

# XN-Series Automated Hematology Analyzer

# Clinical Case Report Vol.2



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# Prefatory note

The examination of peripheral blood provides important information on parameters used for evaluating hematologic conditions and diseases, which is useful in identification of the pathophysiology, definitive diagnosis, and monitoring the course of the disease. Technological advances in automated hematology analyzers have, one after the other, enabled the automated classification of white blood cells, and measurement of reticulocytes and immature platelets (reticulated platelets). Analyzers now have better performance in that they can measure multiple parameters and perform multiple functions, including abnormal cell detection, at higher throughput.

Sysmex Corporation has released the newly developed XN-Series automated hematology analyzers. The new series of analyzers has improved performance of conventional functions and is implemented with some new functions. They have enhanced precision in blood cell counting because of the adoption of a new fluorescent dye for platelet counting (PLT-F channel), a nucleated red blood cell correction function for the white blood cell counts in all specimens (WNR channel), and a newly added measurement mode for specimens with low white blood cell counts (LW mode). In white blood cell differentiation (WDF channel) and abnormal cell detection (WPC channel), optimization of the reagent reaction, signal processing and analysis algorithms have all improved the cell differentiation and detection performance as well as flagging (IP messages). As the system has become more complex, expertise and experience with a number of different cases are needed for ones to be able to properly interpret the data displayed by the highly advanced new multi-parameter, multi-functional analyzers. This booklet was prepared to help the users to better understand the data generated by the new analyzers. It provides case reports on changes during the course of therapy and on abnormal samples. Firstly, it describes the measurement principle for each parameter, the reagent reaction in each channel, the data generated, and their significance. In the following section of Case Reports, clinical laboratory findings and micrographs of peripheral blood smears necessary for understanding the pathogenesis are shown along with the blood cell counts, flags and scattergrams of different XN channels. The case reports show changes in the course of treatment of typical hematologic diseases, and analysis results of abnormal samples that we often come across. In each disease, there is various pathophysiology which is affected by the disease stage and modified by the treatment, and various abnormal samples. Changes in hematologic pathophysiology in the course of treatment of a disease, as also those caused by abnormal samples, have varied effects on the flagging information and the patterns of cell distribution in the scattergrams. The results displayed by the analyzer are compared with the visual differential cell counts and blood cell morphology findings (as seen in micrographs) for assisting interpretation of the XN data. In each case report, the interpretation of blood cell morphology and laboratory findings as well as the basis for the diagnosis are provided. Additional information includes major points to be noted when there is a flag message and a cell distribution abnormality in the scattergram, or when XN data and scattergrams largely differ from the microscopic findings.

In the daily routine of hematologic testing and clinical practice using this analyzer, it is important to interpret the data of each specimen, namely, the anomalies in blood cell counts and cell distribution in the scattergrams, and flagging information, for early detection of abnormal conditions of patient pathophysiology or their samples and thereby adoption of appropriate remedial measures. I hope that each concerned institution would deepen its understanding of the functions of this analyzer and the parameters measured by it, through analyzing many cases and conditions. It would be my pleasure if this booklet is of help in achieving this purpose and contributes to the routine work of blood testing and clinical practice as a reference source.

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# Measurement principles and analysis parameters

#### Whole blood mode

Analysis channel	Principles	Analysis parameter	Meanings
WNR		WBC	White blood cell (leukocyte) count
Dilution 1:61		BASO#	Basophil count
		BASO%	Basophil percent
		NRBC#	Nucleated red blood cell count
		NRBC%	Nucleated red blood cell percent
WDF		NEUT#	Neutrophil count
Dilution 1:61		NEUT%	Neutrophil percent
		LYMPH#	Lymphocyte count
		LYMPH%	Lymphocyte percent
		MONO#	Monocyte count
		MONO%	Monocyte percent
		EO#	Eosinophil count
		EO%	Eosinophil percent
	Elow outomatry mathad	IG#	Immature granulocyte count
	using semiconductor	IG%	Immature granulocyte percent
WPC Dilution 1:61	laser	HPC#	Hematopoietic progenitor cell count
RET		RET# *	Reticulocyte count
Dilution 1:204		RET% *	Reticulocyte percent
		IRF *	Immature reticulocyte fraction
		LFR *	Low fluorescence ratio
		MFR *	Medium fluorescence ratio
		HFR *	High fluorescence ratio
		RET-He *	Reticulocyte hemoglobin equivalent
		PLT-O *	Platelet count (measured by RET channel)
PLT-F	PLT-F		Platelet count (measured by PLT-F channel)
Dilution 1:204		IPF *	Immature platelet fraction
RBC/PLT	Sheath flow DC	RBC	Red blood cell (erythrocyte) count
Dilution 1:498	detection method	НСТ	Hematocrit
		MCV	Mean corpuscular volume
		RDW-SD	Red cell distribution width (standard deviation)
		RDW-CV	Red cell distribution width (coefficient of variation)
		PLT-I	Platelet count (measured by RBC/PLT channel)
		PDW	Platelet distribution width
		MPV	Mean platelet volume
		РСТ	Plateletcrit
		P-LCR	Platelet large cell ratio
RBC/		МСН	Mean corpuscular hemoglobin
PLT & HGB		МСНС	Mean corpuscular hemoglobin concentration
HGB Dilution 1:747	SLS-Hemoglobin Method	HGB	Hemoglobin concentration

(HPC analysis can only be performed if the instrument offers the HPC analysis mode.)

#### $^{\ast}$ These items do not appear with all analyzer types.

#### Body fluid mode

Analysis channel	Principles	Analysis parameter	Meanings
WDF	Flow cytometry method using semiconductor laser	WBC-BF	White blood cell (leukocyte) count
Dilution 1:20		MN#	Mononuclear count
		MN%	Mononuclear percent
		PMN#	Polymorphonuclear count
		PMN%	Polymorphonuclear percent
		TC-BF#	Total nucleated cell count
RBC Dilution 1:498	Sheath flow DC detection method	RBC-BF	Red blood cell (erythrocyte) count

(The body fluid analysis can only be performed if the instrument offers the body fluid mode.)

# **Research parameters**

#### Whole blood mode

Analysis channel	Research parameter	Meanings
WNR	WBC-N	WBC count calculated from the WNR channel.
	TNC-N	The total nuclear cell count (WBC#+NRBC#) calculated from the WNR channel.
	BA-N#	The basophil counts calculated from the WNR channel.
	BA-N%	The basophil percent calculated from the WNR channel.
WDF	WBC-D	WBC count calculated from the WDE channel.
	TNC-D	The total nuclear cell count (WBC#+NRBC#) calculated from the WDF channel.
	NFUT#&	The number of particles obtained by subtracting the IG count from the NEUT count
	NEUT%&	The ratio of the count obtained by subtracting IG# from NEUT# to the WBC count.
	LYMP#&	The number of particles obtained by subtracting the HFLC count from the LYMPH count.
	LYMP%&	The ratio of the count obtained by subtracting HFLC# from LYMPH# to the WBC count.
	HFLC#	The count of the upper LYMPH area of the WDF scattergram.
	HFLC%	The ratio of the count of the upper LYMPH area of the WDF scattergram to the WBC count.
	BA-D#	The basophil counts calculated from the WDF channel.
	BA-D%	The basophil percent calculated from the WDF channel.
	NE-SSC	The lateral scattered light intensity of the NEUT area on the WDF scattergram.
	NE-SFL	The fluorescent light intensity of the NEUT area on the WDF scattergram.
	NE-FSC	The forward scattered light intensity of the NEUT area on the WDF scattergram.
	LY-X	The lateral scattered light intensity of the LYMPH area on the WDF scattergram.
	LY-Y	The fluorescent light intensity of the LYMPH area on the WDF scattergram.
	LY-Z	The forward scattered light intensity of the LYMPH area on the WDF scattergram.
	MO-X	The lateral scattered light intensity of the MONO area on the WDF scattergram.
	MO-Y	The fluorescent light intensity of the MONO area on the WDF scattergram.
	MO-Z	The forward scattered light intensity of the MONO area on the WDF scattergram.
	NE-WX	The lateral scattered light distribution width of the NEUT area on the WDF scattergram.
	NE-WY	The fluorescent light distribution width of the NEUT area on the WDF scattergram.
	NE-WZ	The forward scattered light distribution width of the NEUT area on the WDF scattergram.
	LY-WX	The lateral scattered light distribution width of the LYMPH area on the WDF scattergram.
		The fluorescent light distribution width of the LYMPH area on the WDF scattergram.
		The lotward scattered light distribution width of the LYMPH area on the WDF scattergram.
		The fateral solution light distribution wind of the MONO area on the WDF scattergram.
	MO-W/7	The forward scattered light distribution width of the MONO area on the WDF scattergram.
WDC		The MMC equate relaying the model of the model of the MMC and the MMC and the MMC equation in the MMC equa
WPC	TNC D*	The total purples call count (WPC + WPC channel)
		The total nuclear cent count (WDC++NRDC+) calculated from the WPC channel.
	DDC U. *	Ratio of the cert outre equivalent to the rientacipotent progenitor cert count, tacchared non-the who channels, to white blood certs.
	RBC-He*	The correlation between RBC-Y (the intensity of the lateral fluorescent light of the mature red blood cells) and MCH to convert RBC-Y into [pg] units.
	Delta-He*	Delta-He is calculated by the equation Delta-He = RET-He - RBC-He.
	RET-Y*	The intensity of forward scattered light in the RET area of the RET scattergram.
	RET-RBC-Y*	The intensity of forward scattered light in the RBC (mature red blood cell) area of the RET scattergram.
	RPI*	Reticuloryte Production Index
	RET-UPP*	The court in the UPP area of the RET scattergram.
	RET-TNC*	The count in the TNC area of the RET scattergram.
	Hypo-He*	The ratio of the count in the low level area of the forward scattered light signal in the RBC (mature red blood cell) area of the
	Hyper-He*	The ratio of the count in the high level area of the forward scattered light signal in the RBC (mature red blood cell) area of
		the RET scattergram to mature red blood cells.
	RBC-U	The absolute count calculated from the RET Channel.
	FRC# FRC%*	The absolute count calculated from the count in a specific area below the RBC area in the RET scattergram.
	IPF#*	The platelet count in the IPE area of the PI T-E scattergram
	H-IPF*	The ratio to the total platelet count of the count of platelets that appear in the area of stronger fluorescent light intensity
		Within the IPF on the PLI-F scattergram.
RBC	MicroR	Micro RBC ratio

(HPC analysis can only be performed if the instrument offers the HPC analysis mode.)

\* These items do not appear with all analyzer types.

#### Body fluid mode

Analysis channel	Research Parameter	Meanings
WDF	HF-BF#	The count in the area with stronger fluorescence than the WBC-BF area of the WDF scattergram.
	HF-BF%	HF-BF count divided by WBC-BF and expressed as a percentage.
	NE-BF#	The count in the NEUT area of the WDF scattergram.
	NE-BF%	NE-BF count divided by WBC-BF and expressed as a percentage.
	LY-BF#	The count in the LYMPH area of the WDF scattergram.
	LY-BF%	LY-BF count divided by WBC-BF and expressed as a percentage.
	MO-BF#	The count in the MONO area of the WDF scattergram.
	MO-BF%	MO-BF count divided by WBC-BF and expressed as a percentage.
	EO-BF#	The count appearing in the EO area of the WDF scattergram.
	EO-BF%	The count in the EO area of the WDF scattergram.
RBC	RBC-BF2	RBC in the body fluid mode with a minimum of $100/\mu$ L displayed.

(The body fluid analysis can only be performed if the instrument offers the body fluid mode.)

# Measurement principle of flow cytometry with a semiconductor laser

# Cell analysis based on the principle of flow cytometry

The flow cytometry using a semiconductor laser counts and classifies cells by irradiating them with a 633 nm laser beam and analyzing their forward scattered light (FSC), side scattered light (SSC) and side fluorescent light (SFL). The intensity of the two types of scattered light (FSC and SSC) reflects cell surface structure, particle shape, nucleus form, refractive index and reflectivity of the cells. In general, the FSC signal is stronger for larger cells, and the SSC signal becomes stronger as the intracellular structures become more complex. The intensity of the side fluorescent light mainly reflects the type and amount of nucleic acids and cell organelles. These three signals are used to differentiate and count white blood cells, nucleated red blood cells, reticulocytes, and platelets, and to detect abnormal cells and immature cells with the help of unique digital technology and algorithms.



# Measurement principle of sheath flow DC detection

# **RBC/PLT** channel

#### Reagent CELLPACK DCL/DST

The RBC/PLT channel counts red blood cells and platelets using the sheath flow DC (direct current) detection method. A diluted sample is ejected from the nozzle tip and blood cells pass through the specified path at the center of the aperture enclosed in the sheath fluid. As each blood cell passes through the center of the aperture, blood cell volume information is accurately reflected in the pulse. The cell signals are sensitively captured because of innovations in the unique digital waveform processing technology.



% This is a conceptual drawing.



**PLT** histogram



# Measurement principle of the SLS-hemoglobin method

#### **HGB** channel

#### Reagent CELLPACK DCL/DST, SULFOLYSER

The SLS-hemoglobin method uses sodium lauryl sulfate (SLS) for measuring the hemoglobin concentration. The reaction mechanism of the SLS-hemoglobin method is considered to be as follows:

① Stage 1 (hemolytic reaction between SLS and the red blood cell membrane)

SLS binds to the red blood cell membrane mainly by ionic bonding and partly by hydrophobic bonding. This leads to the solubilization of phospholipids on the red blood cell membrane and causes the leakage of hemoglobin from the inside of the red blood cell by modifying the membrane protein structure.

- ② Stage 2 (change in three-dimensional globin structure by SLS) The free hemoglobin released by hemolysis undergoes a change in its three-dimensional structure due to the bonding between the hydrophobic group of SLS and globin.
- ③ Stage 3 (oxidation of heme iron by oxygen)

Concurrently with the change in the three-dimensional structure of globin, the divalent heme iron is easily changed to trivalent iron by the oxygen bound to the heme iron or dissolved oxygen.

④ Stage 4 (Binding of SLS)

The hydrophilic groups of SLS bind to the trivalent heme iron to form stable SLS-hemoglobin. It has been confirmed that the reactions up to this point are completed within 10 seconds when SULFOLYSER is used.

SLS-hemoglobin shows an absorption curve with the maximum peak at wavelength 535 nm and a shoulder peak at 560 nm. The analyzer irradiates light of 555 nm wavelength and measures the absorption.



% This is a conceptual drawing.

# **Reagent reactions**

#### WNR channel

#### Reagent Lysercell WNR, Fluorocell WNR

The WNR channel counts white blood cells and performs a differential counting of basophils and nucleated red blood cells. While causing hemolysis of red blood cells by the action of the surfactant within it, Lysercell WNR penetrates the cell membrane of white blood cells. This causes changes of the external shape and internal structure depending on the cell characteristics of each white blood cell. This channel differentiates basophils from other white blood cells and counts them by capturing these morphological differences based on changes in light scatter (FSC).

Fluorocell WNR fluorescently stains nucleic acids and cell organelles of white blood cells and nucleated red blood cells. With Lysercell WNR, the stained parts tend to be better preserved and show a stronger fluorescence in white blood cells than in nucleated red blood cells. The WNR channel uses these fluorescence differences to differentiate nucleated red blood cells from white blood cells and thus enables a separate count to be provided for each.

	H	emolys	is Flu	oresce	nce	Side fluorescent light (SFL)	Forward scattered light (FSC)
Basophils		+		+		Strong	Strong
Lymphocytes		+	۲	+	٢		
Monocytes		+		+	B	Medium	Medium
<b>Granulocytes</b> (neutrophils, eosinophils, etc.)		+		+	E		
Nucleated red blood cells		+		+	$\bigcirc$	Weak	Medium
Red blood cells	0	+		+	<b>\$</b> \$	Very weak	Very weak

※ This is a conceptual drawing.





SFL : Side fluorescent light

## WDF channel

#### Reagent Lysercell WDF, Fluorocell WDF

The WDF channel differentiates and counts neutrophils, lymphocytes, monocytes and eosinophils and detects abnormal cells such as immature white blood cells and atypical lymphocytes. Surfactants in Lysercell WDF cause the hemolysis and dissolution of red blood cells and platelets and penetrate the cell membranes of white blood cells. The degree of impact and hence change in cell morphology depends on the individual characteristics of each type of white blood cell. These differences are distinguished using side scattered light. Then the fluorescent dye in Fluorocell WDF enters the cells and stains the nucleic acids and cell organelles. The intensity of fluorescence varies among different types of white blood cells, depending on the type and amount of nucleic acids and cell organelles. It is possible to differentiate and count various cells and flag abnormal cells through the cluster analysis of differences in scattered light and fluorescence with the proprietary algorithm.

	Hemolysis			Staining		Side fluorescent light (SFL)	Side scattered light (SSC)
Lymphocytes		+		<b>→</b>	$\bigcirc$	Medium	Weak
Monocytes		+		+		Medium	Weak
Neutrophils		+		+	B	Weak	Medium
Eosinophils		+		+		Weak	Strong
Atypical lymphocytes		+		+			
Immature white blood cells		+	6	<b>→</b>	6	Medium – Strong	Weak – Medium

※ This is a conceptual drawing.

WDF scattergram



SSC : Side scattered light

## WPC channel

#### Reagent Lysercell WPC, Fluorocell WPC

The WPC channel detects blasts and abnormal cells of the lymphocyte series. Surfactants in Lysercell WPC cause the hemolysis and dissolution of red blood cells and platelets and penetrate the cell membranes of white blood cells. Then the fluorescent dye in Fluorocell WPC enters the cells and stains the nucleic acids and so forth to allow the detection of white blood cells and groups of abnormal cells.

Blasts and abnormal lymphocytic cells have variable characteristics depending on the disorder and individual case, and there may be various differences in contrast to normal cells manifesting in their morphology and their reaction with the surfactants in Lysercell WPC and the fluorescent dye in Fluorocell WPC. These differences in reactivity are reflected by the scattered light and fluorescence intensity, and abnormal cells and cell groups are detected with the proprietary algorithm.

	H	emoly	sis S	Staining Side fluorescer light (SFL)			Forward scattered light (FSC)	Side scattered light (SSC)
Abnormal lymphocytes		+		+		Medium – Strong	Weak	Weak
Blasts		+	6	+	6	Weak – Medium	Strong	Weak
Mature white blood cells		+		+	62	Medium	Weak – Strong	Weak – Strong

\* This is a conceptual drawing.



WPC scattergram

#### WPC (SSC-FSC) scattergram



SSC : Side scattered light

SSC : Side scattered light

# **RET channel**

#### Reagent CELLPACK DFL, Fluorocell RET

In the RET channel, the nucleic acids and so forth in reticulocytes and white blood cells are fluorescently stained with fluorescent dye of Fluorocell RET when treated with CELLPACK DFL. These cells are differentiated from mature red blood cells using the difference in fluorescence intensity.

		Stainin	ıg	Side fluorescent light (SFL)	Forward scattered light (FSC)
White blood cells		<b>→</b>		Strong	Strong
Reticulocytes	R	+	<b>e</b>	Medium	Strong
Red blood cells	0	+	0	Weak	Strong

% This is a conceptual drawing.

**RET scattergram** 



#### SFL : Side fluorescent light

#### **PLT-O scattergram**



SFL : Side fluorescent light

# PLT-F channel

#### Reagent CELLPACK DFL, Fluorocell PLT

In the PLT-F channel, platelets are specifically stained with the Fluorocell PLT fluorescent dye when treated with CELLPACK DFL. The platelets are counted and additionally, the plots in the area with high fluorescence intensities are separated as the immature platelet fraction (IPF). Platelets are clearly distinguished from other blood cells using the difference in forward scattered light and the fluorescence intensity.

	S	tainin	g	Side fluorescent light (SFL)	Forward scattered light (FSC)
Red blood cells	0	+	0	Weak – Medium	Strong
Platelets	rQ	+	Ŕ	Weak – Medium	Weak
<b>IPF</b> (immature platelet fraction)	<u> Ind</u>	+		Medium – Strong	Medium

\* This is a conceptual drawing.

PLT-F scattergram

SFL : Side fluorescent light

# Scattergram patterns that show abnormal cells (whole blood mode)

#### WNR scattergram



WDF scattergram



SSC : Side scattered light

#### WPC (SSC-FSC) scattergram



(high band cell count) Abnormal lymphocytic cells

Blast

Left shift

Atypical lymphocytes

Red blood cell fragments

Nucleated red blood cells

Immature granulocytes

Aggregated platelets

#### WPC scattergram



SSC : Side scattered light

#### **RET scattergram**



SFL : Side fluorescent light

#### PLT-F scattergram



SFL : Side fluorescent light

#### PLT-O scattergram



SFL : Side fluorescent light

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# An example of a normal specimen analysis (whole blood mode)



#### Analysis results (main screen)

Measured parameters: Numerical data for a total of 36 parameters are displayed.

**Research parameters:** The measured values of research parameters are indicated with a gray background on the data browser screen. **Flag(s):** IP messages concerning WBC, RBC and PLT are displayed.

#### Scattergram



PLT histogram

# An example of the analysis of a peripheral blood sample having HPC (HPC mode)

#### Analysis results (main screen)



HPC mode outputs the HPC# and the same analysis parameters as the whole blood mode.

The graphs screen displays the WNR, WDF, RET and PLT-F scattergrams, and the RBC and PLT histograms, as in the whole blood mode (p. 15), but with the WPC(SSC-FSC) scattergram replaced by a scattergram of the type shown below.



Hematopoietic progenitor cells (HPC) that are mobilized into the peripheral blood after stem cell mobilization in peripheral blood stem cell transplantation (PBSCT) can be measured in the HPC mode.

WPC (SSC-FSC) scattergram

The availability of the HPC analysis function depends on the operating environment of the user's analyzer. HPC mode is not available in all regions.

# An example of a body fluid specimen analysis (body fluid mode)

#### Analysis results (main screen)

Ttem	Data	Unit
WBC-BF	0.945	10^9/1
RBC		
Item	Data	Unit
RBC-BF	0.000	10^12/1
		120 22/21
WBC Diffe Item	erential Data	Unit
WBC Diffe Item MN# PMN#	Data 0,278 0,667	Unit 10^9/L 10^9/L
WBC Diffe Item MN# PMN# MN% PMN%	erential Data 0,278 0,667 29,4 70,6	Unit 10^9/L 10^9/L % %



**RBC** histogram

Measured parameters: The numerical data for a total of 7 parameters are displayed.

Research parameters: The measured values of research parameters are indicated with a gray background on the data browser screen. Flag(s): IP messages concerning WBC are displayed.



WBC-BF = MN# + PMN#TC-BF# = WBC-BF + HF-BF#MN = LY-BF + MO-BFPMN = NE-BF + EO-BF\* See pages 4 and 5 for the description of terms.

The body fluid analysis can only be performed if the instrument offers the body fluid mode.

# Scattergram

# IP message parameters

	Message	Meaning	Analysis channel	Judgment method/equation
	Abnormal			
	WBC Abn Scattergram	Abnormal WBC scattergram	WNR, WDF	Based on clustering in WNR and WDF scattergrams. For body fluid analysis, based on clustering in the WDF scattergram and the HF-BF value.
	Neutropenia *	Low neutrophil count	WDF	NEUT# < 1.00 x 10 <sup>9</sup> /L or NEUT% < 0.0 %
	Neutrophilia *	High neutrophil count	WDF	NEUT# > 11.00 x 10 <sup>9</sup> /L or NEUT% > 100.0 %
	Lymphopenia *	Low lymphocyte count	WDF	LYMPH# < 0.80 x 10 <sup>9</sup> /L or LYMPH%< 0.0 %
	Lymphocytosis *	High lymphocyte count	WDF	LYMPH# > 4.00 x 10 <sup>9</sup> /L or LYMPH% > 100.0 %
	Monocytosis *	High monocyte count	WDF	MONO# > 1.00 x 10 <sup>9</sup> /L or MONO% > 100.0 %
	Eosinophilia *	High eosinophil count	WDF	EO# > 0.70 x 10 <sup>9</sup> /L or EO% > 100.0 %
	Basophilia *	High basophil count	WNR	BASO# > 0.20 x 10 <sup>9</sup> /L or BASO% > 100.0 %
	Leukocytopenia *	Low leukocyte count	WNR, WDF	WBC < 2.50 x 10 <sup>9</sup> /L
W	Leukocytosis *	High leukocyte count	WNR, WDF	WBC > 18.00 x 10 <sup>9</sup> /L
B	NRBC Present *	High nucleated RBC count	WNR	NRBC% > 2.0 %
	IG Present *	Increased immature granulocyte	WDF	IG# > 0.10 x 10°/L or IG% > 100.0 %
	Suspect	Dessibility that blasts are		ludged from the presence of Directs (Abril umphe on the
	Blasts/Abn Lympho?	present/Possibility of abnormal lymphocytes	WDF	WDF scattergram.
	Blasts?*1	Possibility that blasts are present	WDF+WPC	Judged from the presence of Blasts on the WDF and WPC scattergrams.
	Abn Lympho?*1	Possibility of abnormal lymphocytes	WDF+WPC	Judged from the presence of AbnLympho on the WDF and WPC scattergrams.
	Left Shift? Possibility of left shift		WDF	Based on the distribution state of the upper right area of the NEUT in the WDF scattergram.
	Atypical Lympho?	Possibility of atypical lymphocytes	WDF, WDF+WPC	Based on the distribution state of the upper area of the lymphocytes in the WDF scattergram.
	Abnormal			
	RBC Abn Distribution	Abnormal RBC distribution	RBC	Arithmetic calculation and numerical comparison
	Dimorphic Population	Multi-peak RBC distribution	RBC	Gap between the high and low points and shape of distribution peak.
	RET Abn Scattergram*2	Abnormal RET scattergram	RET	Clustering in the RET scattergram
	Reticulocytosis*2 *	Reticulocytosis	REI	RE1% > 5.00% or RE1# > 200 x 10 <sup>9</sup> /L
	Anisocytosis *	Anisocytosis	RBC	RDW-SD > 65.0 fL or RDW-CV > 20.0%
	Microcytosis *	Microcytosis	RBC	MCV < /0.01L
	Macrocytosis *	Macrocytosis	RBC	MCV > 110.0fL
R	Hypochromia *	Hypochromia	KBC+HGB	MCHC < 290g/L
В	Anemia *	Ariennia	HGB DBC	
С	Suspect	Erythrocytosis		
	RBC Agglutination?	agglutination	RBC+HGB	Arithmetic calculation and numerical comparison
	Turbidity/HGB Interf?	Possibility of effect on HGB by chylemia	RBC+HGB	Arithmetic calculation and numerical comparison
	Iron Deficiency?	Possibility of iron deficiency	RBC+HGB	Arithmetic calculation and numerical comparison
	HGB Defect?	Possibility of HGB abnormality	RBC	Arithmetic calculation and numerical comparison
	Fragments?	Possibility of fragmented red blood cells	RBC, PLT, RET	Arithmetic calculation and numerical comparison
	Abnormal			
	PLT Abn Distribution	Abnormal PLT distribution	PLT	Arithmetic calculation and numerical comparison
D	PLT Abn Scattergram* <sup>2</sup>	Abnormal PLT scattergram	PLT-F	PLT clustering in the PLT scattergram
L	Thrombocytopenia *	Thrombocytopenia	PLT, RET, PLT-F	PLT# < 60 x 10 <sup>9</sup> /L
Т	Thrombocytosis *	Thrombocytosis	PLT, RET, PLT-F	PLT# > 600 x 10 <sup>9</sup> /L
	Suspect			
	PLI Clumps?	Possibility of PLI clumps	wnk, wdf, plt-F	Judged from the presence of PLI Clumps on the WNR, WDF and PLT-F scattergrams.

\*1 WPC+WDF channel only. These messages do not appear with all analyzer types. \*2 These messages do not appear with all analyzer types. The threshold for assessing a value as abnormal (marked with "%") can be changed.

# Data display and significance

#### When an IP message is displayed

When the sample is judged to be positive [for an abnormality], and one or more of the following IP messages is displayed, the measured value is considered to have low reliability due to the effect of the abnormality, and an asterisk [\*] is displayed on the right side of the data. Moreover, some values are masked [----].

	WBC	NRBC #/%	NEUT #/%	LYMPH #/%	MONO #/%	EO #/%	BASO #/%	IG #/%	WBC-BF TC-BF# PMN#/% MN#/%	RBC RET# HCT MCV MCH MCHC	HGB MCH MCHC	RDW-SD	RDW-CV	RET# RET% IRF LFR MFR HFR	PLT	RET-He	PDW MPV P-LCR PCT	IPF
WBC Abn Scattergram																		
Lymph, Mono (WDF)				*	*													
Neut, Eo (WDF)			*			*		*										
Lymph, Neut (WDF)			*	*				*										
Neut, Mono (WDF)			*		*			*										
Lymph, Baso (WDF)			*	*				*										
Lymph, Eo (WDF)				*		*												
Mono, Eo (WDF)					*	*												
Mono, Baso (WDF)			*		*			*										
Ghost, Neut (WDF)			*	*	*	*	**2	*										
Ghost, baso (WDF)	**2	**2	*	*	*	*	**2	*										
Ghost, Lymph (WDF)	**2	**2	*	*	*	*	**2	*										
Ghost, Eo (WDF)	**2	**2	*	*	*	*	**2	*										
Ghost, WBC (BF)* <sup>3</sup> Ghost or other interference with WBC in body fluid analysis	**2	**2							*									
4DIFF, Baso (WNR)			*				*	*										
4DIFF, Nrbc (WNR)			*	*	*	*	*	*										
Ghost, 4DIFF (WNR)	**1	*	*	*	*	*	*	*										
Ghost, Nrbc (WNR)	**1	*	*	*	*	*	*	*										
5DIFF data calculation not possible	**1	*																
IG fraction								*										
HF-BF high value																		
NRBC Present																		
IG Present																		
Blasts/Abn Lympho?			*	*	*													
Blasts?*4			*	*	*													
Abn Lympho?*4			*	*	*													
Left Shift?			*			*												
Atypical Lympho?			*	*	*													
RBC Abn Distribution																		
MP-Flag										*								
Abnormal RDW-SD										*			*					
Other abnormal distribution										*		*	*					
Dimorphic Population																		
RET Abn Scattergram*4																		
RET abnormal fraction (Deformation)														*		*		
Other than above (RET zone error)														*		*		
Foreign particles mixed in PLT zone (High impact)															*6			
PRC Agglutination?										Ne					<b>.</b>			
Turbidity/HCB Interf2										<b></b>	4							
Iron Deficiency?											1							
HGB Defect?																		
Fragments?																		
Other abnormal distribution																	*	
															₩*7		*	*
PLT Clumps?															Υ <sup></sup>			*
PI T-E not analyzed															**5,6		*	
PI T-E analyzed															**7		*	×
i Ei i ullulyzeu									1								-12	.12

\*1 WBC in the WNR channel.

\*2 WBC in the WDF channel.

\*3 The body fluid analysis can only be performed if the instrument offers the body fluid mode.

\*4 These messages do not appear with all analyzer types.

\*5 PLT in the RBC/PLT channel.

\*6 PLT in the RET channel.

\*7 PLT in the PLT-F channel.

**XN-Series** 

Changes in data with treatment

**XN-Series** 

This was a case of AML (M2) that showed blasts with cup-like concave nuclei. A combination of anticancer chemotherapy was started.

Information from XN-Series (At initial examination)

WDF

WBC	85.90	10 <sup>9</sup> /L	+	
RBC	2.42	$10^{12}/L$	-	
HGB	81	g/L		
HCT	0.248	L/L	-	
MCV	102.5	fL		
MCH	33.5	pg		
MCHC	327	g/L		
PLT	23	10º/L	—	
RDW-SD	55.9	fL	+	
RDW-CV	15.1	%		
PDW	17.3	fL	+	
MPV	11.7	fL		
P-LCR	38.7	%		
PCT	0.0003	L/L	-	
NIDDC				

NEUT	29.27	10º/L	*	34.0	%	*
LYMPH	4.90	10 <sup>9</sup> /L	*	5.7	%	*
MONO	51.33	10º/L	*	59.8	%	*
EO	0.23	10 <sup>9</sup> /L	*	0.3	%	*
BASO	0.17	10º/L	+	0.2	%	
IG	2.03	10º/L	*	2.4	%	*
RET	24.2	10 <sup>9</sup> /L		1.00	%	
IRF	25.9	%				
LFR	74.1	%				
MFR	14.0	%				
HFR	11.9	%				
RET-He	33.8	pg				
IPF	1.9	10 <sup>9</sup> /L		17.0	%	

#### Flags

WBC Flag(s) WBC Abn Scattergram Neutrophilia Lymphocytosis Monocytosis Leukocytosis IG Present Blasts? Left Shift?

#### RBC Flag(s)

RET Abn Scattergram Anemia

**PLT Flag(s)** Thrombocytopenia

















#### Visual differential counts

PB	
Myelobla	ast 82.5
Promyelo	0.0
Myelo	1.5
Meta	0.0
Stab	2.0
Seg	8.5
Lympho	3.0
Mono	2.5
Baso	0.0
Eosino	0.0
At-Ly	0.0
NRBC	1/100WBC

Point

The white blood cell count is markedly high with a high monocyte fraction of 59.8% on the XN analyzer. While blasts are the main components in the visual differential count, the WDF scattergram shows interfered separation. It seems that these cells are plotted near the area where normal monocytes appear (O). In the WPC scattergram, most of the blasts are found in the O area; some are detected as abnormal cells in the WPC and WPC (SSC-FSC) scattergrams (shown in red), with the "Blasts?" flag displayed.

# PB

×400

**XN-Series** 

# Acute myeloid leukemia with maturation (AML-M2 cup-like)

On day 5 of treatment	On day 6 of treatment
WBC         20.02         10 <sup>9</sup> /L         +           NEUT         23.9         %         *           LYMPH         10.1         %         *           MONO         63.4         %         *           EO         2.6         %         *           BASO         0.0         %         *	WBC 1.93 10 <sup>9</sup> /L – NEUT 19.2 % * LYMPH 42.0 % * MONO 38.3 % * EO 0.5 % BASO 0.0 %
Visual differential counts	Visual differential counts
PB         Myeloblast       84.0         Promyelo       0.0         Myelo       0.0         Myelo       0.0         Meta       0.0         Stab       1.0         Seg       4.5         Lympho       8.0         Mono       2.5         Baso       0.0         At-Ly       0.0         NRBC       0.1/100WBC	PB         Myeloblast       44.0         Promyelo       0.0         Myelo       0.0         Myelo       0.0         Meta       0.0         Stab       0.0         Stab       0.0         Seg       9.0         Lympho       45.0         Mono       1.0         Baso       0.0         Eosino       0.0         At-Ly       1.0         NRBC       0.5/100WBC
unit:%	unit:% WPC (ssc-Fsc)

**Blood smear** (May-Giemsa staining) PΒ



#### The major points in XN data

On day 5 of the treatment, the white blood cell count, which was earlier markedly high, still remains high, although with some decrease. The XN white blood cell differential count shows 63.4% monocytes, but this fraction is 2.5% in the visual differential count. It appears that most of the blasts are plotted in the MONO area (green dots) on the WDF scattergram. Both the WPC and WPC (SSC-FSC) scattergrams show clusters (1) believed to represent blasts, as in the initial examination. We can see that the white blood cell count decreases and the aforesaid clusters, believed to represent blasts, gradually clear with progress of the treatment  $(\uparrow)$ .

Blood smear (May-Giemsa staining)



#### Cumulative data

	On day 8	3 of tre	atment			On day 1	3 of tre	eatment
	On day c		activent			On day i	5 01 11	cutificati
WBC NEUT LYMPH MONO EO BASO	0.87 10 <sup>9</sup> /L 33.4 % 57.5 % 8.0 % 1.1 % 0.0 %	- * * * *	WNR E		WBC NEUT LYMPH MONO EO BASO	0.60 10 <sup>9</sup> /L 5.0 % 91.7 % 0.0 % 3.3 % 0.0 %	- * * * *	WNR E
isual diff	erential co	unts	WDF 분	١	/isual diff	erential co	unts	WDF
PB Myeloblas Promyelo Myelo Meta Stab Seg Lympho Mono Baso Eosino At-Ly NRBC	t 6.0 0.0 0.0 1.0 35.0 53.0 4.0 0.0 1.0 0.0 0.0/100WBC		WPC		PB Myeloblas Promyelo Myelo Meta Stab Seg Lympho Mono Baso Eosino At-Ly NRBC	t 0.0 0.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.		wpc ssc
	ur	nit:%	WPC (SSC-FSC)			u	nit:%	WPC (SSC-FSC)

#### Blood smear (May-Giemsa staining) PB



Major points in blood smear micrographs

On day 6 of treatment there is a decrease in white blood cell count, but with the differential being mainly in blasts. Some blasts show denucleation. By day 8, the fraction of blasts comes down to 6% and vacuoles are seen in some blasts. There is a relative increase in the lymphocyte fraction. The blasts are cleared and only lymphocytes are seen on day 13. Bone marrow tests was carried out after this confirm clearance of blasts, thereafter it being judged that complete remission has been attained.

Blood smear (May-Giemsa staining)



**XN-Series** 

This was a case of AML (M3) that showed an increase in abnormal promyelocytes in the peripheral blood. A combination therapy regimen with anticancer agents including all-trans retinoic acid (ATRA) was started.

#### Information from XN-Series (At initial examination)

84.90	10º/L	+	
2.98	$10^{12}/L$		
92	g/L		
0.283	L/L		
95.0	fL		
30.9	pg		
325	g/L		
87	10 <sup>9</sup> /L		
43.5	fL		
12.8	%		
11.5	fL		
11.5 10.3	fL fL		
11.5 10.3 28.0	fL fL %		
11.5 10.3 28.0 0.0009	fL fL % L/L	_	
	2.98 92 0.283 95.0 30.9 325 87 43.5 12.8	2.98 10 <sup>12</sup> /L 92 g/L 0.283 L/L 95.0 fL 30.9 pg 325 g/L 87 10 <sup>9</sup> /L 43.5 fL 12.8 %	2.98 10 <sup>12</sup> /L 92 g/L 0.283 L/L 95.0 fL 30.9 pg 325 g/L 87 10 <sup>9</sup> /L 43.5 fL 12.8 %

NEUT	22.90	10º/L	*	27.0	%	*
LYMPH	26.83	10 <sup>9</sup> /L	*	31.6	%	*
MONO	33.64	10º/L	*	39.6	%	*
EO	0.92	10 <sup>9</sup> /L	*	1.1	%	*
BASO	0.61	10º/L	+	0.7	%	
IG	3.17	10º/L	*	3.7	%	*
RET	19.7	10 <sup>9</sup> /L		0.66	%	
IRF	32.0	%				
LFR	68.0	%				
MFR	14.6	%				
HFR	17.4	%				
RET-He	33.4	pg				
IPF	4.8	10 <sup>9</sup> /L		5.3	%	

#### Flags



**RBC Flag(s)** Anemia

PLT Flag(s)















#### Visual differential counts

PB	
Myelobla	ast 2.0
Promyelo	82.0
Myelo	4.0
Meta	0.0
Seg	0.5
Stab	0.0
Lympho	7.0
Mono	4.5
Baso	0.0
Eosino	0.0
At-Ly	0.0
NRBC	0/100WBC

●Point

RET

The white blood cell count is markedly high and the WDF scattergram shows poor separation of cell clusters. The cluster seems as one cell population, and it is assumed that promyelocytes, which are observed in large numbers in the blood smear, are plotted in this area. The WPC scattergram shows an abnormal pattern, indicating the presence of abnormal cells.



×	4	0	0

#### **XN-Series**

# Acute promyelocytic leukemia (AML-M3 APL)

23.66 10<sup>9</sup>/L + WBC NEUT 76.7 % \* LYMPH 10.8 % \* MONO 11.0 % \* EO 1.3 % \* BASO 0.2 %

On day 4 of treatment

#### Visual differential counts

PB	
Myeloblas	t 1.0
Promyelo	32.0
Myelo	33.0
Meta	10.0
Stab	2.0
Seg	1.0
Lympho	12.0
Mono	9.0
Eosino	0.0
Baso	0.0
At-Ly	0.0
NRBC	0.1/100WBC





unit:%



#### Blood smear (May-Giemsa staining) PB



#### The major points in XN data

The WDF scattergram shows poor separation of white blood cell fractions even on day 4 of the treatment. In the visual differential count, the combined fraction of promyelocytes, myelocytes, and metamyelocytes is more than 70%, and these are believed to have formed the cluster marked with  $\bigcirc$  on the WDF scattergram of day 4. There are no apparent cells in the abnormal cell detection areas of the WPC and WPC (SSC-FSC) scattergrams. But a cluster believed to represent immature granulocytes shows progressive reduction in size ( $\uparrow$ ) along with a decrease in white blood cell count with the treatment.

#### On day 5 of treatment

WBC	8.20	10 <sup>9</sup> /L	
NEUT	51.6	%	*
LYMPH	14.4	%	*
MONO	33.8	%	*
EO	0.1	%	*
BASO	0.1	%	

#### Visual differential counts

PB	
Myeloblast	t 1.0
Promyelo	43.0
Myelo	32.0
Meta	1.0
Stab	0.0
Seg	10.0
Lympho	11.0
Mono	2.0
Eosino	0.0
Baso	0.0
At-Ly	0.0
NRBC	0.4/100WBC







unit:%



#### Blood smear (May-Giemsa staining)



#### Cumulative data

WBC 0.17 10 <sup>9</sup> /L – NEUT 29.5 % * LYMPH 52.9 % * MONO 17.6 % * EO 0.0 % * BASO 0.0 % * Visual differential counts PB	WBC NEUT LYMPH MONO EO BASO <b>Visual diffe</b>	0.79 10 <sup>9</sup> /L – 54.4 % * 38.0 % * 7.6 % * 0.0 % * 0.0 % *	
Visual differential counts	Visual diffe	erential counts	WDF
PB			
Myeloblast0.0Promyelo8.0Myelo2.0Meta0.0	<b>PB</b> Myeloblast Promyelo Myelo Meta	t 0.0 0.0 2.0 0.0	
Stab0.0Seg2.0Lympho76.0Mono12.0Eosino0.0	Stab Seg Lympho Mono Eosino	4.0 49.0 35.0 4.0 0.0	WPC
Baso 0.0 At-Ly 0.0 NRBC 0.0/100WBC	Baso At-Ly NRBC	0.0 6.0 0.0/100WBC	wpc (ssc-fsc)



**Blood smear** (May-Giemsa staining) PΒ



Major points in blood smear micrographs

Blood smear (May-Giemsa staining)





On day 4 of the treatment, there is a decrease in the white blood cell count. The differential white blood cell count shows a trend of differentiation, as seen in the appearance of myelocytes and metamyelocytes. Promyelocytes have few azurophilic granules and a tendency of aggregation of nuclear chromatin. Besides this, irregularities of the nuclei have become more prominent. On day 5, the micrographs show an increase in the neutrophil fraction (10%). The neutrophils show no cytoplasmic granules and the nuclei are irregular, which gives them the appearance of pseudo Pelger nuclei. On day 7, there is a marked decrease in the white blood cell count and a decrease in promyelocytes. The nuclei with anomaly in the promyelocytes have a curved indentations and a tendency to get segmented. The peripheral blood of day 18 shows no promyelocytes but a rise in neutrophils with pseudo Pelger anomaly. In this case, the ATRA therapy induced differentiation of block and the peripheral blood of day 18 shows no promyelocytes but a rise in neutrophils with pseudo Pelger anomaly. In this case, the ATRA therapy induced differentiation of leukemic cells.



**XN-Series** 

This case had microcytic hypochromic anemia. The patient was diagnosed as having iron deficiency anemia because of a low serum level of iron and elevated those TIBC and UIBC, and oral iron therapy was started.

#### Information from XN-Series (At initial examination)

WBC	17.53	10 <sup>9</sup> /L	+	
RBC	3.16	$10^{12}/L$		
HGB	41	g/L	-	
НСТ	0.187	L/L	-	
MCV	59.2	fL	-	
MCH	13.0	pg	-	
MCHC	219	g/L	-	
PLT&F	209	10 <sup>9</sup> /L		
PLT-I	228	10 <sup>9</sup> /L	*	
RDW-SD	52.3	fL		
RDW-CV	26.4	%	+	
PDW		fL		
MPV		fL		
P-LCR		%		
РСТ		L/L		
NRBC	0.17	10 <sup>9</sup> /L		1.0 /100WBC

NEUT	4.42	10º/L	*	25.3	%	*
LYMPH	11.26	10 <sup>9</sup> /L	*	64.2	%	*
MONO	1.32	10º/L	*	7.5	%	*
EO	0.48	10 <sup>9</sup> /L	+	2.7	%	
BASO	0.05	10º/L		0.3	%	
IG	0.05	10º/L	*	0.3	%	*
RET	47.1	10 <sup>9</sup> /L		1.49	%	
IRF	24.2	%				
LFR	75.8	%				
MFR	14.7	%				
HFR	9.5	%				
RET-He	13.6	pg				
IPF	3.3	10 <sup>9</sup> /L		1.6	%	

#### Flags

WBC Flag(s) Lymphocytosis Monocytosis Atypical Lympho? Abn Lympho?

#### RBC Flag(s)

Anisocytosis Microcytosis Hypochromia Anemia Iron Deficiency? Fragments?

PLT Flag(s)

PLT Abn Distribution



#### Point

All red blood cell parameters have low values. In the RET scattergram, the cluster of mature red blood cells is seen in the area with low forward-scattered light intensity (shifted downwards,  $\bigcirc$ ). The low RET-He\* value of 13.6 pg (0.84 fmol) suggests the production of microcytic hypochromic red blood cells. In the RBC histogram, the size distribution curve shows a general leftward shift, which suggests the appearance of microcytic red blood cells. Some of these apparently interfere in the PLT histogram ( $\uparrow$ ), and the "PLT Abn Distribution" flag is displayed.

\*RET-He: Reticulocyte hemoglobin equivalent; its clinical reference range is 30.3 - 36.0 pg (1.88 - 2.23 fmol).

# Iron deficiency anemia

#### At initial examination

RBC	3.16	10 <sup>12</sup> /L	
HGB	41	g/L	-
HCT	0.187	L/L	-
MCV	59.2	fL	-
MCH	13.0	pg	-
MCHC	219	g/L	-
PLT&F	209	10 <sup>9</sup> /L	
PLT-I	228	10 <sup>9</sup> /L	*
RDW-SD	52.3	fL	
RDW-CV	26.4	%	+
PDW		fL	
MPV		fL	
P-LCR		%	
PCT		L/L	
RET#	47.1	10 <sup>9</sup> /L	
RET%	1.49	%	
IRF	24.2	%	
LFR	75.8	%	
MFR	14.7	%	
HFR	9.5	%	
RET-He	13.6	pg	







#### Other tests

Serum iron(µg/dL)	10
TIBC(µg/dL)	445
UIBC(µg/dL)	435

#### PLT Flag(s)

Fragments?

**RBC Flag(s)** 

Anisocytosis

Microcytosis

Hypochromia

Iron Deficiency?

Anemia

Flags

PLT Abn Distribution

#### **Blood smear** (May-Giemsa staining) ΡB

The major points in XN data



#### HGB 53 g/L 0.235 L/L HCT \* 67.5 fL MCV \* MCH 15.2 pg \* 226 g/L MCHC \* PLT&F 492 10<sup>9</sup>/L +PLT-I 492 10<sup>9</sup>/L +76.0 fL RDW-SD \* RDW-CV 34.5 % \* 10.1 fL PDW MPV 9.0 fL P-LCR 17.8 % PCT 0.0044 L/L +RET# 194.9 10<sup>9</sup>/L \* RET% 5.60 % IRF 35.2 % \* LFR 64.8 % \* 16.8 % MFR \* 18.4 % HFR \* RET-He 14.1 pg \*

#### On day 7 of treatment

3.48 10<sup>12</sup>/L \*







#### Flags

RBC

#### RBC Flag(s)

**RBC** Abn Distribution Anisocytosis Microcytosis Hypochromia Anemia Reticulocytosis Iron Deficiency? Fragments?

#### PLT Flag(s)

**Blood smear** (May-Giemsa staining) PR



The hemoglobin level shows an increase with progression of the iron replacement therapy. The RBC histograms on day 7 and day 15 of treatment are bimodal  $(\uparrow)$ , as a result of the increase in normocytic red blood cells. From day 15 to 28, the mature red blood cell cluster in the RET scattergram also is seen shifted towards the area of higher forward-scattered light intensity (1), suggesting recovery of the red blood cell size and the hemoglobin level. However, the iron deficiency appears to be persisting even on day 29 of the treatment, as the RET-He is low at 23.0 pg (1.43 fmol), which suggests the need for continuing the treatment.

#### Cumulative data

#### On day 15 of treatment

RBC	5.17	10 <sup>12</sup> /L	*
HGB	92	g/L	
НСТ	0.386	L/L	*
MCV	74.7	fL	*
MCH	17.8	pg	*
MCHC	238	g/L	*
PLT&F	38	10 <sup>9</sup> /L	-
PLT-I	67	10º/L	*
RDW-SD		fL	
RDW-CV		%	
PDW		fL	
MPV		fL	
P-LCR		%	
PCT		L/L	
RET#	192.8	10 <sup>9</sup> /L	*
RET%	3.73	%	
IRF	26.8	%	*
LFR	73.2	%	*
MFR	17.2	%	*
HFR	9.6	%	*
RET-He	18.3	pg	*









#### Other tests

Serum iron(µg/dL)	105
TIBC(µg/dL)	429
UIBC(µg/dL)	324

NDC	2.11	TO / L	
HGB	112	g/L	
HCT	0.421	L/L	
MCV	77.8	fL	_
MCH	20.7	pg	-
MCHC	266	g/L	-
PLT&F	315	10º/L	
PLT-I	360	10º/L	
RDW-SD	65.6	fL	+
RDW-CV	24.2	%	+
PDW	11.6	fL	*
MPV	9.8	fL	*
P-LCR	24.7	%	*
PCT	0.0035	L/L	*
RET#	40.0	10 <sup>9</sup> /L	
RET%	0.74	%	
IRF	9.8	%	
LFR	90.2	%	
MFR	9.1	%	
HFR	0.7	%	
RET-He	23.0	pg	

RRC

Flags

**RBC Flag(s)** 

Anisocytosis Hypochromia

Fragments?

PLT Flag(s)

RET Abn Scattergram

PLT Abn Distribution

#### On day 29 of treatment

5/11 1012/1







#### Anemia Fragments? PLT Flag(s) PLT Abn Distribution

RBC Abn Distribution

**Dimorphic Population** 

Flags

RBC Flag(s)

Hypochromia

Thrombocytopenia

#### **Blood smear** (May-Giemsa staining) PB



#### Major points in blood smear micrographs

At initial examination, the peripheral blood shows microcytic hypochromic anemia. The smear sample shows a low red blood cell density, and red blood cell anisocytosis. There are microspherocytes, poikilocytes and numerous target cells with enlarged pale central regions. On day 7, the red blood cells have anisocytosis, with microcytic red blood cells and poikilocytes among them, and target cells are fewer compared to the micrograph at initial examination. Moreover, a number of polychromatic red blood cells have appeared. The red blood cells seen on day 15 are of two types, normal and microcytic, suggesting a recovery stage from the anemia. On day 29, the red blood cell density is high and there are fewer microcytic red blood cells, poikilocytes, and target cells, which indicates improvement of the anemia. Although the red blood cell count and hemoglobin level have recovered, MCH and MCHC are still low. Therefore, the iron replacement therapy is continued.

#### **Blood smear** (May-Giemsa staining) PB



**XN-Series** 

This was a case suffered from burn injury all over the body.

Information from XN-Series (At initial examination)

	WBC	13.32	10 <sup>9</sup> /L		
	RBC	4.15	$10^{12}/L$	*	
	HGB	123	g/L		
	НСТ	0.365	L/L	*	
	MCV	88.0	fL	*	
	MCH	29.6	pg	*	
	MCHC	337	g/L	*	
	PLT&F	187	10 <sup>9</sup> /L		
	PLT-I	1012	10 <sup>9</sup> /L	*	
	RDW-SD	57.1	fL	*	
	RDW-CV	19.3	%	*	
	PDW	19.3	fL	+	
	MPV	12.6	fL		
	P-LCR	42.9	%		
	PCT	0.0128	L/L	+	
	NRBC	0.01	10 <sup>9</sup> /L		0.1 /100WBC

NEUT	6.72	10 <sup>9</sup> /L	*	50.5	%	*
LYMPH	5.69	10 <sup>9</sup> /L	*	42.7	%	*
MONO	0.47	109/L	*	3.5	%	*
EO	0.32	10 <sup>9</sup> /L		2.4	%	
BASO	0.12	10º/L	+	0.9	%	
IG	0.10	10º/L	*	0.8	%	*
RET	41.1	10 <sup>9</sup> /L	*	0.99	%	*
IRF	9.1	%	*			
LFR	90.9	%	*			
MFR	6.7	%	*			
HFR	2.4	%	*			
RET-He	27.8	pg	*			
IPF	6.2	10 <sup>9</sup> /L		3.3	%	
FRC	799.2	10 <sup>9</sup> /L	*	19.26	%	*

#### Flags

WBC Flag(s)

Lymphocytosis Abn Lympho?

#### RBC Flag(s)

RBC Abn Distribution RET Abn Scattergram Fragments?

#### PLT Flag(s)



#### Point

In the RET scattergram, the cluster of mature red blood cells is seen extending to the area of low forward-scattered light intensity ( $\bigcirc$ ). The PLT-F scattergram also shows a large number of particles in the Debris area ( $\bigcirc$ ), a feature that reflects the presence of red blood cell fragments. Some influence with the PLT histogram is also seen (1), and the PLT count measured by the impedance method (PLT-I) of 1012×10<sup>9</sup>/L is quite different from PLT-F and the result obtained by the immunological method (CD61<sup>+</sup> 204×10<sup>9</sup>/L). The "Fragments?" flag is displayed on the XN analyzer, and the research parameter FRC%, the proportion of particles in the red blood cell fragments area\* on the RET scattergram, is as high as at 19.26%\*. \*Red blood cell fragments area: The "RBC Fragments" area on the RET scattergram on p. 14.

\*Reference range of FRC%: 0.04 - 0.39%

#### **XN-Series**

## **Burn injury**

Day 1 after injury

RBC	4.15	10 <sup>12</sup> /L	*
HGB	123	g/L	
НСТ	0.365	L/L	*
MCV	88.0	fL	*
MCH	29.6	pg	*
MCHC	337	g/L	*
PLT&F	187	10 <sup>9</sup> /L	
PLT-I	1012	10 <sup>9</sup> /L	*
RDW-SD	57.1	fL	*
RDW-CV	19.3	%	*
PDW	19.3	fL	+
MPV	12.6	fL	
P-LCR	42.9	%	
PCT	0.0128	L/L	+
RET#	41.1	10 <sup>9</sup> /L	*
RET%	0.99	%	*
IRF	9.1	%	*
LFR	90.9	%	*
MFR	6.7	%	*
HFR	2.4	%	*
RET-He	27.8	pg	*
FRC%	19.26	%	*

#### Flags

**RBC Flag(s)** RBC Abn Distribution RET Abn Scattergram Fragments?

PLT Flag(s)







Other tests CD61<sup>+</sup> 204×10<sup>9</sup>/L

RBC	3.35	1012/L	*
HGB	95	g/L	
HCT	0.309	L/L	*
MCV	92.2	fL	*
MCH	28.4	pg	*
MCHC	307	g/L	*
PLT&F	85	10 <sup>9</sup> /L	
PLT-I	476	10 <sup>9</sup> /L	+
RDW-SD	70.7	fL	*
RDW-CV	22.7	%	*
PDW		fL	
MPV		fL	
P-LCR		%	
PCT		L/L	
RET#	66.7	10 <sup>9</sup> /L	*
RET%	1.99	%	
IRF	21.7	%	*
LFR	78.3	%	*
MFR	13.8	%	*
HFR	7.9	%	*
RET-He	26.6	pg	*
FRC%	15.51	%	*

#### Flags

**RBC Flag(s)** RBC Abn Distribution Anisocytosis Anemia RET Abn Scattergram Fragments?

**PLT Flag(s)** PLT Abn Distribution

#### Day 2 after injury

![](_page_36_Picture_15.jpeg)

![](_page_36_Picture_16.jpeg)

![](_page_36_Picture_17.jpeg)

![](_page_36_Figure_18.jpeg)

Other tests

CD61<sup>+</sup> 95×10<sup>9</sup>/L

Blood smear (May-Giemsa staining) PB

![](_page_36_Figure_22.jpeg)

#### ×400

**Blood smear** (May-Giemsa staining) PB

![](_page_36_Picture_25.jpeg)

The major points in XN data

With the passage of days, the number of particles in the red blood cell fragments area of the RET scattergram ( $\uparrow$ ) and the Debris area of the PLT-F scattergram ( $\uparrow$ ) decrease, along with a decrease in FRC% as well. PLT-F measured by the XN analyzer is almost equal to the platelet count estimated by the immunological method that uses CD61, in a series of measurement on different days. PLT-F is not affected by the appearance of red blood cell fragments, demonstrating that the platelet measurements are accurate.

#### Cumulative data

Day 3 after	injury		Day 4 after i	njury
RBC       2.6       10 <sup>12</sup> /L         HGB       75       g/L       -         HCT       0.248       L/L       -         MCV       95.4       fL       -         MCH       28.8       pg       -         MCHC       302       g/L       -         PLT&F       55       10 <sup>9</sup> /L       -	RET	RBC       3.         HGB       9         HCT       0.2         MCV       88         MCH       28         MCHC       3.         PLT&F       9         PLT-I       9	27 10 <sup>12</sup> /L 94 g/L 89 L/L 8.4 fL 8.7 pg 25 g/L 45 10 <sup>9</sup> /L – 79 10 <sup>9</sup> /L	RET
RDW-SD       73.7 fL       +         RDW-CV       22.4 %       +         PDW       fL       +         PDW       fL       +         PDW       fL       +         P-LCR       %       +         PCT       L/L       +         RET#       54.9 10°/L       +         RET%       2.11 %       +         IRF       28.6 %       +         LFR       71.4 %       +         MFR       17.0 %       +         HFR       11.6 %       +         RET-He       28.4 pg       +	PLT-F	RDW-SD       59         RDW-CV       18         PDW          MPV          P-LCR          PCT          RET#       45         RET%       1.4         IRF       23         LFR       76         MFR       16         HFR       77         RET-He       27	9.1 fL + 8.8 % + fL fL % L/L 5.8 10 <sup>9</sup> /L 40 % 3.3 % 6.7 % 6.1 % 7.2 % 7.5 pg	PLT-F
FRC% 14.68 % *		FRC% 4.	31 % *	PLT
Flags RBC Flag(s) Anisocytosis		Flags RBC Flag(s) Anemia		
Anemia RET Abn Scattergram Fragments?	CD61 <sup>+</sup> 75×10 <sup>9</sup> /L	PLT Flag(s) PLT Abn Distr	ibution	CD61 <sup>+</sup> 54×10 <sup>9</sup> /L
PLT Flag(s) PLT Abn Distribution Thrombocytopenia		inrombocyto	реша	

![](_page_37_Picture_2.jpeg)

![](_page_37_Picture_3.jpeg)

![](_page_37_Figure_4.jpeg)

#### Major points in blood smear micrographs

**Blood smear** (May-Giemsa staining) PB

![](_page_37_Figure_7.jpeg)

Red blood cells in peripheral blood show striking anisocytosis on day 1 of the injury. The micrograph shows a number of red blood cell fragments such as platelet-sized micropoikilocytes and microspherocytes. These red blood cell fragments are believed to be produced by degeneration of the cellular membrane proteins in the blood vessels when the skin gets damaged by heat. The red blood cell fragments that appear in such heat injury cases are counted as platelets by the impedance method, resulting in false high values of platelet counts. Visual platelet counting or immunological testing would be required in such cases. On day 2 after the injury, there is an increase in band cells (left shift). Red blood cell fragments like microspherocytes and micropoikilocytes are recognized in as many as seen on day 1. On day 3, micropoikilocytes are decreased in number but the platelet count measured by the impedance method (PLT-I) remains high. On day 4, the smear samples show almost no micropoikilocytes, indicating their disappearance. On day 5, there is no effect of microcytic red blood cells on PLT-I.

**XN-Series** 

Frequently found abnormal specimens

# XN-Series Immature granulocytes

#### Information from XN-Series

	100/1	-	
21.28	10°/L	T	
3.47	$10^{12}/L$		
98	g/L		
0.299	L/L		
86.2	fL		
28.2	pg		
328	g/L		
87	10 <sup>9</sup> /L		
87 46.6	10 <sup>9</sup> /L fL		
87 46.6 15.2	10 <sup>9</sup> /L fL %		
87 46.6 15.2 10.6	10 <sup>9</sup> /L fL % fL		
87 46.6 15.2 10.6 9.3	10 <sup>9</sup> /L fL fL fL		
87 46.6 15.2 10.6 9.3 21.8	10 <sup>9</sup> /L fL fL fL %		
87 46.6 15.2 10.6 9.3 21.8 0.0008	10 <sup>9</sup> /L fL fL fL % L/L	_	
	3.47 98 0.299 86.2 28.2 328	3.47 10 <sup>12</sup> /L 98 g/L 0.299 L/L 86.2 fL 28.2 pg 328 g/L	3.47 10 <sup>12</sup> /L 98 g/L 0.299 L/L 86.2 fL 28.2 pg 328 g/L

NEUT	18.35	10º/L	*	86.2	% *
LYMPH	0.81	10º/L	*	3.8	% *
MONO	1.81	10 <sup>9</sup> /L	*	8.5	% *
EO	0.00	10 <sup>9</sup> /L	*	0.0	% *
BASO	0.31	10º/L	+	1.5	% +
IG	2.74	10º/L	*	12.9	% *
RET	11.5	10º/L		0.33	%
IRF	15.0	%			
LFR	85.0	%			
MFR	11.7	%			
HFR	3.3	%			
RET-He	31.7	pg			
IPF	2.6	10 <sup>9</sup> /L		2.7	%

#### Flags

WBC Flag(s) WBC Abn Scattergram Neutrophilia Monocytosis Basophilia Leukocytosis IG Present Blasts? Left Shift?

**RBC Flag(s)** Anemia

#### PLT Flag(s)

![](_page_40_Figure_8.jpeg)

#### Point

The myelocytes and metamyelocytes seen in the smears are plotted in the IG area ( $\bigcirc$ ) of the WDF scattergram. In the WPC scattergram, the granulocyte cluster extends downwards ( $\uparrow$ ), a feature that reflects the presence of immature granulocytes. The WPC and WPC (SSC-FSC) scattergrams show cells in the abnormal cell detection area ( $\uparrow$ , displayed in red). These could be the blastoid cells seen in small numbers in the smears. The "Blasts?" flag is displayed.

This was a case with malignant lymphoma under anticancer chemotherapy. G-CSF was administered because of neutropenia.

#### **Blood smear** (May-Giemsa staining)

![](_page_41_Picture_4.jpeg)

PΒ

![](_page_41_Picture_6.jpeg)

PΒ

![](_page_41_Picture_8.jpeg)

#### Point

The white blood cell count is elevated. The differential count reveals immature cells including myelocytes, metamyelocytes and blastoid cells. Granulocytic series cells show toxic granules and Döhle bodies.

Based on the above findings, the patient is assumed to be in the bone marrow recovery phase with G-CSF after anticancer chemotherapy.

![](_page_41_Picture_12.jpeg)

![](_page_41_Picture_13.jpeg)

#### Visual differential counts

PB	
Myeloblast	0.0
Promyelo	0.0
Myelo	23.0
Meta	11.0
Stab	5.0
Seg	49.0
Lympho	10.0
Mono	1.0
Baso	0.0
Eosino	0.0
At-Ly	0.0
Other*	1.0
NRBC 1/10	OWBC
*Blasto	id cells

unit:%

#### Information from XN-Series

WBC	3.21	10 <sup>9</sup> /L		
RBC	2.07	$10^{12}/L$	—	
HGB	62	g/L	-	
HCT	0.193	L/L	—	
MCV	93.2	fL		
MCH	30.0	pg		
MCHC	321	g/L		
PLT	26	10 <sup>9</sup> /L	-	
RDW-SD	54.3	fL	+	
RDW-CV	15.8	%		
PDW	11.3	fL		
MPV	11.5	fL		
P-LCR	34.9	%		
РСТ	0.0003	L/L	-	
NRBC	0.00	10 <sup>9</sup> /L		0.0 /100WBC

NEUT	2.79	10 <sup>9</sup> /L	*	86.9	%	*
LYMPH	0.32	10 <sup>9</sup> /L	*	10.0	%	*
MONO	0.05	10 <sup>9</sup> /L	*	1.6	%	*
EO	0.02	10 <sup>9</sup> /L		0.6	%	
BASO	0.03	10º/L		0.9	%	
IG	0.70	10 <sup>9</sup> /L	*	21.8	%	*
RET	1.2	10 <sup>9</sup> /L		0.06	%	
IRF	8.0	%				
LFR	92.0	%				
MFR	8.0	%				
HFR	0.0	%				
RET-He		pg				
IPF	0.7	10 <sup>9</sup> /L		3.4	%	

#### Flags

WBC Flag(s) Lymphopenia

IG Present Blasts?

RBC Flag(s) Anemia

**PLT Flag(s)** Thrombocytopenia

![](_page_42_Figure_10.jpeg)

#### Point

In the WNR scattergram, some white blood cells are seen in the area of high fluorescence intensity (O). It is possible that the mononuclear neutrophils with the pseudo-Pelger anomaly seen in the smears are plotted in this area. This matches with the large number of cells seen plotted in the IG area of the WDF scattergram, the high IG% of 21.8%, and the presence of myelocytes in the smears. The NEUT cluster has been shifted slightly to the direction of weaker side-scattered light intensity (**↑**). This is believed to be due to a reduction in granules in the neutrophils and a low degree of segmentation of their nuclei.

The pseudo-Pelger anomaly was detected in peripheral blood neutrophils during chemotherapy with anticancer agents administered based on the diagnosis of acute myelogenous leukemia.

#### **Blood smear** (May-Giemsa staining)

ΡB

![](_page_43_Picture_5.jpeg)

#### Visual differential counts

#### PB

Myelobla	ast 0.0
Promyel	0.0
Myelo	29.0
Meta	3.0
Stab	4.0
Seg	39.0
Lympho	12.0
Mono	13.0
Baso	0.0
Eosino	0.0
At-Ly	0.0
NRBC	1/100WBC

unit:%

![](_page_43_Picture_11.jpeg)

#### ●Point

Peripheral blood shows anemia and thrombocytopenia. Neutrophils have the pseudo-Pelger anomaly with no nuclear segmentation. There are neutrophils with two-lobed pseudo-Pelger anomaly. The nuclei are round-shaped with highly dense nuclear chromatin, and the cytoplasm has Döhle bodies (1).

The pseudo-Pelger anomaly disappears with an increase in the white blood cell count and is therefore supposed to be a transient occurrence during the bone marrow recovery phase.

#### Information from XN-Series

WBC	35.36	10º/L	+	
RBC	2.72	$10^{12}/L$		
HGB	82	g/L		
HCT	0.258	L/L	-	
MCV	94.9	fL		
MCH	30.1	pg		
MCHC	318	g/L		
DIT	196	$10^{9}/l$		
1 61	1)0	TO / L		
RDW-SD	55.5	fL	+	
RDW-SD	55.5 16.2	fL %	+++	
RDW-SD RDW-CV PDW	55.5 16.2 9.9	fL % fL	+ +	
RDW-SD RDW-CV PDW MPV	55.5 16.2 9.9 9.7	fL % fL fL	+ +	
RDW-SD RDW-CV PDW MPV P-LCR	55.5 16.2 9.9 9.7 21.8	fL % fL fL %	+ +	
RDW-SD RDW-CV PDW MPV P-LCR PCT	55.5 16.2 9.9 9.7 21.8 0.0019	fL % fL fL % L/L	+ +	

NEUT	34.27	10º/L	+	97.0 % +
LYMPH	0.53	10 <sup>9</sup> /L	_	1.5 % -
MONO	0.04	10 <sup>9</sup> /L		0.1 %
EO	0.01	10 <sup>9</sup> /L		0.0 %
BASO	0.51	10º/L	+	1.4 % +
IG	7.70	10º/L	+	21.8 %
RET	14.7	10 <sup>9</sup> /L		0.54 %
IRF	2.7	%		
LFR	97.3	%		
MFR	2.0	%		
HFR	0.7	%		
RET-He	34.5	pg		
IPF	4.6	10 <sup>9</sup> /L		2.1 %

#### Flags

WBC Flag(s) Neutrophilia Lymphopenia Basophilia

Leukocytosis

IG Present

**RBC Flag(s)** Anemia

PLT Flag(s)

![](_page_44_Figure_10.jpeg)

#### Point

There is an increase in the white blood cell count, and more than 95% of the white blood cells are neutrophils. The NEUT cluster of the WDF scattergram is extending to the high fluorescence intensity area. The giant hypersegmented neutrophils seen on the smears are supposed to be plotted in the IG area (O) because of their higher fluorescence intensity and higher side-scattered light intensity than normal neutrophils. The "IG Present" flag is displayed, and the IG% is as high as at 21.8%.

This was a case of malignant lymphoma in which the patient received anticancer chemotherapy. Giant neutrophils appeared in the peripheral blood after G-CSF administration.

#### Blood smear (May-Giemsa staining)

PB

Visual differential counts

PB

![](_page_45_Picture_6.jpeg)

#### Point

Peripheral blood shows an increase in neutrophils. Some of them are giant neutrophils of about twice the size of normal neutrophils and their nuclei are segmented with 6-9 lobes. These neutrophils disappear after a few days, suggesting that their appearance is transient in the bone marrow recovery phase.

Hypersegmented neutrophils appear in megaloblastic anemia, myelodysplastic syndrome (MDS), chronic administration of anticancer drug (folic acid antagonist) and other conditions.

#### PB

ost 0.0
0.0
0.0
0.0
1.0
98.0
1.0
0.0
0.0
0.0
0.0
0/100WBC

unit:%

#### Information from XN-Series

WBC	492.30	10 <sup>9</sup> /L	@	
RBC	3.91	$10^{12}/L$		
HGB	100	g/L		
HCT	0.331	L/L		
MCV	84.7	fL	-	
MCH	25.6	pg	-	
MCHC	302	g/L	-	
PLT&F	9	10 <sup>9</sup> /L	-	
PLT-I	35	10 <sup>9</sup> /L	-	
RDW-SD	52.2	fL		
RDW-CV	/ 17.5	%	+	
PDW	21.4	fL	*	
MPV	11.6	fL	*	
P-LCR	40.0	%	*	
PCT	0.0004	L/L	*	
NRBC	0.18	10 <sup>9</sup> /L		0.0 /100WBC

NEUT	22.97	10º/L	*	4.6	%	*
LYMPH	381.87	10º/L	*	77.6	%	*
MONO	76.13	10 <sup>9</sup> /L	*	15.5	%	*
EO	0.04	10 <sup>9</sup> /L		0.0	%	
BASO	11.29	10º/L	+	2.3	%	+
IG	2.03	10º/L	*	0.4	%	*
RET	10.6	10 <sup>9</sup> /L		0.27	%	
IRF	21.6	%				
LFR	78.4	%				
MFR	17.2	%				
HFR	4.4	%				
RET-He	30.9	pg				
IPF	1.8	10 <sup>9</sup> /L	*	19.5	%	*

#### Flags

WBC Flag(s)
WBC Abn Scattergram
Neutrophilia
Lymphocytosis
Monocytosis
Basophilia
Leukocytosis
IG Present
Blasts?
Abn Lympho?

**RBC Flag(s)** RET Abn Scattergram

PLT Flag(s) PLT Abn Distribution Thrombocytopenia

![](_page_46_Figure_9.jpeg)

#### Point

The separation of WBC and BASO is unclear in the WNR scattergram. The cluster separation is also poor in the WDF scattergram. The "WBC Abn Scattergram" flag is displayed. In the visual differential count, 96% are blastoid cells and some of them are plotted in the abnormal cell detection areas (shown in red) of the WPC and WPC (SSC-FSC) scattergrams. The smear shows fragments of blastoid cells, and these may have affected the platelet counts as their size is similar to platelets. The PLT-F scattergram has some particles, believed to be white blood cell fragments, plotted in the high fluorescence intensity area ( $\uparrow$ ). The "PLT Abn Distribution" flag, which suggests abnormality in the PLT histogram, is displayed. The PLT-I, the platelet count determined by the impedance method, of 35 ×10<sup>9</sup>/L, possibly includes white blood cell fragments. However, the true value is not known because the results of the immunological methods are not available in this case.

The patient presented at the hospital with headache as the chief complaint. Peripheral blood showed an increased white blood cell count, anemia and a low platelet count.

#### Blood smear (May-Giemsa staining)

PΒ

![](_page_47_Picture_5.jpeg)

Visual differential counts

PB

![](_page_47_Picture_7.jpeg)

#### Point

The white blood cell count is markedly increased, and 96% of the white blood cells are blastoid cells. This case is thought to be an acute leukemia. Leukemia cells are destructed and there are a large number of nuclear shadows. There are also a number of platelet-sized white blood cell fragments (**↑**) derived from destructed blasts.

### DD

PD	
Myelobla	ast 0.0
Promyel	o.0
Myelo	0.0
Meta	0.0
Stab	0.0
Seg	1.0
Lympho	3.0
Mono	0.0
Baso	0.0
Eosino	0.0
At-Ly	0.0
Other*	96.0
NRBC	1/100WBC
*В	lastoid cells

unit:%

#### Information from XN-Series

WBC	297.85	10 <sup>9</sup> /L	+	
RBC	3.15	$10^{12}/L$		
HGB	104	g/L		
HCT	0.354	L/L		
MCV	112.4	fL	+	
MCH	33.0	pg		
MCHC	294	g/L	-	
PLT&F	7	10 <sup>9</sup> /L	*	
PLT-I	25	10 <sup>9</sup> /L	—	
RDW-SD	72.3	fL	+	
RDW-CV	18.2	%	+	
PDW	9.7	fL	*	
MPV	7.7	fL	*	
P-LCR	7.6	%	*	
PCT	0.0002	L/L	*	
NRBC	0.92	10 <sup>9</sup> /L		0.3 /100WBC

NEUT	40.49	10 <sup>9</sup> /L	*	13.6 % *
LYMPH	162.89	10º/L	*	54.7 % *
MONO	91.77	10º/L	*	30.8 % *
EO	0.13	10º/L	*	0.0 % *
BASO	2.57	10º/L	+	0.9 %
IG	2.55	10º/L	*	0.9 % *
RET	26.5	10 <sup>9</sup> /L		0.84 %
IRF	20.8	%		
LFR	79.2	%		
MFR	17.2	%		
HFR	3.6	%		
RET-He	32.3	pg		
IPF	0.5	10 <sup>9</sup> /L	*	7.6 % *

#### Flags

WBC Flag(s) WBC Abn Scattergram Neutrophilia Lymphocytosis Monocytosis Basophilia Leukocytosis IG Present Blasts? Left Shift? Abn Lympho?

#### RBC Flag(s)

Anisocytosis Macrocytosis RET Abn Scattergram

**PLT Flag(s)** Thrombocytopenia PLT Clumps?

![](_page_48_Figure_10.jpeg)

#### Point

The white blood cell series scattergrams are similar to those of the white blood cell fragments (1) case described before. Thus, the separation of WBC and BASO is unclear in the WNR scattergram, and cluster separation is poor in the WDF scattergram. The "WBC Abn Scattergram" flag is displayed. The visual differential count shows 94% blastoid cells, and some of them are plotted in the abnormal cell detection areas (shown in red) of the WPC and WPC (SSC-FSC) scattergrams. The smear samples show fragments of blastoid cells, with a size varying in the range of platelet size to red blood cell size. The PLT-F scattergram has an abnormal cluster in an area close to WBC. The white blood cell fragments of red blood cell size are believed to be plotted in this area ( $\uparrow$ ). But there is no false counting of smaller fragments as platelets (the blue cluster at top right represents white blood cells). The PLT-I, the platelet count determined by the impedance method, of 25×10<sup>9</sup>/L, suggests the possible effect of the white blood cell fragments.

While the patient was followed up for B-ALL, the peripheral blood showed an increase in blastoid cells.

#### Blood smear (May-Giemsa staining)

PB

" PB

![](_page_49_Picture_6.jpeg)

#### ×400

#### Visual differential counts

PB		
Myeloblast	0.0	Other tests
Promyelo	0.0	CD61 <sup>+</sup> 15×10 <sup>9</sup> /L
Myelo	0.0	
Meta	0.2	
Stab	1.0	
Seg	2.5	
Lympho	2.0	
Mono	0.0	
Baso	0.0	
Eosino	0.0	
At-Ly	0.0	
Other*	94.0	
NRBC 1	/100WBC	
*Blas	stoid cells	

unit:%

#### Point

The peripheral blood showed a marked increase in the white blood cell count, anemia, and a reduced platelet count. Blastoid cell fraction is 94%. The blastoid cells are of medium size and have a high N/C ratio. The nuclear chromatin has a delicate net-like appearance with a few nucleoli. There are a number of collapsed cells. White blood cell fragments with the size of platelet (↑) destructed blasts and red blood cell (↑) are seen.

White blood cell fragments are generally seen when there is a marked increase in leukemic blasts. This sometimes causes falsely high platelet counts when they are counted by the impedance method.

#### Information from XN-Series

WBC	10.69	10º/L		
RBC	3.42	$10^{12}/L$		
HGB	140	g/L		
НСТ	0.426	L/L		
MCV	124.6	fL	+	
MCH	40.9	pg	+	
MCHC	329	g/L		
PLT	185	10 <sup>9</sup> /L		
RDW-SD	70.5	fL	+	
RDW-CV	15.4	%		
PDW	13.6	fL		
MPV	11.5	fL		
P-LCR	36.8	%		
РСТ	0.0021	L/L		
NRBC	1.12	10 <sup>9</sup> /L		10.5 /100WBC

NEUT	6.88	10 <sup>9</sup> /L	*	64.4	%	*
LYMPH	3.06	10 <sup>9</sup> /L	*	28.6	%	*
MONO	0.63	10 <sup>9</sup> /L	*	5.9	%	*
EO	0.08	10 <sup>9</sup> /L		0.7	%	
BASO	0.04	10º/L		0.4	%	
IG	0.16	10º/L	*	1.5	%	*
DET	2524	109/1		7 2 0		
REI	252.4	10°/L		1.38	%	
IRF	45.7	10 <sup>5</sup> /L		/.38	%	
IRF LFR	45.7 54.3	10 <sup>5</sup> 7L %		/.38	%	
IRF LFR MFR	252.4 45.7 54.3 19.6	10 <sup>3</sup> 7L % % %		/.38	%	
IRF LFR MFR HFR	45.7 54.3 19.6 26.1	10 <sup>3</sup> /L % % %		/.38	%	
RET IRF LFR MFR HFR RET-He	252.4 45.7 54.3 19.6 26.1 36.6	10"/L % % % % pg		/.38	%	
REI IRF LFR MFR HFR RET-He IPF	252.4 45.7 54.3 19.6 26.1 36.6 4.8	10°7L % % % % pg 10°7/L		3.1	%	

#### Flags

WBC Flag(s) WBC Abn Scattergram

NRBC Present IG Present Blasts?

#### RBC Flag(s)

Anisocytosis Macrocytosis RET Abn Scattergram Reticulocytosis

#### PLT Flag(s)

![](_page_50_Figure_10.jpeg)

#### Point

The WNR scattergram shows the presence of erythroblasts ( $\bigcirc$ ), which agrees with the results of the visual differential counting. The Howell-Jolly bodies seen in the smears have not affected the NRBC measurements. The WDF scattergram shows a shifting of all the clusters towards the weaker fluorescence intensity direction, and has poor cluster separation. The WPC scattergram has clusters representing monocytes ( $\uparrow$ ) and granulocytes ( $\uparrow$ ) in the areas of weaker fluorescence intensity than in adults. This suggests that the white blood cells of newborns differ from those of adults in their reaction to the reagents. The reticulocyte fraction is as high as at 7.38%, which is a characteristic of newborns. These characteristics can be seen on the RET scattergram as well (pink to red dots,  $\bigcirc$ ).

This was case of a baby, tested on the day of birth

#### Blood smear (May-Giemsa staining)

PΒ

![](_page_51_Picture_5.jpeg)

Visual differential counts

PB

![](_page_51_Picture_7.jpeg)

#### Point

Red blood cells show anisocytosis. There are many polychromatic red blood cells, and also Howell-Jolly bodies (1). There are some erythroblasts (11/100 WBC). It is normal for the blood of newborns to have erythroblasts. Full term babies have 2/100 WBC on average while premature babies tend to have higher numbers.

#### PR

I D		
Myelob	last 0.0	)
Promye	lo 0.0	)
Myelo	0.0	)
Meta	0.0	)
Stab	0.5	5
Seg	58.0	)
Lympho	) 32.0	)
Mono	9.0	)
Baso	0.5	5
Eosino	0.0	)
At-Ly	0.0	)
NRBC	11/100WBC	-

unit:%

#### Information from XN-Series

WBC	15.88	10º/L	+	
RBC	3.26	$10^{12}/L$		
HGB	101	g/L		
HCT	0.309	L/L		
MCV	94.8	fL		
MCH	31.0	pg		
MCHC	327	g/L		
PLT	16	$10^{9}/l$	_	
	70	10 / 2		
RDW-SD	55.6	fL	+	
RDW-SD RDW-CV	55.6 17.0	fL %	++	
RDW-SD RDW-CV PDW	55.6 17.0	fL % fL	+ +	
RDW-SD RDW-CV PDW MPV	55.6 17.0 	fL % fL fL	++	
RDW-SD RDW-CV PDW MPV P-LCR	55.6 17.0 	fL % fL fL %	++	
RDW-SD RDW-CV PDW MPV P-LCR PCT	55.6 17.0 	fL % fL fL % L/L	++	

NEUT	8.06 10 <sup>9</sup>	/L *	50.8	%	*
LYMPH	2.35 10 <sup>9</sup>	/L *	14.8	%	*
MONO	5.40 10º	/L *	34.0	%	*
EO	0.00 10 <sup>9</sup>	/L	0.0	%	
BASO	0.07 10 <sup>9</sup>	/L	0.4	%	
IG	1.19 10 <sup>9</sup>	/L *	7.5	%	*
RET	20.2 10 <sup>9</sup>	/L	0.62	%	
IRF	25.2 %				
LFR	74.8 %				
MFR	21.2 %				
HFR	4.0 %				
RET-He	25.6 pg				
IPF	7.2 10 <sup>9</sup>	/L	40.2	%	

#### Flags

WBC Flag(s)

Monocytosis NRBC Present IG Present Blasts?

RBC Flag(s)

PLT Flag(s) PLT Abn Distribution Thrombocytopenia

![](_page_52_Figure_10.jpeg)

#### Point

The WNR scattergram confirms the presence of erythroblasts ( $\uparrow$ ). The XN analysis value of NRBC is slightly lower than the visual differential count. The smears show the presence of basophilic to orthochromatic erythroblasts, and a part of these erythroblasts are plotted in the WBC area in the WNR scattergram, which makes the XN data on erythroblasts falsely low. The more immature the erythroblasts, the larger they are, and thus the NRBC are plotted in the area of higher forward-scattered light intensity ( $\uparrow$ ). The WDF scattergram shows a cell population, believed to be erythroblasts, below the LYMPH cluster ( $\bigcirc$ ). Although there is interference with the LYMPH cluster, the lymphocyte count displayed by the XN analyzer is the value corrected by subtracting the NRBC count.

The patient experienced common cold for a several days. Blood tests showed anemia and thrombocytopenia.

#### Blood smear (May-Giemsa staining)

![](_page_53_Figure_4.jpeg)

#### Point

The peripheral blood shows leucocytosis with blastoid cells(0.5%), anemia and thrombocytopenia. Erythroblasts are also present (28/100 WBC). The erythroblasts have dysplastic morphology: irregular nuclei, basophilic immature cytoplasm and megaloblastoid changes. Giant platelets are occasionally seen. Some of neutrophils are giant neutrophils with few granules and hypersegmented nuclei. The bone marrow is normocellular with a low M/E ratio of 0.4, suggesting an increase in erythroblast lineage cells. The count of blast cells is not increased(1.5%). Megaloblastoid changes are seen in many of the erythroblasts. The neutrophils show hypogranulation. Serum level of vitamin B12 is elevated and that of folic acid is in the normal range.

Based on these findings, the patient is diagnosed with myelodysplastic syndrome (MDS - refractory cytopenia with multilineage dysplasia).

#### Visual differential counts

PB			BM			
Myeloblast	0.0	Other tests	NCC(×10 <sup>4</sup> /µL)	N.T.	Pro Erb	0.0
Promyelo	0.0	LD(U/L)	Megakaryo(/µL	) N.T.	Baso Erb	1.1
Myelo	1.0	1211(110-219)	Myeloblast	1.5	Poly Erb	55.8
Meta	0.0	Vitamin B12(pg/mL)	Promyelo	1.6	Orth Erb	3.6
Stab	1.5	6161(233-914)	Myelo	5.4	M:E ratio	0.4
Seg	57.5	Folic acid(ng/mL)	Meta	2.1		
Lympho	11.0	11.8(3.6-12.9)	Stab	1.3	Chromoso	me analysis
Mono	27.0		Seg	10.7	G-band	46,XY, [8]
Baso	0.5		Eosino	0.3		
Eosino	1.0		Baso	0.2		
EUSITIU	1.0		Lympho	9.9		
At-Ly	0.0		Mono	6.3		
Other*	0.5		Plasma	0.2		
NRBC 28/10	DOWBC		Macrophage	0.0		
*Blastc	id cells		Megakaryo.	0.0		

unit:%

#### Information from XN-Series

WBC	5.44	10 <sup>9</sup> /L		
RBC	2.28	$10^{12}/L$	*	
HGB	66	g/L	-	
HCT	0.24	L/L	*	
MCV	105.3	fL	*	
MCH	28.9	pg	*	
MCHC	275	g/L	*	
PLT	323	10º/L		
RDW-SD		fL		
RDW-CV		%		
	107	-		
FDVV	10.7	fL		
MPV	10.7	fL fL		
MPV P-LCR	10.7 10.2 25.5	fL fL %		
MPV P-LCR PCT	10.7 10.2 25.5 0.0033	fL fL % L/L		

NEUT	3.14	10º/L		57.8	%	
LYMPH	1.54	10º/L		28.3	%	
MONO	0.18	10º/L		3.3	%	
EO	0.53	10º/L	+	9.7	%	+
BASO	0.05	10 <sup>9</sup> /L		0.9	%	
IG	0.02	10 <sup>9</sup> /L		0.4	%	
RET	219.6	10 <sup>9</sup> /L	*	9.63	%	
IRF	32.4	%	*			
LFR	67.6	%	*			
MFR	17.5	%	*			
HFR	14.9	%	*			
RET-He	23.8	pg	*			
IPF	6.8	10 <sup>9</sup> /L		2.2	%	

#### Flags

WBC Flag(s)

**RBC Flag(s)** RBC Abn Distribution Dimorphic Population Hypochromia Anemia Reticulocytosis

#### PLT Flag(s)

![](_page_54_Figure_8.jpeg)

#### Point

The reticulocyte fraction is as high as at 9.63%. The white blood cell series and PLT-F scattergrams show no abnormalities, but an increase in reticulocytes is confirmed by the RET scattergram (pink to red dots,  $\bigcirc$ ). Mature red blood cells and reticulocyte clusters show a slight downward shift ( $\uparrow$ ), suggesting the presence of hypochromic red blood cells. The RET-He of 23.8 pg (1.48 fmol) is also slightly low. The RBC histogram is bimodal. The "Dimorphic Population" flag is displayed. Recovery of hematopoiesis brought about by iron replacement therapy is believed to be causing the anisocytosis.

This was a case of iron deficiency anemia. The patient showed an increase in the reticulocyte count while on iron replacement therapy

#### Blood smear (May-Giemsa staining)

![](_page_55_Picture_4.jpeg)

#### Visual differential counts

#### PB

Myelobla	ist 0.0
Promyelo	0.0
Myelo	0.0
Meta	0.0
Stab	0.0
Seg	54.0
Lympho	34.0
Mono	5.0
Baso	1.0
Eosino	6.0
At-Ly	0.0
NRBC	1/100WBC

unit:%

![](_page_55_Picture_9.jpeg)

#### Point

Peripheral blood test shows anemia. Red blood cells have severe anisocytosis and the red blood cell density is low. Blue-tinged polychromatic red blood cells are prominent. Besides this, tear-drop cells, elliptocytes and target cells are also seen.

The increase in polychromatic red blood cells is believed to have occurred as the recovery of the erythropoiesis with the iron replacement therapy.

#### Information from XN-Series

WBC	27.36	10º/L	+	
RBC	2.84	$10^{12}/L$		
HGB	85	g/L		
HCT	0.304	L/L		
MCV	107.0	fL		
МСН	29.9	pg		
MCHC	280	g/L	-	
PLT	109	10 <sup>9</sup> /L		
RDW-SD	67.0	fL	+	
RDW-CV	23.8	%	+	
PDW		fL		
MPV		fL		
P-LCR		%		
PCT		L/L		
NRBC	1.22	10 <sup>9</sup> /L		4.5 /100WBC

NEUT	26.57	10º/L	+	97.1 9	% +
LYMPH	0.25	10º/L	_	0.9	% –
MONO	0.51	10º/L		1.9	%
EO	0.00	10º/L		0.0	%
BASO	0.03	10º/L		0.1	%
IG	0.42	10º/L		1.5 9	%
RET	184.9	10 <sup>9</sup> /L	*	6.51	% *
IRF	25.1	%	*		
LFR	74.9	%	*		
MFR	13.3	%	*		
HFR	11.8	%	*		
RET-He	32.7	pg	*		
IPF	15.4	10 <sup>9</sup> /L		15.4	%
FRC	77.5	10 <sup>9</sup> /L		2.73	%

#### Flags

WBC Flag(s) Neutrophilia Lymphopenia Leukocytosis NRBC Present IG Present

#### **RBC Flag(s)**

Anisocytosis Hypochromia Anemia RET Abn Scattergram Reticulocytosis Fragments?

**PLT Flag(s)** PLT Abn Distribution

![](_page_56_Figure_9.jpeg)

#### Point

The WNR scattergram clearly shows the presence of erythroblasts ( $\bigcirc$ ), which agrees with the visual differential count. The WDF scattergram has a cell population ( $\bigcirc$ ), which is believed to be of erythroblasts, below the LYMPH cluster. The mature red blood cell cluster in the RET scattergram extends to the area of weaker forward-scattered light intensity ( $\bigcirc$ ), which suggests the presence of small red blood cells, but these are believed to be only the red blood cell fragments seen in the smears. The "Fragments?" flag is displayed. The XN research parameter FRC%, which corresponds to the particle count in the area where red blood cell fragments are plotted, is 2.73%. This is consistent with the visual differential count data.

This was a case wherein red blood cell fragments were detected in the peripheral blood smear samples.

#### Blood smear (May-Giemsa staining)

![](_page_57_Picture_4.jpeg)

РВ

#### Point

The peripheral blood shows leucocytosis, anemia and mild thrombocytopenia. The neutrophil fraction is increased and erythroblasts are recognized. Red blood cell morphology shows anisocytosis, and poikilocytosis including a large number of red blood cell fragments like crescent-shaped cells and helmet cells. Besides these, target-shaped red blood cells and Howell-Jolly bodies are also seen.

Microangiopathic hemolytic anemia (MHA), disseminated intravascular coagulation (DIC) syndrome, thrombotic thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS) should be considered when red blood cell fragments are detected in the peripheral blood. This case is diagnosed as DIC because of thrombocytopenia and an elevated level fibrin degradation products (FDP).

РВ				
	• ;	07	3.	bo
0	21	0	6	6
DC	10	0	~	-
0	600	0	0	
		2.	-	
2	300	200	-	
				5
-0			-0	

#### ×600

#### Visual differential counts

PB	
Myelobla	ast 0.0
Promyel	o.0
Myelo	0.0
Meta	0.0
Stab	4.0
Seg	93.0
Lympho	0.0
Mono	3.0
Baso	0.0
Eosino	0.0
At-Ly	0.0
NRBC	5/100WBC
Red bloc	d cell fragments
3	0/1000RBC

Coagulation te	est
PT(sec)	15.6 (9.3-13.8)
PT%	59
INR	1.38
APTT(sec)	33 (25-36)
D-Dimer(µg/mL)	49.9 (<1.0)
total FDP(µg/mL)	52.3 (<5.0)

unit:%

Μ R Η Η Ν Ν Ν Ρ Ρ R R Ρ Ν P Ρ Ν

		He	moly	/sis			Res	ampl	ing of	fblood	
WBC RBC HGB HCT MCV MCH MCHC PLT&F PLT-I	13.84 1 4.54 1 141 g 0.417 1 31.1 g 338 g 239 1 375 1	10 <sup>9</sup> /L 10 <sup>12</sup> /L g/L L/L fL pg g/L 10 <sup>9</sup> /L		WNR	SFL	WBC RBC HGB HCT MCV MCH MCHC PLT&F PLT-I	13.31 10 4.69 10 145 g/l 0.436 L/l 93.0 fL 30.9 pg 333 g/l 236 10 250 10	<sup>9</sup> /L <sup>12</sup> /L - - <sup>9</sup> /L		WNR	
RDW-SD RDW-CV PDW MPV P-LCR PCT (	45.5 f 13.5 d 16.5 f 11.7 f 37.5 d	fL % fL fL % L/L	+		ssc	RDW-SD RDW-CV PDW MPV P-LCR PCT	45.6 fL 13.3 % 11.3 fL 10.2 fL 26.5 % 0.0026 L/I	_			station of the state of the sta
NRBC# NRBC% NEUT% LYMPH% MONO% EO% BASO%	0.00 2 0.0 / 84.1 0 7.7 0 6.6 0 1.3 0 0.3 0	10 <sup>9</sup> /L /100WBC % % % %	+	RET		NRBC# NRBC% NEUT% LYMPH% MONO% EO% BASO%	0.00 10 0.0 /10 84.4 % 8.3 % 6.5 % 0.3 % 0.5 %	97L DWBC	 + -	RET	
IG# IG%	0.17 1 1.2 c	10 <sup>9</sup> /L %		PLT-F	SFL	IG# IG%	0.20 10 1.5 %	9/L	_	PLT-F	2
RET# RET% IRF LFR MFR HFR RET-He	53.1 2 1.17 4 96.6 4 3.4 4 0.0 4 24.6 j	10 <sup>9</sup> /L % % % % pg		RBC	SFL	RET# RET% IRF LFR MFR HFR RET-He	38.5 10 0.82 % 2.6 % 97.4 % 2.5 % 0.1 % 32.7 pg	<sup>9</sup> /L		RBC	s
FRC%	2.29	%	_			FRC%	0.00 %		_		
					1250ñL						

#### Point

In the initial test (sample hemolyzed), the RET scattergram shows a large number of dots in the weak forward-scattered light intensity area (O), whereas the PLT-F scattergram shows a large number of particles plotted in the Debris area (O). These are believed to be red blood cell ghosts characteristically seen in hemolyzed samples. The PLT histogram is also affected (O). In the retest (resampled blood), the RET and PLT-F scattergrams are free of the abnormal particles seen in the initial test, and the histograms are also normal.

When the platelet count is measured by the impedance method, red blood cell ghosts are sometimes counted as platelets, giving rise to false high platelet counts. There is difference between the platelet count (375×10<sup>9</sup>/L) measured by the impedance method (PLT-I) and the PLT-F in the initial XN analysis. PLT-F has about the same value as obtained by the immunological method that uses CD61 (244×10<sup>9</sup>/L). Thus, the platelet fraction is accurately measured without being affected by the red blood cell ghosts. The platelet count by PLT-I of 250×10<sup>9</sup>/L for the resampled specimen is not very different.

This was a case with severely hemolyzed sample.

#### Blood smear (May-Giemsa staining)

PΒ

![](_page_59_Picture_5.jpeg)

![](_page_59_Picture_6.jpeg)

#### Point

Some of the red blood cells have a weakly stained cytoplasm and irregular shapes. Red blood cell fragments of platelet size are recognized (<sup>↑</sup>).

×400

In persons with narrow blood vessels, which make blood sampling difficult, the red blood cell receives greater stress than normal when blood are taken, and this sometimes leads to their hemolysis. It is necessary to note that hemolytic samples often show false high platelet counts because of red blood cell ghosts and red blood cell fragments.

#### Visual differential counts

PB					
Myeloblast	0.0	Other tests			
Promyelo	0.0	Hemolys	sis	Resampling of b	lood
Myelo	0.0	CD61+(×10 <sup>9</sup> /L)	244	CD61+(×10 <sup>9</sup> /L)	257
Meta	0.0	FRC(%)	2.29	FRC(%)	0.00
Stab	0.0	K+(mEq/L)	8.4	к+(mEq/L)	4.3
Seg	54.0	Hemolytic inde	x* 21	Hemolytic index	* 0
Lympho	34.0			*Hitachi 770	0
Mono	5.0				
Baso	1.0				
Eosino	6.0				
At-Ly	0.0				
NRBC 1/1	00WBC				

unit:%

# Hemagglutination

WBC	6.13	10 <sup>9</sup> /L	
RBC	1.09	$10^{12}/L$	*
HGB	92	g/L	*
НСТ	0.109	L/L	*
MCV	100.0	fL	*
MCH	84.4	pg	*
MCHC	844	g/L	*
PLT	235	10º/L	
RDW-SD	56.0	fL	*
RDW-CV	25.4	%	*
PDW	14.2	fL	
MPV	12.1	fL	
P-LCR	39.0	%	
PCT (	0.0028	L/L	
NRBC#	0.00	10 <sup>9</sup> /L	
NRBC%	0.0	/100WBC	
NEUT%	73.1	%	+
LYMPH%	21.0	%	
MONO%	4.1	%	
EO%	1.0	%	
BASO%	0.8	%	
IG#	0.05	10º/L	
IG%	0.8	%	
RET#	33.7	10 <sup>9</sup> /L	*
RET%	3.09	%	*
IRF	25.5	%	*
LFR	74.5	%	*
MFR	17.2	%	*
HFR	8.3	%	*
RET-He	26.9	pg	*
RBC-O	3.45	10 <sup>12</sup> /L	*

Before warming

![](_page_60_Picture_4.jpeg)

WBC	6.89	10 <sup>9</sup> /L	
RBC	2.60	$10^{12}/L$	>
HGB	93	g/L	2
HCT	0.249	L/L	2
MCV	95.8	fL	2
MCH	35.8	pg	>
MCHC	373	g/L	>
PLT	292	10 <sup>9</sup> /L	
RDW-SD	53.1	fL	2
RDW-CV	23.1	%	2
PDW	16.9	fL	
MPV	12.6	fL	
P-LCR	42.4	%	
PCT (	0.0037	L/L	-
NRBC#	0.00	10 <sup>9</sup> /L	
NRBC%	0.0	/100WBC	
NEUT%	73.7	%	_
LYMPH%	20.5	%	
MONO%	3.6	%	
EO%	1.2	%	
BASO%	1.0	%	
IG#	0.06	10º/L	
IG%	0.9	%	
RET#	77.2	10 <sup>9</sup> /L	>
RET%	2.97	%	>
IRF	24.8	%	>
LFR	75.2	%	2
MFR	16.6	%	2
HFR	8.2	%	>
RET-He	27.4	pg	>
RBC-O	3.40	10 <sup>12</sup> /L	
Flags			

**RBC Flag(s)** 

Anisocytosis

Fragments?

RET Abn Scattergram

Turbidity/HGB Interf?

Anemia

#### After warming

![](_page_60_Picture_7.jpeg)

![](_page_60_Picture_8.jpeg)

![](_page_60_Figure_10.jpeg)

#### Flags

#### RBC Flag(s)

Anisocytosis Anemia RET Abn Scattergram RBC Agglutination? Turbidity/HGB Interf? Fragments?

![](_page_60_Picture_14.jpeg)

#### Point

The WNR and WDF scattergrams of the peripheral blood before and after warming do not show any abnormality. But the RBC and HCT are abnormally low and MCH and MCHC are abnormally high before warming, which is characteristic of hemagglutination. The RBC histogram shows a small bump ( $\uparrow$ ) at 150-200 fL, suggesting the presence of aggregated masses of red blood cells. After warming, the red blood cell count increases, and MCH and MCHC comes down to near-normal levels. However, there is a possibility that the influence on the measurement of red blood cells remains, because the above-mentioned small bump is still recognized after warming in the RBC histogram. The XN analyzer measures the red blood cell count in the RET channel as well, the reaction chamber of which is warmed. In this case, the RBC-O of the unwarmed sample measured in the RET channel is  $3.45 \times 10^{12}$ /L, and of the warmed sample is  $3.40 \times 10^{12}$ /L, which are about the same effect in some cases as making the measurement after warming the sample in a water bath, etc., generating near a correct value as needed.

This was a case with autoimmune hemolytic anemia.

#### Blood smear (May-Giemsa staining)

![](_page_61_Figure_4.jpeg)

#### Point

Red blood cells of the peripheral blood are forming aggregated masses by piling up in an irregular manner.

The peripheral blood smear after warming to 37°C shows dissociation of red blood cell aggregates, suggesting a diagnosis of cold agglutinin-mediated autoimmune hemolytic anemia.

#### Visual differential counts

PB		Other tests
Myelobla	ist 0.0	LD(U/L) 313
Promyelo	0.0	T-Bil(mg/dL) 1.4
Myelo	0.0	D-Bil(mg/dL) 0.4
Meta	0.0	Haptoglobin(mg/dL) <10
Stab	0.0	Cold agglutination
Seg	73.0	65536 times(+)
Lympho	21.0	
Mono	5.0	
Baso	0.0	
Eosino	1.0	
At-Ly	0.0	
NRBC	0/100WBC	

unit:%

#### Information from XN-Series

WBC	5.29	10 <sup>9</sup> /L		
RBC	4.49	$10^{12}/L$		
HGB	135	g/L		
HCT	0.457	L/L		
MCV	101.8	fL		
MCH	30.1	pg		
MCHC	295	g/L	-	
PLT&F	39	10 <sup>9</sup> /L	-	
DI TI	2.4	100/1		
PLI-I	34	10º/L	*	
RDW-SD	55.0	fL	*	
RDW-SD RDW-CV	34 55.0 14.6	fL %	+	
RDW-SD RDW-CV PDW	55.0 14.6	fL % fL	+	
PLI-I RDW-SD RDW-CV PDW MPV	34 55.0 14.6 	fL % fL fL fL	+	
PLI-I RDW-SD RDW-CV PDW MPV P-LCR	34 55.0 14.6 	fL % fL fL %	+	
PLI-I RDW-SD RDW-CV PDW MPV P-LCR PCT	34 55.0 14.6 	fL % fL fL fL % L/L	+	

NEUT	3.28 10 <sup>9</sup> /L	62.0 %
LYMPH	1.57 10 <sup>9</sup> /L	29.7 %
MONO	0.26 10 <sup>9</sup> /L	4.9 %
EO	0.15 10 <sup>9</sup> /L	2.8 %
BASO	0.03 10 <sup>9</sup> /L	0.6 %
IG	0.01 10 <sup>9</sup> /L	0.2 %
RET	38.6 10 <sup>9</sup> /L	0.86 %
IRF	10.0 %	
LFR	90.0 %	
MFR	8.5 %	
HFR	1.5 %	
RET-He	28.3 pg	
IPF	17.6 10 <sup>9</sup> /L	45.2 %

#### Flags

WBC Flag(s)

RBC Flag(s)

#### PLT Flag(s)

PLT Abn Distribution Thrombocytopenia

![](_page_62_Figure_10.jpeg)

#### Point

This is a case with a reduced platelet count because of an *MYH9* gene abnormality. The PLT-F scattergram shows dots up to the area of large cell size and high fluorescence intensity ( $\bigcirc$ ). The PLT histogram shows an abnormal pattern, and the "PLT Abn Distribution" flag is displayed. The platelet count determined by the impedance method (PLT-I) is 34×10<sup>9</sup>/L, which is slightly lower than the 46×10<sup>9</sup>/L count, determined by the immunological method that uses CD61. It appears that some of the giant platelets seen in the smears are not counted as platelets in PLT-I.

The patient was diagnosed with thrombocytopenia and was referred to our hospital.

#### Blood smear (May-Giemsa staining)

PΒ

![](_page_63_Picture_5.jpeg)

Visual differential counts

PΒ

![](_page_63_Picture_7.jpeg)

#### ●Point

Peripheral blood shows a low platelet count. The platelet size is basically large and many giant platelets are seen. Although some Döhle-like inclusions (1) are seen in neutrophils, they are small in size and have indistinct boundaries with the cytoplasm, which are a different morphology than usually seen in the May-Hegglin anomaly. Another type of MYH9 gene abnormality such as Fechtner syndrome is suspected based on macrothrombocytopenia and poorly defined inclusions in the white blood cells.

РВ		
Myelobla	ast 0.0	Other tests
Promyel	0.0 c	CD61 <sup>+</sup> 46×10 <sup>9</sup> /L
Myelo	0.0	
Meta	0.0	
Stab	0.0	
Seg	61.0	
Lympho	26.0	
Mono	9.0	
Baso	1.0	
Eosino	3.0	
At-Ly	0.0	
NRBC	0/100WBC	

unit:%

#### Information from XN-Series

WRC				
VVDC	4.08	10 <sup>9</sup> /L		
RBC	2.87	$10^{12}/L$		
HGB	86	g/L		
HCT	0.272	L/L		
MCV	94.8	fL		
MCH	30.0	pg		
MCHC	316	g/L		
PLT&F	23	10 <sup>9</sup> /L	-	
PLT-I	16	10 <sup>9</sup> /L	*	
RDW-SD	48.7	fL		
RDW-SD RDW-CV	48.7 14.4	fL %		
RDW-SD RDW-CV PDW	48.7 14.4	fL % fL		
RDW-SD RDW-CV PDW MPV	48.7 14.4 	fL % fL fL		
RDW-SD RDW-CV PDW MPV P-LCR	48.7 14.4 	fL % fL fL %		
RDW-SD RDW-CV PDW MPV P-LCR PCT	48.7 14.4 	fL % fL fL % L/L		

1.54	10 <sup>9</sup> /L	*	37.7 % *
1.01	10 <sup>9</sup> /L	*	24.8 % *
1.19	10 <sup>9</sup> /L	*	29.2 % *
0.27	10 <sup>9</sup> /L		6.6 % +
0.07	10 <sup>9</sup> /L		1.7 % +
0.39	10º/L	*	9.6 % *
4.0	10 <sup>9</sup> /L		0.14 %
12.5	%		
87.5	%		
6.8	%		
5.7	%		
32.4	pg		
9.1	10 <sup>9</sup> /L		39.7 %
	1.54 1.01 1.19 0.27 0.07 0.39 4.0 12.5 87.5 6.8 5.7 32.4 9.1	1.54 10 <sup>9</sup> /L 1.01 10 <sup>9</sup> /L 1.19 10 <sup>9</sup> /L 0.27 10 <sup>9</sup> /L 0.07 10 <sup>9</sup> /L 0.39 10 <sup>9</sup> /L 12.5 % 87.5 % 6.8 % 5.7 % 32.4 pg 9.1 10 <sup>9</sup> /L	1.54 10 <sup>9</sup> /L * 1.01 10 <sup>9</sup> /L * 1.19 10 <sup>9</sup> /L * 0.27 10 <sup>9</sup> /L / 0.07 10 <sup>9</sup> /L / 0.39 10 <sup>9</sup> /L * 4.0 10 <sup>9</sup> /L / 12.5 % / 87.5 % / 6.8 % / 5.7 % / 32.4 pg // 9.1 10 <sup>9</sup> /L /

#### Flags

#### WBC Flag(s)

WBC Abn Scattergram Monocytosis IG Present Blasts?

**RBC Flag(s)** Anemia

PLT Flag(s)

PLT Abn Distribution Thrombocytopenia

![](_page_64_Figure_11.jpeg)

#### Point

This is a case of MDS-related thrombocytopenia. As in the case before, the PLT-F scattergram has dots extending to the large cell size and high fluorescence intensity area ( $\bigcirc$ ). This agrees with the finding of a large number of giant platelets in the smears. The PLT histogram has an abnormal pattern and the "PLT Abn Distribution" flag is displayed. The PLT-I, the platelet count determined by the impedance method, of 16×10<sup>9</sup>/L, is slightly low compared to PLT-F.

This was a case with MDS (RAEB-1). The peripheral blood had an increased number of blasts.

#### Blood smear (May-Giemsa staining)

![](_page_65_Figure_4.jpeg)

#### Point

The peripheral blood shows anemia and thrombocytopenia. The blast fraction is 57%. The blasts are of medium size with a slightly low N/C ratio. Their nuclei are round shaped with the fine nuclear chromatin, and a few nucleoli are seen. The platelets are large in size, and many of them are double the size of red blood cells. Erythroblasts and megakaryocytoid cell (1) are also seen. Neutrophils have pseudo-Pelger anomaly and fewer granules. The bone marrow is normocellular with

60.3% blasts. The blasts are medium to large in size, with a slightly low N/C ratio. Their nuclei are irregular in shape, the nuclear chromatin is fine to slightly coarse, and distinct nucleoli are seen. The cell margins are irregular and have projections. Erythroblasts show megaloblastoid changes, and neutrophils have pseudo-Pelger anomaly and reduced number of granules. Micromegakaryocytes are also present (**†**).

Based on these findings, AML with myelodysplasia-related changes is diagnosed.

#### Visual differential counts

459(100-219)

РВ		BM			
Myelobla	st 57.0	NCC(×10 <sup>4</sup> /µL)	9.7	Pro Erb	0.1
Promyelc	2.0	Megakaryo(/µĹ	) <15	Baso Erb	0.6
Myelo	0.0	Myeloblast	60.3	Poly Erb	6.0
Meta	1.0	Promyelo	3.3	Orth Erb	0.0
Stab	3.0	Myelo	4.4	M:E ratio	11.3
Seg	16.0	Meta	0.8		
Lympho	18.0	Stab	0.6	Chromosome	analysis
Mono	3.0	Seg	2.5	G-band	
Baso	0.0	Eosino	3.4	46,XY,-3,add	(5)
Eosino	0.0	Baso	0.5	(q11.2),-7,-2	0,
At-Ly	0.0	Lympho	12.1	+3mar [19] /	46,XY [1]
NRBC	2/100WBC	Mono	1.2		
Mgk	3/100WBC	Plasma	1.1		
		Macrophage	1.6		
Other te	sts	Megakaryo	1.5		
LD(U/L)					

#### Cell surface antigen expressions (BM)

T-Cell		Myeloid	
CD2	2.2	CD11b	5.0
CD4	15	CD13	72.0
CD5	1.3	CD14	9.3
	1.2	CD33	65.2
CD7	ŏ./	CD36	29.0
CD8	5.2	CD64	15.3
B-Cell		CD117	38.0
	33	MPO	43.0
CDIJ	ر.ر	Others	
NK-Cell		CD34	65.0
CD56	23.0	CD38	72.0
		CD41b	12.0
		CD61	17
		Gly-A	1.7
		HIA-DR	36.4
			50.4
		CD45	gating
			unit:%

#### Point

FCM: The granulocyte markers CD13, CD33, CD36, CD117, and MPO are positive in the bone marrow blasts. Also CD56 and NK cell marker are weakly expressed. Chromosome analysis reveals abnormalities, including -7, in 19/20 cells.

#### Information from XN-Series

WBC	5.10	10º/L		
RBC	4.68	$10^{12}/L$		
HGB	137	g/L		
HCT	0.417	L/L		
MCV	89.1	fL		
MCH	29.3	pg		
MCHC	329	g/L		
PLT&F	17	10 <sup>9</sup> /L	*	
PLT-I	14	10 <sup>9</sup> /L	_	
RDW-SD	39.9	fL		
RDW-CV	123	0/2		
	12.0	70		
PDW	18.4	fL	*	
PDW MPV	18.4 12.4	fL fL	*	
PDW MPV P-LCR	18.4 12.4 48.2	fL fL %	* * *	
PDW MPV P-LCR PCT	18.4 12.4 48.2 0.0002	fL fL % L/L	* * *	

NEUT	3.20 1	.0 <sup>9</sup> /L		62.7	%
LYMPH	1.41 1	.0 <sup>9</sup> /L		27.6	%
MONO	0.36 1	.0 <sup>9</sup> /L		7.1	%
EO	0.10 1	.0º/L		2.0	%
BASO	0.03 1	.0 <sup>9</sup> /L		0.6	%
IG	0.02 1	.0º/L		0.4	%
RET	88.5 1	.0º/L		1.89	%
IRF	7.3 %	6			
LFR	92.7 %	6			
MFR	7.0 %	6			
HFR	0.3 %	6			
RET-He	29.6 p	g			
IPF	2.6 1	.0 <sup>9</sup> /L	*	15.0	% *

#### Flags

WBC Flag(s)

RBC Flag(s)

**PLT Flag(s)** Thrombocytopenia PLT Clumps?

![](_page_66_Figure_9.jpeg)

#### Point

This is a case of EDTA-dependent pseudothrombocytopenia. The "PLT Clumps?" flag is displayed, suggesting platelet aggregation. The analysis results are normal except for a low platelet count. Both the platelet counts, i.e., PLT-I measured by the impedance method and PLT-F are low. The accurate platelet count remains unknown because there are aggregated masses in the smears as well. When it is clear that the pseudothrombocytopenia is EDTA-dependent, measurement after resampling such as by changing the anticoagulant to sodium citrate become necessary.

The patient was pointed out with thrombocytopenia in a regular health checkup and was referred to our hospital.

PB (tail end of the smear)

#### Blood smear (May-Giemsa staining)

![](_page_67_Picture_4.jpeg)

#### Visual differential counts

PB	
Myelobla	ast 0.0
Promyel	0.0 c
Myelo	0.0
Meta	0.0
Stab	0.0
Seg	58.0
Lympho	37.0
Mono	4.0
Baso	0.0
Eosino	1.0
At-Ly	0.0
NRBC	0/100WBC

unit:%

WDF (FSCW-FSC)
PLT-F (FSCW-FSC)

Normal

The present case
Normal
The present case

Image: transmission of tran

#### Point

In the XN analyzer, the PLT Clumps? detection is made on the basis of the WNR, WDF (FSCW-FSC) and PLT-F (FSCW-FSC) scattergrams. A broadening of FSCW (the forward-scattered light width) range is a characteristic feature seen when there is platelet aggregation (1). In this patient, this characteristic is easy to detect in the PLT-F (FSCW-FSC) scattergram. \* The PLT Clumps? detection area of WNR: "PLT Clumps" detection area of the WNR scattergram on p. 14

Point

×400

Small aggregates of platelets are seen in the parts of the smear that are normally observed. In addition, many large aggregates of platelets are seen at the feathered edge of the smear. EDTA-dependent pseudothrombocytopenia is suspected because there is no fibrin deposition, which is usually present in coagulated blood samples as a cause of reduced platelet counts.

#### Information from XN-Series

WBC	31.84	10 <sup>9</sup> /L	+	
RBC	1.72	$10^{12}/L$	—	
HGB	58	g/L	—	
НСТ	0.202	L/L	—	
MCV	117.4	fL	+	
MCH	33.7	pg		
MCHC	287	g/L	—	
PLT	87	10 <sup>9</sup> /L		
RDW-SD	86.9	fL	+	
RDW-CV	20.4	%	+	
PDW	11.9	fL		
MPV	10.5	fL		
P-LCR	29.0	%		
РСТ	0.0009	L/L	-	
NRBC	0.44	10 <sup>9</sup> /L		1.4 /100WBC

NEUT	30.52	10º/L	*	95.8 % *
LYMPH	0.83	10º/L	-	2.6 % -
MONO	0.39	10º/L		1.2 %
EO	0.02	10º/L	*	0.1 % *
BASO	0.08	10 <sup>9</sup> /L		0.3 %
IG	2.14	10º/L	*	6.7 % *
RET	34.7	10 <sup>9</sup> /L		2.02 %
IRF	17.9	%		
LFR	82.1	%		
MFR	9.3	%		
HFR	8.6	%		
RET-He	24.7	pg		
IPF	4.2	10 <sup>9</sup> /L		4.5 %

#### Flags

WBC Flag(s)
Neutrophilia
Leukocytosis
IG Present
Left Shift?

#### RBC Flag(s)

Anisocytosis Macrocytosis Hypochromia Anemia

#### PLT Flag(s)

![](_page_68_Figure_9.jpeg)

#### Point

The presence of erythroblasts can be confirmed by the WNR scattergram ( $\bigcirc$ ). The WDF scattergram has a cell population ( $\bigcirc$ ) which is believed to consist of erythroblasts below the LYMPH cluster. The white blood cell count is increased and the neutrophil fraction is more than 90%, which is characteristic for bacterial infection. The stab cells and neutrophils phagocytosing the bacteria seen on the smear are believed to be plotted in the detection area of "Left Shift?" flag, slightly higher than the NEUT cluster ( $\uparrow$ ).

The patient was brought in as an emergency case because of disturbed consciousness. Bacteria were detected in the peripheral blood smear.

#### Blood smear (May-Giemsa staining)

PΒ

![](_page_69_Picture_5.jpeg)

![](_page_69_Picture_6.jpeg)

#### Point

Peripheral blood shows leucocytosis, anemia and thrombocytopenia. Among the white blood cells, there is an increase in stab neutrophils and there are immature white blood cells as well. Neutrophils have toxic granules, Döhle bodies and vacuolar degeneration in the cytoplasm. Some neutrophils are seen in the process of phagocytosing the bacteria (<sup>↑</sup>). Gram-positive cocci are detected in blood cultures.

![](_page_69_Picture_9.jpeg)

×600

#### Visual differential counts

<b>PB</b> Myeloblast	
Mveloblast	
INIGETODIUSE	0.0
Promyelo	0.0
Myelo	4.5
Meta	1.0
Stab	29.0
Seg	64.0
Lympho	0.5
Mono	1.0
Baso	0.0
Eosino	0.0
At-Ly	0.0
NRBC 2/10	DOWBC
Other tests	
CRP(mg/dL)	23.44

unit:%

**XN-Series**