AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MYCOLOGY

Efficacy of T2 Magnetic Resonance Assay in Monitoring Candidemia after Initiation of Antifungal Therapy: the Serial Therapeutic and Antifungal Monitoring Protocol (STAMP) Trial

Eleftherios Mylonakis,[a] Ioannis M. Zacharioudakis,[b] Cornelius J. Clancy,[c] M. Hong Nguyen,[c] Peter G. Pappas[d]

[a]Infectious Diseases Division, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

[b]Department of Medicine, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

[c]University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

[d]University of Alabama at Birmingham, Birmingham, Alabama, USA

ABSTRACT The performance of blood culture for monitoring candidemia clearance is hampered by its low sensitivity, especially during antifungal therapy. The T2 magnetic resonance (T2MR) assay combines magnetic resonance with nanotechnology to identify whole *Candida* species cells. A multicenter clinical trial studied the performance of T2MR in monitoring candidemia clearance compared to blood culture. Adults with a blood culture positive for yeast were enrolled and had blood cultures and T2MR testing performed on prespecified days. Thirty-one patients completed the trial. Thirteen of the 31 patients (41.9%) had at least one positive surveillance T2MR and/or blood culture result. All positive blood cultures (7/7 [100%]) had an accompanying positive T2MR result with concordance in the identified *Candida* sp., while only 7/23 (30.4%) T2MR results had an accompanying positive blood culture. There was one case of discordance in species identification between T2MR and the preenrollment blood culture with evidence to support deep-seated infection by the *Candida* spp. detected by the T2MR assay. Based on the log rank test, there was a statistically significant improvement in posttreatment surveillance using the T2MR assay compared to blood culture ($P = 0.004$). Limitations of the study include the small sample size and lack of outcome data. In conclusion, the T2MR assay significantly outperformed blood cultures for monitoring the clearance of candidemia in patients receiving antifungal therapy and may be useful in determining adequate source control, timing for deescalation, and optimal duration of treatment. However, further studies are needed to determine the viability of *Candida* species cells detected by the T2MR assay and correlate the results with patient outcomes. (This study is registered at ClinicalTrials.gov under registration number NCT02163889.)

KEYWORDS candidemia, invasive candidiasis, molecular diagnostics, monitor, T2 *Candida*, T2MR

Invasive candidiasis is a health care-associated infection with high morbidity and a mortality rate exceeding 40% (1–3). Optimal antifungal therapy and control of the source of infection constitute the cornerstones for successful treatment (4, 5). As part of optimal antifungal therapy, the most recent Infectious Diseases Society of America (IDSA) clinical practice guidelines support follow-up blood cultures every 24 to 48 h in order to monitor clearance of candidemia, determine the need for further interventions for source control, and guide deescalation and the total duration of therapy (6). However, the low sensitivity, suppression by antifungal therapy, and prolonged time to result (3 to 5 days) deem blood cultures a suboptimal tool to guide the treatment of candidemia.

Received 9 November 2017 **Returned for modification** 4 December 2017 **Accepted** 17 January 2018

Accepted manuscript posted online 24 January 2018

Citation Mylonakis E, Zacharioudakis IM, Clancy CJ, Nguyen MH, Pappas PG. 2018. Efficacy of T2 magnetic resonance assay in monitoring candidemia after initiation of antifungal therapy: the Serial Therapeutic and Antifungal Monitoring Protocol (STAMP) trial. J Clin Microbiol 56:e01756-17. https://doi.org/10.1128/JCM.01756-17.

Editor David W. Warnock

Address correspondence to Eleftherios Mylonakis, emylonakis@lifespan.org.

M.H.N. and P.G.P. contributed equally to the study.

More specifically, despite the reliance on blood culture as a monitoring standard to guide therapy, blood cultures perform poorly for detecting invasive candidiasis. In studies comparing their performance with postmortem autopsy results of patients proven to have invasive candidiasis, the sensitivity of blood cultures ranged from 21 to 71% (7). Moreover, blood cultures are heavily influenced by the initiation of antifungal therapy. For example, in a study examining the performance of two commonly used blood culture systems in seeded blood culture bottles, the addition of therapeutic levels of antifungal agents halved the detection rate of *Candida* species (8, 9). Even fungus-specific blood cultures have not been shown to outperform regular blood cultures in clinical trials (10–12); also, they are rarely used in clinical practice and are not included in the recently published guidelines for monitoring of candidemia.

The T2 Magnetic Resonance (T2MR) technology platform combines magnetic resonance with nanotechnology to identify whole *Candida* cells within 3 to 5 h of processing a sample (8, 13). The T2MR assay directly analyzes whole-blood specimens to identify *Candida* spp. without the need for prior isolation of *Candida* cells, with a specificity of 99.4% and sensitivity of 91.1% (14). Importantly, early *in vitro* interference studies for exogenous substances have shown that the T2MR assay is not suppressed by the presence of antifungal agents (8, 15). The purpose of this multicenter prospective clinical trial, designated the Serial Therapeutic and Antifungal Monitoring Protocol (STAMP) trial (registered at ClinicalTrials.gov under registration no. NCT02163889), was to investigate the performance of the T2MR assay as a monitoring tool for posttherapy clearance of candidemia compared to blood cultures.

MATERIALS AND METHODS

T2 magnetic resonance assay. The T2MR is a qualitative assay that utilizes the magnetic resonance-based approach used in magnetic resonance imaging (MRI) technology and is run automatically on the T2Dx instrument. Details about this technology can be found in reference 8. In brief, T2Dx lyses *Candida* cells by mechanical bead beating, uses pan-*Candida* PCR primers to amplify the internal transcribed spacer 2 (ITS2) region within the *Candida* ribosomal DNA operon, introduces superparamagnetic nanoparticles coated with binding agents that target species-specific capture probes nested within the pan-*Candida* amplicons into whole-blood samples, and detects and provides species-level identification by measuring the magnetic resonance signal produced as the result of the agglomeration of the superparamagnetic particles. The T2MR assay has been designed to detect intact *Candida* cells and not circulating DNA due to the unclear clinical relevance of circulating pathogen DNA as a marker of infection (8).

In order to monitor for the presence of inhibitors, T2MR processes an internal control with each clinical specimen. If the internal control is invalid and there are no positive T2MR signals, an "invalid" result is displayed, indicating the possible existence of an inhibitor that interferes with *Candida* detection. The T2MR assay is designed to detect 5 *Candida* spp.: *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida glabrata*, and *Candida parapsilosis*. The T2MR results are grouped, and one of 3 results is reported: *C. albicans*/*C. tropicalis*, *C. krusei*/*C. glabrata*, or *C. parapsilosis* on the basis of antifungal resistance patterns of the aforementioned *Candida* species (6).

Trial design. The STAMP clinical trial was conducted between September 2014 and April 2017 at 3 centers in the United States. Patients age 18 to 95 years with a blood culture positive for yeast receiving or scheduled to receive antifungal therapy within 12 h from the positive blood culture result were eligible for participation in the study. Patients had to be enrolled within 36 h from the positive blood culture result. Patients receiving any novel drug compound within 30 days prior to potential enrollment were excluded from the study. In cases where the yeast identified in the preenrollment blood culture was different from the 5 *Candida* spp. targeted by the T2MR technology, patients were withdrawn from the study and no further blood draws were performed. The institutional review board of each center approved the study protocol. Written informed consent was obtained from all patients.

Sample collection and outcomes. A set of aerobic and anaerobic blood cultures and 3 whole-blood T2MR specimens (tubes A, B, and C) were collected from each participant on the day of enrollment (day 0) and then on days 3, 5, and 7 or until hospital discharge (Fig. 1). Only the data of patients who had at least 2 surveillance sets of T2MR specimens and blood cultures collected were used in the study analysis. A window of 1 day was allowed for study visits to account for weekends and holidays. Patients who were discharged before having 2 specimens collected were excluded from the study. The T2MR results were not used for clinical decision-making, and the antifungal regimen was determined in accordance with routine institutional practice.

T2MR specimens and blood cultures were collected at the same time from the same anatomical collection site either through a peripheral venipuncture or from a central line or port. T2MR clinical specimens were collected in K2 EDTA plastic blood collection Vacutainers. Tube A was stored at room temperature (20°C to 25°C) and was analyzed within 12 h of collection. Tube B was refrigerated up to 72 h until tube A was successfully run and was then frozen if not tested. Tube C was maintained in frozen

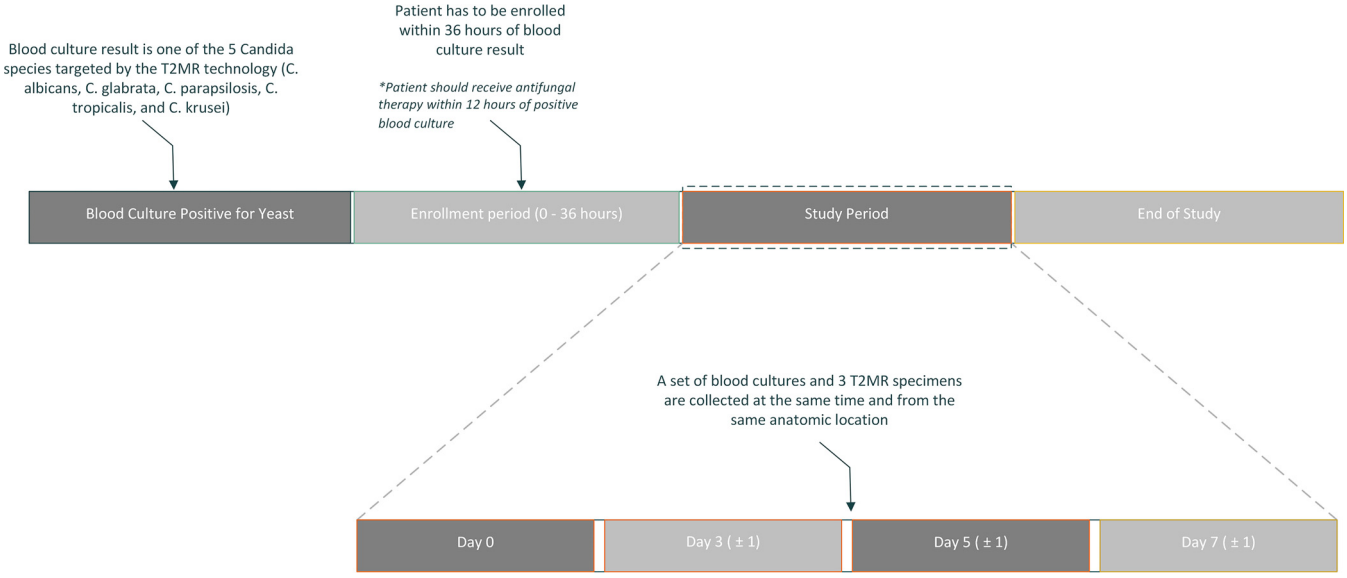

FIG 1 Graphic representation of study design.

storage (−70°C to −80°C) immediately after collection. The blood cultures were processed in accordance with routine institutional practice for a period of 5 days or until a positive blood culture result was reported, whichever occurred first. The BacT/Alert 3D, Bactec FX, and VersaTREK blood culture systems were used in the participating study centers. In cases of positive blood cultures, the species of the bloodstream isolate was identified with matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), Vitek 2, Microscan, or a peptide nucleic acid (PNA) probe, in accordance with routine institutional practice.

Statistical analysis. The collected data were represented as lifetime data, and the Kaplan-Meier estimator was used to measure the length of time patients remained candidemic on the 2 diagnostic methods. A log rank test was used to compare the above-mentioned distributions of the two samples. Categorical data were presented as relative frequencies and were compared using the chi-square test. Statistical significance was set at 0.05. Statistical analysis was performed using the Stata version 14 software package (Stata Corporation, College Station, TX, USA).

RESULTS

Overall, 188 patients were screened, of whom 42 patients met the inclusion criteria and consented to participate in the clinical trial. Among those, 6 patients had either an inadequate number of samples collected or samples were collected outside the prespecified window, 3 patients had blood cultures with *Candida* spp. other than the 5 detected by the T2MR assay (1 *Candida lusitaniae*, 1 *Candida guilliermondii*, and 1 *Candida dubliniensis*), 1 withdrew from the study, and 1 eventually grew *Trichosporon asahii*, initially identified as yeast on the preenrollment blood culture, and was excluded from the study (Fig. 2). Thirty-one patients completed the study, and their data were used for the study analysis. All patients had a single *Candida* sp. isolated from their preenrollment blood culture, except for 1 patient with both *C. albicans* and *C. parapsilosis*. The frequencies of isolated *Candida* spp. among the 31 patients were *C. glabrata*, 12/31 (38.7%), *C. albicans*, 11/31 (35.5%), *C. tropicalis*, 4/31 (12.9%), *C. parapsilosis*, 2/31 (6.5%), *C. krusei*, 1/31 (3.2%), and *C. albicans*/*C. parapsilosis*, 1/31 (3.2%) (Table 1).

In 18 patients (58.1%), all surveillance T2MR specimens and blood cultures collected for the purposes of the study were negative. Patient data for the remaining 13 patients (41.9%), who had at least one positive surveillance blood culture or T2MR test result, are outlined in Table 1. In total, out of the 93 sets of blood cultures and T2MR specimens that were collected, 7 blood cultures (7.5%) versus 23 T2MR specimens (24.7%) were positive ($P = 0.001$) in 4 (12.9%) and 13 (41.9%) unique patients, respectively ($P = 0.01$) (Table 1). Of note, all positive surveillance blood cultures had a positive accompanying T2MR result with concordance in the identified *Candida* sp. (7/7 [100%]), compared to only 7/23 (30.4%) positive T2MR results with an accompanying positive blood culture

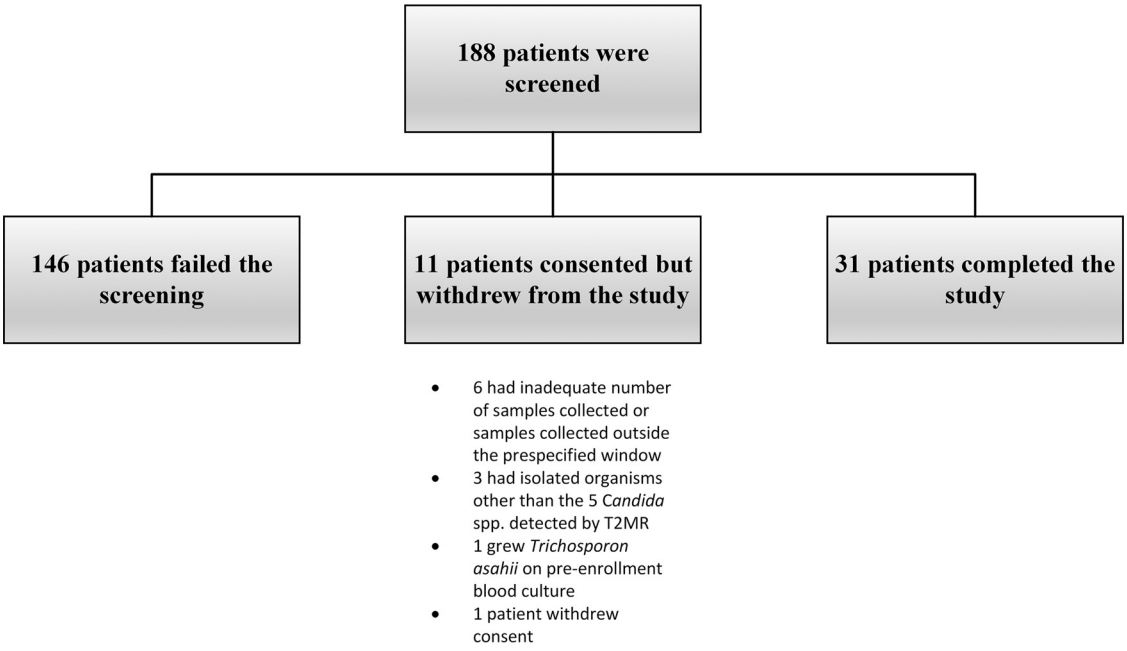

FIG 2 Flow chart of patients in the study.

(Table 1). In 4 out of 13 patients, T2MR specimens and blood cultures were not collected on at least one of the prespecified study visits (on one visit in patients 5, 8, and 11 and on two visits in patient 13). This was due to visits falling on holiday weekends or due to device malfunction. In patient 3, the T2MR assay result was invalid on day 7 on both tubes A and B.

By the end of the first surveillance week, candidemia was still detected in 18.2% of patients (2/11) by the T2MR assay (patients 2 and 4) versus 0% by the blood cultures. The Kaplan-Meier curves showing the length of time that the 31 patients remained candidemic by the 2 diagnostic methods are presented in Fig. 3. Based on the log rank hypothesis, which was used to compare the time-to-negative-result distributions for the 2 surveillance methods, there was a statistically significant improvement in post-treatment surveillance using the T2MR test compared to the regular blood cultures (chi-square, 8.2; $P = 0.004$) (Fig. 3).

Given the intermittent presence of *Candida* species cells in the blood of patients with invasive candidiasis, we reviewed the data of the patients with positive T2MR results to analyze the frequency of detection of patients with ongoing infection during each one of the sampling days (Table 1). Among the total of 13 patients with positive T2MR results during the study, 9 patients had a positive result on day 0 (Table 1). The addition of a second blood draw on day 3 allowed the detection of 3 more patients with ongoing infection who had a negative T2MR result on day 0 (patients 1, 9, and 13), increasing the detection rate by 33.3%. On day 5, another patient with active infection but negative prior specimens was identified (patient 10), but that patient had a different *Candida* sp. (*C. albicans*) detected from the preenrollment blood culture (*C. glabrata*), with evidence to suggest that this represented a new deep-seated infection, with *C. albicans* isolated from a biloma drainage culture. Sampling on day 7 did not identify any patients who did not already have positive results from previous blood draws.

DISCUSSION

Even though clinical practice guidelines support the use of follow-up cultures in order to monitor the clearance of candidemia and guide therapy (6), blood cultures are a suboptimal tool to guide the treatment of candidemia. In the STAMP trial, we studied the performance of the T2MR assay as a monitoring tool for mycologic response to

TABLE 1 Baseline characteristics of study participants with positive surveillance T2MR and/or blood culture results and individual T2MR and blood culture results for subjects with at least 1 positive surveillance test result over the first week of monitoring[a]

Study ID[b]	Age (yr)	Acute medical condition(s)[c]	Patient location[d]	Time of enrollment from day of positive culture	Antifungal therapy prior to enrollment[e]	Central line/ port at the time of enrollment?	*Candida* sp(p). in preenrollment blood culture, time from culture positivity for yeast to species identification	Time of removal of central lines
1	42	Abdominal surgery	MICU	20 h	FLC started on day of positive culture	Yes	*C. glabrata*, 6 days	Between days 3 and 5
2	56	CV disease, IDDM, renal insufficiency	CCU	8 h	MFG started on day of positive culture	Yes	*C. tropicalis*, 3 days	Between days 0 and 3
3	51	Colon cancer	Medical ward	28 h	MFG started on day of positive culture	Yes	*C. albicans*, 4 days	Between days 0 and 3
4	23	Severe burns, abdominal surgery, severe neutropenia	MICU	26 h	MFG started on day of positive culture	Yes	*C. tropicalis*, 3 days	Between days 5 and 7
5	54	Bowel transplant, on steroid tx, TPN dependent	SICU	28 h	FLC started on day of positive culture and was changed to CAS the following day	Yes	*C. tropicalis*, 5 days	Between days 0 and 5[h]
6	66	Pancreatic cancer, abdominal surgery, mild neutropenia	Surgical oncology	5 days 6 h[i]	CAS started on the 3rd day after positive culture	Yes	*C. glabrata*, 14 days	Between days 0 and 3
7	35	CV disease, IVDU	Cardiology	9 h	CAS started on day of positive culture	No	*C. parapsilosis*, 2 days	No central line in place at the time of study enrollment
8	42	FAP, abdominal surgery	SICU	25 h	FLC started on day of positive culture	Yes	*C. albicans*, 5 days	Between days 0 and 3
9	47	Sickle cell disease, CV disease	Medical ward	23 h	FLC started on the 1st day after positive culture	Yes	*C. albicans*, 1 day	Not removed
10	51	CV disease, abdominal surgery	Medical ward	34 h	VCZ started on the 2nd day after positive culture	No	*C. glabrata*, 2 h	No central line in place at the time of study enrollment
11	58	CV disease, IDDM, spinal surgery	Medical ward	21 h	FLC started 2 days prior to positive culture	Yes	*C. glabrata*, 15 h	Between days 0 and 5[h]
12	28	Brain tumor, CV disease	Medical ward	6 h	CAS started on day of positive culture	Yes	*C. albicans*, 2 h; *C. parapsilosis*, 7 h	Between days 0 and 3
13	25	Not available	Medical ward	15 h	CAS started on day of positive culture	Yes	*C. albicans*, 12 h	Not removed

[a]Days are defined from study enrollment.
[b]ID, identification.
[c]CV, cardiovascular; IDDM, insulin-dependent diabetes mellitus; tx, therapy; TPN, total parenteral nutrition; IVDU, intravenous drug use; FAP, familial adenomatous polyposis.
[d]MICU, medical intensive care unit; CCU, coronary care unit; SICU, surgical intensive care unit.
[e]FLC, fluconazole; MFG, micafungin; CAS, caspofungin; LAB, liposomal amphotericin B; VCZ, voriconazole.
[f]Samples were collected within a +1-day window from the prespecified study visit to account for weekends and holidays.
[g]Samples were collected within a −1-day window from the prespecified study visit to account for weekends and holidays.
[h]Day 3 visit was not performed.
[i]Protocol deviation regarding timing of study enrollment.
[j]A, *C. albicans*; P, *C. parapsilosis*; T, *C. tropicalis*; A/T, *C. albicans*/*C. tropicalis*; K/G, *C. krusei*/*C. glabrata*.

antifungal therapy in patients with candidemia. We followed 31 candidemic patients during their treatment with antifungal agents. All positive surveillance blood cultures had an accompanying positive T2MR result with concordance in the identified *Candida* sp., while only 7/23 (30.4%) T2MR results had an accompanying positive blood culture. Interestingly, we found a statistically significant improvement in posttreatment surveillance using the T2MR test compared to the regular blood cultures based on the Kaplan-Meier curves, with 18.2% of patients (2/11) remaining candidemic by the end of the first surveillance week based on the T2MR assay compared to none based on the blood cultures. All T2MR results were in agreement in terms of species-level identification both with the paired and the preenrollment blood cultures, except for one patient (patient 10) for whom the T2MR sample on day 5 was positive for a different *Candida* sp. from the preenrollment blood culture, with evidence to support deep-seated infection from this species.

This study provides evidence that the T2MR assay might outperform blood cultures in monitoring the clearance of *Candida* spp. in candidemic patients who are on

TABLE 1 (Continued)

Antifungal therapy during study enrollment	Test result by day and type[i]							
	Day 0		Day 3		Day 5		Day 7	
	T2MR	Blood culture	T2MR	Blood culture	T2MR	Blood culture	T2MR	Blood culture
FLC changed to MFG on day 5	Negative	Negative	Positive K/G[f]	Negative[f]	Negative	Negative	Negative	Negative
MFG changed to FLC on day 2	Positive A/T	Negative	Negative[f]	Negative[f]	Positive A/T	Negative	Positive A/T	Negative
MFG changed to FLC on day 3	Positive A/T	Positive A	Positive A/T[g]	Positive A[g]	Negative[f]	Negative[f]	Invalid	Negative
MFG changed to FLC on day 5	Positive A/T	Negative	Negative[f]	Negative[f]	Positive A/T	Negative	Positive A/T	Negative
CAS	Positive A/T	Positive T	Not done	Not done	Negative	Negative	Negative[g]	Negative[g]
CAS changed to FLC on day 0	Positive K/G	Negative	Positive K/G[g]	Negative[g]	Positive K/G[f]	Negative[f]	Negative[f]	Negative[f]
CAS changed to FLC on day 2 and FLC to LAB on day 7	Positive P	Positive P	Positive P[f]	Positive P[f]	Positive P	Positive P	Negative[g]	Negative[g]
FLC	Positive A/T	Positive A	Negative	Negative	Not done	Not done	Negative	Negative
FLC changed to CAS on day 1 and back to FLC on day 5	Negative	Negative	Positive A/T[f]	Negative[f]	Positive A/T	Negative	Negative	Negative
VCZ changed to CAS on day 0	Negative	Negative	Negative[g]	Negative[g]	Positive A/T[g]	Negative[g]	Negative	Negative
CAS	Positive K/G	Negative	Not done	Not done	Negative[f]	Negative[f]	Negative[f]	Negative[f]
CAS changed to LAB on day 1	Positive A/T	Negative	Negative	Negative	Negative[f]	Negative[f]	Negative[f]	Negative[f]
CAS	Negative	Negative	Positive A/T	Negative	Not collected	Not collected	Not collected	Not collected

antifungal treatment. This could be at least partially explained by the fact that T2MR results are not suppressed by antifungal agents. Indeed, published studies have demonstrated a decrease in the performance of blood cultures in detecting candidemia in the presence of therapeutic levels of antifungal agents (9). While the T2MR assay detects whole cells and not cell fragments or free DNA, *in vitro* studies suggested that the result is not inhibited by antifungal agents (8). This observation was also supported

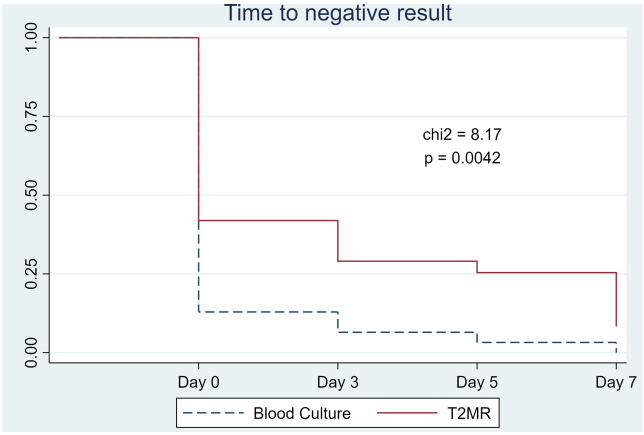

FIG 3 Kaplan-Meier diagram for the first week of surveillance. chi2, chi-square test.

in the current clinical trial by the fact that none of the 31 patients who were on antifungal treatment had persistently invalid T2MR results that, as explained in Materials and Methods, would indicate the potential inhibition of the T2MR assay by the presence of therapeutic levels of antifungal agents.

Another factor that may have contributed to the significantly improved performance of the T2MR assay could be the long time to positivity for specific *Candida* spp., such as *C. glabrata*, that frequently exceeds the typical 5-day period during which cultures are processed (16, 17). This becomes particularly important, as in an expanding number of clinical centers, non-*albicans Candida* spp. represent >50% of clinical isolates (18). Indeed, in this trial, *C. glabrata* was detected in almost 39% of patients (12/31) and was the most frequent isolate among the enrolled patients. All subsequent surveillance blood cultures of those patients were negative, in comparison to 5 positive surveillance T2MR results in 3 different patients (patients 1, 6, and 11).

The 2016 IDSA guidelines recommend empirical treatment with an intravenous echinocandin and subsequent transitioning to oral fluconazole as soon as surveillance cultures are negative (for patients who are clinically stable and have a fluconazole-susceptible isolate). Based on studies using data from autopsy-proven cases of invasive candidiasis, we know that the sensitivity of blood cultures for diagnosing invasive candidiasis is roughly 50%, in a large part because of the intermittent nature of candidemia in deep-seated infections (7). In this study, a requirement of two consecutive negative T2MR results to document the clearance of *Candida* from the bloodstream increased the possibility of detecting ongoing fungemia by 33.3%. This seems to indicate that more than one T2MR result would be needed to demonstrate candidemia clearance. However, future clinical trials with a collection of surveillance samples for longer periods are needed to determine the required number of negative results that would be enough to justify a mycologic response to therapy.

The rapid turnaround time of the T2MR assay (mean time to negative result, 4.2 ± 0.9 h; mean time to species identification, 4.4 ± 1.0 h [14]) compared to the 5 days that blood cultures take to finalize can hasten the adjustment of antifungal therapy by 3 days, even when requiring 2 consecutively negative T2MR results within 48 h and 1 negative blood culture to document clearance. Echinocandins, the recommended first-line treatment for candidemia, are generally well tolerated (19, 20). Deescalation to another class of antifungals can reduce the risk of resistance development, since echinocandin resistance is almost always associated with previous exposure and prolonged treatment courses (21). The faster transition to oral therapy would also be expected to have implications in reducing hospital costs by reducing the length of stay, among other reasons (22).

Additionally, the rapid turnaround time of the T2MR assay could allow for timely adjustment of the treatment plan. Mortality in candidemia is closely linked to source control, with mortality rates approaching 100% in patients with septic shock without timely source control (4). Central venous catheters (CVCs) are not always the source of infection (6). Persistently positive T2MR results in patients with a CVC may, however, be the determining factor in a decision to remove a CVC. Further, consistently positive results in a patient after CVC removal may point to deep-seated infection and alert the clinician to the need for an additional diagnostic work-up to rule out visceral candidiasis. Indeed, in our study, we observed one patient with a positive T2MR result 5 days after enrollment, following 2 negative surveillance T2MR specimens, and this correlated with the diagnosis of *Candida* biloma (patient 10). Even though in this case the bile drainage culture was already finalized as being positive for *C. albicans* 2 days prior to the collection of the T2MR sample, this finding indicates that the T2MR assay can detect deep-seated candidiasis and potentially guide treatment decisions in cases where deep culture data are not available.

It should be noted that using the T2MR assay for monitoring candidemia is limited by the ability to recognize only 5 *Candida* species. This is particularly important in the era of increasing prevalence of other *Candida* spp., especially *Candida auris*, worldwide. However, based on worldwide registry data, over 92% of invasive disease is still caused

by the 5 *Candida* spp. detected by the T2MR assay (23). Indeed, out of the 43 patients who consented to participate in the study, only 3 (7%) patients were excluded due to the isolation of *Candida* spp. other than the ones detected by the T2MR assay. Another potential shortcoming of the T2MR assay is that it may detect nonviable whole *Candida* cells after initiation of antifungal therapy. This potentially explains the intermittent positive T2MR results (i.e., patients 2 and 4). Furthermore, the intermittent release of *Candida* cells into the blood of patients with deep-seated candidiasis or the different *Candida* burden during the disease course would be another plausible explanation, but further studies are needed to justify this. Weaknesses of the study include the small sample size and the fact that we did not use the T2MR results to guide clinical decision-making, so as to correlate the results with patient outcomes. Thus, it is uncertain whether incorporating T2MR monitoring into clinical practice for the management of known candidemia leads to improved clinical outcomes, a decrease in the duration of intravenous antifungal treatment, side effects from antifungals, and the emergence of resistant *Candida* spp.; this is to be shown in future studies. Furthermore, despite some evidence in this study that T2MR might be able to detect at least some cases of deep-seated candidiasis, future studies are needed to determine the performance of the T2MR assay with invasive candidiasis without candidemia. The above-mentioned concerns should be addressed in well-designed clinical trials which will incorporate T2MR monitoring into critical management decisions among patients with proven and suspected candidemia.

The incorporation of the T2MR assay in daily practice is anticipated to pose financial challenges to the hospital budget (24, 25). Bilir et al. studied the economic impact of incorporating the T2MR assay in diagnostic protocols in certain high-risk patient populations with a 3% prevalence of disease, such as critical care admissions, solid organ transplantation, hematopoietic stem cell transplantation, and oncology patients (26). The diagnostic strategy that incorporated the T2MR assay was estimated to result in potential annual savings of $5,858,448 and a 47.6% reduction of cost in a 500-bed hospital, with the savings mostly driven by a reduction in mortality and days of hospital stay.

In conclusion, the finding of this clinical trial that almost one-third of patients had ongoing fungemia after the first negative blood culture indicates the need to revisit the way we currently determine the duration of therapy starting from the first negative blood culture. The STAMP trial provides strong evidence that among patients on antifungal therapy, the T2MR assay can be used to detect ongoing candidemia in a more timely fashion than the traditionally used blood cultures. The T2MR assay has the potential to be a monitoring tool for patients with invasive candidiasis and, given its quick turnaround, may provide actionable information to adjust treatment.

ACKNOWLEDGMENTS

This work was supported by T2 Biosystems.

E.M. has received grant support from Boehringer Ingelheim, T2 Biosystems, and Astellas and has served on an advisory board for Astellas. M.H.N. has received grants from the National Institutes of Health and investigator-initiated research grants from Merck, Astellas, and Cidara. P.G.P. has received grants/research support from T2 Biosystems, Cidara, Scynexis, IMMY Diagnostics, Astellas, Gilead, and Merck and is on an *ad hoc* scientific advisory committee for T2 Biosystems, Merck, Astellas, Amplyx, Viamet, Matinas, and Gilead. All other authors report no potential conflicts.

REFERENCES

1. Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J, Ray SM, Thompson DL, Wilson LE, Fridkin SK, Emerging Infections Program Healthcare-Associated Infections, Antimicrobial Use Prevalence Survey Team. 2014. Multistate point-prevalence survey of health care-associated infections. N Engl J Med 370:1198–1208. https://doi.org/10.1056/NEJMoa1306801.
2. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M. 2012. The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis 73:45–48. https://doi.org/10.1016/j.diagmicrobio.2012.02.001.
3. Arvanitis M, Anagnostou T, Fuchs BB, Caliendo AM, Mylonakis E. 2014. Molecular and nonmolecular diagnostic methods for invasive fungal infections. Clin Microbiol Rev 27:490–526. https://doi.org/10.1128/CMR.00091-13.
4. Kollef M, Micek S, Hampton N, Doherty JA, Kumar A. 2012. Septic shock attributed to Candida infection: importance of empiric therapy and

source control. Clin Infect Dis 54:1739–1746. https://doi.org/10.1093/cid/cis305.
5. Morrell M, Fraser VJ, Kollef MH. 2005. Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother 49:3640–3645. https://doi.org/10.1128/AAC.49.9.3640-3645.2005.
6. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. 2016. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis 62:e1–e50. https://doi.org/10.1093/cid/civ1194.
7. Clancy CJ, Nguyen MH. 2013. Finding the "missing 50%" of invasive candidiasis: how nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis 56:1284–1292. https://doi.org/10.1093/cid/cit006.
8. Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D, Blanco M, Demas V, Skewis LR, Anagnostou T, Coleman JJ, Wellman P, Mylonakis E, Lowery TJ. 2013. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med 5:182ra154. https://doi.org/10.1126/scitranslmed.3005377.
9. Riedel S, Eisinger SW, Dam L, Stamper PD, Carroll KC. 2011. Comparison of BD Bactec Plus Aerobic/F medium to VersaTREK Redox 1 blood culture medium for detection of Candida spp. in seeded blood culture specimens containing therapeutic levels of antifungal agents. J Clin Microbiol 49:1524–1529. https://doi.org/10.1128/JCM.02260-10.
10. Morrell RM, Jr, Wasilauskas BL, Steffee CH. 1996. Performance of fungal blood cultures by using the Isolator collection system: is it cost-effective? J Clin Microbiol 34:3040–3043.
11. Mess T, Daar ES. 1997. Utility of fungal blood cultures for patients with AIDS. Clin Infect Dis 25:1350–1353. https://doi.org/10.1086/516140.
12. Vetter E, Torgerson C, Feuker A, Hughes J, Harmsen S, Schleck C, Horstmeier C, Roberts G, Cockerill F, III. 2001. Comparison of the Bactec MYCO/F Lytic bottle to the isolator tube, Bactec Plus Aerobic F/bottle, and Bactec Anaerobic Lytic/10 bottle and comparison of the Bactec Plus Aerobic F/bottle to the Isolator tube for recovery of bacteria, mycobacteria, and fungi from blood. J Clin Microbiol 39:4380–4386. https://doi.org/10.1128/JCM.39.12.4380-4386.2001.
13. Zervou FN, Zacharioudakis IM, Kurpewski J, Mylonakis E. 2017. T2 magnetic resonance for fungal diagnosis. Methods Mol Biol 1508:305–319. https://doi.org/10.1007/978-1-4939-6515-1_18.
14. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, Groeger JS, Judson MA, Vinagre YM, Heard SO, Zervou FN, Zacharioudakis IM, Kontoyiannis DP, Pappas PG. 2015. T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis 60:892–899. https://doi.org/10.1093/cid/ciu959.
15. Clancy CJ, Peter PG, Vazquez J, Judson M, Kontoyiannis DP, Thompson GR, Garey KW, Reboli A, Greenberg R, Apewokin S, Lyons M, Ostrosky-Zeichner L, Wu A, Tobin E, Nguyen MH, Caliendo AM. Detecting Infections Rapidly and Easily for Candidemia Trial-2 (DIRECT2): a prospective, multicenter study of the T2Candida Panel. Clin Infect Dis, in press.
16. Lai CC, Wang CY, Liu WL, Huang YT, Hsueh PR. 2012. Time to positivity of blood cultures of different Candida species causing fungaemia. J Med Microbiol 61:701–704. https://doi.org/10.1099/jmm.0.038166-0.
17. Huang L, Zhang YY, Sun LY. 2013. Time to positivity of blood culture can predict different Candida species instead of pathogen concentration in candidemia. Eur J Clin Microbiol Infect Dis 32:917–922. https://doi.org/10.1007/s10096-013-1826-8.
18. Pfaller MA, Jones RN, Castanheira M. 2014. Regional data analysis of *Candida* non-*albicans* strains collected in United States medical sites over a 6-year period, 20062011. Mycoses 57:602–611. https://doi.org/10.1111/myc.12206.
19. Aguilar-Zapata D, Petraitiene R, Petraitis V. 2015. Echinocandins: the expanding antifungal armamentarium. Clin Infect Dis 61(Suppl 6):S604–S611. https://doi.org/10.1093/cid/civ814.
20. Walsh TJ, Teppler H, Donowitz GR, Maertens JA, Baden LR, Dmoszynska A, Cornely OA, Bourque MR, Lupinacci RJ, Sable CA, dePauw BE. 2004. Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. N Engl J Med 351:1391–1402. https://doi.org/10.1056/NEJMoa040446.
21. Shields RK, Nguyen MH, Clancy CJ. 2015. Clinical perspectives on echinocandin resistance among Candida species. Curr Opin Infect Dis 28:514–522. https://doi.org/10.1097/QCO.0000000000000215.
22. Arnold HM, Micek ST, Shorr AF, Zilberberg MD, Labelle AJ, Kothari S, Kollef MH. 2010. Hospital resource utilization and costs of inappropriate treatment of candidemia. Pharmacotherapy 30:361–368. https://doi.org/10.1592/phco.30.4.361.
23. Guinea J. 2014. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect 20(Suppl 6):S5–S10. https://doi.org/10.1111/1469-0691.12539.
24. Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA, Groeger JS, Judson MA, Vinagre YM, Heard SO, Zervou FN, Zacharioudakis IM, Kontoyiannis DP, Pappas PG. 2015. Reply to Bauer and Goff. Clin Infect Dis 61:487–488. https://doi.org/10.1093/cid/civ357.
25. Bauer KA, Goff DA. 2015. When diagnostic technology is ahead of the hospital budget: what is antimicrobial stewardship to do? Clin Infect Dis 61:486–487. https://doi.org/10.1093/cid/civ355.
26. Bilir SP, Ferrufino CP, Pfaller MA, Munakata J. 2015. The economic impact of rapid *Candida* species identification by T2Candida among high-risk patients. Future Microbiol 10:1133–1144. https://doi.org/10.2217/fmb.15.29.