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TRANSPLANT INFECTIOUS DISEASE

CLINICOPATHOLOGIC CONFERENCE

Clinicopathologic conference: Bloodstream infection in an allogeneic hamatopoietic cell transplant: Thinking beyond the usual

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Abstract

This case involves a 53-year-old female with concurrent acute myeloid leukemia (AML) and multiple myeloma. She underwent cytarabine and daunorubicin (7+3) induction chemotherapy followed by cytarabine (HiDAC) consolidation, with an early AML relapse requiring azacitidine and venetoclax therapy. She achieved complete remission and incomplete count recovery. Following fludarabine, melphalan, and thymoglobulin induction chemotherapy, she underwent an allogeneic stem cell transplant with failure to engraft, requiring autologous stem cell rescue, buffy coat, and granulocyte transfusions, eventually presenting with a diffuse skin rash consistent with Steven-Johnson syndrome and toxic epidermal necrolysis, persistent neutropenic fevers and positive blood cultures.

KEYWORDS
immunocompromised, hematopoietic stem cell transplant, bloodstream infection

1 | INITIAL PRESENTATION (KIM YEOH)

A 53-year-old woman living in metropolitan Melbourne, Australia with concurrent acute myeloid leukemia (AML) and multiple myeloma underwent cytarabine and daunorubicin (7+3) induction

List of Abbreviations: AML, acute myeloid leukemia; HCT, hematopoietic cell transplant; ITS, internal transcribed spacer; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; rRNA, ribosomal RNA; SJS, Steven-Johnson syndrome; TENS, toxic epidermal necrolysis.

chemotherapy followed by cytarabine (HiDAC) consolidation. She achieved complete remission and commenced lenalidomide maintenance therapy, however this was ceased after 1 month due to myelosuppression. Eleven months following her initial diagnosis, her AML relapsed and she underwent three cycles of azacitadine and venetoclax therapy, achieving complete remission, however with persistent neutropenia and thrombocytopaenia. She had a neutrophil count of $<0.5 \times 10^9$/L for 1 month, followed by a neutropenia of $<1.0 \times 10^9$/L in the 2 months prior to her admission for her allogeneic hematopoietic cell transplant (HCT). Medications on admission included posaconazole which she was taking 4 months prior to admission, with a serum trough level of 0.77 mg/L. On Day −7 prior to her transplant, reduced-intensity conditioning chemotherapy was initiated with fludarabine, melphalan, and thymoglobulin (Figure 1). On Day −2 she developed febrile neutropenia with no clear focus of infection and empiric piperacillin-tazobactam was started. She was afebrile on the day of HCT (Day 0) with a neutrophil count of 0.0×10^9/L and platelet count of 14×10^9/L. On Day 5, she developed an erythematous macular rash over her chest, neck, and abdomen. She developed recurrent neutropenic fever on Day 7 and empiric antibiotic treatment was transitioned to meropenem. Over the following days, her rash progressed with widespread erythematous plaques over her back, buttocks, vulva, ocular, and oral mucosa, and she had erosions to her back and axillae. She had a body surface area involvement of 45% with 12% epidermal detachment. A skin punch biopsy performed on Day 11 revealed intradermal blisters roofed by necrotic epidermis, consistent with Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis (TENS), with the most likely culprits being either piperacillin-tazobactam or lamotrigine. Pulsed-dose methylprednisolone and intravenous immunoglobulin were initiated as cyclosporin, diprosone, dermeze topical cream, and intraocular steroids.

Two weeks following her HCT, she remained pancytopenic without any signs of engraftment. Over the following few weeks, she developed a number of bloodstream infections, including the following[1]: candidemia due to *Candida glabrata* on blood cultures obtained on Days 18 and 20, treated initially with caspofungin, followed by anidulafungin, in the context of a developing transaminitis[2]; bacteremia due to *Staphylococcus capitis* on Days 31 and 33, for which daptomycin was started in addition to the meropenem[3]; bacteremia due to *Cupriavidus pauculus* on Days 34 and 36, for which a retained femoral central venous catheter guidewire was removed and ciprofloxacin was started. As she had failed to engraft a month post-transplant, on Day 36 she underwent an autologous stem cell rescue. Due to her profound and prolonged neutropenia, she received a buffy coat and granulocytes on Days 40 and 41. On Day 43, whilst still receiving daptomycin, meropenem, ciprofloxacin, and anidulafungin, she developed further fevers with blood cultures obtained revealing gram-positive round sporangia of various shapes and sizes with endospores resembling a 'spoked wheel' (Figure 2A–C). A calcofluor stain revealed sporangia of various shapes and sizes with the absence of budding (Figure 2D). A Biofire FilmArray BCID Panel (Biofire) for blood cultures did not detect yeast targets and a serum cryptococcal antigen was negative.

Question 1 (Dionysios Neofytos)

What is your differential diagnosis?

A. Antibiotic-affected *Candida* spp.
B. *Saccharomyces* spp.
C. *Malassezia* spp.
D. *Prototheca* spp.
E. Dimorphic fungus
F. Other

2 | DIFFERENTIAL DIAGNOSIS AND INTERPRETATION OF MICROBIOLOGICAL RESULTS (CORNELIA LASS-FLÖRL)

My first thought was of a breakthrough fungal infection, including posaconazole-resistant *Candida glabrata* as the patient previously had a *C. glabrata* infection whilst on posaconazole. The Day 43 blood culture Gram stain showed a yeast-like structure on microscopy. So, my initial thought is one of *Candida* spp. However, the Biofire FilmArray was negative for yeast targets. Internal transcribed spacer (ITS) sequencing would be my next investigation. In the meantime, I would have started an echinocandin. Without hyphae on microscopy, it is unlikely to be a *Saccharomyces* spp. and it is not *Malassezia* spp., as this would be very small and is a very atypical organism. The microscopy showing

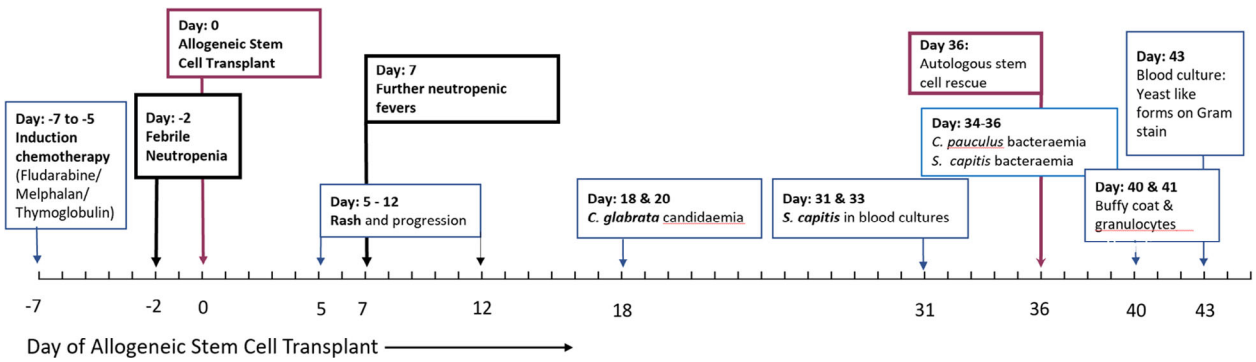

FIGURE 1 Timeline of events in relation to the day of allogeneic stem cell transplant.

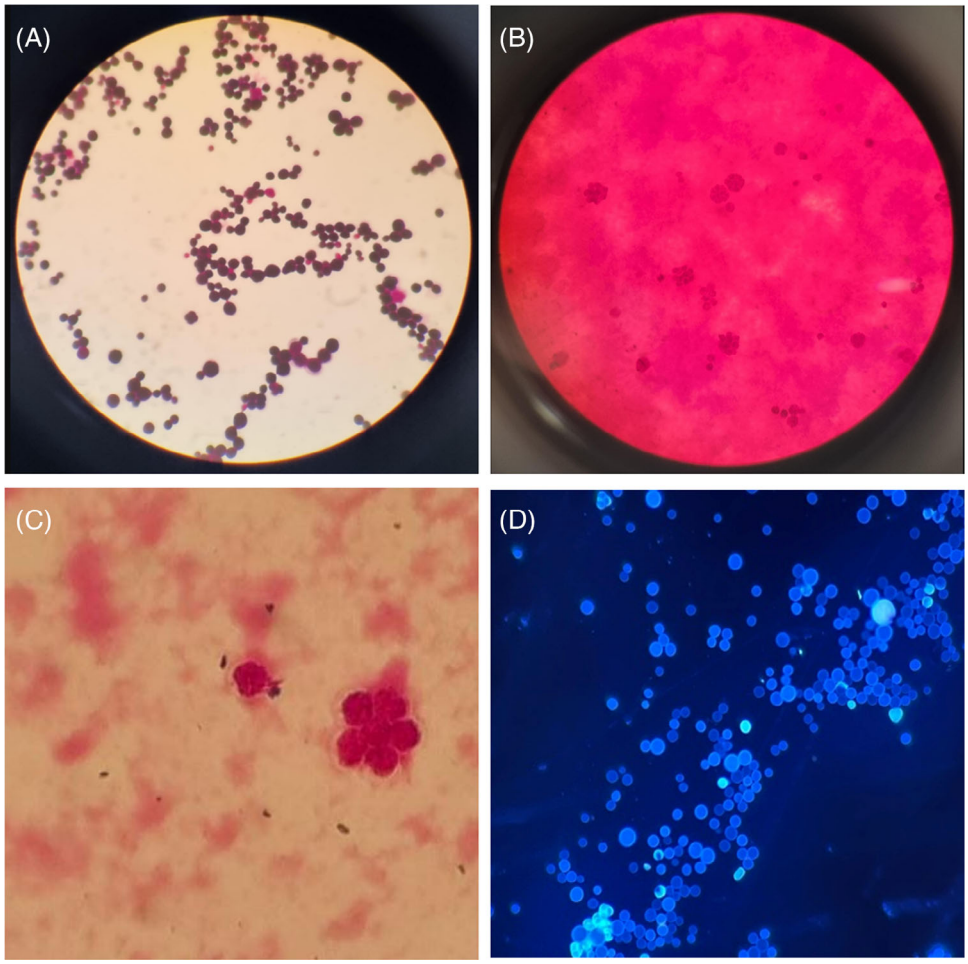

FIGURE 2 Microscopy of the organism: (A–C) Gram stain of blood culture broth that became positive on Day 43 post allogeneic hematopoietic cell transplant (HCT), revealing sporangia of various shapes and sizes. Arrow demonstrating classic "morula" pattern with endospores radially arranged. (D) Calcofluor stain of blood culture broth on Day 43 post-HCT demonstrating sporangia of various shapes and sizes with the absence of budding.

the morula-like structure is very specific for *Prototheca* spp. My answer would therefore be a suspicion of *Prototheca* spp. infection.

3 | FURTHER MICROBIOLOGICAL RESULTS (KIM YEOH)

The blood cultures were inoculated under aerobic conditions at 37°C on both chocolate agar and horse blood agar plates. At 24 h, a growth of fine cream yeast-like colonies was noted (Figure 3), but they were not able to be identified on the horse blood agar (MALDI-TOF) and VITEK yeast ID card (bioMérieux), likely secondary to a mixed culture. However, over the subsequent few days, a new blood culture was flagged positive and easily identified as *C glabrata* on MALDI-TOF. Three days later, there was growth on Sabouraud agar and chocolate plates with colonies of two distinct morphologies: one of a yeast-like organism white smooth-edged colony and the other of a dry, irregular-edged morphology.

Question 2

Based on this additional information, how would your differential diagnosis change?

A. Antibiotic-affected *Candida* spp.
B. *Saccharomyces* spp.
C. *Malassezia* spp.
D. *Prototheca* spp.
E. Dimorphic fungus
F. Other

4 | INTERPRETATION OF FURTHER MICROBIOLOGICAL RESULTS (FRÉDÉRIC LAMOTH)

This is a highly immunosuppressed patient with prolonged neutropenia. She is on broad-spectrum antifungal prophylaxis with posaconazole, as well as prolonged broad-spectrum anti-bacterial therapy, which may induce some imbalance of the normal microbiota of the skin and

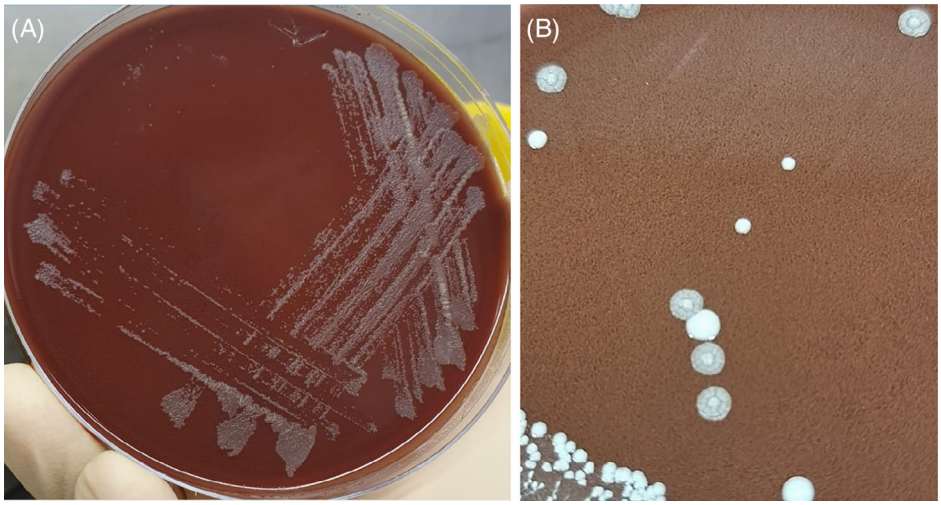

FIGURE 3 Chocolate agar Day 3 of incubation: (A, B) dry white to cream yeast-like colonies, identified as *Prototheca* spp.

VIDEO 1 Video recording of clinicopathologic conference- Bloodstream infection in an allogeneic hematopoietic cell transplant recipient: Thinking beyond the usual. Clockwise from top left: Primary presenter- Kim Yeoh. Moderator- Dionysios Neofytos. Speakers- Cornelia Lass-Florl and Frederic Lamoth.

gut, with a selection of some unusual pathogens. She has impressive skin lesions disrupting the skin barrier, and possibly leading to the introduction of pathogens, which do not look like a typical *Candida* spp. or *Saccharomyces* spp. The Gram stains show Gram-positive yeast-like structures, without filamentations or beading forms. There is also a typical structure called "morula". The agar plates show after only 24 h, a slimy film covering the plates, which after three days forms greyish, slimy, shiny colonies. *Candida* species would be possible as a potential skin organism, however, this would be likely identified on MALDI-TOF, and the plates are not typical for *Candida* spp. *Saccharomyces* spp. are more likely to be of the gut than skin origin and the grey slimy appearance on the plates is not consistent with *Saccharomyces* spp. *Malassezia* spp. is possible from the skin origin, but typically on Gram-stain, you would see forms multiplying by binary fission which is not evidenced here. *Malassezia* would not grow on standard culture media as it is a lipophilic yeast, that needs to grow on specific media usually with olive oil, except for *Malassezia pachydermatis*, which may grow on the plates. *Prototheca* is possible, with a skin portal of entry the presence of a morula, and a thin slimy appearance on plates. Regarding dimorphic fungi, the time of incubation is extremely short for a dimorphic fungus and microscopy is not consistent with this. My answer is *Prototheca* based on the "morula"-like structure and the exclusion of the other options.

5 | FINAL DIAGNOSIS AND CLINICAL PROGRESS (KIM YEOH)

Organism identification of *Prototheca wickerhamii* was confirmed on VITEK 2 yeast ID card (bioMérieux) and via sequencing of the D1/D2

region of the 28S Ribosomal RNA (rRNA) gene.[1] Liposomal amphotericin B was commenced, however, the patient deteriorated clinically three days later and the decision was made with her family to pursue comfort care.

6 | DISCUSSION ON PRIOR EXPERIENCE OF PATIENTS WITH PROTOTHECA AND TREATMENT OF CHOICE (DIONYSIS NEOFYTOS, CORNELIA LASS-FLÖRL AND FRÉDÉRIC LAMOTH)

Dr. Neofytos and Dr. Lamoth have never seen a case of *Prototheca*. In the past 10 years, Professor Lass-Flörl has seen three cases. Sixty percent of the audience had never heard of *Prototheca* previously, with 17% of the audience having seen a patient with *Prototheca* infection. Diagnosis in the laboratory can be challenging. If MALDI-TOF or VITEK fails to identify a yeast-like growth on typical agar, if there is a clinical suspicion of *Prototheca*, repeat microscopy would be advised to look for "morula"-like structures, consistent with *Prototheca*.

Lamoth: For a microbiologist, when facing a possible fungemia, *Candida* is usually thought of, as it represents 95% of fungaemias, followed by *Cryptococcus*, other yeasts, and rarely filamentous fungi. However, when there is an unexpected result, it would be prudent to seek advice from a mycologist with careful screening of microscopic slides to alert you that this is not a *Candida* or yeast and to search for more atypical causes of infection.

Lass-Flörl: *Prototheca* is a skin commensal. In particular, for heavily immunosuppressed patients, one should consider looking for *Prototheca* spp. even if a skin swab is positive for yeast-like organisms. *Prototheca wickheramii* and *Protocheca zopfii* are both on the MALDI-TOF database.

Question 3

What treatment would you recommend?

A. Posaconazole, amphotericin-B
B. Meropenem and levofloxacin
C. Amphotericin-B and doxycycline
D. Caspofungin and posaconazole
E. Meropenem, amikacin, and daptomycin
F. Other

7 | TREATMENT CONSIDERATIONS (FRÉDÉRIC LAMOTH AND CORNELIA LASS-FLÖRL)

Lamoth: The *Prototheca* in this patient was a breakthrough infection with posaconazole. With *Prototheca* spp. there is variable susceptibility to azoles. It is usually sensitive to amphotericin B. *Prototheca* spp. are not susceptible to echinocandins, as *Prototheca* does not have β-glucan in their cell wall. Therefore an amphotericin B-based formulation would be appropriate. Additionally, there is a synergistic action with doxycycline.

Lass-Flörl: On review of the literature, amphotericin B should be one of the most important treatments plus an additional anti-fungal or antibiotic. A first-line recommendation is high-dose lipid-formulation amphotericin B (5 mg/kg daily).

8 | DISCUSSION (KIM YEOH)

Prototheca is an aerobic opportunistic unicellular non-budding alga. It is ubiquitous in the environment. Human infection is rare and classically affects profoundly immunocompromised hosts, however, cases have been described in the immunocompetent host as well.[2] *Prototheca wickerhamii* is the main pathogenic species affecting humans.[2] The patient described was susceptible to prothothecosis, in the context of several well-described, recognized risk factors.[2–4] She was immunocompromised due to her underlying hematological malignancy, administration of high-dose corticosteroids, and prolonged neutropenia in the setting of an allogeneic HCT and failure to engraft.[2,5,6] Her prolonged neutropenia was a contributing factor as qualitative and quantitative defects in neutrophil function compromise the host defense against *Prototheca* species.[7–9] Furthermore, as with our patient, infections caused by bacteria, viruses, and fungi are often reported concurrently with *Prototheca* infection, indicative of the individual's net state of immunosuppression.[6] Other risk factors included her widespread loss of epithelial barrier secondary to SJS and TENS, as well as the presence of prosthetic line-related material.[2,4,10]

Microscopically, *Prototheca* species are 3–10 μm in diameter and form a characteristic "morula"-like ("daisy") pattern of sporangia with endospores radially arranged.[2,4,5] It can take up to 72 h of incubation at 30 or 37°C in aerobic conditions on standard culture media (e.g. blood agar) to detect visible growth of this organism. *Prototheca wickerhamii* is the species most commonly identified in infections in humans and typically displays a hemispheric colony with smooth margins, whereas *Prototheca zopfii* typically has flat colonies with a rough margin.[2] Biochemical methods of identification include auxanographic carbohydrate assimilation assays or semi-automated phenotypic methods (eg VITEK).[5,11] Although MALDI-TOF (Bruker and bioMérieux) can identify *Prototheca* to species level, this was not successful in this case, possibly as clinical isolates of *Prototheca* are not well represented in the databases.[5,12,13] Molecular methods including ribosomal ITS and 28S rRNA sequencing are frequently required to confirm the diagnosis.[14]

Antimicrobial susceptibility testing is not recommended for *Prototheca* species.[5] In vitro antifungal susceptibility can be performed, however there is no direct correlation between in vitro activity and clinical response. Reproducibility is poor and there is an absence of standardized methods and interpretive breakpoints.[2,5,15] Hence, there are no standardized treatment protocols for protothecosis, and evidence for various treatment regimens is limited to case reports and case series.[4,5,15] Treatment is recommended for all cases unless infection is relatively mild and contraindications relatively strong.[5,14] For mild disease (e.g. limited skin infection), oral itraconazole or fluconazole is recommended. For serious disease, intravenous amphotericin B is recommended and if this fails, in vitro data suggest that the addition

of tetracycline may provide a synergistic effect.[2,4,5,9,10,16] Additionally, source control, if feasible, is an important aspect of management.[2]

Outcomes for patients with protothecosis are influenced by the type or extent of disease and whether the patient is immunocompromised.[5] More severe infections tend to occur in immunocompromised individuals.[15] In a review of 211 cases by Todd et al (2018), outcomes of patients with localized skin infection were: 78% of cases experienced cure or improvement, there was 1% mortality and 21% of patients had unknown or unevaluable outcomes or had a recurrence or progression of the disease. For patients with disseminated disease, there was a 56% mortality rate and a 33% cure or improvement rate. There were 19 deaths, with 16 of these patients being immunocompromised and eight of these 16 patients had disseminated disease.[5]

AUTHOR CONTRIBUTIONS

Kim Yeoh was the primary author of the manuscript and the primary speaker for the clinicopathologic conference. Monica A Slavin was the primary physician involved in the clinical care of the patient. Kim Yeoh and Eloise Williams were involved in the work-up and microbiological testing of the patient's microbiological samples. Monica A Slavin and Eloise Williams provided supervision for, and contributed to the writing, reviewing and editing of the manuscript (case presentation and literature review sections). Dionysios Neofytos was the primary supervisor for, and moderator of the clinicopathologic conference. Cornelia Lass-Florl and Frederic Lamoth contributed to the writing, reviewing and editing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Frédéric Lamoth: Research funding from Gilead, MSD, Pfizer, and Novartis. Speaker honoraria from Gilead, MSD, Pfizer, and Mundipharma. All contracts were made with and fees paid to his institution (CHUV).

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable- no new data were generated.

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