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SINGLE CASE REPORT

WILEY mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Candida-reactive T cells for the diagnosis of invasive *Candida* infection of the lumbar vertebral spine

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Summary

Invasive *Candida* infection is the fourth most common bloodstream infection. Blood cultures are the current gold standard diagnostic method, however, false negatives remain a clinical challenge. We developed a new technique measuring *Candida*-reactive T cells as diagnostic read-out for invasive *Candida* infection. In a pilot study, we followed the treatment course of a patient with an invasive *Candida* infection of the lumbar vertebral spine. We present the case of a 56-year-old patient with HIV-associated Burkitt lymphoma who developed septic shock during chemotherapy-induced neutropenia. For the first time, we provide flow cytometry-based diagnostics with *Candida*-reactive T cells for invasive candidiasis with comprehensive MRI imaging. The *Candida*-reactive T cell assay has potential to complement current diagnostic assays for invasive *Candida* infection and thus to support targeted treatment.

KEYWORDS
Candida spondylodiscitis, candidaemia, CD154, flow cytometry, invasive candidiasis, osteomyelitis

1 | INTRODUCTION

Invasive *Candida* infection (ICI) comprises both candidaemia and invasive candidiasis and it is the fourth most common infection among hospitalised patients.[1] *Candida* spp. are opportunistic fungal pathogens and are part of the gastrointestinal flora, but may also cause ICI comprising both candidaemia and invasive candidiasis in immunocompromised patients. Once candidaemia has occurred, *Candida* spp. may disseminate leading to secondary deep-seated infections in liver, spleen, kidneys, eyes, the central nervous system, bones and joints.[2] Incidence rates for ICI differ between 0.19 and 2.5 per 1000 admissions and attributable mortality ranges from 38% to 49%.[2-4]

Current diagnostics of ICI consists of direct tests, such as culture or histopathological analyses. Blood cultures are the gold standard diagnostic method; however, false negatives remain a clinical challenge.[5] Proof of deep organ ICI often requires surgical biopsy which is often contraindicated due to underlying medical conditions. Indirect tests, such as *Candida* mannan antigen, anti-mannan

antibodies and β1,3-D-glucan, exist, however, for interpretation of test results knowledge of pitfalls is important. β-1,3-D-glucan is part of most fungal cells and it is released into blood during invasive fungal diseases. Therefore, it is rather a panfungal diagnostic method not specific for *Candida* species.[5,6] Furthermore, false-positivity of the β-1,3-D-glucan assay remains a clinical challenge.[7,8] *Candida* mannan/anti-mannan testing do not facilitate discrimination between different *Candida* species.[2] PCR-based assays applied upon blood and tissue samples for the diagnosis of ICI are now entering clinical diagnostics, but lack of validation and standardisation have hampered their widespread use.[5,9]

We developed a novel technique measuring *Candida*-reactive, CD69-CD154 double-positive, $CD4^+$ T cells as diagnostic read-out for ICI allowing the identification of *Candida* spp. and thus facilitating targeted treatment.

2 | CASE REPORT

A 56-year-old male patient with HIV-associated Burkitt lymphoma developed septic shock during chemotherapy-induced neutropenia. Peripheral and central venous line blood cultures grew *C. albicans* susceptible to echinocandins, azoles and amphotericin B. Candidaemia was treated with anidulafungin 200/100 mg intravenously for 7 days followed by fluconazole 400 mg intravenously according to current guidelines.[10] Blood cultures were negative from day 1 onwards. The central venous catheter was removed and eye involvement was ruled out. During chemotherapy, posaconazole 200 mg oral solution was administered thrice daily due to aspergillosis suspicious nodular infiltrates found in a chest CT. As the patient recovered, he was discharged to the outpatient clinic and posaconazole was stopped (Figure 1).

One month after discharge blood analyses showed elevated C-reactive Protein (CRP) between 70 and 350 mg/L. Several blood cultures remained sterile.

Four months later, the patient presented with severe lower back pain and hypoesthesia following the left L5 and S1 dermatome. A positron emission tomography computed tomography with [^{18}F] 2-fluoro-2-desoxy-D-glucose (FDG-PET/CT) revealed endplate erosion as well as increased uptake at L3/4 and within the adjacent paravertebral soft tissue indicating an inflammatory process. Magnetic resonance imaging (MRI) confirmed spondylodiscitis and paraspinal psoas abscess (Figure 2). Further blood cultures were negative. Cultures from computer tomography (CT)-guided biopsies of the paraspinal psoas abscess and the spinal disc L3/4 grew pan-susceptible *C. albicans*. Antifungal therapy with anidulafungin 200/100 mg/d was begun and vertebral bone resection and dorsal and ventral spinal fusion were done. The patient recovered unremarkably and at 3 weeks, anidulafungin was replaced with fluconazole 800 mg/d for a total treatment duration of 6 months.

Eight months later, the patient developed profound lower back pain and sub-febrile temperatures. CRP was slightly elevated with 18 mg/L (limit <5 mg/L). Central and peripheral blood cultures and serum β1,3-D-glucan remained negative. An enhanced MRI of the lumbar spine revealed paraspinal abscesses and CT-guided biopsies of the abscesses and the intervertebral disc L 3/4 were performed. PCR was positive for *C. albicans*, but there was no growth in tissue culture. Surgical debridement was performed and tissue culture of surgical samples of L3 and L4 revealed pan-susceptible *C. albicans*. Foreign material was removed and the patient received both, ventral and dorsal spinal fusion. Afterwards, antifungal treatment with caspofungin 70/50 mg was initiated for 8 weeks.

We developed an assay measuring *Candida*-reactive $CD4^+$ T cells as new diagnostic read-out for ICI allowing the identification of *Candida* species. By stimulating peripheral blood monocular cells (PBMC) in vitro with lysate from either *C. albicans*, *Candida glabrata*,

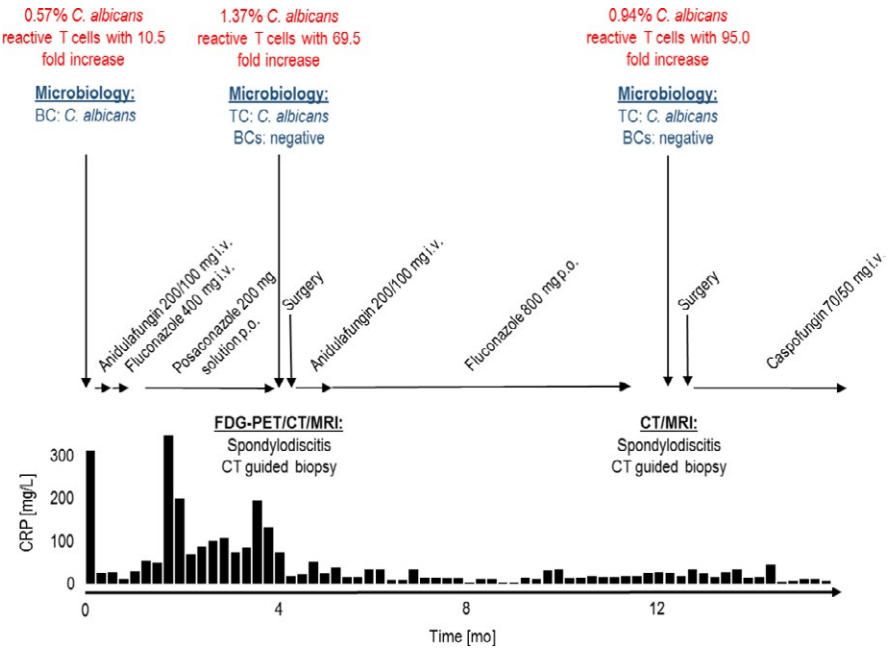

FIGURE 1 Diagnosis and treatment course. CRP, C-reactive Protein (upper limit of normal <5 mg/L); BC, blood culture; i.v., intravenous; FDG-PET/CT, [^{18}F] 2-fluoro-2-desoxy-D-glucose positron emission tomography/computer tomography; MRI, magnetic resonance imaging; TC, tissue culture obtained during either computer tomography-guided biopsy or surgery; p.o., per os

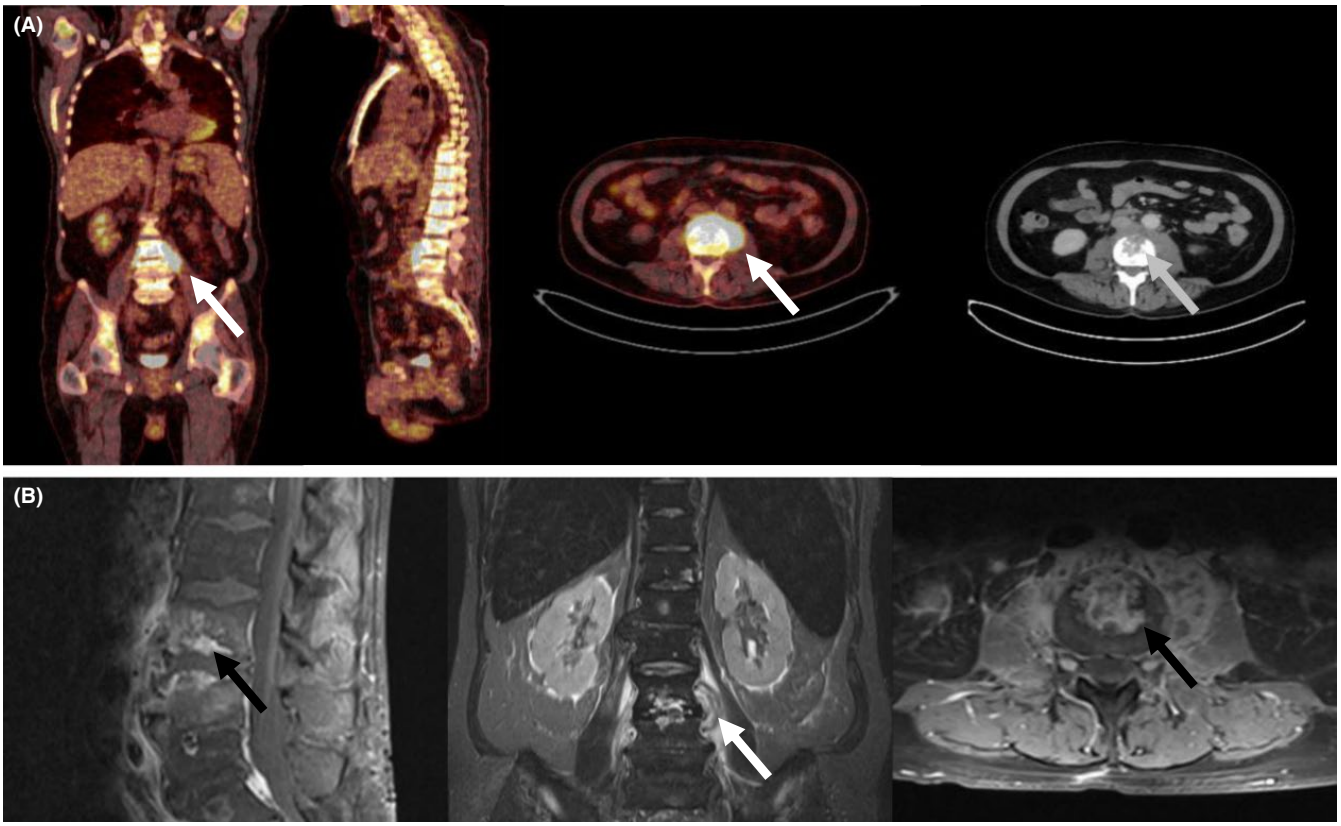

FIGURE 2 Imaging studies of *Candida* spondylodiscitis and paravertebral abscess. (A) Positron emission tomography computer tomography with [^{18}F] 2-fluoro-desoxy-D-glucose (FDG-PET/CT) shows pathological FDG uptake in the l3-l4 vertebrae including the left psoas muscle (white arrows), as well as bone destruction (grey arrow). (B) From left to right: sagittal, coronal and axial magnetic resonance imaging (MRI) of the lumbar spine revealed abnormal signal/enhancement of the L3-4 vertebral marrow (black arrow) including intervertebral space and paraspinal soft tissues (white arrow) indicative for spondylodiscitis and adjacent psoas abscess

Candida parapsilosis, *Candida tropicalis* or *Candida krusei*. CD4$^+$ T cells specifically reactive for these *Candida* spp. were detected based on the upregulation of CD154 (CD40L) and CD69 by flow cytometry firstly described by Bacher et al[11] for mould-reactive T cells. Cut-off values for a positive response for each *Candida* spp. were defined as 0.4% CD69$^+$/CD154$^+$ cells among CD4$^+$ T cells with a simultaneous threefold increase compared to unstimulated CD4$^+$ T cells.

When blood cultures were positive for *C. albicans*, we detected an elevation of 0.57% CD69$^+$/CD154$^+$ *C. albicans* reactive cells among CD4$^+$ T cells with a simultaneous 10.5-fold increase. Four months later, when the *Candida* spondylodiscitis was diagnosed, we determined ICI by measuring 1.37% CD69$^+$/CD154$^+$ *C. albicans* reactive cells among CD4$^+$ T cells with a simultaneous 69.5-fold increase. Further 8 months later, we measured elevated yields of 0.94% CD69$^+$/CD154$^+$ *C. albicans* reactive cells among CD4$^+$ T cells with a simultaneous 95-fold increase (Figure 3).

2.1 | Ethical approval and consent to participate

The patient participated in the biomaterial repository protocol—Improving Diagnosis of Severe Infections of Immunocompromised Patients (ISI) of the University Hospital of Cologne (local ethics identifier 08-160).

3 | DISCUSSION

ICI is, as gold standard, proven by tissue and blood culture, or histopathology.[5] However, blood cultures may remain negative and biopsies carry a significant risk of adverse events and are often contraindicated in the case of thrombocytopenia or coagulopathy.[5]

The *Candida*-reactive T cell assay results correlated with candidaemia and correctly identified the pathogen-causing invasive candidiasis to species level. Bacher and colleagues established the technique of fungus-reactive T cells as a diagnostic read-out for invasive fungal infections due to *Aspergillus* spp. and *Mucorales* as a timely detection of those difficult-to-diagnose fungal pathogens enabling the initiation of a targeted treatment.[11,12]

Wurster and colleagues connected the variability in *Aspergillus fumigatus* reactive T cell frequencies to domestic and work environmental mould exposure, so that in general, cut offs for positivity concerning invasive fungal infection have to be cautiously chosen.[13]

Limitation to the *Candida*-reactive T cell assay are autofluorescence of patient cells and insufficient counts of T cells or antigen presenting cells.[12] Furthermore, the novel *Candida*-reactive T cell assay does not allow susceptibility testing.

The *Candida*-reactive T cell assay has potential to complement current diagnostics for ICI if biopsies or cultures remain negative or

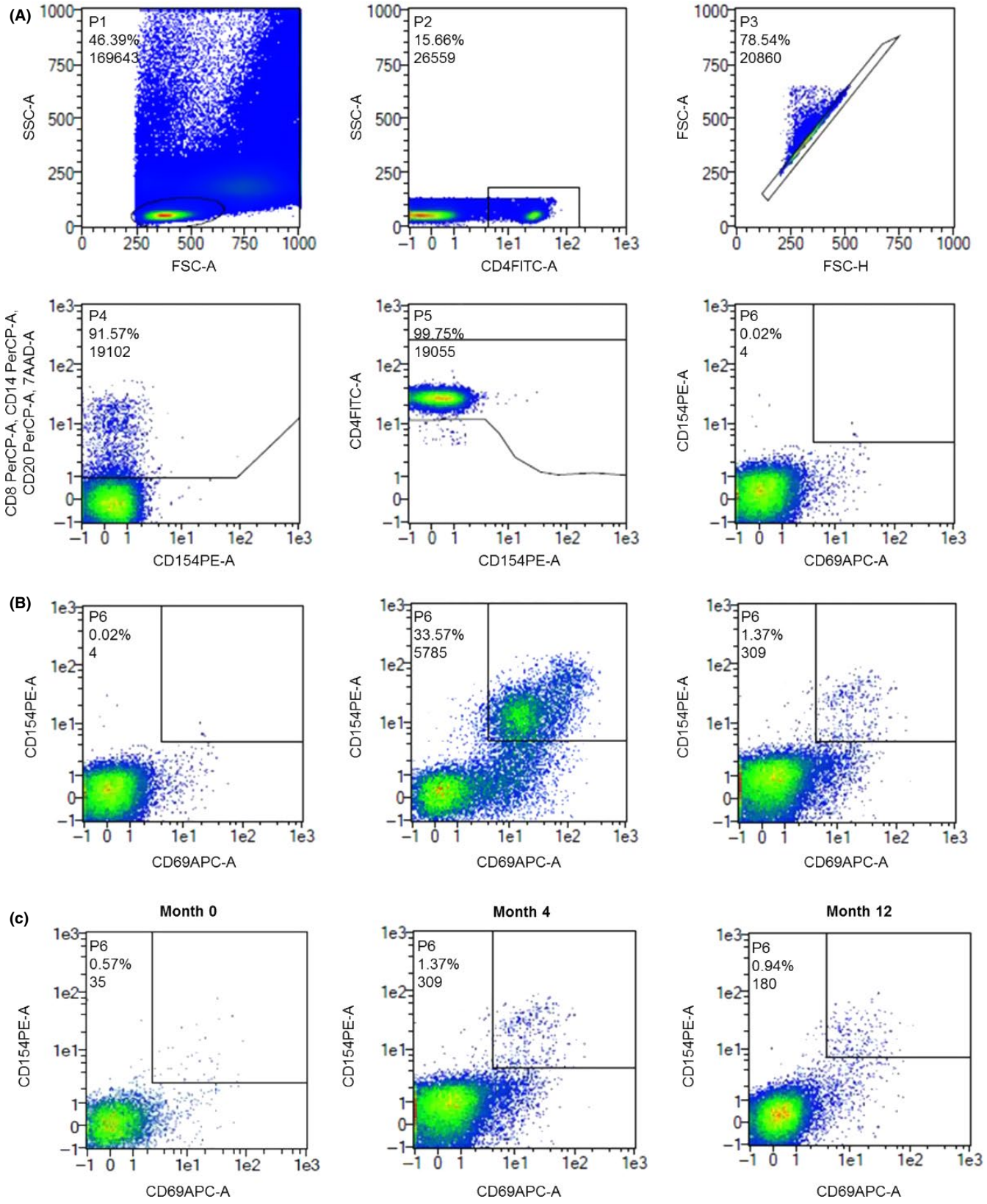

FIGURE 3 Flow Cytometry-Gating strategy and detection of *Candida albicans* reactive T cells. Cell frequencies (%) and absolute cell (n) count are given. (A) Gating strategy. Negative control, unstimulated $CD4^+$ T cells, show no CD69/CD154 expression. (B) Detection of *Candida albicans* reactive T cells. From left to right. Negative control; positive control (staphylococcal enterotoxin B-stimulated $CD4^+$ T cells show CD69-cd154 double-expression); antigen-stimulated probe (CD69-CD154 double-expression of *Candida albicans* reactive $CD4^+$ T cells after antigen stimulation with *Candida albicans* lysate). (C) *Candida albicans* reactive T cells at baseline, at 4 and 12 months. CD69-CD154 double-expression of *Candida albicans* reactive $CD4^+$ T cells after antigen stimulation with *Candida albicans* lysate

cannot be obtained. The *Candida* T cell assay is a novel, non-invasive surrogate marker for ICI, identified *C. albicans* as pathogen and supported targeted treatment.

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CONFLICT OF INTEREST

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AUTHORSHIP/CONTRIBUTION

FCK has contributed towards collection and assembly of data, data analysis and interpretation, manuscript writing and final manuscript approval. OAC has contributed towards collection and assembly of data, data analysis and interpretation, manuscript writing and final manuscript approval. HW has contributed *Candida* strains for mechanical lysis, BDG-data, data analysis and interpretation, manuscript writing and final manuscript approval. DHC has contributed towards interpretation of radiographic data, manuscript writing and final manuscript approval. AR has contributed towards data analysis and interpretation, manuscript writing and final manuscript approval. PK has contributed towards collection and assembly of data, data analysis and interpretation, manuscript writing and final manuscript approval.

PREVIOUS PRESENTATIONS

Meetings where the information has previously been presented: Preliminary data of this case report have been presented at the Annual Meeting of the German Center for Infection Research (DZIF) 2016, Cologne, Germany and at the Frühjahrstagung der Sektion Antimykotische Therapie of the Paul-Ehrlich Gesellschaft für Chemotherapie e.V. (PEG) 2017, Bonn, Germany.

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