Korean J Lab Med 2010;30:540-6
DOI 10.3343/kjlm.2010.30.6.540

Differential Blast Counts Obtained by Automated Blood Cell Analyzers

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Background: Automated blood cell analyzers often read leukemic blasts as normal cells. In this study, we evaluated the 5-part differential patterns of blasts using automated analyzers to determine if they can differentiate among blast types.

Methods: Blood samples containing 10% or more blasts were collected from patients with acute leukemia (N=175). The 5-part differential count was conducted using DxH 800 (Beckman Coulter, USA) and XE-2100 analyzers (Sysmex Co., Japan), and the results were compared with manual differential counts, which was used as a reference method.

Results: The DxH 800 reported the 5-part white blood cell differential count in 98.9% of the cases. The XE-2100 provided an invalid automated differential count in 72% of the cases. Both analyzers counted most lymphoblasts as lymphocytes and most myeloblasts as monocytes. In 11 cases, the DxH 800 reported a 5-part differential count without a blast flag.

Conclusions: Some automated analyzers are able to recognize and count blasts according to their characteristic cell types. Therefore, complete blood counts obtained automatically can provide valuable data for making provisional decisions regarding the lineage of leukemia cells before further investigation. *(Korean J Lab Med 2010;30:540-6)*

Key Words : *Blast, Blood cell analyzer, Leukocyte differential, Manual differential*

INTRODUCTION

Manual differential counting is considered as the gold standard for the accurate identification of cells in the peripheral blood [1]. However, this method is both labor- and time-intensive [1, 2]. Additionally, the low cell counts noted following chemotherapy frequently complicate attempts to obtain a sufficient number of cells to render a meaningful manual differential count [3]. Therefore, complete blood counts (CBCs) and white blood cell (WBC) differentials conducted using automated blood cell analyzers have replaced the traditional manual differential count method for the initial screening and detection of hematologic abnormalities in modern hospitals and clinics [4, 5]. The automated analyzers are capable of detecting the presence of abnormal cell populations and provide cautionary flags. With improved performance and flagging capabilities, CBCs are frequently employed as an effective screening tool to detect the presence of hematologic abnormalities such as leukemia [6-8].

When the preliminary diagnosis of leukemia is made with CBC and manual slide reviews, clinical hematologists generally require crude distinctions between AML and ALL. Although automated analyzers can detect the presence of blasts, they are unable to determine the lin-

Received : April 22, 2010 Manuscript No : KJLM10-069
Revision received : October 11, 2010
Accepted : October 13, 2010
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*This study was partially supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST) (2009-0087946).

ISSN 1598-6535

eage of leukemia [8]. Moreover, determination of the lineage of leukemia via immunophenotypic, molecular, or cytogenetic analyses is a time-consuming process.

If the automated differential results vary according to the type of blasts, the CBC can provide us with valuable data to make provisional decisions about the lineage of leukemia. In this study, we assessed the patterns of blasts counted using 2 automated analyzers-the DxH 800 (Beckman Coulter, Miami, FL, USA) and the XE-2100 (Sysmex Co., Kobe, Japan)-and compared the results with manual differential counts.

MATERIALS AND METHODS

1. Specimens

Venous whole blood specimens treated with K3-EDTA were evaluated in this study. Specimens were obtained from the routine workload of the clinical laboratory of the Seoul St. Mary's Hospital, College of Medicine, over a 6-month period (from July 2009 to February 2010). Leukemic promyelocytes and promonocytes were included in the blast counts. In order to assess the pattern of blast counts obtained by the analyzers, blood sample specimens were selected if the percentage of blasts was a minimum of 10% of the total WBCs. During the 6-month period, a total of 175 peripheral blood specimens exhibited blast counts of 10% or more on routine manual differential counts. The diagnosis and classification of the patients were made on the basis of the 2008 WHO classification [9] by morphologic, immunophenotypic, cytogenetic, and molecular genetic findings of the bone marrow. All the patients had been previously or newly diagnosed as having acute leukemia. The specimens were then divided into 4 groups according to the lineage of the blasts: the lymphoblast group, myeloblast group, monoblast group, and promyelocyte group. There were 28 cases of ALL in the lymphoblast group, including 3 T-cell ALL (T-ALL) cases and 25 B-cell ALL (B-ALL) cases; 113 cases of AML in the myeloblast group, which consisted of cases other than acute monocytic or myelomonocytic leukemia (AMoL) and acute promyelocytic leukemia (APL); 24 cases of AMoL in the monoblast group, including 14 cases of acute myelomonocytic leukemia and 10 cases of acute monoblastic/monocytic leukemia; and 10 cases of APL in the promyelocyte group.

2. WBC Differential

The 5-part automated WBC differential was conducted using both the DxH 800 and the XE-2100 within 6 hr after blood collection. For the validation of the results from both the analyzers, 200-cell manual differential counts were obtained by 2 experienced technologists using the corresponding Wright-stained blood smears. The results were confirmed by a hematopathologist. The automated WBC differential counts were compared with the manual differential counts, and the cell type identifications of the blast populations made by the analyzers were determined. The different cell types were defined when the automated WBC differential count was over 10% higher than the manual differential count in a certain cell type.

3. Flagging of blasts

The DxH 800 system flagged the presence of blasts. The blast flags were triggered when abnormal cells were detected in the blast region on the cytogram [10], which is determined by volume and conductivity. In the DxH 800 system, the blast region is divided into 3 different regions: lymphoblasts (LYB), monoblasts (MOB), and myeloblasts (NEB). The XE-2100 system expressed suspicion for the presence of blasts or abnormal cells as Q-flag values (0-300 arbitrary units) [11]. Although the Q-flag setting recommended by the manufacturer is 100 for each parameter, we adjusted the blast and abnormal lymphocyte/lymphoblast Q-flag setting to 200 and 300, respectively, in order to ameliorate the risk of false positives. Therefore, the flagging results from the XE-2100 system were not included in this study. The blast suspect flags generated by the DxH 800 apparatus were compared with the results of the manual differential count.

RESULTS

1. Automated differential count

The DxH 800 reported CBC results with the 5-part WBC differential count in 98.9% (173/175) of the cases containing blasts. Only 1 AML case and 1 AMoL case were expressed as invalid and marked as "----." In the differential count results with the DxH 800 and XE-2100, a message of "impossible to analyze (----)" was displayed after classification when the classified cells were plotted out of the DIFF channel scattergram. The XE-2100 provided an invalid automated differential count in 72% (126/175) of the cases (Table 1).

With the DxH 800, lymphoblasts were counted as lymphocytes in 85.7% (24/28) of ALL cases. The blasts in 3 cases were counted as lymphocytes mixed with neutrophils or monocytes. In only 1 case of the lymphoblast group, the blasts were counted as monocytes. The blasts exhibited irregularly shaped nuclei relative to the lymphoblasts of the other ALL cases, and many of them had abundant cytoplasm and contained many vacuoles (Fig. 1). The XE-2100 generated invalid results in half of the cases. In the remaining cases with valid results, the majority of the lymphoblasts (39.3%, 11/28) were counted as lymphocytes, and the rest of them were counted as lymphocytes mixed with monocytes.

In the AML group, 1 case (0.9%, 1/113) yielded an invalid result with the DxH 800. In the remaining AML cases with valid results, myeloblasts were counted as monocytes in 57.5% (65/113) of the cases. Blasts were read as lymphocytes in 20 cases (17.7%, 20/113) and as neutrophils in 15 cases (13.3%, 15/113). In 14 cases (12.4%), a mixed pattern was observed, including 10 cases where the blasts

Table 1. Automated differential count results of peripheral blood samples containing 10% or more blasts obtained using the DxH 800 and XE-2100 according to the type of cells which were counted as blasts (% of valid results)

Diagnosis		Cases							Invalid	Total
		LY	NE	MO	LY+NE	MO+NE	LY+MO	LY+NE+MO		
ALL	DxH 800	24 (85.7%)		1 (3.6%)	2 (7.1%)		1 (3.6%)			28
	XE-2100	11 (39.3%)					3 (10.7%)		14 (50%)	
AML	DxH 800	20 (17.7%)	15 (13.3%)	65 (57.5%)	1 (0.9%)	1 (0.9%)	10 (8.8%)		1 (0.9%)	113
	XE-2100	4 (3.5%)		11 (9.7%)	1 (0.9%)	1 (0.9%)	10 (8.8%)	2 (1.8%)	84 (74.3%)	
AMoL	DxH 800	7 (29.1%)	3 (12.5%)	10 (41.7%)			3 (12.5%)		1 (4.2%)	24
	XE-2100	1 (4.2%)			1 (4.2%)		2 (8.3%)		20 (83.3%)	
APL	DxH 800		6 (60.0%)	4 (40%)						10
	XE-2100			2 (20%)					8 (80%)	

Abbreviations: LY, lymphocyte; NE, neutrophil; MO, monocyte; AMoL, acute monocytic or myelomonocytic leukemia; APL, acute promyelocytic leukemia.

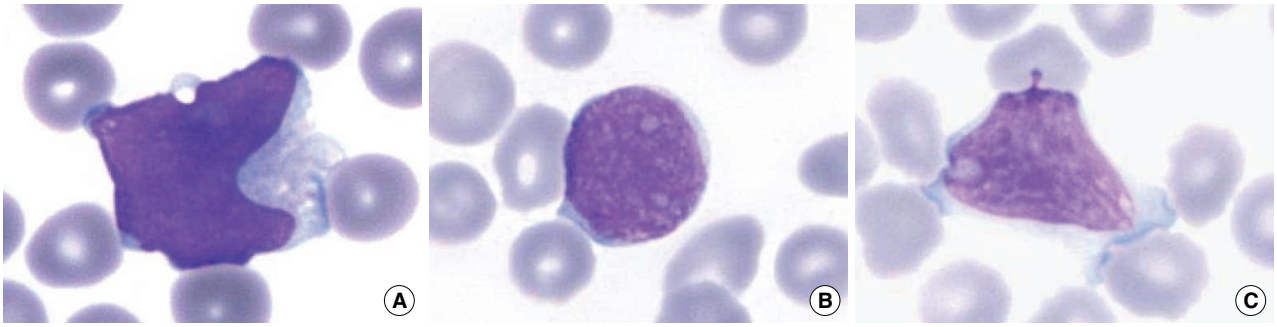

Fig. 1. (A) A lymphoblast from an ALL case in which the blasts were counted as monocytes in the DxH 800; it showed irregularly shaped nuclei and cytoplasmic vacuoles. (B) A lymphoblast from a case of ALL in which the blasts were counted as lymphocytes in the DxH 800; it showed round nuclei with dispersed chromatin. (C) A lymphoblast from a case of T-ALL that did not generate blast flags; it showed coarse chromatin and abundant cytoplasm. All photomicrographs are ×1,000 magnification.

were counted as monocytes and lymphocytes. The XE-2100 generated invalid results in 74.3% of the cases. The myeloblasts of the remaining cases were counted largely as monocytes (11/113, 9.7%). Blasts were counted as lymphocytes mixed with monocytes in 10 cases (10/113, 8.8%).

In the AMoL group, 1 case (4.2%, 1/24) produced invalid results. In the remaining AMoL cases with valid results, leukemic cells were largely counted as monocytes (41.7%, 10/24); in 3 cases, blasts were counted as monocytes and lymphocytes. Blasts were read as lymphocytes in 7 cases (29.1%) and as neutrophils in 3 cases (12.5%). The leukemic cells of myelomonocytic leukemia cases were counted primarily as lymphocytes (64.3%, 9/14). In contrast, the leukemic cells of only 1 case of monoblastic/monocytic leukemia were counted as lymphocytes (10%, 1/10). The XE-2100 provided no valid results in the majority of cases of AMoL (20/24, 83.3%).

Leukemic promyelocytes of APL cases were counted as neutrophils in 60.0% (6/10) of the cases and as monocytes in 40.0% (4/10) of the cases. No distinct morphological differences between these 2 groups were noted. The XE-2100 system generated no valid results in most of the cases of APL (8/10, 80%).

2. Blast flags of the DxH 800

The blast flags of lymphoblasts exhibited a distribution pattern similar to that of the automated differential count. In most of the cases of lymphoblasts (85.7%, 24/28), blast flags were generated, mainly as LYB (71.4%, 20/28). The lymphoblasts in 2 of the 3 T-ALL cases were counted as lymphocytes without blast flags, and they exhibited low N/C ratios and coarse chromatin structures (Fig. 1). The 2 ALL cases in which the blasts were counted as neutrophils and lymphocytes exhibited NEB flags, and the 1 case in which blasts were counted as monocytes exhibited an MOB flag.

Unlike in the automated differential count, myeloblasts were simultaneously flagged primarily as both LYB and MOB (47.8%, 54/113). This result was more frequently observed compared to the count proportion of LY and MO from the automated differential count (8.8%). The incidence rates of NEB and LYB flags were 16.0% (18/113) and 13.3% (15/113), respectively. This result was similar to the count proportion of NE and LY from the automated differential count (13.4% and 17.9%, respectively). Monoblasts of the AMoL group were flagged as both LYB and MOB (29.2%, 7/24), LYB (25.0%, 6/24), or NEB (16.7%, 4/24) (Table 2).

The total incidence of MOB (58.3%, 14/24) was similar to the count proportion of MO from the automated differential counts (54.2%, 13/24). The leukemic promyelocytes from APL were flagged principally as NEB (50%, 5/10) and MOB (40%, 4/10). The incidence rates were similar to the count proportions of NE and MO from the automated differential counts (60.0% and 40.0%, respectively).

No blast flags were generated in 11 cases. Among the 11 cases, 5 generated variant lymphocyte flags, and 4 generated left-shift and immature granulocyte flags. In 2 cases, no abnormal WBC flags were generated (Table 3). One of these cases had a low WBC count (2,099/μL) and a low blast percentage (19%), but the other had a high

Table 2. Blast flag results of the peripheral blood samples containing 10% or more blasts obtained using DxH 800 according to the final diagnosis

Diagnosis	Coulter suspect message								
	LYB	NEB	MOB	LYB, NEB	MOB, NEB	LYB, MOB	LYB, NEB, MOB	No blast flag	Total
ALL	20 (71.4%)	2 (7.1%)	1 (3.6%)	1 (3.6%)				4 (14.3%)	28
AML	14 (13.3%)	18 (16%)	12 (9.7%)	2 (1.8%)	3 (2.7%)	54 (47.8%)	3 (2.7%)	7 (6.2%)	113
AMoL	6 (25.0%)	4 (16.7%)	3 (12.5%)		1 (4.2%)	7 (29.2%)	3 (12.5%)		24
APL		5 (50%)	4 (40%)		1 (10 %)				10

Abbreviations: LYB, blasts in the lymphocyte region; NEB, blasts in the neutrophil region; MOB; blasts in the monocyte region; AMoL, acute monocytic or myelomonocytic leukemia; APL, acute promyelocytic leukemia.

Table 3. Total WBC count, percentage of blasts, the cell types that were counted as blasts, and WBC flags in the cases without blast flags by DxH 800

Diagnosis	WBC (/μL), blast %	Blasts counted as	WBC flag
ALL	24.662 (87)	LY	VLY
ALL	25.507 (15)	LY	LS, IG
ALL	16.404 (31)	LY	LS, IG
ALL	3.069 (24)	LY	VLY
AML	21.822 (94)	LY	
AML	9.348 (86)	NE	VLY
AML	9.182 (93)	NE	LS, IG
AML	3.880 (11)	LY	VLY
AML	2.798 (86)	LY+MO	LS, IG
AML	2.424 (23)	MO	VLY
AML	2.099 (19)	Invalid	

Abbreviations: WBC, white blood cell; LY, lymphocyte; VLY, variant lymphocyte; LS, left shift; IG, Immature granulocytes; NE, neutrophil; MO, monocyte.

WBC count (21,822/μL) and a high blast proportion (94%).

DISCUSSION

Advances in differential WBC technology have focused principally on the accurate identification of small numbers of circulating abnormal cells and increased flagging efficiency. However, automated analyzers do not generally count abnormal cells separately from the normal cell population. The DxH 800 analyzer counted blasts into 5 differential cell types in the vast majority (98.9%) of the specimens, unlike the XE-2100 system, which provided invalid differential reports in 72% of the cases. Nevertheless, it counted the remaining cases in a pattern similar to that of the DxH 800.

The DxH 800 principally counted lymphoblasts as lymphocytes (85.7%) and myeloblasts as monocytes (57.5%). The XE-2100 reported more than half of the cases as invalid (i.e., 50% of the lymphoblast cases and 74.3% of the myeloblast cases). The difference between the values obtained with the 2 analyzers could be related to the difference between the differential algorithms. The XE-2100 produces a differential WBC count in 2 distinct channels (WBC/BASO- and DIFF-channel). Cells are examined by the light beam from a semi-conductor laser and are differentiated by 2 out of 3 captured signals: forward scatter of which the intensity is equivalent to cell volume, side scatter for the cellular contents, and side fluorescence yielding the amount of RNA and DNA present in the cell [12]. The DxH 800 collects the data using variable angles of light scatter for differential WBC counts. In all, 7 distinct parameter measurements were performed for each cellular event. There are 3 scatter (lower median angle light scatter, upper median angle light scatter, and median angle light scatter) measurements that determine granularity and membrane surface, in addition to volume and conductivity. The axial light loss measurement that analyzes cellular transparency and low angle light scatter is a cellular complexity index [13, 14].

With the DxH 800, blasts of only 1 case of the lymphoblast group were counted as monocytes. The blasts exhibited irregularly shaped nuclei compared to the lymphoblasts of the other cases of the ALL group, and they had relatively abundant cytoplasm, which contained a number of vacuoles. This means that the cell types determined by the analyzer reflect the morphology, rather than the origins, of the cells. Lymphocytes were the second most frequently determined cell type of the myeloblasts identified by the analyzers. This may be attributable to the simple morphology of the myeloblasts that do not contain granules. In the AMoL group, monoblasts from acute monoblastic leukemia were counted as neutrophils and the promonocytes and leukemic monocytes from acute monocytic leukemia were counted as monocytes. Therefore, when the other laboratory findings are consistent with the results of AMoL, the diagnosis may be either monoblastic or monocytic leukemia according to the cell type designated by the analyzer. In APL, the leukemic promyelocytes were included primarily in the monocytes or neutrophils, and not in both cell populations. However, the morphology of APL counted as monocytes exhibited no distinct morphologic differences from those of the APL counted as neutrophils on blood film examinations.

Some automated analyzers, including the DxH 800, the GEN-S system (Beckman Coulter), and the LH 750 (Beckman Coulter), generate different blast flags for different blast types. In our study, the blast flags of lymphoblasts

exhibited distributions similar to the results from the automated differential counts. However, most myeloblasts simultaneously generated both MOB and LYB flags. Many types of myeloblasts are supposed to be located over the MOB and LYB region on the blast flag cytogram. The blast flag was reported in 91.2% (115/126) of all the cases in our study. Moreover, all 11 cases without blast flags fell within the criteria for the slide review, as suggested by the International Consensus Group for Hematology [15]. The actual sensitivity of blast detection would be expected to be lower than that reported in our study, because we selected only samples that contained 10% or more blasts. However, we can conclude that the manual differential counts and the application of stringent slide review criteria remain essential whenever a hematologic disorder is suspected, even in cases in which blast flags are not generated.

Interestingly, 2 of the 3 cases of T-ALL in our study generated no blast flags. These 2 cases had high N/C ratios, coarse chromatin patterns, and were counted as lymphocytes. Further studies with more specimens will be necessary to determine whether the type of blast is related to the blast flag sensitivity, or simply related to their morphology. Our data show that differential WBC reports from automatic cell analyzers should be interpreted with great care, with extra attention paid to flags, because the majority of leukemic blasts may be counted as monocytosis, lymphocytosis, or neutrophilia.

In most cases, the results of the blast flags were consistent with those of the 5-part differential counts. In a few cases involving discordance, the 5-part differential results more accurately reflected the actual blast type in the final diagnosis. Many leukemic blasts, particularly myeloblasts, simultaneously generated different types of blast flags. We can conclude that blast flags are sufficient for reporting the presence of blasts but cannot be used to distinguish the accurate lineages of blasts. However, among the 92 cases that yielded MOB flags, only 1 case was ALL (Table 2). Therefore, the blast suspect messages containing MOB flags could be helpful to exclude ALL before slide review.

In conclusion, 5-part differential cell types of the blasts determined by the automated analyzers are characteristic of the different blast types. Although the presence of blasts can be almost completely detected by assessing the flags and conducting strict manual reviews of the slides, the diagnosis of leukemia is still delayed because of the decision regarding lineage. In the XE-2100, the 5-part differential result of abnormal cell populations may not be generated. However in the DxH 800, the 5-part differential of the abnormal blasts can be generated regardless of flags, and it may prove helpful in the differential diagnosis of acute leukemias prior to additional investigation. For more accurate distinctions of lineages, further analysis will be necessary to determine the differences in cell population data between normal cells and leukemic blasts.

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