Automatic Analysis of Normal Bone Marrow Blood Cells Using the XE-2100 Automated Hematology Analyzer

Hisako Shibata,¹ Takahisa Yamane,^{1*} Ryousuke Yamamura,¹ Kensuke Ohta,¹ Takayuki Takubo,¹ Tomio Kamitani,² and Masayuki Hino¹

¹*Clinical Hematology and Clinical Diagnostics, Graduate School of Medicine, Osaka City University, Osaka, Japan*

²*Internal Medicine, Wakakoukai Hospital, Osaka, Japan*

The bone marrow aspiration test conventionally has been performed by visual methods, using a light microscope, because automatic blood cell analyzers cannot adequately capture erythroblasts and immature granulocytes (IGs) (Tatsumi et al.: *Osaka City Med J* 1988;34:135–146; Tatsumi et al.: *Am J Clin Pathol* 1986;86:50–54). With the development of the XE-2100 automatic blood cell analyzer (Sysmex Corporation, Kobe, Japan) in 1999, the classification of erythroblasts and IGs by means of flow cytometry (Zini et al.: *Infus Ther Transfus Med* 2001;28:277–279; Briggs et al.: *Sysmex J Int* 1999;9:113–119) became possible. In the present study we classified cells in 65 bone marrow aspiration specimens

by the microscopic method and with the XE2100, and compared the results. A good correlation was found in the nucleated red blood cell (NRBC), white blood cell (WBC), and total nucleated cell (TNC) counts; the myeloid/erythroid ratio (M/E ratio); neutrophils, lymphocytes, eosinophils, and IGs in the immature myeloid information (IMI) channel; and the total cell count. These items can all be analyzed in about 54 sec with the XE2100, which is faster than with the microscopic method. Therefore, analysis of bone marrow aspiration specimens with this apparatus appears to be very useful for clinical screening as well as laboratory testing. *J. Clin. Lab. Anal.* 17:12–17, 2003. © 2003 Wiley-Liss, Inc.

Key words: bone marrow; automated hematology analyzer; nucleated red blood cell; immature information channel

INTRODUCTION

For the differentiation of myeloid cells, manual methods involving the use of optical microscopes conventionally have been used. Since bone marrow contains various cells of different sizes and nuclear shapes, it has been difficult to identify each type of cell using conventional automated hematology analyzers (1,2). These were intended to identify neutrophils, lymphocytes, and monocytes in peripheral blood, and are not capable of identifying myeloid cells—especially erythroblasts and IGs. Analyzers that are capable of white blood cell (WBC) five-differential measurement, which is currently used for general purposes, still cannot clearly differentiate between erythroblasts and lymphocytes because of the close similarity in size and nuclear shape of these two types of cells. On the basis of previous reports (3,4) that the XE-2100 automated hematology analyzer (Sysmex Corporation, Kobe, Japan) is capable of identifying erythroblasts, the

present study was conducted to ascertain its feasibility for myeloid cell analysis.

MATERIALS AND METHODS

Subjects

Specimens were collected by bone marrow puncture at the Osaka City University Hospital Hematology Clinic and Wakakoukai Hospital between June and October 2001. From among them, 65 specimens were selected for the current XE-2100 analysis after optical-microscopically

*Correspondence to: Takahisa Yamane, Department of Clinical Hematology, Osaka City University Medical School, 1-4-3, Asahimachi, Abeno-ku, Osaka 545-8586, Japan. E-mail: yamane@msic.med.osaka-cu.ac.jp

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screening out those with aplasia in erythroblasts, granulocytes or lymphocytes and those with abnormal cells.

XE-2100

The XE-2100 differentiates five types of WBCs and determines nucleated red blood cells (NRBCs), reticulocytes, and platelets by flow cytometry, emitting a semiconductor laser beam, and detecting three optical signals: forward scatter, side scatter, and side fluorescence (Fig. 1). The WBCs and NRBCs are analyzed as described below.

WBC and basophil measurement

The acidic lysing reagent (Stromatolyser-FBII) lyses RBCs and selectively suppresses the release of granules from basophils so that the basophils are shown separately from other leukocytes in the scattergram. After flow cytometry, the WBC/basophil scattergram is obtained from the detected forward- and side-scatter signals. Analysis of this scattergram provides the WBC and basophil counts.

WBC four-differential measurement

A surfactant in Stromatolyser-4DL causes lysis or destruction of RBCs and platelets, and slightly damages WBC membranes. Through the damaged portions in the WBC membranes, a dye contained in another reagent, Stromatolyser-4DS (which is added at the same time), migrates into the cells, staining deoxyribonucleic and ribonucleic acids. After flow cytometry, the four-differential scattergram is obtained from the detected side scatter and fluorescence signals. Analysis of this scattergram provides four-differential measurement of lymphocytes, monocytes, eosinophils, and neutrophils (including basophils) (5,6).

Immature WBC measurement using the immature myeloid information (IMI) channel

Immature WBCs are detected in the IMI channel, which uses a specific reagent and a combination of radiofrequency (RF) and direct current (DC) detection methods. This specific reagent contains a polyoxyethylene-series non-ionic surfactant and sulfur-containing amino acid that fixes intracellular components and

Fig. 1. An illustration showing relevant data screens from the XE-2100. **A:** Differential channel (blue: neutrophil; green: monocyte; pink: lymphocyte; red: eosinophil; purple: ghost). **B:** Basophil channel (white: basophil; blue: WBC; purple: ghost). **C:** IMI channel (red: IG; purple: ghost). **D:** NRBC channel (pink: NRBC; blue: WBC; purple: ghost).

membranes of blood cells, and an anionic surfactant that damages cell membranes and causes shrinkage of RBC ghosts and platelets. Although all blood cells are damaged by the surfactant, the degree of damage is known to depend on the lipid content or components in each cell. This specific reagent destroys mature WBCs, which contain abundant lipids, while immature WBCs are left intact due to their low lipid content. When a WBC measurement is carried out with such specimens in the IMI channel (which uses the RF/DC detection method), the exposed nuclei of mature WBCs are distributed in the ghost region, while immature WBCs are distributed at the bottom of the scattergram. This occurs because the immaturity of the nuclei and the scarcity of intracellular components result in low densities and thus small RF pulses. As a result, immature WBCs are detected, being distinguishable from mature neutrophils, lymphocytes, and monocytes.

NRBC measurement

The Stromatolyser-NR lysing reagent lyses RBCs and exposes the nuclei of NRBCs. The Stromatolyser-NR staining solution, which is added at the same time, stains WBCs specifically. After flow cytometry, the NRBC scattergram is obtained from the detected forward scatter and side fluorescence signals. Analysis of this scattergram provides the NRBC count (7–11).

Since conventional automated hematology analyzers could not distinguish NRBCs from WBCs, it was necessary to identify NRBCs visually and adjust the results properly in order to obtain accurate WBC counts. Although NRBCs and WBCs are not distinguished in the XE-2100 WBC/basophil scattergram, subtracting the NRBC value obtained from the NRBC scattergram automatically provides a more precise WBC count.

Blood Cell Differentiation

Differentiation by the XE-2100

According to previous research (3,12,13), interference by fat particles often falsely increases the total cell count. Goossens et al. (14) analyzed bone marrow samples that had been washed with phosphate-buffered saline (PBS) in order to eliminate interference by microfibers and fat droplets. In the present study we prepared the cleaned specimens according to the method of Bentley et al. (15), as follows. Bone marrow fluid (1.0 mL) was collected by puncture from ilia into an EDTA-2K-added test tube, and four types of specimens were prepared for measurement: the original liquid

specimen, fivefold- and 10-fold-diluted specimens in PBS, and the cleaned specimen (which was cleaned to remove fat globules from the bone marrow blood). The cleaned specimens were prepared by diluting 100 μ L of bone marrow puncture specimen with 10 mL of sodium chloride solution buffered with boric acid, centrifuging it at 3,500 rpm for 15 min, removing the supernatant, and adding 1 mL of PBS to the remaining pellet. To verify that the differential count was not changed by the procedure, we prepared the smears from the original liquid specimen and the cleaned specimen, and counted them by optical microscopic observation. We thus ensured that the procedure was adequate for the removal of fat. The effects of dilution were corrected by applying the ratio of the hemoglobin value of the original liquid specimen against that of a diluted solution to the measurement results. These measurements were carried out within 5 hr of collecting the bone marrow specimens.

Manual differentiation

Smears were prepared immediately from the bone marrow puncture specimen and stained by May-Grünwald-Giemsa. Differentiation was carried out on 500 cells by optical microscopic observation. For counting of nucleated cells, the EDTA-2K-added bone marrow puncture specimens were diluted 50 times with Türk's solution, and Thoma-Zeiss counting chambers were used.

The following differentiable items were examined for comparison: eosinophils, basophils, neutrophils (stab and segmented cells), immature neutrophils (promyelocytes, myelocytes, and metamyelocytes), myeloblasts, monocytes, lymphocytes, erythroid cells, myeloid/erythroid ratio (M/E ratio), WBC count, and total nucleated cell (TNC) count.

Statistical Analysis

The XE-2100 results and manual results were examined and compared by regression analysis. The Bearman method was employed for correlation coefficient testing. Differences were considered significant at $P \leq 0.05$. Values are expressed as mean $\pm$ standard deviation.

RESULTS

The XE-2100 measurements had the best correlation with the manual measurements when the cleaned specimens were used, compared with the other specimens (the original liquid, fivefold dilution, and 10-fold dilution). In a comparison of the XE-2100 and manual measure-

ments, good correlations were found for NRBC, WBC, TNC, M/E ratio, neutrophils, lymphocytes, eosinophils, immature granulocytes (IGs; in the IMI channel), and total WBC count. However, no significant correlation was found for basophils, monocytes, blasts, or left-shifted clusters (metamyelocyte+stab) in the IMI channel.

Percent neutrophils in the differential channel had a better correlation with manual percent (myelocyte + metamyelocyte + stab + segmented) than with manual percent (stab + segmented). The percent total in the IMI channel had the best correlation with the manual percent (myeloblast + promyelocyte + myelocyte) (see Tables 1–4 for results).

DISCUSSION

The use of bone marrow puncture has become increasingly important clinically for diagnosing various blood diseases and monitoring the effects on bone marrow of chemotherapy in patients with malignant diseases. However, bone marrow puncture smears are usually evaluated by manual methods using optical microscopes, which requires considerable time for staining and differentiation processes. Since conventional automated hematology analyzers can not identify erythroblasts, it has been impossible to accurately differentiate myeloid cells (1,2). Automating the hematological analysis of bone marrow puncture specimens

TABLE 1. Comparison of bone marrow analysis parameters for the XE-2100 and the manual measurement

	XE-2100				
	Original	5-time dilution	10-time dilution	Cleaned	Manual
NRBC(%)	22.8±8.2	23.9±9.4	25.1±10.1	19.1±9.4	24.4±11.2
TNC(×10 ⁴ /μl)	9.7±8.1	13.6±25.3	11.1±9.5	9.3±9.3	9.8±8.8
M/E ratio	2.4±2.3	2.6±2.1	2.6±2.4	3.8±3.6	3.0±3.3
WBC(BASO)(×10 ⁴ /μl)	7.5±6.6	8.0±7.1	8.3±7.4	7.4±7.6	7.5±7.1
WBC(DIFF) (×10 ⁴ /μl)	6.9±6.9	7.0±7.4	7.2±7.8	7.7±8.2	
Neutrophils	40.0±13.3	46.7±15.3	46.7±15.5	52.6±11.0	30.7±9.3a 49.6±11.6b
Lymphocytes	14.9±8.7	16.5±9.0	15.3±11.0	15.6±10.4	14.3±8.4
Monocytes	8.5±1.9	8.3±2.7	7.8±3.0	7.9±3.1	3.4±2.4
Eosinophils	2.2±1.5	2.1±1.6	2.2±1.6	2.3±1.8	2.5±2.4
Basophils	10.7±5.5	4.8±2.0±	4.1±1.8	4.5±2.5	0.24±0.4

Each value indicates the mean ± standard deviation.

^astab + seg.

^bmyelocyte + metamyelocyte + stab + seg.

NRBC, nucleated red blood cell; TNC, total nucleated cell; M/E, myeloid/erythroid; WBC, white blood cell; BASO, BASO channel; DIFF, DIFF channel.

TABLE 2. Coefficients of correlation between the XE-2100 and the manual measurement at four types of specimens prepared for measurement for bone marrow analysis parameters

	Correlation coefficient			
	Original	5-time dilution	10-time dilution	Cleaned
NRBC	0.65	0.68	0.62	0.91
TNC	0.85	0.50	0.83	0.90
M/E ratio	0.34	0.69	0.79	0.94
WBC(BASO)	0.86	0.91	0.84	0.94
WBC(DIFF)	0.91	0.92	0.90	0.95
Neutrophils ^a vs. stab + seg ^b	0.26	0.14	0.12	0.43
Neutrophils ^a vs. myelo + meta + stab + seg ^b	0.38	0.41	0.39	0.75
Lymphocytes	0.45	0.41	0.34	0.87
Monocytes	0.13	0.06	0.08	0.13
Eosinophils	0.41	0.66	0.70	0.71
Basophils	0.13	0.00	0.00	0.30

^aXE-2100.

^bManual measurement. NRBC, nucleated red blood cell; TNC, total nucleated cell; M/E, myeloid/erythroid; WBC, white blood cell; BASO, BASO channel; DIFF, DIFF channel.

TABLE 3. Comparison of bone marrow analysis parameters for the XE-2100 and the manual measurement

XE-2100/manual measurement	XE-2100				Manual measurement
	Original	5-time dilution	10-time dilution	Cleaned	
DIFF IG/myelo + metamyelo + stab	9.8 ± 3.7	13.3 ± 5.4	13.9 ± 5.8	14.2 ± 5.6	14.3 ± 11.3
IMI blast/myeloblast + other	0.31 ± 0.51	0.13 ± 0.23	0.13 ± 0.21	0.99 ± 1.32	0.67 ± 0.53
IMI IG/promyelo + myelo	11.1 ± 7.9	16.2 ± 7.6	16.2 ± 8.6	17.4 ± 7.0	14.0 ± 6.3
IMI LS/metamyelo + stab	4.9 ± 7.0	2.1 ± 2.0	1.6 ± 1.8	2.5 ± 3.0	20.9 ± 7.4

DIFF, DIFF channel; IG, immature granulocyte; IMI, immature information channel; LS, left shift; myelo, myelocyte; meta, metamyelocyte; promyelo, promyelocyte

TABLE 4. Coefficients of correlation between the XE-2100 and the manual measurement at four types of specimens prepared for measurement for bone marrow analysis parameters

XE-2100/ manual measurement	Correlation coefficient			
	Original	5-time dilution	10-time dilution	Cleaned
DIFF IG/myelo + metamyelo + stab	0.72	0.81	0.79	0.85
IMI blast/myeloblast + other	0.02	0.00	0.08	0.24
IMI IG/promyelo + myelo	0.17	0.68	0.67	0.69
IMI LS/metamyelo + stab	0.07	0.12	0.10	0.16

DIFF, DIFF channel; IG, immature granulocyte; IMI, immature information channel; LS, left shift; myelo, myelocyte; meta, metamyelocyte; promyelo, promyelocyte

will markedly reduce the time required for myeloid cell differentiation. The Sysmex XE-2100 utilizes flow cytometry and is capable of determining erythroblasts and immature cells, as well as WBC five-differential measurement (3,4). In the current study we tested the feasibility of automatic differentiation by an automated hematology analyzer, using 65 bone marrow aspiration specimens.

Both manual measurements and automatic measurements with the XE-2100 were taken for original liquid specimens, fivefold- and 10-fold-diluted specimens, and cleaned specimens. The correlation with manual measurements was best with the cleaned specimens, probably because the cleaning process eliminated the effects of fat globules, which were otherwise counted as blood cells (13–17). Since fat globules do not contain many components, they are particularly detectable by the forward scatter. Therefore, the differential channel, which does not use forward scatter signals for detection, provides information that is not affected by fat globules, and exhibits a better correlation with manual results for total WBC count than the basophil channel.

It has been reported for peripheral blood that neutrophils can be classified into stab and segmented cells in the differential channel. However, for bone marrow liquid, the manual results better matched the sum of myelocytes, metamyelocytes, stab cells, and

segmented cell values than the sum of stab cell and segmented cell values alone. This appears to be the case because the neutrophil count in the differential channel is obtained by subtracting the sum of the basophils, monocytes, lymphocytes, and eosinophils from the total WBC count. Therefore, the neutrophil count in the differential channel obtained by measuring bone marrow liquid in the XE-2100 must include myelocytes and metamyelocytes as well as stab and segmented cells.

The values for basophils, monocytes, and blasts (in the IMI channel) exhibited no significant correlation with the manual measurements. The proportions of these cells are so low that it may be impossible to determine the significance of correlation between automatic and manual measurements (18,19).

Although it has been reported that the LS value in the IMI channel includes stab cells and metamyelocytes, no significant correlation was found with manual measurements. There are two possible reasons for this. First, manual differentiation of metamyelocytes markedly depends on subjective decisions made by operators, and therefore some metamyelocytes are counted as myelocytes. Second, some of the cells destroyed by changes caused by time or temperature could pass to the ghost region, which is positioned right above the left shift (LS) region in the IMI scattergram. Some reports have recommended that measurement should be carried

out within 4 hr of blood collection, because the IMI channel is prone to change over time (20). Probably for the same reason, the total value in the IMI channel best matched the myeloblast + neutrophil (promyelocyte + myelocyte) value, exhibiting a slightly better correlation than with the myeloblast + other + neutrophil (promyelocyte + myelocyte + metamyelocyte + stab) value, which is conventionally reported as the total in the IMI channel. The differential channel is more stable over time than the IMI channel, and the IG (myelocyte + metamyelocyte + stab) region in the differential scattergram was distinctly separate from the ghost region, indicating that the IG (myelocyte + metamyelocyte + stab) data in the differential channel were acceptable (21).

Previous studies of automated bone marrow analysis by a hematology analyzer, the Cell-Dyn 4000 (which provides a mechanism for removal of fat globules) showed that the total cell count does not falsely increase when original liquid specimens are used (17). However, the XE-2100 is superior to the Cell-Dyn 4000 from the standpoint of correlation (22).

The XE-2100 was found to be equivalent to manual measurement in bone marrow blood cell differentiation when cleaned specimens were used. The XE-2100 can complete the entire measurement process in about 54 sec, allowing remarkably quick differentiation of bone marrow puncture specimens compared with manual measurement. Automatic analysis with the XE-2100 may be a useful means of screening bone marrow blood.

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