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진단혈액학 LMO

불안정헤모글로빈을 Sysmex 자동혈구분석기에서 백혈구 이상 산포도를 통해 발견할 수 있는 단서

A Clue to Discovering Unstable Hemoglobin Variants via Abnormal WBC Differential Scattergrams Using the Sysmex Automated Hematology Analyzer

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Incidentally, hemoglobin (Hb) variants can be detected using HbA1c tests in clinical laboratories. We found 38 patients with Hb variants after reviewing a total of 29,398 HbA1c test results from January 2017 to December 2017. While reviewing the complete blood count results of the patients (N=36) using the Sysmex XN-9000 analyzer (Sysmex, Japan), 35 patients were flagged as unremarkable with respect to differential white blood cell (WBC) counts. However, 1 patient with a normal WBC count did not obtain a differential WBC count while being flagged for an abnormal WBC scattergram in the white blood cell differential (WDF) channel. The WBC histogram showed an abnormally low fluorescent signal in the WDF channel; however, the differential WBC count was normal upon microscopic examination. After testing the patient's buffy coat suspended in normal saline and removing red blood cells (RBCs), the WBC scattergram and differential WBC count returned to normal. This finding suggests that the presence of a patient's RBCs may affect WBC scattergrams and Hb variants may interfere with the fluorescent dye in the differential WBC count. Therefore, when an abnormal WBC scattergram with an abnormally low fluorescent signal is encountered on the Sysmex XN-9000 analyzer, the presence of an Hb variant can be suspected.

Key Words: Hemoglobin variant, Leukocytes differential, Automated hematology analyzer

The presence of hemoglobin (Hb) variants can have variable clinical symptoms ranging from none to mild or compensated hemolysis to severe hemolytic anemia [1]. Hb variants can be detected using isoelectric focusing or high-performance liquid chromatography (HPLC) and confirmed by sequencing the globin genes. Incidentally, Hb variants can also be identified using HbA1c measurements. At our institution, 38 patients were found to have Hb variants from a total of 29,398 HbA1c results obtained between January 2017 and December 2017 using two different types of HbA1c measuring instruments (Bio-Rad D100, Bio-Rad Laboratories, Hercules, CA, USA; and Tosoh G8 System, Tosoh, Tokyo, Japan). Out of 36 patients whose complete blood count (CBC) results were determined using the Sysmex XN-9000 automated hematology analyzer (Sysmex, Kobe, Japan), 1 patient was flagged for an abnormal white blood cell (WBC) scattergram with an abnormally low fluorescent signal. However, this patient did not obtain a differential WBC count, whereas the remaining 35 patients were flagged unremarkably with respect to differential WBC counts. Generally, abnormal WBC scattergrams in Sysmex analyzers are flagged when WBC clusters are observed or more than a certain level of cell dots between cell clusters are present, which is caused by an interference in the white blood cell differential (WDF) channel. The purpose of this study was to present a possible cause for

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the abnormal WBC scattergram in the patient with the unstable Hb variant.

The 41-year-old male patient, with a history of antiphospholipid and Evans syndromes, was admitted to our hospital due to left forearm pain after receiving intravenous nutritional therapy. CBC testing reported a WBC count of 8.8×10^9/L, Hb of 13.0 g/dL, and platelet count of 100×10^9/L. Interestingly, the Sysmex XN-9000 analyzer could not perform a differential WBC count nor flag the abnormal WBC scattergram. This scattergram demonstrated an abnormally low fluorescent signal without differentiating compartments in the WDF channel (Fig. 1A). The differential WBC count was normal under microscopic examination and showed the following results: 55% neutrophils, 33% lymphocytes, 9% monocytes, and 3% eosinophils. Signs of hemolysis were observed based on the following laboratory findings: reticulocyte count 16%, haptoglobin <10 mg/dL, lactate dehydrogenase 385 IU/L (reference interval 106–211 IU/L), and total bilirubin 2.5 mg/dL. The histograms from the HbA1c measuring instruments showed variant peaks with an extremely low HbA1c level (2.4–2.5%). Glycated albumin was observed to be 9.9%, which approximately converts to an HbA1c of 4.0% [2]. Subsequent Hb electrophoresis (Sebia CAPILLARYS 2, Lisses, France) showed an elevated HbA2 fraction (3.9%) with multiple variant fractions. Altogether, a diagnosis of an unstable Hb variant was made.

In order to demonstrate Hb interference in this study, we isolated the patient's buffy coat suspension (red blood cells [RBCs] excluded), and performed a differential WBC count using the Sysmex XN-9000 analyzer. In contrast to the initial results, the WBC scattergram changed to a normal pattern, showing similar differential WBC counts as those from the manual differential counts via microscopy (Fig. 1B). Furthermore, we replaced the patient's plasma with normal saline and consequently obtained an abnormal WBC scattergram that was similar to the initial findings (Fig. 1C). A sample that contained the RBCs from the patient and the buffy coat of a normal individual showed a persistently abnormal pattern on the WBC scattergram (Fig. 1D). Altogether, these results suggest that the presence of a patient's RBCs may affect WBC scattergrams and that Hb variants may interfere with the fluorescent dye in the differential WBC counts. In order to confirm the analytical interference in the differential WBC count using the

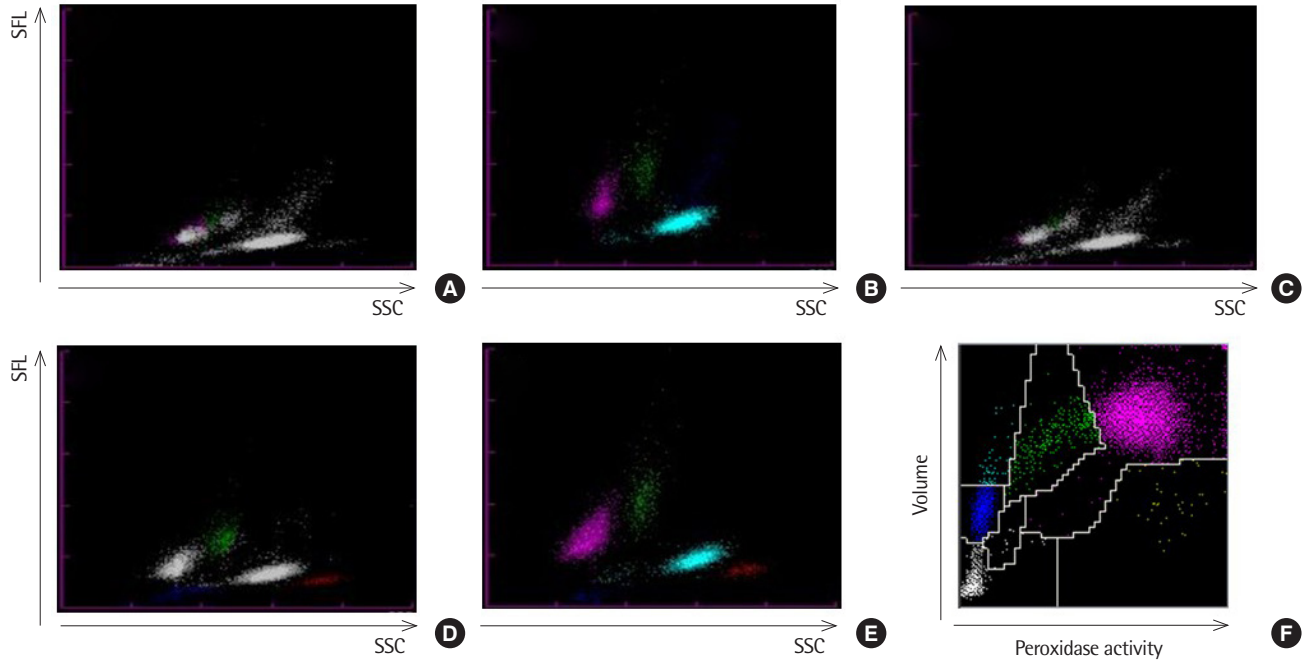

Fig. 1. WBC scattergrams from the automated hematology analyzers. (A), (B), (C), (D), and (E) were performed on the Sysmex XN-9000 analyzer and (F) on the ADVIA 2120i analyzer. (A) Scattergram of the white blood cell differential (WDF) channel of the patient. (B) Scattergram of the patient's buffy coat suspension in phosphate buffered saline after removing RBCs. (C) Scattergram of the patient's WBCs and RBCs containing normal saline instead of plasma. (D) Scattergram of a sample containing a normal control's WBCs and the patient's RBCs. (E) Scattergram of the normal control. (F) WBC scattergram of the patient.
Abbreviations: SFL, side fluorescent light; SSC, side scattered light.

Table 1. Hematologic characteristics of the cases with Hb variants exhibiting abnormal WBC scattergrams detected using a Sysmex series analyzer and from literature reviews

Reference	Case no.	Sex	Age (yr)	Hb (g/dL)	MCV (fL)	RDW (%)	RET ($\times 10^9$/L)	HbA2 (%)	Hb variant
This study	1	M	41	13.0	104.1	14.1	679.8	3.9	NT
3	2	F	37	*	*	*	*	2.8	Leiden
4	3	F	52	*	*	15.2	*	41.8	G-Ferrara
5	4	M	76	*	*	*	*	*	M Dothan
6	5-1	F	72	12.1	80.9	17.9	144.9	3.0	Indianapolis
6	5-2	M	44	13.3	88.1	15.7	258.5	3.1	Indianapolis
6	5-3	F	40	13.6	86.9	13.1	128.0	3.3	Indianapolis
6	5-4	F	36	14.2	92.1	13.3	187.3	3.1	Indianapolis
6	6-1	M	45	14.9	97.1	13.5	161.4	3.8	Köln
6	6-2	M	8	11.9	95.4	15.3	183.0	3.4	Köln
6	7	M	14	13.0	86.8	14.3	235.0	3.6	Köln
6	8	F	59	14.7	97.9	13.6	87.7	2.8	Himeji
6	9	M	81	16.3	93.9	14.9	137.9	3.5	HBB: C.173A > T

*Data not presented in the journal.
Abbreviations: Hb, hemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; RET, reticulocyte; NT, not tested; M, male; F, female.

Sysmex XN-9000 analyzer, we also performed a differential WBC count using another instrument (ADVIA 2120i, Siemens Healthcare Diagnostics, Erlangen, Germany). This instrument performs cytochemical-based differential WBC counts using myeloperoxidase staining. Contrary to the results from the Sysmex XN-9000 analyzer, the differential WBC counts were normal in the myeloperoxidase channel using the ADVIA 2120i (Fig. 1F). Therefore, the presence of unstable Hb may affect results differently between analyzers due to the reagents and methods used to stain WBC.

According to previous reports, the abnormal WBC scattergram with the abnormally low fluorescent signal observed using the Sysmex analyzer may have been caused by unstable Hb interference released in the WDF channel during incomplete RBC lysis [3-6]. The polymethine fluorescence dye used for differential WBC count in the Sysmex analyzer binds to some Hb variants with a greater affinity when compared to the nucleic acids of WBCs [3]. It may also reduce WBC permeability to the dye and therefore, reduce WBC staining, resulting in abnormally low fluorescence signals in the WDF channel [6]. Previous reports have described the diagnosis of unstable Hb variants detected in the WDF channel using automated hematology analyzers such as the Sysmex XE series [3-6] (Table 1). The reported cases had relatively normal laboratory results without severe hemolytic anemia. All the cases displayed a similar, distinctive, low-fluorescence WBC scattergram pattern irrespective of the Hb variant type, suggesting unstable Hb as a cause for the generation of abnormal WBC scattergrams in Sysmex analyzers using fluorescence flow cytometry.

In conclusion, when an abnormal WBC scattergram with an abnormally low fluorescent signal is encountered on the Sysmex XN-9000 analyzer, the presence of an Hb variant can be suspected.

요 약

헤모글로빈 변이형은 임상검사실에서 당화혈색소(HbA1c) 검사를 하는 도중 우연히 발견될 수 있다. 우리는 2017년 1월부터 12월까지 시행한 HbA1c 검사 29,398개의 결과를 검토하여 38명의 헤모글로빈 변이형을 발견했다. 이 중 Sysmex XN-9000 (Sysmex, Japan)으로 검사한 36명의 전체 혈구계산 결과를 검토하여, 35명에서는 백혈구 감별계산과 관련한 특이적인 소견이 없음을 확인하였다. 그러나 백혈구 수치는 정상인 한 명의 환자에서 기기 백혈구 감별계산 결과를 얻을 수 없었고, 백혈구 감별 채널(WDF 채널)에서 백혈구 이상 산포도라는 플래그를 보였다. WDF 채널의 히스토그램에서 비정상적으로 낮은 형광강도가 관찰되었다. 현미경으로 검사한 결과 백혈구 감별계산은 정상이었다. 환자의 적혈구를 제거 후 버피층을 식염수에 부유시켜 검사한 결과 백혈구 수치와 감별계산은 정상으로 측정되었다. 이와 같은 발견은 환자의 적혈구가 백혈구 감별계산에 영향을 주었고 헤모글로빈 변이형이 백혈구 감별을 위해 사용하는 형광염색제에 간섭을 주었을 가능성을 시사한다. 따라서, Sysmex XN-9000에서 비정상적으로 낮은 형광강도를 보이면서 백혈구 이상 산포도라는 플래그를 띄우면 헤모글로빈 변이형의 존재 가능성을 고려해 볼 수 있다.

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AUTHORS' DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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