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Integrating omics techniques and culture-independent systems may improve the detection of persistent candidemia: data from an observational study

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Abstract

Introduction Blood cultures have low sensitivity for candidemia. Sensitivity can be improved by the culture-independent system T2 Magnetic Resonance (T2). SeptiCyte RAPID is a host response assay quantifying the risk of infection-related inflammation through a scoring system (SeptiScore). We investigate the performance of SeptiScore in detecting persistent candidemia as defined by conventional cultures and T2.

Methods This is a prospective multicentre observational study on patients with candidemia. Blood cultures and blood samples for assessment by T2 and SeptiCyte were collected for 4 consecutive days after the index culture. The performance of SeptiScore was explored to predict persistent candidemia as defined by (1) positive follow-up blood culture (2) either positive follow-up blood culture or T2 sample.

Results 10 patients were enrolled including 34 blood collections assessed with the 3 methods. Overall, 4/34 (12%) follow-up blood cultures and 6/34 (18%) T2 samples were positive. A mixed model showed significantly higher SeptiScores associated with persistent candidemia when this was defined as either a positive follow-up blood culture or T2 sample (0.82, 95%CI 0.06 to 1.58) but not when this was defined as a positive follow-up blood culture only (-0.57, 95%CI -1.28 to 0.14). ROC curve for detection of persistent candidemia by SeptiScore at day 1 follow-up showed an AUC of 0.85 (95%CI 0.52-1.00) when candidemia was defined by positive follow-up blood culture, and an AUC of 1.00 (95%CI 1.00–1.00) when candidemia was defined according to both methods.

Conclusion Integrating transcriptome profiling with culture-independent systems and conventional cultures may increase our ability to diagnose persistent candidemia.

Keywords Candidemia, Blood culture, Rapid diagnostic tests, T2 magnetic resonance, Host-response, Omics

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Introduction

Persistent candidemia is associated with high mortality [1]. Follow-up blood cultures (BCs) are a cornerstone of the management of patients with candidemia, to establish treatment duration and assess the need for source control [2]. BCs however are known to have a low sensitivity for *Candida* spp., with up to 50% of cases missed [3], advocating for more sensitive tests.

The T2 Magnetic Resonance (T2) is a culture-independent system based on a miniaturised magnetic resonance able to detect microbial DNA in the bloodstream with a higher sensitivity compared to conventional cultures [4, 5]. Specifically, in the T2, the microbial DNA amplified by PCR binds by hybridization to probes enriched with superparamagnetic nanoparticles, which allow the identification of the amplicons by changes in the magnetic field with results available in a few hours. Of note, this system enables the detection of intact microbial cells rather than freely circulating DNA, with a limit of detection of 1 CFU/mL [6].

SeptiCyte RAPID is a host-response assay based on transcriptomics able to differentiate sepsis from infection-negative systemic inflammation, representing a novel automated version of a previous manual test (SeptiCyte LAB). SeptiCyte RAPID quantifies the transcription of 2 genes (PLAC8 and PLA2G7) involved in the host-response to infection and stratifies patients according to the likelihood of infection-related inflammation, assigning them a score from 1 to 15 (SeptiScore). SeptiCyte is approved for use in critically ill patients, where it has an Area Under the Receiver Operating Characteristic (ROC) curve (AUC) for the detection of infectious vs. sterile inflammation (sepsis) ranging between 0.68 and 0.95 according to different studies, and a turnaround time of 1 h [7, 8]. No data about the use of SeptiCyte in patients with candidemia are available in the literature.

We hypothesised that during the follow-up days after an index episode of candidemia, higher SeptiScores would be associated with the persistence of *Candida* spp. in the bloodstream while lower scores would be found in case of bloodstream clearance, potentially suggesting the use of SeptiCyte to predict persistent candidemia. We also hypothesised that the use of T2 together with conventional BCs would increase the detection of candidemia, further improving the performance of SeptiScore for the same scope. Hence, in this study we investigate the integrated performance of SeptiCyte, BC and T2 to detect persistent candidemia.

Methods

Study design

This is a prospective multicentre observational study conducted at 3 sites in Australia (The Royal Brisbane and Women's Hospital, Redcliffe Hospital, and Caboolture Hospital) from January 2022 to March 2023, enrolling patients with proven candidemia. After obtaining consent, each patient had research blood samples collected daily for 4 consecutive days since the onset of the candidemia (index positive BC), including BC (1 set), 1 sample for T2 assessment and 1 sample for SeptiCyte assessment.

Definitions

Persistent candidemia at a specific day of follow-up was defined as follows: (1) by the presence of positive BC at that day of follow-up; (2) by the presence of either positive BC or T2 sample at that day of follow-up.

Laboratory methods

BC bottles were incubated in the BacT/Alert Virtuo (bioMérieux). After flagging positive, a Gram stain was performed, and BC media was sub-cultured into agar plates. Microorganisms' identification was performed with the MALDI-TOF MS (VITEK MS, bioMérieux). Whole blood samples for T2 assessment were collected into 4 mL EDTA tubes. Two mL of blood was pipetted into the T2 cartridges which were loaded into the instrument. Blood for assessment by SeptiCyte was collected into PAXgene tubes. SeptiCyte was run on the Idylla platform. Blood samples for T2 and SeptiCyte were either run fresh within 24 h from collection or frozen at -80 °C and run retrospectively after thawing at room temperature.

Statistical analysis

Categorical variables are presented as frequency and proportion (%), continuous variables as median and interquartile range (IQR). Performance of T2 vs. BC was described in terms of agreement. We used a mixed-effect model with random intercepts by patient ID and a fixed effect for day to assess the association of SeptiScore with persistent candidemia. ROC curves were also built to assess the performance of SeptiScore to diagnose persistent candidemia at day 1 follow-up. SeptiScore values according to BC/T2 results at day 1 were compared with the Wilcoxon rank-sum test. Statistical analysis was performed with Stata 17 [9].

Ethical approval

Approval was granted to this study by the Royal Brisbane and Women's Hospital Ethics Committee (HREC/2021/QRBW/70126) and ratified by The University of Queensland (2021/HE000073).

Results

After obtaining consent, 10 patients were included in the study, including 34 blood collections assessed with the 3 methods (BC, T2 and SeptiCyte). Patients' characteristics are reported in Supplementary Table 1. All patients were

male. *C. albicans* was the most frequent species (40%) causing candidemia, followed by *C. glabrata* (30%). Most infections had urinary source (70%) and no patient was diagnosed with metastatic localisation of infection. One patient required admission to the Intensive Care Unit for inotropes support at infection onset. In-hospital mortality was 30%.

Overall, 4/10 patients had positive follow-up BCs, and 3/10 patients had positive follow-up T2 samples. Specifically, 4/34 (12%) follow-up BCs were positive, the remaining 30/34 (88%) were negative. Among follow-up T2 samples, 6/34 (18%) were positive, 25/34 (73%) were negative and 3/34 (9%) gave an invalid result due to failure of internal control. 2 samples were positive by both BC and T2. One patient had one positive BC at follow-up but no positive T2 samples at follow-up. In this patient the sample for assessment by the T2 was collected few hours after the BC due to logistic delays in the admitting ward, while in all other cases the samples for assessment by the 3 methods were collected within the same blood draw. Overall, according to our previously reported definitions of persistent candidemia, 4 blood collections met the definition of persistent candidemia according to positive follow-up BC, and 8 blood collections met the definition of persistent candidemia according to the presence of either positive follow-up BC or T2 sample. Table 1 summarises BC and T2 results, at each day of follow-up for each patient. Supplementary Table 2 reports rates of agreement between T2 and BC. SeptiScore results at each day of follow-up for each patient are reported in the Table 1. Overall, SeptiScores ranged between 4.2 and 9.7.

The association between SeptiScore and the presence of persistent candidemia was explored. When persistent candidemia was defined by the presence of positive BC at follow-up, the mixed model did not show any significant difference between mean SeptiScores in samples with or without persistent candidemia (-0.57, 95%CI -1.28 to 0.14). Conversely, the mixed model showed significantly higher mean SeptiScores in samples with persistent candidemia when this was defined as either positive BC or T2 (0.82, 95%CI 0.06 to 1.58).

Day 1 follow-up had the highest amount of positive results for both tests (BC and T2). Specifically, nine blood collections assessed with the 3 systems were available at day 1 follow-up (1 patient missed the blood collection that day): of those, 2/9 samples met the definition of persistent candidemia according to positive follow-up BC, 4/9 according to either BC or T2 (Table 1). When looking at day 1 follow-up, SeptiScores in samples with persistent candidemia defined by positive BC only was not found significantly higher compared to samples without persistent candidemia (median 8.8, IQR 7.8–9.7 vs. 6.8, IQR 6.1–8.8, $p=0.14$), corresponding to an AUC for persistent candidemia by SeptiScore of 0.85 (95%CI

Table 1 BC, T2 and SeptiScore results at each day of follow-up in the 10 enrolled patients

Pt	BC results				T2 results				Combined T2 and BC results*				SeptiScore			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
1	.	neg	neg	.	.	neg	neg	.	.	neg	neg	.	.	4.4	4.2	.
2	neg	neg	neg	POSITIVE	POSITIVE	POSITIVE	neg	neg	POSITIVE	POSITIVE	neg	POSITIVE	8.9	7.8	5.9	5.6
3	POSITIVE	neg	neg	neg	neg	neg	neg	neg	POSITIVE	neg	neg	neg	9.7	8.8	7.9	7.5
4	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	6.4	5.5	5.2	5.4
5	neg	neg	neg	neg	neg	invalid	neg	neg	neg	neg	neg	neg	6.8	5.7	6.0	6.0
6	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	6.0	5.7	5.4	6.0
7	neg	POSITIVE	.	.	POSITIVE	POSITIVE	.	.	POSITIVE	POSITIVE	.	.	8.8	7.8	.	.
8	neg	neg	neg	.	neg	neg	neg	.	neg	neg	neg	.	6.1	6.4	6.3	.
9	POSITIVE	neg	neg	neg	POSITIVE	neg	POSITIVE	invalid	POSITIVE	neg	POSITIVE	neg	7.8	7.8	8.9	8.7
10	neg	neg	neg	.	invalid	neg	neg	neg	neg	neg	neg	.	7.6	6.5	6.7	6.5

*POSITIVE = positive by either T2 or BC; *neg = negative by both methods (including T2 invalid results)

0.52-1.00) (Fig. 1a). Conversely, when persistent candidemia was defined by either positive BC or T2 sample, higher scores were observed in samples with vs. without persistent candidemia (median 8.9, IQR 8.3–9.3 vs. 6.4, IQR 6.1–6.8, $p=0.014$) corresponding to an AUC of 1.00 (95%CI 1.00–1.00) (Fig. 1b).

Discussion

Our study shows how the integration of conventional testing with novel culture-independent systems and host-response technologies may increase our ability to diagnose persistent candidemia. Previous studies showed how T2 is more sensitive compared to conventional cultures. Specifically, the performance of the T2 on follow-up samples from an index episode of candidemia has been assessed by a large multicentre trial showing how T2 was more likely to be positive than BC in patients receiving antifungals [4]. Similarly, the STAMP trial assessed the role of T2 for monitoring the clearance of candidemia, again showing how only 30% of positive T2 follow-up samples were accompanied by positive BCs [5], and in-vitro studies also confirmed how the T2 performance is not affected by the inoculation of antifungals into BC vials [10]. A preliminary report subsequently suggested that T2 may be able to predict adverse outcomes in patients with proven candidemia [11]. Nonetheless, whether the persistence in the bloodstream of fungal DNA rather than of live culturable microorganisms, may have clinical and prognostic significance is yet to be confirmed in large studies.

Our results confirm a higher positivity rate of T2 compared to BC on follow-up samples of a small group of patients with candidemia. Nonetheless, we also detected some BC+/T2– results highlighting how T2 false negative results exist. One of the two T2+/BC negative discordant cases we detected, may be explained by the delayed collection of the T2 sample compared to the BC, due to logistic delays in the ward. All other daily blood collections were however performed within the same draw. The T2 false negative results highlight the limitations in the accuracy of the T2 technology, suggesting caution in using T2 results to rule out infection or support early step-down of antimicrobials, but rather highlighting its usefulness in combination with, rather than in place of, conventional BCs. A high rate of invalid results was also observed in our study (3/34, 9%), possibly due to the use of frozen rather than fresh samples.

Given the limitations of conventional cultures in diagnosing candidemia [3] and in the perspective of exploiting synergism between novel and conventional diagnostic approaches, we investigated whether SeptiScore could also support clinicians to this scope. Interestingly, SeptiScore showed a better performance in predicting persistent candidemia when this was defined according to either T2 or BC positivity, compared to when persistent candidemia was defined by the use of BC only. This suggests how patients with persistently positive T2 samples in the context of an episode of candidemia may have a high host inflammatory response as measured by the SeptiCyte, again supporting the interpretation of the "T2emia" as a true BSI rather than as the mere persistence of fungal DNA lacking clinical significance, or a false positive result. At the same time, our results suggest a potential use of SeptiScore for detecting persistent candidemia, with higher performance when used in combination with both conventional cultures and novel culture independent testing rather than in association with conventional systems only. Future studies on larger samples size may clarify the performance of this integrated diagnostic approach and its potential clinical applications.

Overall, our observations open interesting research questions on the opportunity of redefining our current

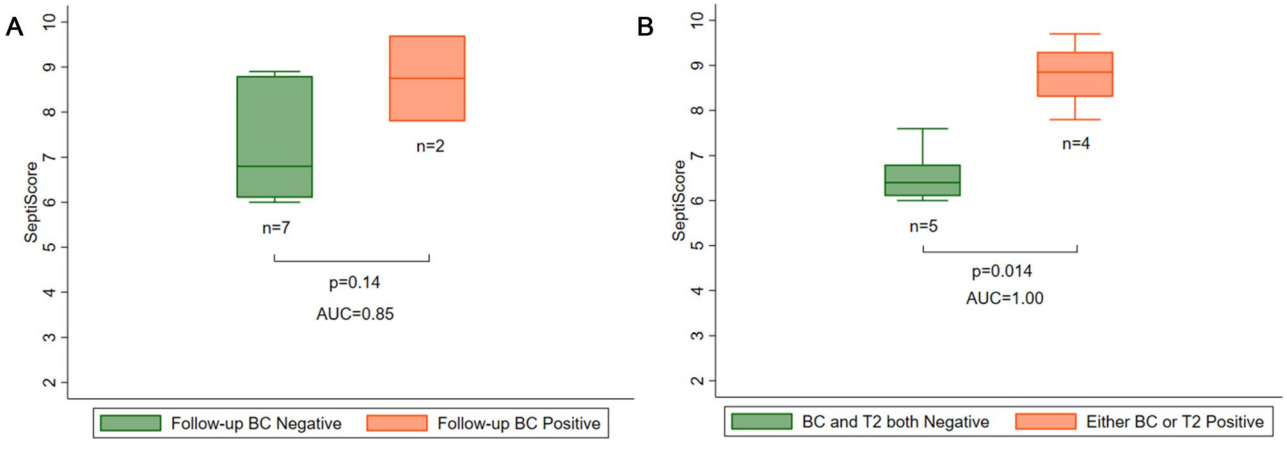

Fig. 1 Box and whisker plots showing the distribution of SeptiScores at day 1 follow-up according to persistent candidemia defined as positive follow-up BC (**a**) and as either positive BC or T2 sample (**b**). (**a**): SeptiScores (day 1) according to persistent candidemia defined by follow-up BC result. (**b**) SeptiScores (day 1) according to persistent candidemia defined by follow-up BC/T2 results

gold standard for the diagnosis of candidemia, and of BSI in general, in an era of rapidly evolving diagnostics. Both culture independent microbiological techniques, and the omics technologies have the potential to transform the diagnostic approach to BSI and sepsis [12].

Our study has several limitations. Firstly, it was a preliminary clinical observation on a very small sample size. Secondly, our definition of persistent candidemia was based on results of single days. However, some patients had intermittent positivity of BC or T2 over the 4 days of follow-up and whether to interpret negative BC/T2 results before a new positivity as true negatives may be questionable. Indeed, we could argue that the clearance of the bloodstream had not happened yet, although the possibility of intermittent candidemia still exists. Thirdly, none of our patients were diagnosed with metastatic infection, so we were unable to explore any association between SeptiScore and metastatic dissemination of candidemia. A fourth limitation pertains to our classification of T2 invalid results as negative when candidemia was diagnosed based on results of both BC and T2. Lastly, we built ROC curves at day 1 only because that day had the highest number of positive results for both tests.

In conclusion, our study suggests how utilising both conventional culture and culture-independent systems may enhance diagnosis of persistent candidemia. Further studies are needed to clarify the role of transcriptome profiling for the diagnosis of candidemia and how this could be integrated with microbiological testing to improve patients' outcomes.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-024-00736-w.

Supplementary Material 1

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Author contributions

DLP and AMP designed the study. AMP, KOC, NR and AT contributed to screening and enrolling eligible patients. HB run the SeptiCyte tests. AMP performed data analysis and wrote the main version of the manuscript. MC supervised statistical analysis and data interpretation. DLP supervised data interpretation and the manuscript drafting. All authors substantially contributed to revising the manuscript and approved its final version.

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Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Approval was granted to this study by the Royal Brisbane and Women's Hospital Ethics Committee (HREC/2021/QRBW/70126) and ratified by the University of Queensland (2021/HE000073). Patients were enrolled after consent was obtained by study team members from patients or their representatives.

Consent for publication

Not applicable.

Competing interests

Competing interests: DLP has research funding from Shionogi, Merck, bioMerieux, BioVersys and Pfizer and has received consulting fees from the AMR Action Fund, CARB-X, Aurobac, Pfizer, Merck, Cepheid, bioMerieux and Spero. PNAH reports research grants from Gilead, has served on advisory boards for OpGen, Merck and Sandoz, and has received honoraria from OpGen, Sandoz, Pfizer and bioMerieux. The other authors declare no conflict of interest.

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