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ORIGINAL ARTICLE

Effects of *Talaromyces marneffei* on Complete Blood Count by a Sysmex XN-9000 Analyzer

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SUMMARY

***Background:* *Talaromyces marneffei* (*T. marneffei*) infection detected in the peripheral blood smears has been described by several reports. We studied the effects of *T. marneffei* in peripheral blood samples on complete blood count (CBC) using a Sysmex XN-9000 analyzer.**
***Methods:* In a simulated *T. marneffei* infection model, blood samples with and without infectious diseases were selected, with high, medium, and low levels of white blood cell (WBC) and platelet (PLT) count, respectively. All samples were detected immediately and after a warm bath of 37°C for 2 hours.**
***Results:* WBC count of all samples was significantly increased by *T. marneffei* from a certain concentration and higher. For all samples, the effect of *T. marneffei* on WBC count after warm bath was significantly reduced compared to that on immediate WBC count from 4 - 6 x 10^9/L *T. Marneffei* and higher ($p < 0.05$). The presence of *T. marneffei* in all blood samples did not affect the results of PLT count. For all samples, the obvious effects of *T. marneffei* on WBC differential (WDF) and white cell nucleated red blood cell (WNR) scatter plots were from 4 - 6 x 10^9 *T Marneffei* and higher.**
***Conclusions:* As a kind of intracellular yeast, *T. marneffei* may affect WBC count, NRBC count, and WBC differential count of peripheral blood samples when the yeast concentration is (4 - 6) x 10^9 *T Marneffei* and higher. Moreover, the unique scatter plot cloud on WDF and WNR scatter plots caused by *T. marneffei*, may become an important clue pointing toward *T. marneffei* in peripheral blood.**
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KEYWORDS

Talaromyces marneffei, Sysmex XN-9000 analyzer, complete blood count, white blood cell count, WBC differential count

INTRODUCTION

The wide application of automated hematology analyzers has contributed to a great improvement in hematology due to timely and correct results. However, some fictitious results relevant to automated hematology analyzers were reported. It was reported that spuriously elevated PLT and/or WBC counts were caused by fungal spores in the peripheral circulating blood [1-4]. As an important opportunistic fungal pathogen, *T. Marneffei* may lead to disseminated infection in immunocompromised patients, especially in endemic areas (southern China, Taiwan, and southeast Asia), and the prevailing evidence strongly showed an expansion of the traditional endemic areas [5-7]. For patients with serious talaromycosis, *T. marneffei* can be present in the peripheral blood [8-10]. Xiaocheng Luo et al. reported obvious false results on CBC due to interference of numerous extracellular *T. marneffei* in the peripheral blood impacting the measurement by a Sysmex XN-9000 analyzer [11]. Given yeast of *T. marneffei* with intracellular presence and unique morphological features of cross-wall formation [12], for the first time, we investigated the effect of *T. marneffei* with different concentrations on CBC by a Sysmex XN-9000 analyzer. In addition, in order to simulate the patients without and with infectious diseases under human body environmental conditions, we also analyzed the measurement results of samples after 2 hours of warm bath at 37°C. This study was approved by the Ethics Committee of Medical Research and Clinical Trials of Baise People's Hospital (reference number: LW2022072701).

MATERIALS AND METHODS

Subjects

For the purpose of measuring CBC results, the peripheral circulating blood specimens were collected by BD vacutainer EDTA-K2 tubes (Zhong Xing, China). Blood samples from patients without and with infectious diseases were used, with high (WBC 11.8 x 10^9/L, PLT 532 x 10^9/L), medium (WBC 4.7 x 10^9/L, PLT 206 x 10^9/L), and low (WBC 2.0 x 10^9/L, PLT 40 x 10^9/L), and high (WBC 13.2 x 10^9/L, PLT 351.0 x 10^9/L), medium (WBC 7.0 x 10^9/L, PLT 68 x 10^9/L), and low (WBC 2.2 x 10^9/L, PLT 17 x 10^9/L) levels of WBC and PLT, respectively, which were all from inpatient specimens for blood routine tests. The infectious diseases are defined as resulting from bacteria, fungi, viruses, parasites and other pathogens, and their products result in local or systemic inflammation of the patients, with diverse clinical manifestations and high mortality. Non-infectious diseases are the diseases with evidence of no infectious diseases. All patients aged 18 or above. Patients without infectious diseases were diagnosed with trauma (with high levels of WBC and PLT), chronic obstructive pulmonary disease (with medium levels of WBC and PLT), and hepatocirrhosis (with low levels of WBC and PLT). Patients with infectious diseases were diagnosed with severe pneumonia (with high levels of WBC and PLT), bacterial pneumonia (with medium levels of WBC and PLT), and sepsis (with low levels of WBC and PLT), respectively. These values represented relatively high, moderate, low levels of WBC and PLT among the hospitalized patients that day, which were subject to the constraints, only represented a relative level.

Instruments

The tested instrument was a Sysmex XN-9000 analyzer, which determines WBC by information of forward-scattered light and side-scattered light detected by a semiconductor laser. In order to separate basophils from other forms of WBC, the reagent of red cell lysis, which can selectively suppress the degranulation of basophils, is selected. In the WDF channel, WBC is categorized based on side-scattered light and fluorescence intensity after a fluorescent stain of deoxyribonucleic acid and ribonucleic acid, and classified into five groups: neutrophils, monocytes, lymphocytes, eosinophils, and basophils.

Yeast of *T. marneffei*

Positive specimen of *T. marneffei* was obtained from blood culture specimen of an AIDS patient in People's Hospital of Baise City and identified by direct microscopic examination, isolation culture, and mass spectrometry. *T. marneffei* was transferred to the Sabouraud dextrose agar plates and cultured at 37°C for 3 days. The colonies were identified as yeast by microscopic examination and confirmed as *T. marneffei* by mass spectrometry, thus contaminated yeasts were excluded. The fungal colonies were picked and suspended in 200 µL sterile 0.9% sodium chloride using an electric vortex. Given that the size of *T. Malneffei* is close to that of platelets, we counted *T. Malneffei* under the microscope using a blood cell counting plate by reference to manual PLT counting method. The concentration of yeast suspensions was adjusted to a standard of approximately (4 - 6) x 10^{11}/L. By four ten-fold dilutions of the undiluted yeast suspensions, the final yeast concentrations were prepared as (4 - 6) x 10^{11}/L, (4 - 6) x 10^{10}/L, (4 - 6) x 10^9/L, (4 - 6) x 10^8/L, (4 - 6) x 10^7/L.

Simulated *T. marneffei*

Each blood sample was divided into 6 tubes, each of which contained 360 µL peripheral circulating blood, then added 40 µL yeast suspensions with the prepared different yeast concentrations or sterile 0.9% sodium chloride. The yeast concentration in the samples were (4 - 6) x 10^{10}/L, (4 - 6) x 10^9/L, (4 - 6) x 10^8/L, (4 - 6) x 10^7/L, (4 - 6) x 10^6/L, 0/L, respectively. There was a blank in each case, containing equivalent volume of normal saline instead of yeast suspensions.

CBC

The Sysmex XN-9000 analyzer was operated by the experienced technologists of clinical laboratory in accordance with standard laboratory procedures, and the technologists were blinded to the code of the samples. The laboratory has obtained ISO 15189 quality capability certification, which ensures the Sysmex XN-9000 analyzer we used satisfies the quality certification requirements, including repeatability index verification. The indoor quality control (QC) of the day is in the control value range. All samples were measured immediately and after a warm bath of 37℃ for 2 hours. Significant increase was defined as WBC or PLT count increment greater than 20% compared to the samples without *T. marneffei*.

Statistical analysis

Statistical analyses were operated using SPSS version 25.0. $p < 0.05$ identified statistically significant values and were indicated as mean ± SD. A paired *t*-test was used for comparison between groups.

RESULTS

T Marneffei in all blood samples did not affect the results of red blood cell (RBC) count and hemoglobin on the Sysmex XN-9000 analyzer, regardless of the concentrations of *T. marneffei*. For patients without infectious disease, WBC counts both immediate measurement and measurement after warm bath were significantly affected by *T. marneffei*, and the deviating counts were seen from (4 - 6) x 10^9/L *T Marneffei* and higher (Figure 1A). For patients with infectious disease, WBC counts affected by *T. marneffei* were the same, except for the high-level sample, of which WBC count after warm bath was significantly affected by *T. marneffei* from concentrations of up to (4 - 6) x 10^{10}/L (Figure 1B). For all samples, the effect of *T. marneffei* on WBC count after warm bath (16.98 ± 8.82) was significantly reduced compared to that on immediate WBC count (20.63 ± 12.10) from (4 - 6) x 10^9/L *T Marneffei* and higher ($p < 0.05$).

The presence of *T. marneffei* in all blood samples did not affect the results of PLT count (Figure 2C, D). For all samples, the obvious effects of *T. marneffei* on WDF and WNR scatter plots were from (4 - 6) x 10^9 *T Marneffei* and higher. With the increase of *T. marneffei* concentration, the range and intensity of abnormal scatter cloud caused by *T. marneffei* increased. The evident abnormal cloud on WDF scatter plot caused by *T. marneffei* was on the right of the debris area and below the WBC area (lymphocytes, monocytes and some neutrophils). In addition, the abnormal scatter was also present in the NRBC and the basophils areas, which can be easily observed on the WNR scatter plot (Figure 3). Given that peripheral blood smears of all samples showed no NRBC and less than 1.0% basophils, dots caused by *T. marneffei* can be misclassified as NRBC and basophils, which may lead to a spurious increase in NRBC and basophils. Blood smears made immediately from the low-level samples and high-level samples at (4 - 6) x 10^{10}/L *T. marneffei* showed WBC, and scattered or clustered *T. marneffei* (Figure 4. a: low level sample, c: high level sample). After 2 hours of 37℃ water bath, almost all *T. marneffei* were engulfed by neutrophils (Figure 3. b: low level sample, d: high level sample).

DISCUSSION

It is well known that phagocytosis of neutrophils and macrophages can prevent *T. marneffei* infection. A warm bath of 37℃ for 2 hours simulates the human body environment to the greatest extent because of high phagocytosis of *T. marneffei*. In our experiment for all samples, WBC counts both immediate measurement and measurement after warm bath were significantly influenced by *T. marneffei* from a certain *T. marneffei* concentration and higher. The likely reason was that a slight aggregation of *T. marneffei* was formed in the process during being engulfed, which was mistaken for WBC by the analyzer. For the high-level sample of the patient with infectious diseases, WBC count after warm bath was affected by (4 - 6) x 10^{10}/L *T. marneffei*. However, for the other samples, the deviating counts were seen from (4 - 6) x 10^9/L *T Marneffei* and higher. The possible reason was that the numerous neutrophils in the high-level sample of the patient with infectious diseases were in a stressed state and could engulf more *T. marneffei* after a warm bath. In addition, the effect of *T. marneffei* on WBC count after warm bath was significantly reduced compared to that on immediate WBC count. This is mainly because of the powerful phagocytosis of neutrophils against *T. marneffei* after warm bath, just like what happens in the patients.

Our experiment indicated that the presence of *T. marneffei* in all blood samples did not affect the results of PLT count. In fact, PLT are much more numerous than WBC, therefore, the presence of *T. marneffei* will not affect the results of PLT count unless the patient develops a severe neutropenia [11].

The Sysmex XN-9000 analyzer uses flow cytometry with semiconductor laser for WBC count and differential. The WDF channel is used for WBC differential count, whereas WNR channel is mainly used for NRBC count. For all samples, the obvious effects of *T. marneffei* on WDF and WNR scatter plots were from (4 - 6) x 10^9/L *T. Marneffei* and higher. With the increase of *T. marneffei* yeast concentration, the range and intensity of abnormal scatter cloud caused by *T. marneffei* increased. The evident abnormal cloud on WDF and WNR scatter plots caused by *T. marneffei* is closely related to the unique morphology structure of *T. marneffei* and may be a clue to the detection of *T. marneffei*. In addition, the abnormal cloud was also present in the NRBC and the basophils areas, which can be easily observed on the WNR scatter plot. Therefore, the abnor-

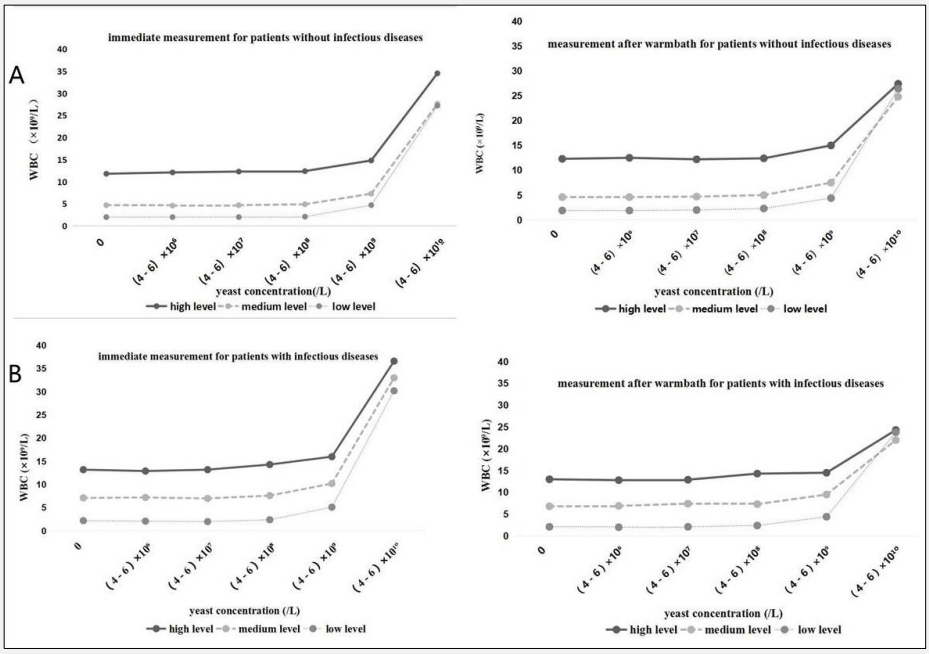

Figure 1. A. For patients without infectious disease, WBC count both immediate measurement and measurement after warm bath was significantly affected by *T. marneffei*, and the deviating counts were seen from (4 - 6) x 10^9/L *T. Marneffei* and higher. B. For patients with infectious disease, WBC counts affected by *T. marneffei* were the same, except for the high-level sample, of which WBC count after warm bath was significantly affected by *T. marneffei* from concentrations of up to (4 - 6) x 10^{10}/L.

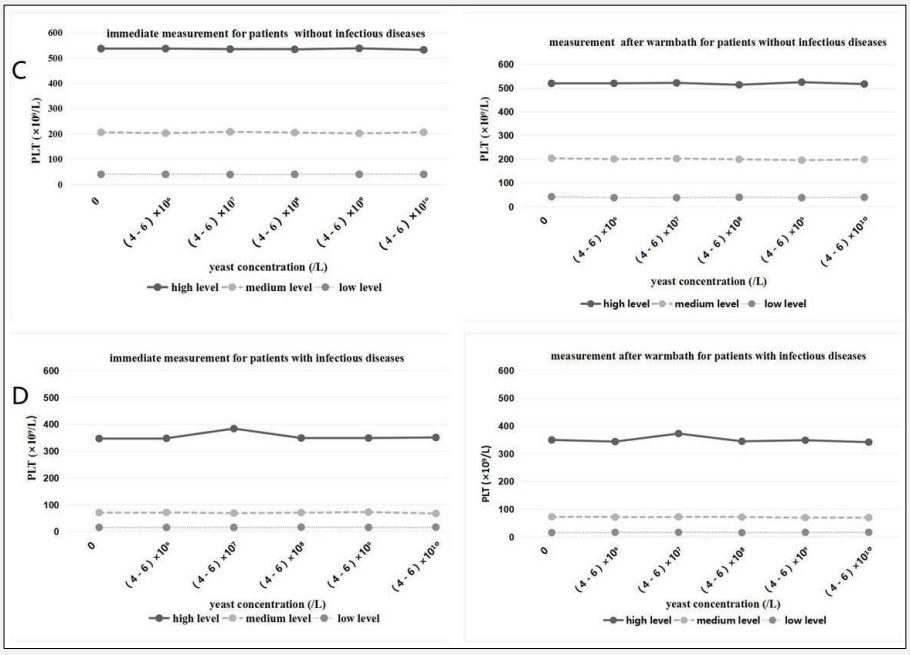

Figure 2. C, D: The presence of *T. marneffei* yeast in all blood samples did not affect the results of PLT count.

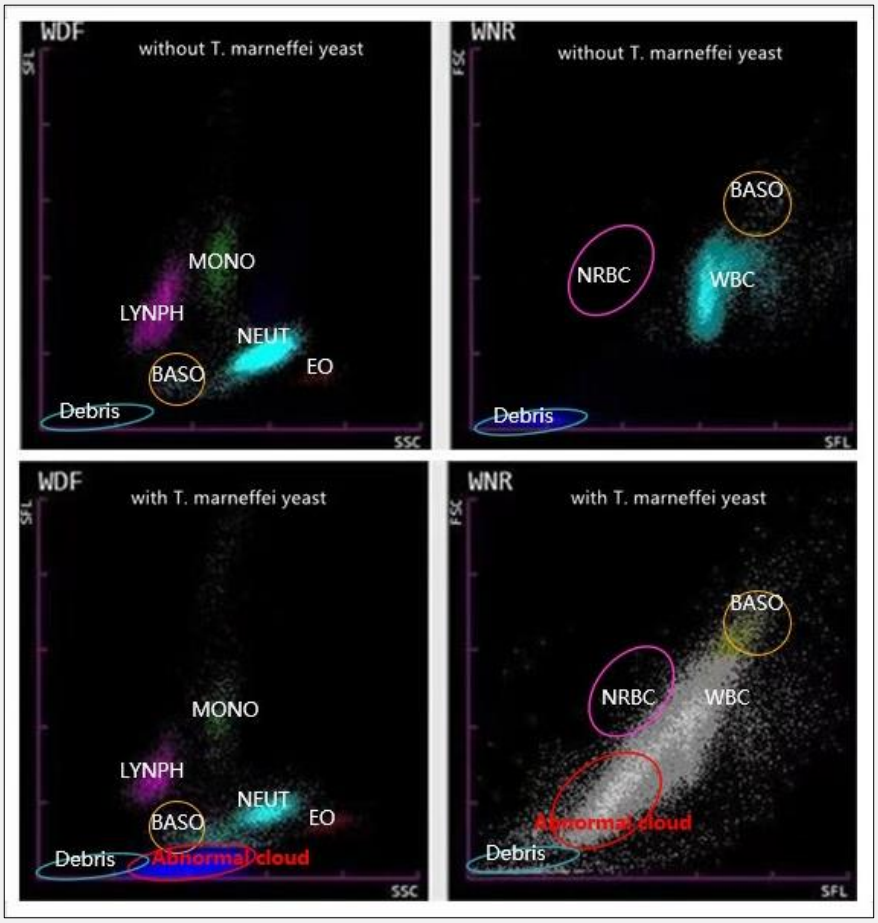

Figure 3. The evident abnormal cloud on WDF scatter plot caused by *T. marneffei* was on the right of the debris area and below the WBC (lymphocytes, monocytes and some neutrophils) area, which is closely related to the unique morphology structure of *T. marneffei* and may be a clue pointing toward *T. marneffei*.

In addition, the abnormal cloud was also present in the NRBC and the basophils areas, which can be easily observed on the WNR scatter plot.

mal cloud resulting from *T. marneffei* is mainly misclassified as NRBC and basophils, which may lead to a spurious increase in NRBC and basophils. It was reported that often NRBC appear in the peripheral circulating blood due to the bone marrow barrier being disrupted by *T. marneffei* infection. Hematology analyzers automatically correct WBC count using NRBC detected, therefore the false elevation of NRBC can increase the effect of *T. marneffei* on WBC count [11]. Unfortunately, we were unable to simulate NRBC in specimens and measure the extent of the *T. marneffei* effect on NRBC, which was also a defect in our experiment and worth paying attention to in our future work.

CONCLUSION

As a kind of intracellular yeast, *T. marneffei* may affect WBC count, NRBC count, and WBC differential count of peripheral blood samples when the yeast concentration is (4 - 6) x 10^9 *T. Marneffei* and higher, which is consistent with our observation of the previous cases of *T. marneffei* infection in peripheral blood. Moreover, the unique scatter plot cloud on WDF and WNR scatter plots caused by *T. marneffei* may become an important clue pointing toward *T. marneffei* in peripheral blood. Laboratory staff should be aware that spurious WBC elevation and erroneous WBC differential count may be present in samples containing *T. marneffei*, and during this situation, peripheral blood smears by microscopy should be reviewed to correct underlying false CBC results.

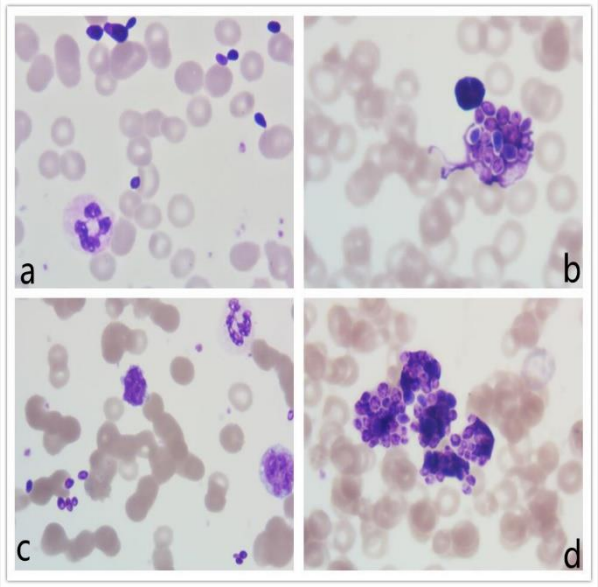

Figure 4. a: Blood smears made immediately from the low level sample at (4 - 6) x 10^{10}/L *T. marneffei*, showed a few white blood cells and scattered or clustered *T. marneffei*. b: After 2 hours of 37°C water bath, almost all *T. marneffei* were engulfed by a neutrophil. c: Blood smears made immediately from the high-level sample at (4 - 6) x 10^{10}/L *T. marneffei*, showed white blood cells and *T. marneffei* both clustered and scattered. d: After 2 hours of 37°C water bath, almost all *T. marneffei* were engulfed by neutrophils.

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Declaration of Interest:
None of the authors have a conflict of interest to disclose.

References:

1. Latif S, Veillon DM, Brown D, et al. Spurious automated platelet count. Enumeration of yeast forms as platelets by the cell-DYN 4,000. Am J Clin Pathol 2003;120(6):882-5. (PMID: 14671977)

2. Branda JA, Kratz A. Effects of yeast on automated cell counting. Am J Clin Pathol 2006;126(2):248-54. (PMID: 16891201)

3. Lesesve JF, Khalifa MA, Denoyes R, Braun F. Peripheral blood candidosis infection leading to spurious platelet and white blood cell counts. Int J Lab Hematol 2009;31(5):572-6. (PMID: 18498388)

4. Kim HR, Park BR, Lee MK. Effects of bacteria and yeast on WBC counting in three automated hematology counters. Ann Hematol 2008;87(7):557-62. (PMID: 18301891)

5. Kawila R, Chaiwarith R, Supparatpinyo K. Clinical and laboratory characteristics of penicilliosis marneffei among patients with and without HIV infection in Northern Thailand: a retrospective study. BMC Infect Dis 2013,5;13:464. (PMID: 24094273)

6. Cao C, Xi L, Chaturvedi V. Talaromycosis (Penicilliosis) Due to Talaromyces (Penicillium) marneffei: Insights into the Clinical Trends of a Major Fungal Disease 60 Years After the Discovery of the Pathogen. Mycopathologia 2019;184(6):709-20. (PMID: 31811603)

7. Lee PP, Chan KW, Lee TL, et al. Penicilliosis in children without HIV infection-are they immunodeficient? Clin Infect Dis 2012, 15;54(2):e8-e19. (PMID: 22065867)

8. Jiang X, Zhou D. Diagnosis of Penicillium marneffei infection from a blood film. Br J Haematol 2015;171(5):670. (PMID: 26250957)

9. Othman J, Brown CM. Talaromyces marneffei and dysplastic neutrophils on blood smear in newly diagnosed HIV. Blood 2018, 11;131(2):269. (PMID: 29326352)

10. Norasethasopon T, Boonyagars L. Talaromycosis. N Engl J Med 2021;384(13):1261. (PMID: 33789014)

11. Luo X, Lin J, Gao H, et al. Fatal pancytopenia masked by Talaromyces marneffei. Int J Lab Hematol 2022;44(5):812-4. (PMID: 35244336)

12. Vanittanakom N, Cooper CR Jr, Fisher MC, Sirisanthana T. Penicillium marneffei infection and recent advances in the epidemiology and molecular biology aspects. Clin Microbiol Rev 2006; 19(1):95-110. (PMID: 16418525)