

## CASE REPORT

# Mindray BC-6900 Scattergram Analysis Combined with Peripheral Blood Smear in the Diagnosis of *T. Marneffei* Infection in an HIV-Positive Patient: a Case Report

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### SUMMARY

**Background:** Rapid and accurate diagnosis of HIV-positive patients with *Talaromyces marneffei* (*T. marneffei*) infections remains challenging. A 60-year-old woman came to our inpatient department presenting with hematuria, abdominal pain, and diarrhea for one week. The patient had a past medical history of Acquired Immune Deficiency Syndrome (AIDS). The patient's stool was watery and the color of soy sauce. The patient was without fever, cough, and skin lesions.

**Methods:** The blood routine was performed with a Mindray BC-6900 hematology analyzer.

**Results:** Blood routine showed leukocytosis with neutrophilia and basophils and the WBC/DIFF scattergram showed a cluster of neutrophils connected with a monocyte and lymphocyte cluster and an additional cluster of immature granulocytes and heterotypic lymphocytes or primitive cells. Surprisingly, the peripheral blood film evaluation revealed small round-to-ovoid yeast cells within the cytoplasm of neutrophils. A *T. marneffei* infection was suspected and anti-fungal therapy was initiated. The patient's diarrhea improved after treatment with amphotericin B for two days. A second blood routine showed a normal number of leukocytes and basophils and a diminished cluster of immature granulocytes and heterotypic lymphocytes or primitive cells. After one week, blood cultures had grown *T. marneffei*.

**Conclusions:** The WBC/DIFF scattergram obtained from a Mindray BC-6900 analyzer provided significant hints to enhance diagnosis of *T. marneffei* when combined with results of a peripheral blood smear.

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### KEY WORDS

WBC/DIFF scattergram, *Talaromyces marneffei*, peripheral blood smear, case report

### LIST OF ABBREVIATIONS

AIDS - acquired immunodeficiency syndrome

HIV - human immunodeficiency virus

WBC - white blood cell

PMN - polymorphonuclear

RBC - red blood cell

MN - mononuclear cell

*T. marneffei* - *Talaromyces marneffei*

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## INTRODUCTION

*Talaromyces marneffeii*, also known as *Penicillium marneffeii*, is a dimorphic fungus primarily among human immunodeficiency virus (HIV)-infected patients living in Southeast Asia [1]. *T. marneffeii* infections are associated with a high mortality rate due to delayed diagnoses and multiple clinical manifestations [2]. Although various methods have been used to diagnose *T. marneffeii*, including serodiagnostics and nucleic acid assays, laboratory diagnosis relies mainly on microscopic examination of the fungus with confirmation by culture [3], which is the gold standard for fungal identification. However, initial identification of *T. marneffeii* infection can be difficult, especially for patients with atypical manifestations.

Absolute peripheral white blood cell (WBC) counts and differential (DIFF) have been used for many years to help discriminate between acute infectious and non-infectious diseases. Although there are no uniformly accepted criteria for the rational explanation of outliers, the WBC/DIFF counts can deliver powerful supportive data for clinical decision making. Nowadays, the WBC/DIFF count is determined by an automated hematology analyzer, and has become a routine laboratory test to evaluate hospitalized patients [4]. According to a previous study, WBC/DIFF-scattergrams acquired from automated hematological analyzers were useful in diagnosing [5]. However, using WBC/DIFF scattergrams to assist in diagnosing fungal infections has rarely been reported.

Here, we report a 60-year-old female patient with non-specific fever and skin lesions, who was suspected to have acute bacterial dysentery according to her clinical symptoms. However, she was presumptively diagnosed as *T. marneffeii* infection within minutes after a WBC/DIFF blood routine combined with a peripheral blood smear examination. A few days later, the diagnosis of *T. marneffeii* infection was confirmed by fungal culture.

## CASE PRESENTATION

A 60-year-old woman presented with a one-week history of diarrhea and hematuria for three days. She was known to be seropositive patient for HIV and denied any history of hypertension, diabetes, or heart disease. A physical examination revealed a normal bowel sound and body temperature, no cutaneous lesions, and lymphadenectasis. She had no other obvious abnormalities. Based on her clinical symptoms, the initial diagnosis of this patient was abdominal pain and diarrhea. Differential diagnoses, including acute bacillary dysentery and hemorrhagic necrotizing enteritis, were under consideration and needed further confirmation.

Laboratory testing on admission showed that the patient's leukocyte level in a routine blood examination was  $11.77 \times 10^9$  leukocytes/L. The levels of her neutrophils ( $9.32 \times 10^9$  neutrophils/L) and basophils ( $0.35 \times$

$10^9$  basophils/L) were increased (Table 1). Interestingly, the Mindray WBC/DIFF scattergram showed an additional cluster of immature granulocytes and heterotypic lymphocytes or primitive cells, which were located above a neutrophil cluster and a monocyte cluster, respectively (Figure 1B). Moreover, the cluster of neutrophils was closer to the cluster of monocytes and lymphocytes compared with those seen in a normal blood sample (Figure 1A). A double-bind manual differential count of 200 nucleated cells on the blood smear revealed round-to-ovoid yeast cells with central septa, some detached (Figure 2A) and others located predominantly within the cytoplasm of neutrophils (Figure 2B). Striking neutrophilic dysplasia with abnormal forms and binucleate cells were observed (Figure 2B, C). Considering the patient had AIDS and was vulnerable to *T. marneffeii* infection and *T. marneffeii* was seen on the blood smear, the physicians reached a diagnosis of *T. marneffeii* infection, and anti-fungal treatment with amphotericin B was initiated.

After two days of treatment with amphotericin B, the patient's condition improved. A routine blood examination revealed the number of leukocytes and basophils had reverted to a normal level (Table 1), and the WBC/DIFF scattergram was markedly different from the first one as the clusters of immature granulocytes and heterotypic lymphocytes or primitive cells had diminished (Figure 1C). Seven days later, the blood culture grew fungi, which were whitish colonies producing a red-wine-colored diffusible pigment on Sabouraud glucose agar (Figure 2D - F). Thus, the physicians diagnosed the patient with *T. marneffeii* and HIV co-infection. A continuous anti-fungal therapy was prescribed.

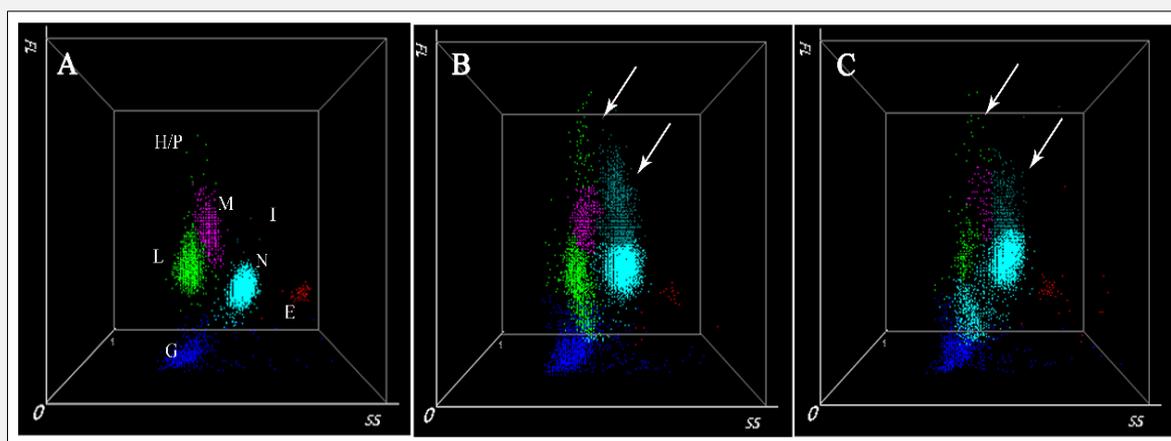
## DISCUSSION

BC-6900 (Mindray, Shenzhen-China), a hematology analyzer, can perform body fluids (BFs) analysis in dedicated module. BC-6900 provides numerous default parameters including leukocyte (WBC-BF), total nucleated cells (TC-BF), polymorphonuclear cells (PMN), and different cell count for mononuclear cells (MN), which provide quantification of RBC, WBC, and TC in BF analysis by using fluorescent flow cytometry with hydrodynamic focusing. In BF mode, all nucleated cells were classified by using forward scatter, laser side scatter, and fluorescence analysis, and cells are clustered in a three-dimensional scattergram (3D) according to their internal complexity (SS axis), nucleic acid content (FL axis), and size (FS axis) [6]. Previous studies have demonstrated that Mindray BC-6900, an automated hematology analyzer provides accurate and rapid counts in clinically relevant concentration ranges [7]. Furthermore, abnormal WBC scattergrams obtained from the hematology analyzer provides a clue to the diagnosis of malaria [5].

In the case described here, the patient came to our hospital with only a symptom of diarrhea, which was not

**Table 1. Comparison of before and after anti-fungal treatment in differential leukocyte counts.**

Analyte	First blood sample (Prior-treatment)	Second blood sample (Posttreatment)	Deltas	Unit	Reference interval
Leukocyte	11.77	9.18	-3.590	10 <sup>9</sup> /L	3.50 - 9.50
Neutrophils	9.32	7.78	-1.540	10 <sup>9</sup> /L	1.80 - 6.30
Lymphocytes	1.73	0.23	-1.500	10 <sup>9</sup> /L	1.10 - 3.20
Monocytes	0.34	0.07	-0.270	10 <sup>9</sup> /L	0.10 - 0.60
Eosinophils	0.03	0.05	0.020	10 <sup>9</sup> /L	0.02 - 0.52
Basophils	0.35	0.05	-0.300	10 <sup>9</sup> /L	0.0 - 0.06
Neutrophils	79.2	95.2	16.00	%	50.0 - 70.0
Lymphocytes	14.7	2.8	-11.90	%	20.0 - 50.0
Monocytes	2.9	0.8	-2.10	%	3.0 - 10.0
Eosinophils	0.2	0.6	0.40	%	0.40 - 8.0
Basophils	3.0	0.6	-2.40	%	0.0 - 1.0

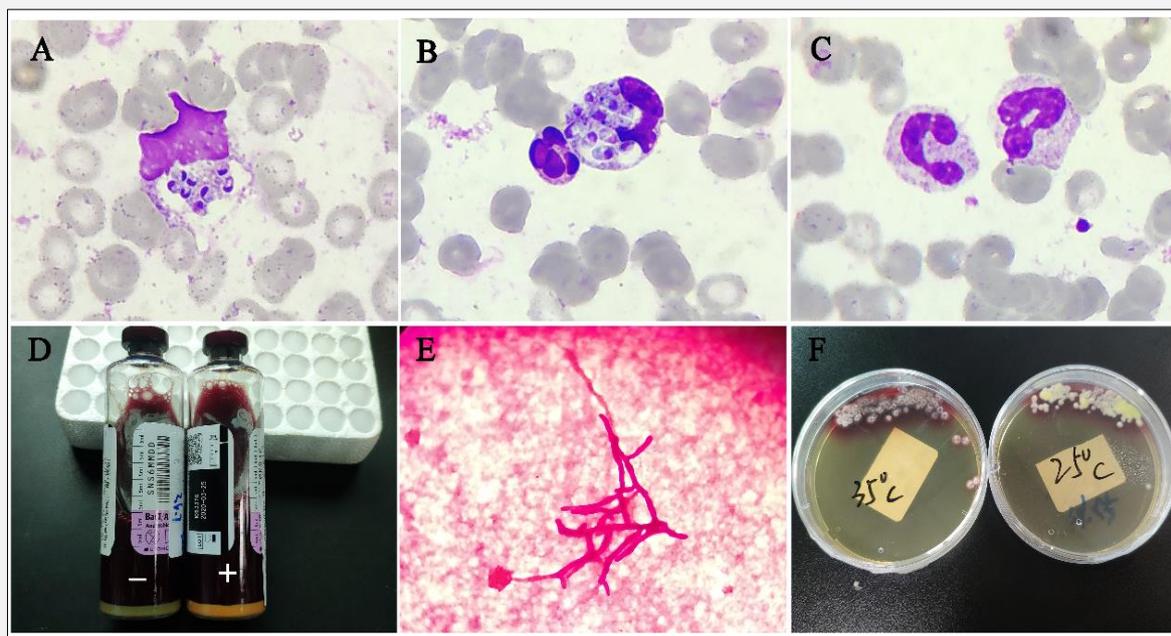
**Figure 1. Mindray BC-6900 scattergrams of leukogram.**

A scattergram of a normal blood sample shows no additional cluster on I and H/P domains of the WBC/DIFF channel (A). The WBC/DIFF scattergram (B) shows an additional cluster (white arrow) on the neutrophil cluster and monocyte cluster. The cluster of immature granulocytes and heterotypic lymphocytes or primitive cells were diminished two days after the initiation of the anti-fungal treatment (C). N - indicates neutrophils, L - lymphocytes, M - monocytes, E - eosinophils, I - immature cells, H/P - heterotypic lymphocytes and primitive cells, G - debris cells or ghost, SS - scattered light signal, FL - fluorescence light.

the most typical symptom of *T. marneffeii* infection [1]. However, WBC/DIFF scattergram showed an additional cluster of heterotypic lymphocytes and immature granulocytes, which were related closely with infection [8], leading us to a careful microscopic review of a peripheral blood film. Then we found the suspected *T. marneffeii* and treated promptly with amphotericin B. Finally, the patient's diarrhea improved two days after the initia-

tion of the treatment, and the culture medium grew *T. marneffeii*, confirming the previously suspected diagnosis of *T. marneffeii* infection.

Nowadays, an automated hematology analyzer is an ordinary instrument operated by a laboratory technician in daily clinical practice, which has improved quality and reduced cost due to the characteristics of precision and less labor-intensive.



**Figure 2. Microscopic and mycological findings.**

The microscopic examination (A, B) revealed some small (3 - 7  $\mu\text{m}$ ) round-to-ovoid yeast-like cells with occasional central septa in the neutrophil cytoplasm and extracellular domain, neutrophilic dysplasia (C) with large abnormal morphology and frequent small nuclear fragments (Wright's-stain, x 1000). Blood culture grew *T. marneffei* (D, E), and a colony (F) produced a wine-red pigment, which diffused into Sabouraud glucose agar plate during incubation.

In summary, this case showed for the first time that WBC/DIFF scattergram obtained from BC-6900 combined with peripheral blood film has potential utility for the rapid, accurate, and economic diagnosis of *T. marneffei*. In addition, the present report may indicate a possible correlation between additional clusters of heterotypic lymphocytes or immature granulocytes and fungal infections, but further investigations with well-designed studies and on a larger scale are needed.

#### **Ethics Approval and Consent to Participate:**

Not applicable.

#### **Consent for Publication:**

We informed the patient and acquired the written consent for publication of this case report. The data supporting our findings can be found in Liuzhou Municipal Liutie Central Hospital internal network database.

#### **Availability of Data and Materials:**

Materials including all relevant raw data described in the manuscript, will be freely available to any researchers wanting to use them for non-commercial purposes, without breaching participant confidentiality.

#### **Declaration of Interest:**

The authors declare no competing interests.

#### **Source of Funds:**

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