



## CASE REPORT

## Intraleukocytic Yeast Inclusions and Toxic Granulation Neutrophils on Peripheral Blood Smear: An Interesting Synergy between Hematology and Microbiology

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### Abstract

The presence of yeast neutrophil inclusions was observed and discussed several times in other reports; moreover some works demonstrated how Toxic Granulation Neutrophils (TGNs) are especially helpful in predicting acute bacterial infection, while the development of candidaemia-related TGNs was rarely described and in-depth.

We describe two occasional findings of neutrophil inclusions and marked TGNs respectively due to *Candida tropicalis* and *Candida guilliermondii* on peripheral blood smear.

We proved how the microscopic observation of marked toxic granulations can afford to suspect a systemic microbial infection without the potential to discriminate between bacterial or fungal infections.

### Keywords

*Candida tropicalis*, *Candida guilliermondii*, Candidemia, Toxic granulations, Peripheral blood smear, Sepsis

### Introduction

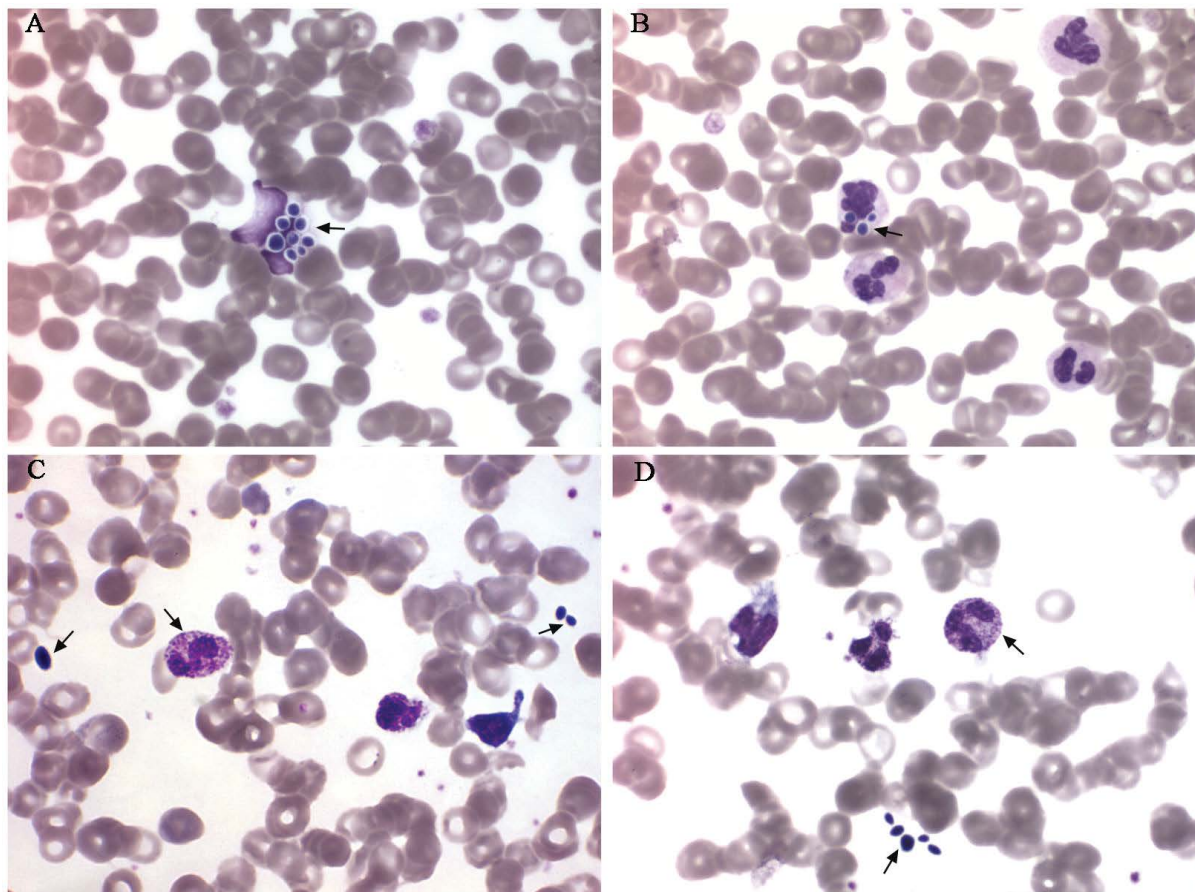
Fungal pathogens, in particular *Candida* species, have become a major cause of nosocomial infection [1]. Blood cultures are limited for diagnosing invasive candidiasis

by poor sensitivity and slow turn-around time [2] while  $\beta$ -D-glucan detection demonstrates variable sensitivity depending on the cut-off diagnostic value and on the *Candida* species under consideration. This last presents several false positive results due to albumin and/or immunoglobulin administration, Gram positive bacteraemia or haemodialysis [3].  $\beta$ -D-glucan, mannan antigen detection and polymerase chain reaction are often expensive and time consuming, furthermore their low specificity could lead to an excessive use of antifungals agents increasing fungal resistance and care costs [4].

A general consensus has not been reached on the usefulness of any of these methods, except for blood culture and histological examination [5].

Yeast with pseudohyphae or those that have been phagocytized by white blood cells are coincidentally found in peripheral blood smears. Nevertheless, the clinical diagnostic value and outcome of candidaemia diagnosed from peripheral blood smears are unclear [6].

We describe two occasional findings of neutrophil



**Figure 1:** Microscopy of peripheral blood smear (May-Grünwald Stain), 100x objective.

Panel A: Neutrophil breakup with phagocytosed yeasts (case 1); Panel B: Intraleukocytic yeast inclusions (case 2); Panel C: Toxic granulation neutrophils and free yeast (case 1); Panel D: Toxic granulation neutrophils and free yeast (case 2).

inclusions and toxic granulation neutrophils (TGNs) respectively due to *Candida tropicalis* and *Candida guilliermondii* on peripheral blood smear (Figure 1).

### Case 1

A 44-year-old man with an history of Focal Segmental Glomerulosclerosis and subjected to hemodialysis for 22 years after a kidney transplant rejection, was hospitalized in the nephrology department due to a lithiasic cholecystitis. A Central Venous Catheter (CVC) was placed and the patient underwent cholecystectomy. Two days after surgery he showed shaking chills and fever of 38.5 °C. Furthermore laboratory investigations highlighted a leukocyte count of  $2.8 \times 10^9/L$  (74% neutrophils) and C-reactive protein of 47.4 mg/L (reference < 10.0).

Peripheral blood smear (May-Grünwald Stain) revealed a neutrophil breakup probably induced by phagocytosed yeasts (Panel A) and TGNs (Panel C) induced by candidaemia.

Blood cultures confirmed the blood smear findings revealing the presence of *Candida tropicalis*. CVC was removed and its cultural test resulted positive for the same fungal species. The patient was successfully submitted to antifungal therapy with caspofungin for 14 days.

### Case 2

A 50-year-old female patient suffering for HBV-related cirrhosis, was admitted in the medicine unit due to an ascites associated with jaundice and a reduction of the liver function. Two days after hospital admission the patient developed fever (38.2 °C) unresponsive to wide spectrum antibiotic therapy (meropenem) while laboratory data highlighted a leukocyte count of  $8.9 \times 10^9/L$  (88% neutrophils), C-reactive protein of 91.9 mg/L (reference < 10) and Procalcitonin of 0.48 ng/mL (reference < 0.05).

On the same day peripheral blood smear (May-Grünwald Stain) revealed some yeast neutrophils inclusions (Panel B) and numerous TGNs induced by candidaemia (Panel D).

*Candida guilliermondii* infection was confirmed by blood cultures results, and the speciation of isolates were performed by biochemical tests. The patient was successfully submitted to antifungal therapy with fluconazole for 14 days.

### Discussion

Previous reports have suggested that peripheral blood smears may be useful for the detection of disseminated yeast infection [5] especially in case

of central venous lines related candidaemia [6]. The microscopic examination of the blood smear often shows a very low sensitivity, otherwise in case of positivity it allows shorter time to diagnosis compared to blood cultures turn-around time. The presence of yeast neutrophil inclusions was observed and discussed several times in other reports [7]; moreover these works demonstrated how TGNs are especially helpful in predicting acute bacterial infection [7], while the development of candidaemia-related TGNs was rarely described and in-depth.

TGNs is the term used when the normally faint stippled granules in neutrophils stain an intense reddish violet which is a consequence of activity against bacteria or proteins and is observed in serious infections, toxic or drug effects, or autoimmune processes (e.g., chronic polyarthritis) [8].

Normal bone marrow granulocyte maturation is associated with progressive decreases of azurophilic granule enzymes (myeloperoxidase, defensins, lysozyme, azurocidin, etc.). On the contrary, the origin of TGNs has been considered to be related to abnormal neutrophil maturation with persistence of azurophilic granules, containing acid mucosubstance which stains more prominently than under normal circumstances [9].

The acidic mucosubstance accumulated in the toxic granules has the potential to acidify phagosomes enhancing bactericidal activity, since bacteria in phagosomes are killed more effectively at pH of 5.5 than of 7 [10]. Our blood smears confirmed the findings of Kabutomori, et al. [11] highlighting TGNs only within neutrophils without phagocytic yeast inclusions; It is probable that these granules prepare the phagocytosis and disappear upon the occurrence of the same.

Luo, et al. [12] studied with a Sysmex XE-5000 automated hematology analyzer, the inner granules or vacuoles of neutrophils by sideward scatter light (displayed as neut-X), the cellular nucleic acid (DNA and RNA) content by sideward fluorescence light (displayed as neut-Y) and the vector sum of neut-X and neut-Y (displayed as neut-Z).

The authors [12] demonstrated a lower tendency to the development of TGNs in case of candidaemia compared to bacterial sepsis, especially those induced by gram-negative bacteria.

Our reports clearly showed the development of marked toxic granules even during the acute phase of *Candida* spp. bloodstream infection. In addition, the analysis of medical records and laboratory data

excluded other non-infectious conditions as possible causes of TGNs development.

The microscopic observation of marked TGNs can afford to suspect a systemic microbial infections without the potential to discriminate between bacterial or fungal infections.

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