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CASE REPORT

Detection Challenges in Myelodysplastic Syndromes: a Case Report of Missed Detection of Nucleated Red Blood Cells

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SUMMARY

Background: **Nucleated red blood cells (NRBCs) play a crucial role in automated blood analysis, particularly in the diagnosis and monitoring of blood disorders. This study examines the limitations of the Sysmex XN-9000 fully automated modular hematology analyzer in detecting nucleated red blood cells (NRBCs) in patients with myelodysplastic syndromes (MDS).**
Methods: **Peripheral blood samples from a patient diagnosed with MDS were analyzed using the XN-9000 analyzer. Manual digital microscopy was employed for validation and detailed cell counts.**
Results: **The XN-9000 incorrectly identified nucleated erythrocytes as monocytes and failed to detect an abnormal quantity of NRBCs. Manual review revealed a significantly lower white blood cell count compared to the automated count, and a notable increase in NRBCs alongside a decrease in monocyte percentage.**
Conclusions: **Fully automated hematology analyzers may have limitations in detecting certain hematopathological conditions, and manual microscopy is essential to ensure diagnostic completeness and accuracy.**
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KEYWORDS

nucleated red blood cells (NRBCs), myelodysplastic syndromes (MDS), Sysmex XN-9000, hematology analyzer, manual microscopy, leukocyte misclassification

INTRODUCTION

Nucleated red blood cells (NRBCs) are immature red blood cells containing a nucleus and serve as important indicators of bone marrow activity, providing insights into the maturation process of erythrocytes. In the peripheral blood of healthy adults, only mature erythrocytes are typically present; the presence of NRBCs, however, indicates underlying pathology. Therefore, the detection of NRBCs is critical for diagnosing and monitoring a variety of hematologic disorders, including hematopoietic dysfunction, various types of anemia, leukemia, and bone marrow infiltration [1]. Furthermore, NRBCs can predict changes in clinical status and mortality among critically ill patients, thereby enhancing their critical role in clinical decision-making [2].

Fully automated hematology analyzers, as core equipment in clinical laboratories, provide critical hematological parameters through high-speed and accurate automated testing, which is essential for disease diagnosis, treatment monitoring, and health assessment. In routine blood analysis, fully automated hematology analyzers play a pivotal role in rapidly providing a comprehensive blood cell count, including nucleated red blood cells (NRBCs). However, these instruments may sometimes fail to accurately identify NRBCs, leading to potential misdiagnoses and treatment delays [3]. The detection of NRBCs is crucial in hematology analysis, with its significance extending beyond the cell count alone. The presence of NRBCs can affect the interpretation of other blood parameters, especially the white blood cell count. This misclassification may interfere with the diagnosis and monitoring of hematologic disorders, as NRBCs can be incorrectly classified as leukocytes, resulting in elevated white blood cell counts [4]. Therefore, accurate detection of NRBCs is essential to ensure reliable assessment of hematologic health and appropriate clinical management of patients. This study aims to investigate a case in which a fully automated modular hematology analyzer failed to detect NRBCs in myelodysplastic syndromes, emphasizing the importance of understanding the limitations of current analytical techniques and the critical role of manual review in ensuring accurate diagnostic results.

CASE REPORT

The patient was a 65-year-old man who had been diagnosed with myelodysplastic syndrome for over one year and was admitted to the hospital due to malaise lasting five days. He was admitted due to worsening anemia accompanied by diarrhea. A bone marrow aspiration examination revealed pronounced nucleated cell hyperplasia, predominantly consisting of immature erythrocytes with pathological changes; primitive cells accounted for 1.5% of the total nucleated cells. Flow cytometry results indicated an increased percentage of nucleated erythrocytes, comprising 72.10% of the total nucleated cell count. In the side scatter/forward scatter (SSC/FSC) analysis, the cells exhibited a large cytoplasmic volume, with 9.6% identified as CD117+CD105+, indicating early-stage juvenile erythrocytes. No myeloid primitive cells were detected (Lower Limit of Detection: 0.006%). The preliminary diagnosis upon admission was myelosuppression following decitabine chemotherapy, severe anemia, and myelodysplastic syndrome. After admission, routine blood analysis using the XN-9000 fully automated modular blood analyzer showed a white blood cell count of 18.27 x 10^9/L, a neutrophil percentage of 5.0%, a lymphocyte percentage of 50.8%, and an abnormally high monocyte percentage of 40.9%. Nucleated red blood cells were not detected in the automated count of 100 cells. Upon meticulous manual microscopic re-examination, the corrected results indicated a white blood cell count of 11.40 x 10^9/L, with an increase in neutrophil percentage to 37.0%, a lymphocyte percentage of 58.0%, and a significant decrease in monocyte percentage to 5.0%. Additionally, 61 nucleated erythrocytes were identified in the retested count of 100 cells (Table 1). This significant discrepancy in counts, in particular concerning the percentage of monocytes and the failure to identify nucleated erythrocytes, underscores the limitations of automated instrumental detection.

The WDF channel scatter plot from the XN-9000 Hematology Analyzer displayed abnormal results, characterized by poorly delineated cell populations and incorrectly colored cell scatters. Typically, the scatter plot should present clear classifications, showing five distinct groups. However, in this case, the scatter plot exhibited gray fused scatters, which could not be accurately classified (Figure 1). The WNR channel scatter plot of the XN-9000 hematology analyzer is normally capable of identifying nucleated red blood cells, which appear as purple-red clusters. Nonetheless, the instrument failed to identify nucleated red blood cells in this study, and the blue cluster representing white blood cells also exhibited abnormalities (Figure 1). Manual digital microscopy revealed a significant number of nucleated erythrocytes in the peripheral blood, with these cells generally exhibiting larger sizes (Figure 2). These results indicate that the automatic detection function of the instrument did not accurately recognize and classify nucleated erythrocytes, thereby compromising the accuracy of the test results.

DISCUSSION

Sample collection and handling are critical factors affecting test results. Improper blood collection techniques, hemolysis of blood samples, and imbalance in the ratio of anticoagulant to blood may lead to bias in test results. When confronted with abnormal test results, timely verification of specimen information and confirmation of specimen status are critical steps in ruling out the influence of sample factors. In this case, we found that the test results were abnormal, and we immediately reviewed the information of the specimen, including the preparation of the patient, the standardization of blood collection, the type and concentration of the anticoagulant, as well as the condition of the specimen and the conditions of storage and transportation. The suitability of the samples was ensured, thus eliminating the possibility of sample factors affecting the test results.

To further analyze the reasons leading to the current results, the detection mechanism of XN-9000 and its limitations need to be considered. XN-9000 adopts advanced flow cytometry technology with laser flow cytometry, and analyzes the cell characteristics through fluorescent dyes and complex algorithms to differentiate between different types of blood cells [5]. Special hemolytic agents and fluorescent dyes are used in the

Table 1. Comparison of XN-9000 blood routine automatic reporting results and manual retesting results.

	Instrument results	Manual reporting of results
White blood cell count (/L)	18.27×10^9	11.40×10^9
Hemoglobin (g/L)	67	67
Platelet count (/L)	80×10^9	80×10^9
Erythrocyte count (/L)	1.92×10^{12}	1.92×10^{12}
Percentage of neutrophils (%)	5.0	37.0
Percentage of lymphocytes (%)	50.8	58.0
Percentage of monocytes (%)	40.9	5.0
Erythrocyte pressure (L/L)	0.193	0.193
Mean erythrocyte volume (fL)	100.5	100.5
Mean hemoglobin volume (pg)	34.9	34.9
Mean hemoglobin concentration (g/L)	347	347
Erythrocyte distribution width (%)	18.9	18.9
Mean platelet distribution width (%)	13.4	13.4
Platelet pressure (%)	0.100	0.100
Mean platelet volume (fL)	12.0	12.0
Nucleated red blood cell count (cells/100)	0	61

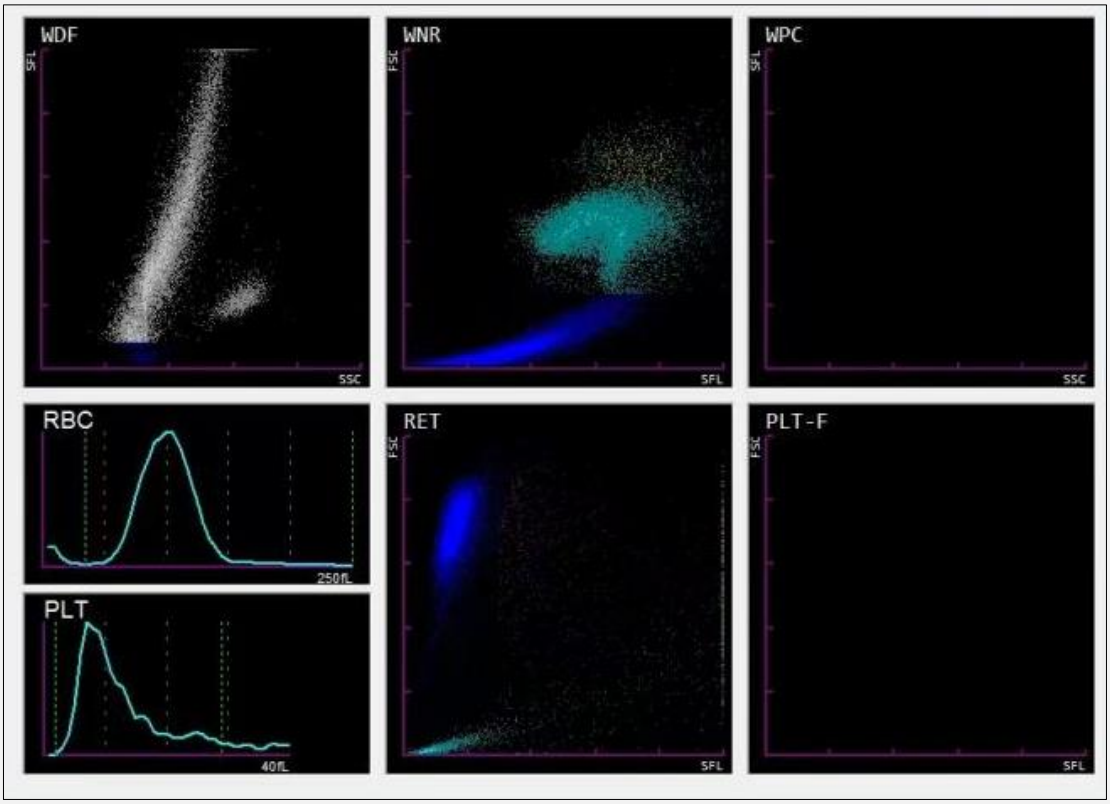

Figure 1. Scatterplot of subjects measured by XN9000.

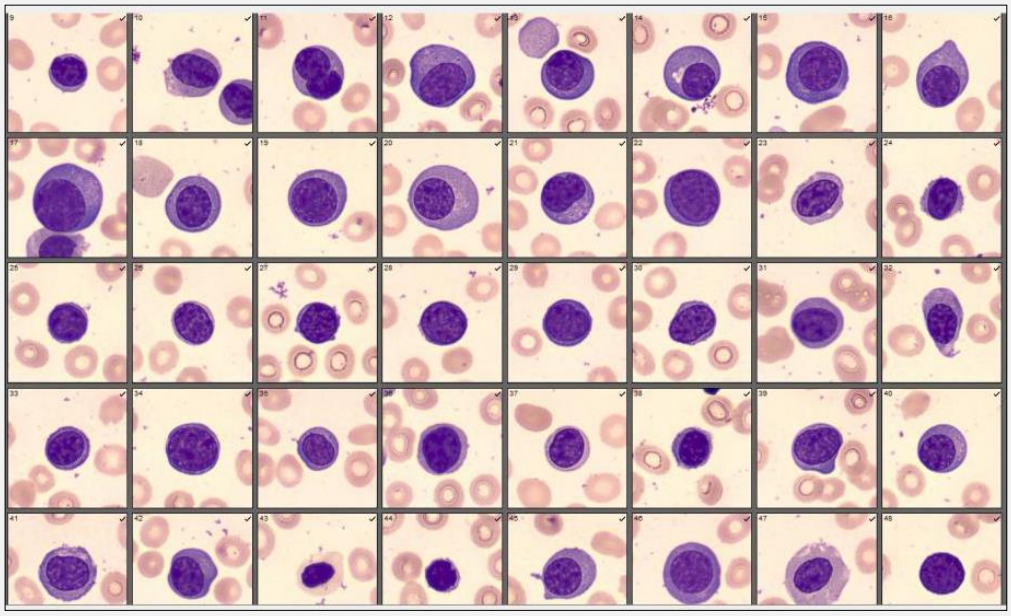

Figure 2. Artificial digitized microscopy images of peripheral blood.

WNR channel, and the naive cells are larger in size, the cell membrane is not easy to penetrate and stain with the dyes, and the reaction to the reagents is weak, which makes them present a larger volume and lower fluorescence intensity in the scatter plot, so that they can be recognized. NRBCs were simultaneously counted during the complete blood count, automatically prompting correction of the white blood cell count when NRBC positivity was detected [6]. The number of NRBCs is significantly increased in patients with myelodysplastic syndromes [7], and immature nucleated red blood cells entering the peripheral blood are mistaken for leukocytes, resulting in a high white blood cell count. In the present patient, in the pathologic situation of MDS, manual microscopy showed that most of the NRBCs were morphologically oversized, and the flow results suggested that the cells were cytosolic in the SSC/FSC position, which is a proto-early-stage juvenile erythrocyte. The enlarged NRBCs were similar to monocytes in terms of volume and nucleoplasmic ratio, which caused the instrument to misclassify most of the nucleated erythrocytes as monocytes. On the other hand, in MDS patients with active cell proliferation [8], some NRBCs may contain richer nucleic acid material than normal, resulting in increased fluorescence intensity detected beyond the instrument's internally predefined threshold range for NRBC fluorescence intensity detection, and may be incorrectly categorized as monocytes, similar to the impedance method of distinguishing between platelets and erythrocytes: For example, the upper limit of platelet volume defaults to 30 fL in the instrument, and cells above this volume will no longer be counted as platelets. Therefore, when encountering NRBCs with high fluorescence intensity, it is not possible to accurately differentiate between NRBCs and monocytes, resulting in NRBCs not being counted. In addition, excessive numbers of a patient's NRBCs that may exceed the instrument's maximum linear range of detection can lead to misses or errors, which require a higher level of sensitivity and specificity from the analyzer.

Reviewing the course of the disease, we noted an abnormal detection of NRBCs in the peripheral blood along with myelosuppressive side effects after the patient received decitabine chemotherapy. Decitabine, a DNA methylation transferase inhibitor and deoxycytidine analog, induces increased apoptosis [9] and alters the expression of cell surface molecules [10], which may lead to altered permeability of the cell's nuclear membrane [11], thus allowing easier access of stains to the interior of the nucleus, resulting in changes in staining properties. Decitabine also inhibits cell proliferation, alters the expression of cell cycle-related genes, increases the proportion of cells in the G phase, affects the production of mitochondrial autophagosomes, and induces mitochondrial damage [12]. In addition, mitochondrial autophagy critically regulates mitochondrial mass and cellular homeostasis [13], which, in turn, may affect the uptake and retention of stains by the nucleus. Overall, decitabine may affect the staining properties of cells by in-

ducing apoptosis, blocking the cell cycle, and altering gene expression and the expression of cell surface molecules, which may increase cellular permeability to stains. This effect may lead to enhanced staining of instruments in the detection of nucleated erythrocytes, incorrectly identifying them as leukocytes with higher fluorescence intensity, such as monocytes, and failing to accurately identify and count nucleated erythrocytes. These findings have important implications for optimizing and monitoring the treatment of patients with MDS.

This study emphasizes the importance of manual microscopy in blood analysis, highlights the limitations of fully automated hematology analyzers in detecting NRBCs in MDS, and proposes strategies for enhancing test accuracy. It aims to provide a valuable reference for examiners and clinicians to optimize test report results and improve the quality of clinical diagnosis.

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Ethical Approval:
The research has complied with all relevant national regulations and institutional policies and has been approved by Ethics Committee of the First Affiliated Hospital of Shihezi University (KJ-2023-400-01).

Declaration of Interest:
Authors state no conflict of interest.

References:

1. Anastasiadi AT, Arvaniti VZ, Hudson KE, et al. Exploring unconventional attributes of red blood cells and their potential applications in biomedicine. Protein Cell 2024;15(5):315-30. (PMID: 38270470)

2. Pikora K, Kretowska-Grunwald A, Krawczuk-Rybak M, Sawicka-Zukowska M. Diagnostic Value and Prognostic Significance of Nucleated Red Blood Cells (NRBCs) in Selected Medical Conditions. Cells 2023;12(14):1817. (PMID: 37508482)

3. Chinudomwong P, Khongjaroensakun N, Chatachote B, Chaothai N, Paisooksantivatana K. Improving the efficiency of the autoverification workflow for nucleated red blood cell reporting in the hematology laboratory. Int J Lab Hematol 2021;43(6):1373-8. (PMID: 34237189)

4. May JE, Marques MB, Reddy VVB, Gangaraju R. Three neglected numbers in the CBC: the RDW, MPV, and NRBC count. Cleve Clin J Med 2019;86(3):167-72. (PMID: 30849034)

5. Lauwers M, Verschelden G, Boero C, Baleine M, Kerrels V, Cantinieaux B. Optimization of the Transwell® assay for the analysis of neutrophil chemotaxis using flow cytometry to refine the clinical investigation of immunodeficient patients. Clin Immunol 2022;238:108994. (PMID: 35390547)

6. Kaido M, Takagi Y, Kono M, et al. Investigation of morphological changes for the discrimination of nucleated red blood cells and other leukocytes in Sysmex XN hematology analyzer scattergrams using transmission electron microscopy. Pract Lab Med 2017;8:70-6. (PMID: 28856231)

7. Tsagarakis NJ, Paterakis G, Papadhimitriou SI, et al. "Bone marrow aspirate automated counts on hematology analyzers: formulating a scoring system based on hematology parameters, to discriminate reactive versus myelodysplastic syndrome-related bone marrows". Int J Lab Hematol 2019;41(4):542-9. (PMID: 31102331)

8. Tanaka TN, Bejar R. MDS overlap disorders and diagnostic boundaries. Blood 2019;133(10):1086-95. (PMID: 30670443)

9. Gutierrez WR, Scherer A, Rytlewski JD, et al. Augmenting chemotherapy with low-dose decitabine through an immune-independent mechanism. JCI Insight 2022;7(22):e159419. (PMID: 36227698)

10. Zhang R, Lu X, Tang LV, et al. Comparative analysis of Decitabine intensified BUCY2 and BUCY2 conditioning regimen for high-risk MDS patients undergoing allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2022;57(7): 1063-71. (PMID: 35459877)

11. Lindenboim L, Zohar H, Worman HJ, Stein R. The nuclear envelope: target and mediator of the apoptotic process. Cell Death Discov 2020;6:29. (PMID: 32351716)

12. Zhang G, Gao X, Zhao X, et al. Decitabine inhibits the proliferation of human T-cell acute lymphoblastic leukemia MOLT4 cells and promotes apoptosis partly by regulating the PI3K/AKT/mTOR pathway. Oncol Lett 2021;21(5):340. (PMID: 33747197)

13. Sun Y, Cao Y, Wan H, et al. A mitophagy sensor PPTC7 controls BNIP3 and NIX degradation to regulate mitochondrial mass. Mol Cell 2024;84(2):327-344.e9. (PMID: 38151018)