5.5	[%]
EO	0.2	3.8	[%]
BA	0.0	0.6	[%]
RBC	5.18	[10⁶/μL]	
HGB	15.7	[g/dL]	
HCT	47.3	[%]	
MCV	91.3	[fL]	
MCH	30.3	[pg]	
MCHC	33.2	[g/dL]	
RDW	11.9	[%]	
PLT	198	[10³/μL]	
PCT	0.14	[%]	
MPV	7.1	[fL]	
PDW	16.6	[%]	





Scattergrams

*"Particle distribution pattern" setting is used for displaying histograms in this book.

Data interpretation

How to read the obtained data is explained in this space.

Morphology

Smear picture(s)

Doctor's comment

Findings based on the manual differential are stated in this space.

Manual differential

Blast
Promyelocyte
Myelocyte
Metamyelocyte
Band
Seg
Lymphocyte
Atypical Ly
Monocyte
Eosinophil
Basophil
Other
Total
NRBC/100WBC
RBC/ other findings

Monocytosis

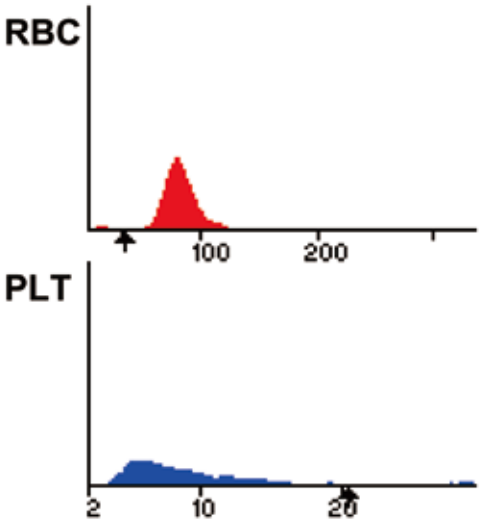
Information from Celltac

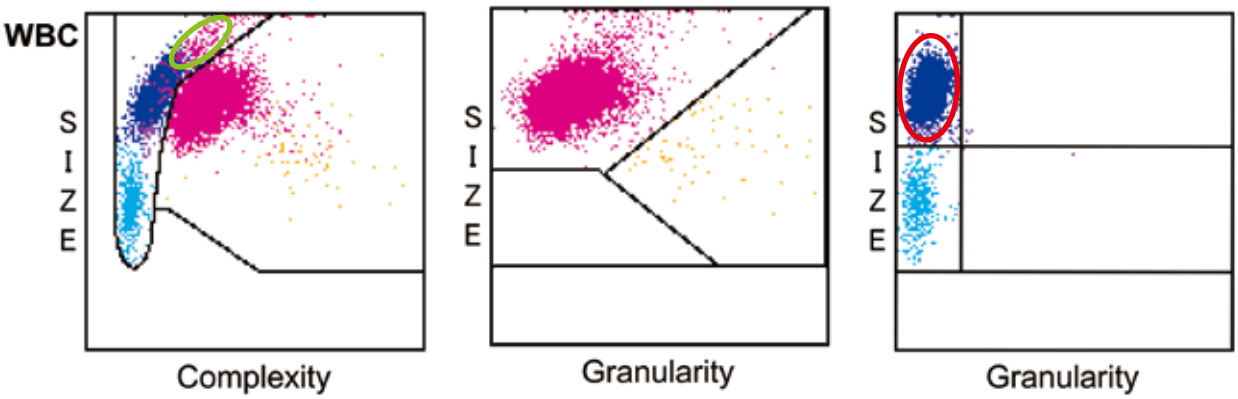
WBC	16.4 H	[10³ /μL]	
NE	12.0 *	73.1 *	[%]
LY	0.7	4.4 L	[%]
MO	3.5 *	21.2 *	[%]
EO	0.1	0.7	[%]
BA	0.1 *	0.6 *	[%]
RBC	3.21 L	[10⁶ /μL]	
HGB	9.7 L	[g/dL]	
HCT	30.7 L	[%]	
MCV	95.6	[fL]	
MCH	30.2	[pg]	
MCHC	31.6	[g/dL]	
RDW	15.7	[%]	
PLT	107 L	[10³ /μL]	
PCT	0.08 L	[%]	
MPV	7.3	[fL]	
PDW	17.7	[%]	

[WBC FLAG]
- Immature Granulocyte
- Left Shift
- Neutrophilia
- Lymphopenia
- Monocytosis

[RBC FLAG]
- Anemia

[PLT FLAG]

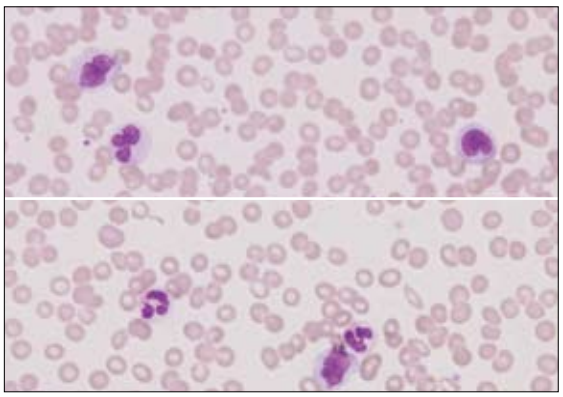




Data interpretation

A large population was displayed around the MO area (○) on the S-G (LY/MO/BA) scattergram, and it triggered the Monocytosis flag. The numeric data showed a relative and absolute monocytosis (21% and 3.5 × 10³/μL). In this case, immature granulocytes appear around the higher FSS and lower FLS intensity area – the Immature Granulocytes flag detection area (○). The monocytosis was confirmed by manual differential (MO: 26.0%).

Morphology



Doctor's comment

Leukocytosis and a higher ratio of monocytes (26.0%) were confirmed by microscopic examination. The absolute monocyte count is also increased. Among the mature monocytes, a few immature monocytes were observed. A few immature granulocytes, including metamyelocytes, were seen. When there are more than 1,000/μL monocytes in the peripheral circulation, a differential diagnosis of monocytic leukemia or myelomonocytic leukemia may be suspected, although immunological flow cytometric tests and bone marrow examination may be necessary for confirmation.

Manual differential

Blast	
Promyelocyte	
Myelocyte	
Metamyelocyte	1.5%
Band	2.5%
Seg	66.0%
Lymphocyte	3.0%
Atypical Ly	
Monocyte	26.0%
Eosinophil	0.5%
Basophil	0.5%
Other	
Total	200
NRBC/100WBC	
RBC/ other findings	ANISO (+) POIKIL (+) POLY (+)

Neutrophilia

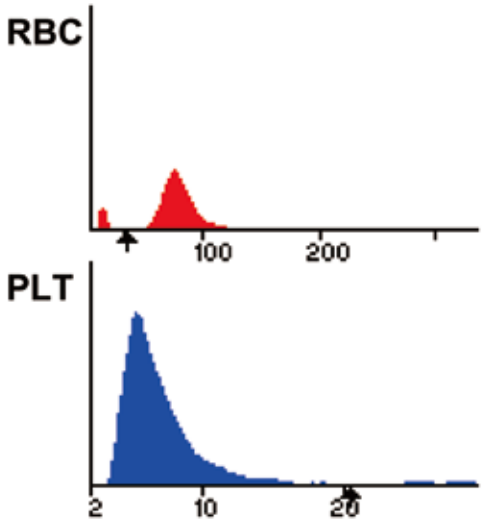
Information from Celltac

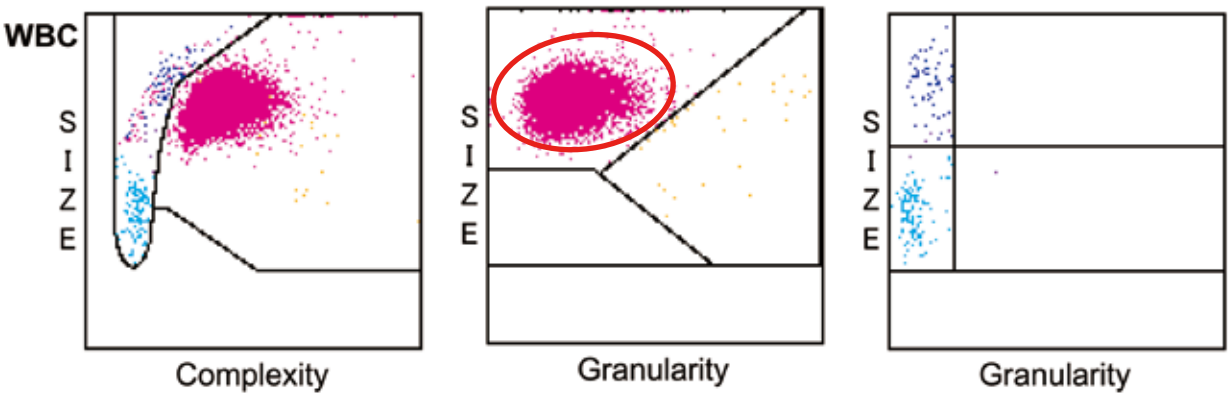
WBC	26.0 H	[10^3 /μL]	
NE	25.4 H	97.6 H	[%]
LY	0.3 L	1.3 L	[%]
MO	0.2	0.7	[%]
EO	0.1	0.3	[%]
BA	0.0	0.1	[%]
RBC	2.68 L	[10^6 /μL]	
HGB	8.1 L	[g/dL]	
HCT	25.2 L	[%]	
MCV	94.0	[fL]	
MCH	30.2	[pg]	
MCHC	32.1	[g/dL]	
RDW	16.2	[%]	
PLT	472 H	[10^3 /μL]	
PCT	0.18	[%]	
MPV	3.9 L	[fL]	
PDW	16.9	[%]	

[WBC FLAG]
Leukocytosis
Neutrophilia
Lymphopenia

[RBC FLAG]
Anemia

[PLT FLAG]

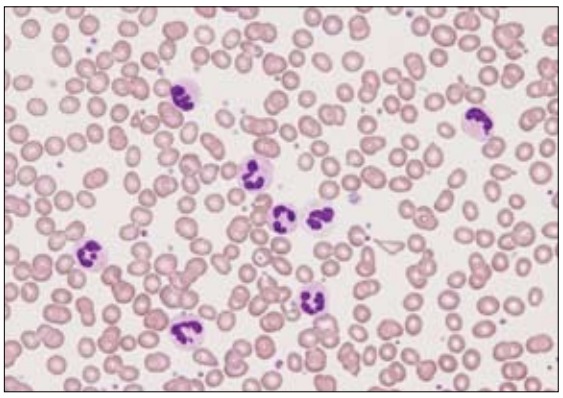




Data interpretation

A distinct, large population was displayed in the NE area on the S-G (NE/EO) scattergram (○), and it triggered the Neutrophilia flag. The numeric data showed an increased relative proportion of neutrophils (97.6%): the high total WBC count triggered the Leukocytosis flag. This sample demonstrates neutrophilia and agrees with the visual differential results (Seg: 96.5%).

Morphology



Doctor's comment

This blood film shows leukocytosis with an increased proportion of segmented neutrophils. There are low or normal numbers of lymphocytes and monocytes. No immature granulocytes or other morphological abnormality was found.

Manual differential

Blast	
Promyelocyte	
Myelocyte	
Metamyelocyte	
Band	2.5%
Seg	96.5%
Lymphocyte	1.0%
Atypical Ly	
Monocyte	
Eosinophil	
Basophil	
Other	
Total	200
NRBC/100WBC	
RBC/ other findings	ANISO (+), POIKIL (+)

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Myeloblast

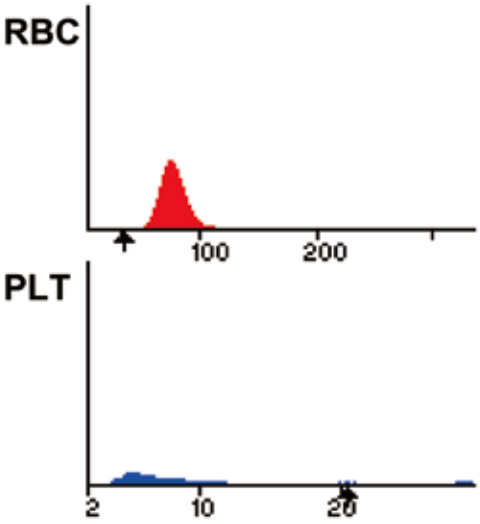
Information from Celltac

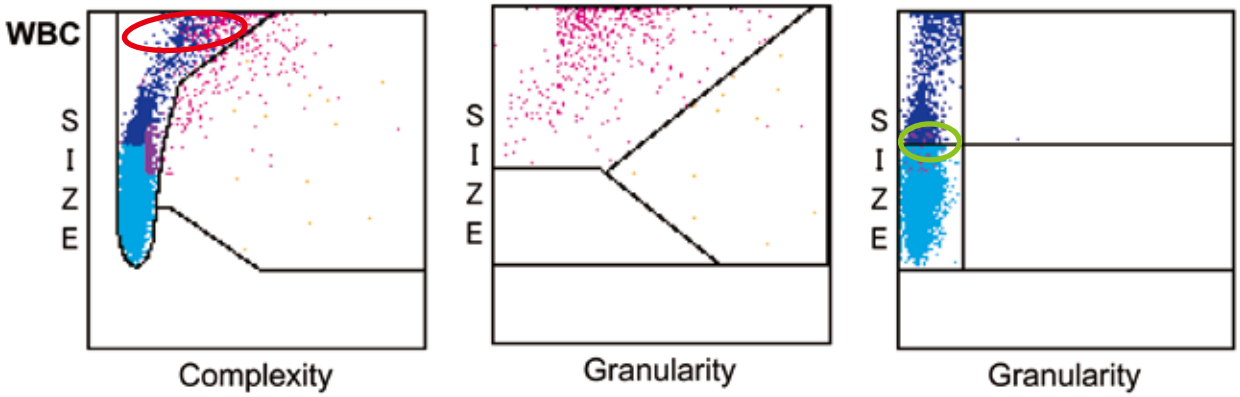
WBC	11.1 H	[10^3 /μL]	
NE	0.5 *	4.7 *	[%]
LY	8.6 *	77.3 *	[%]
MO	1.6 *	14.6 *	[%]
EO	0.0 *	0.1 *	[%]
BA	0.4 *	3.3 *	[%]
RBC	2.81 L	[10^6 /μL]	
HGB	8.3 L	[g/dL]	
HCT	25.7 L	[%]	
MCV	91.5	[fL]	
MCH	29.5	[pg]	
MCHC	32.3	[g/dL]	
RDW	14.7	[%]	
PLT	39 L	[10^3 /μL]	
PCT	0.03 L	[%]	
MPV	7.1	[fL]	
PDW	19.2 H	[%]	

[WBC FLAG]
- Blasts
- Immature Granulocyte
- Left Shift
- Ly-Mo Interference
- Neutropenia
- Lymphocytosis
- Monocytosis
- Basophilia

[RBC FLAG]
- Anemia

[PLT FLAG]
- Thrombocytopenia

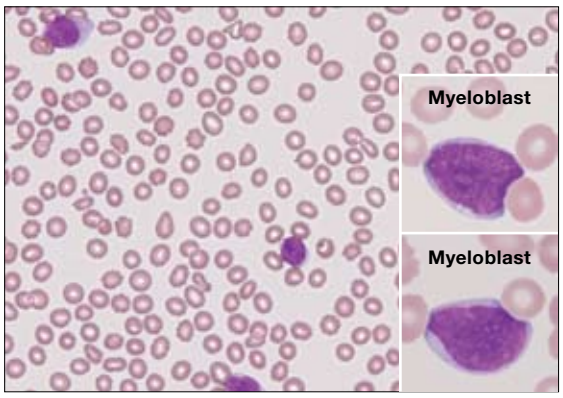




Data interpretation

A large population was confirmed in the higher FSS and lower SDS intensity area on the S-C (MAIN) scattergram including the Blasts flag detection area (○) and it triggered the flag. In this case, blast cells appear around the mononuclear cell area on the S-G (LY/MO/BA) scattergram (○), and make the subpopulations in the area unclear leading to the Ly-Mo Interference. Manual counting confirmed that most of the leukocytes were in fact myeloblasts (Blast: 88.0%).

Morphology



Doctor's comment

A large number of blast cells were confirmed by microscopic film examination. These medium-sized blasts show a range of nuclear: cytoplasmic ratios, fine nuclear chromatin, and clear nucleoli. Some of them have basophilic cytoplasm and irregular nuclear membranes: some cells have a few granules. Mature neutrophils are reduced and a small number of immature granulocytes are observed. Acute myelocytic leukemia (AML) is suspected, and therefore bone marrow examination and further tests are required.

Manual differential

Blast	88.0%
Promyelocyte	1.0%
Myelocyte	
Metamyelocyte	
Band	
Seg	1.5%
Lymphocyte	8.5%
Atypical Ly	0.5%
Monocyte	0.5%
Eosinophil	
Basophil	
Other	
Total	200
NRBC/100WBC	1
RBC/ other findings	ANISO (+) POIKIL

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Immature Granulocyte

Information from Celltac

WBC	6.8		[10^3 /μL]
NE	3.0 *	44.3 *	[%]
LY	1.6 *	22.9 *	[%]
MO	2.0 *	29.1 *	[%]
EO	0.1 *	1.9 *	[%]
BA	0.1 *	1.8 *	[%]

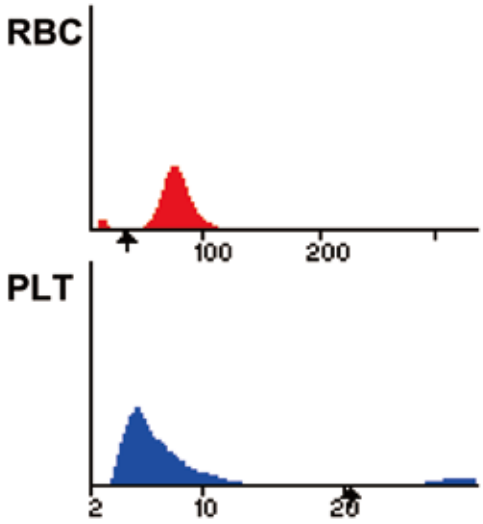
RBC	2.82 L	[10^6 /μL]
HGB	8.3 L	[g/dL]
HCT	26.1 L	[%]
MCV	92.6	[fL]
MCH	29.4	[pg]
MCHC	31.8	[g/dL]
RDW	16.1	[%]

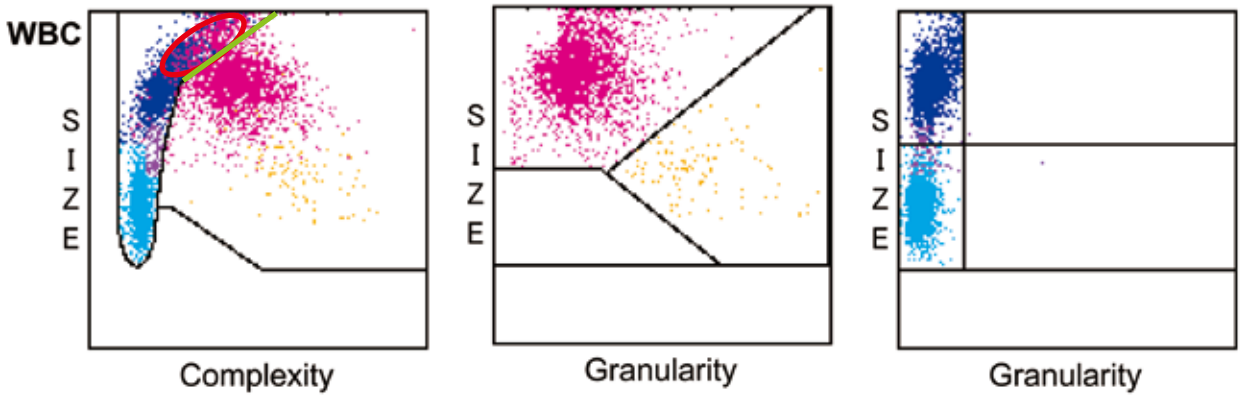
PLT	195	[10^3 /μL]
PCT	0.07 L	[%]
MPV	3.8 L	[fL]
PDW	16.9	[%]

[WBC FLAG]
Blasts
Immature Granulocyte
Left Shift
Monocytosis

[RBC FLAG]
Anemia

[PLT FLAG]

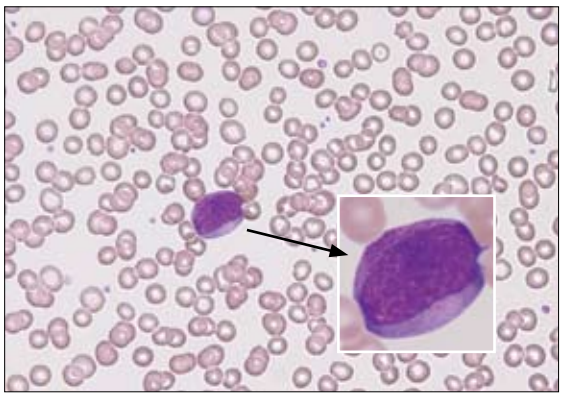




Data interpretation

Immature neutrohils and immature granulocytes confirmed with microscopic blood film examination seemed to appear around the Left Shift area (○) and reach the Immature Granulocyte area (○). This patient was confirmed as exhibiting left shift by the band cell percentage (13.5%).

Morphology



Doctor's comment

The leukocytes counted on the blood film fell within the normal range, but many neutrophils showed a left shift and there were occasional promyelocytes, myelocytes, and metamyelocytes. A small number of blasts were observed. Some mature neutrophils exhibited toxic granulation or Döhle bodies. A relatively high percentage of monocytes was seen and occasional NRBCs. In this case, it is not possible to differentiate between a hematological disorder and a leukemoid reaction. Further tests (bone marrow biopsy and cell markers) and clinical examination is indicated.

Manual differential

Blast	1.0%
Promyelocyte	1.0%
Myelocyte	2.5%
Metamyelocyte	3.0%
Band	13.5%
Seg	30.0%
Lymphocyte	24.0%
Atypical Ly	0.5%
Monocyte	23.0%
Eosinophil	1.0%
Basophil	0.5%
Other	
Total	200
NRBC/100WBC	2
RBC/ other findings	ANISO (+), POLY (+), TOXIC (+), DOHLE (+)

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Basophilia

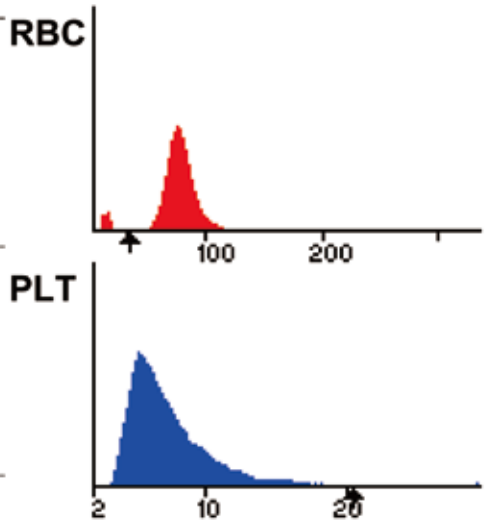
Information from Celltac

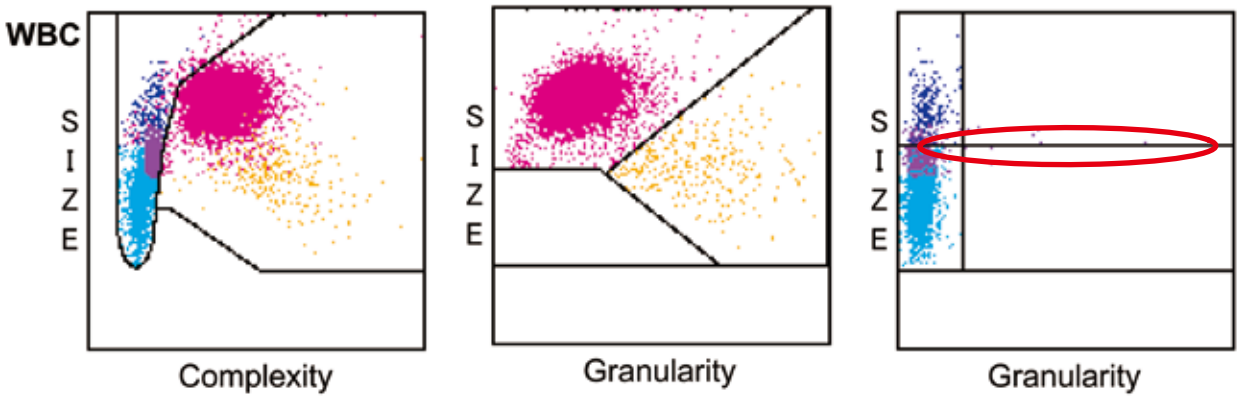
WBC	10.7 H	[10^3 /μL]
NE	7.9 *	73.5 * [%]
LY	1.7 *	16.0 * [%]
MO	0.2 *	2.2 * [%]
EO	0.4 *	3.3 * [%]
BA	0.5 *	5.0 * [%]
RBC	4.28	[10^6 /μL]
HGB	12.4	[g/dL]
HCT	38.6	[%]
MCV	90.2	[fL]
MCH	29.0	[pg]
MCHC	32.1	[g/dL]
RDW	14.1	[%]
PLT	467 H	[10^3 /μL]
PCT	0.22	[%]
MPV	4.7 L	[fL]
PDW	16.7	[%]

[WBC FLAG]
Blasts
Atypical Lymphocytes
Ly-Mo Interference
Basophilia

[RBC FLAG]

[PLT FLAG]

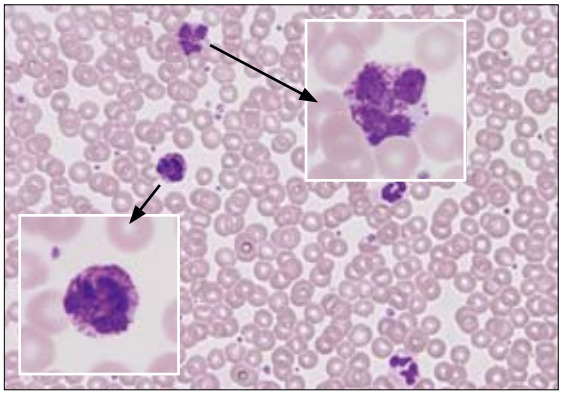




Data interpretation

A large distinct population was confirmed in the BA area on the S-G (MO/BA) scattergram (○), and it triggered the Basophilia flag.

Morphology



Doctor's comment

On the blood film, increased leukocytes with a higher ratio of basophils were confirmed. Basophilia is often seen in cases of chronic myelocytic leukemia (CML) and occasionally myeloproliferative leukemia. Therefore further testing and clinical assessment is necessary. Neither blasts nor immature granulocytes were seen.

Manual differential

Blast	
Promyelocyte	
Myelocyte	
Metamyelocyte	
Band	
Seg	68.5%
Lymphocyte	16.0%
Atypical Ly	
Monocyte	1.5%
Eosinophil	4.0%
Basophil	10.0%
Other	
Total	200
NRBC/100WBC	
RBC/ other findings	

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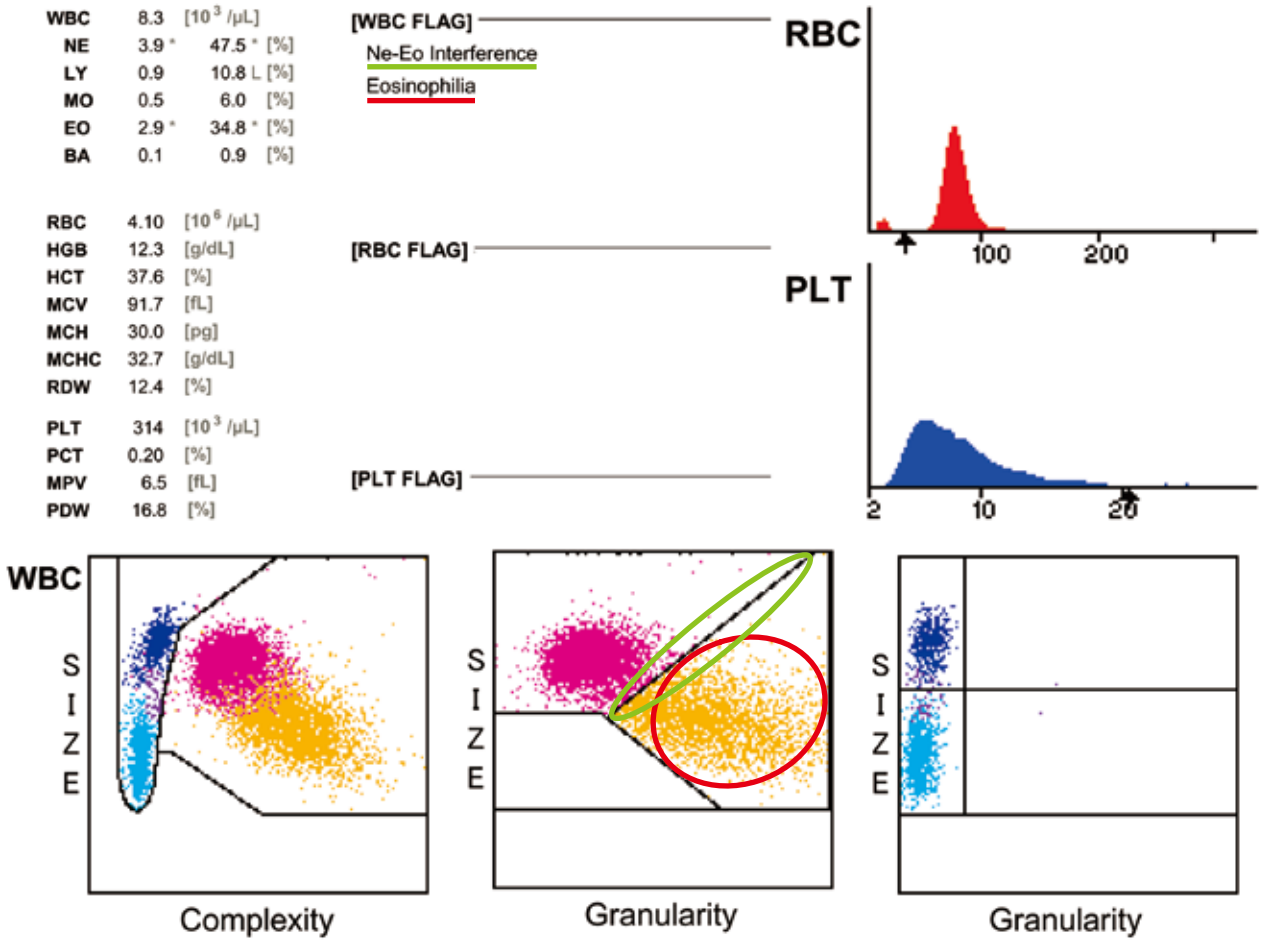
Eosinophilia

Information from Celltac

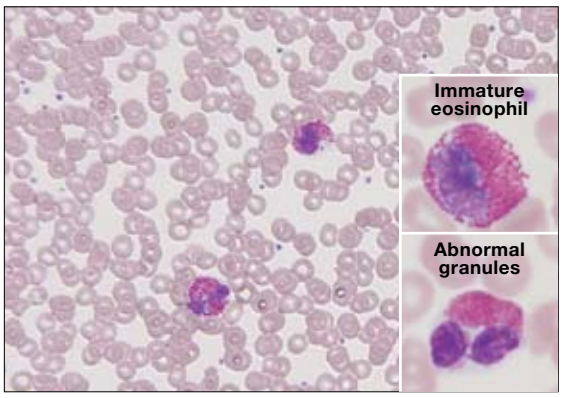
WBC	8.3	[10^3 /μL]
NE	3.9 *	47.5 * [%]
LY	0.9	10.8 L [%]
MO	0.5	6.0 [%]
EO	2.9 *	34.8 * [%]
BA	0.1	0.9 [%]
RBC	4.10	[10^6 /μL]
HGB	12.3	[g/dL]
HCT	37.6	[%]
MCV	91.7	[fL]
MCH	30.0	[pg]
MCHC	32.7	[g/dL]
RDW	12.4	[%]
PLT	314	[10^3 /μL]
PCT	0.20	[%]
MPV	6.5	[fL]
PDW	16.8	[%]



Data interpretation

A large population was seen in the EO area on the S-G (NE/EO) scattergram (○), and it triggered the Eosinophilia flag. The numeric data showed a relative and absolute eosinophilia (34.8% and 2.9 × 10^3/μL). Since a number of immature and degranulated eosinophils appear around NE and EO areas, these two subpopulations overlap and generate the Ne-Eo Interference flag. This patient's eosinophilia was confirmed by microscopic examination and manual differential (EO: 24.5%).

Morphology



Doctor's comment

A higher ratio of eosinophils was confirmed by microscopic analysis. Among the mature eosinophils with a segmented nucleus, a few immature eosinophils were observed. Eosinophilia may be a reactive response triggered by several causes, such as bronchial asthma, parasitic infestation and drug allergy. Importantly this may also be accompanied by hypereosinophilic syndrome or malignancy and therefore requires further tests and clinical assessment.

Manual differential

Blast	
Promyelocyte	
Myelocyte	
Metamyelocyte	
Band	1.0%
Seg	56.5%
Lymphocyte	12.0%
Atypical Ly	0.5%
Monocyte	4.5%
Eosinophil	24.5%
Basophil	1.0%
Other	
Total	200
NRBC/100WBC	
RBC/ other findings	ANISO (+)

NIHON KOHDEN

NIHON KOHDEN CORPORATION
1-31-4 Nishiochiai, Shinjuku-ku, Tokyo 161-8560, Japan
Phone +81 (3) 5996-8036 Fax +81 (3) 5996-8100
www.nihonkohden.com

•Reviewed by
Mitsuru Murata, MD, PhD
Department of Laboratory Medicine, School of Medicine, Keio University

•Data and interpretation provided by
Takayuki Mitsuhashi, MD, PhD
Department of Laboratory Medicine, School of Medicine, Keio University

Nobuko Shimizu, MT
Central Clinical Laboratory, Keio University Hospital

Naoko Katagiri, MT
Central Clinical Laboratory, Keio University Hospital

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