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Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

Molecular diagnosis of fungal infections: where are we?

23 April 2018, 09:00–11:00
Hall R

CANDIDA

Brunella Posteraro, Rome, Italy

Why should we detect *Candida*?

Candida species are among the most common causes of nosocomial bloodstream infections

Candidemia and other forms of invasive candidiasis (IC) represent a life-threatening condition in immunocompromised and critically ill patients

Candidemia risk factors for hospitalized patients

Risk Factor	Possible role in infection
Antimicrobial agents (number and duration)[b]	Provide vascular access and promote fungal colonization
Glucocorticoids	Immunosuppression
Age (<1 year, >70 years)	Immunosuppression
Chemotherapy[b]	Immunosuppression Mucosal disruption
Malignancy	Immunosuppression
Colonization[b]	Translocation across mucosa
Gastric acid suppression	Colonization and translocation
Indwelling catheter[b]	Direct vascular access
Central venous catheter	Contaminated product (device or infusate)
Pressure transducer	
Port	
Total parenteral nutrition	Direct vascular access Hyperglycemia Contaminated infusate
Neutropenia (<500/mm^3)[b]	Immunosuppression
Surgery (abdominal)	Route of infection Mucosal disruption Direct vascular access
Mechanical ventilation	Route of infection
Renal failure/hemodialysis[b]	Route of infection Immunosuppression
Solid organ transplant	Route of infection Mucosal disruption Direct vascular access Immunosuppression
Hospital or ICU stay	Exposure to pathogens Exposure to additional risk factors
Severity of underlying illness	Invasive procedure

Candida infection is the third leading cause of catheter-associated bloodstream infection (BSI) in the United States

The excess mortality (49%) and length of stay (30 days) in hospital exceed that of most healthcare-associated infections (HAI). Likewise, the costs associated with *Candida* infection are among the highest of any HAI

Each day of delay in the initiation of antifungal therapy is associated with a 50% increase in mortality and an additional US$5000 in healthcare costs associated with candidemia

Pfaller and Castanheira, Med Mycol 2016

The risk for candidemia in the hospital is a continuum

- General risk factors upon admission to hospital
  - Hematologic malignancy
  - Neutropenia
  - Abdominal surgery
  - Solid organ transplant
  - Premature infant
  - Older adult (>70 years of age)
- Specific exposures that further increase risk (OR odds ratio)
  - Intensive Care Unit stay >7 days (OR, 9.73)
  - Central venous catheter (OR, 7.23)
  - Dialysis (OR, 18.13)
  - Antibiotics (OR, 1.73 per antibiotic class)
  - Total parenteral nutrition (OR, 8.87)
  - Colonization (OR, 10.37)

While this infection typically affects individuals with considerable comorbidities who have prolonged hospitalization, specific additional exposures have been recognized as independent risk factors among high-risk groups of patients

Pfaller and Castanheira, Med Mycol 2016

Which is the state-of-art for the diagnosis of IC?

Across the spectrum of IC, blood cultures are negative in ~50% of IC, which includes candidemia complicated by deep-seated infection or deep-seated candidiasis that is not associated with candidemia.

Generally, low burdens of *Candida* cells and slow turnaround times are the main limitations of cultures, which provides a rationale for aggressive empirical usage of antimicrobial agents.

Which is the state-of-art for the diagnosis of IC?

In 2011, *Pfeiffer et al.* used the lysis-centrifugation blood culture system to show that

- median concentration of *Candida* organisms was 1 (mode 0.1, range 0.1 to >1000) cfu/mL, and
- >50% of cultures had ≤1 cfu/mL

at the time of first positive blood culture for 152 consecutive patients with candidemia (*J Clin Microbiol, 2011*).

These findings supported a rationale for the emergence of non-culture (molecular) diagnostic systems with greater ability to detect organisms in the blood.

PCR assays for *Candida*

Despite the wide availability of commercial and in-house tests, none of existing PCR-assays for *Candida* is FDA-cleared to date.

However, multiplex formats capable of detecting not only *Candida* but also other fungi and/or bacteria have been investigated.

The lack of standardization continues to be a major issue, which has hindered the laboratory adoption of PCR-based assays for detection of *Candida* species directly in clinical samples.

PCR diagnosis of invasive candidiasis: systematic review and meta-analysis

T Avni, L Leibovici, and M Paul (*J Clin Microbiol 2011, 49:665–670*)

A total of 54 studies, including almost 5000 patients tested by blood-based PCR, were analyzed.

- Pooled sensitivity and specificity for proven or probable IC *vs.* at-risk controls were 95% (95% CI: 82–98%) and 92% (95% CI: 87–98%), respectively.
- Pooled sensitivity and specificity for proven, probable or possible IC *vs.* at-risk controls were 73% (95% CI: 58–83%) and 95% (95% CI: 92–97%), respectively.

PCR diagnosis of invasive candidiasis: systematic review and meta-analysis

T Avni, L Leibovici, and M Paul (*J Clin Microbiol 2011, 49:665–670*)

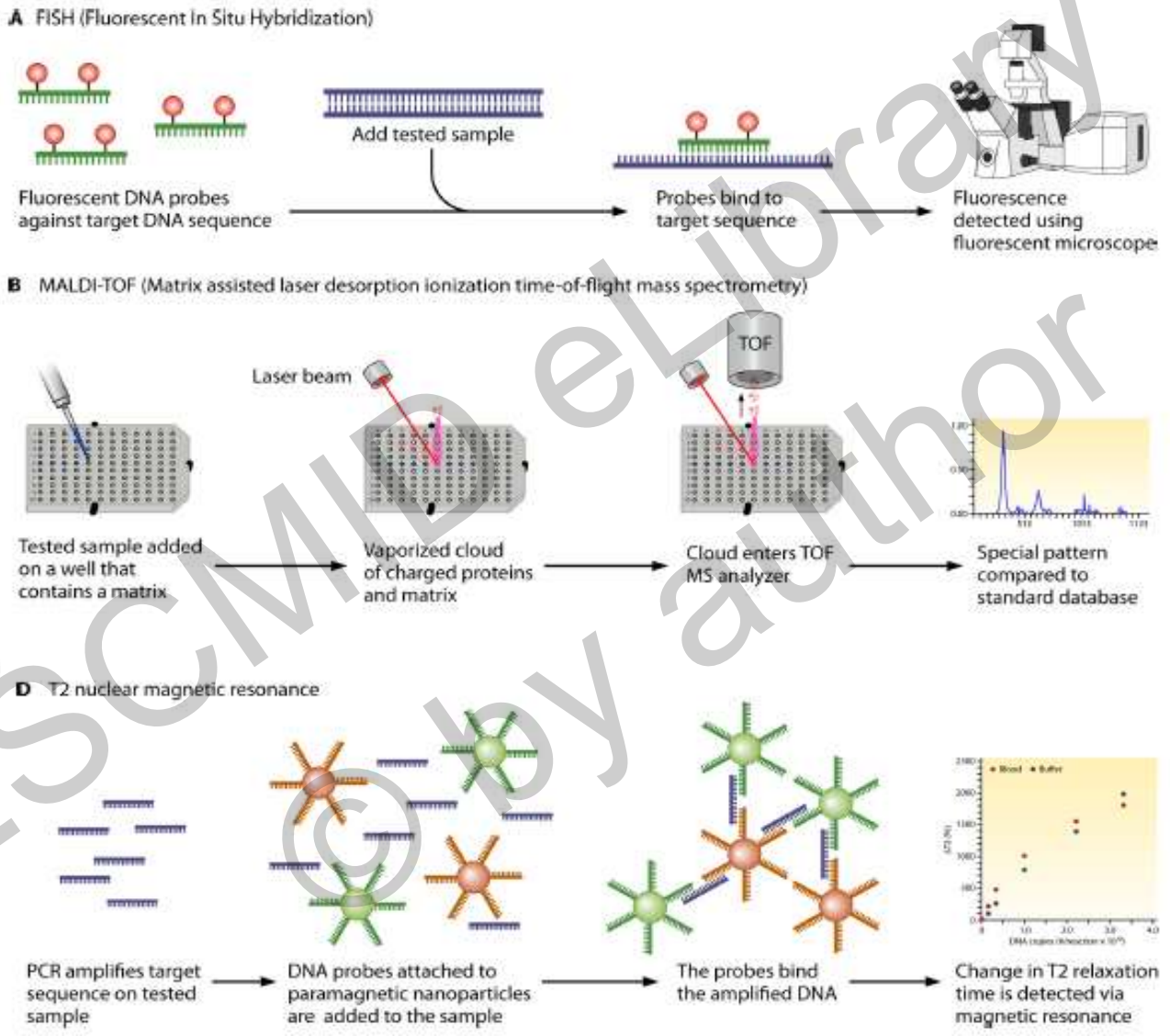
Higher PCR-assay sensitivities were observed with:

- whole blood (rather than serum samples)
- panfungal ribosomal DNA (rDNA) or cytochrome P450 gene as targets
- *Candida*-specific assays rather than broader multiplex assays (which detect a selection of multiple pathogens in a single session)
- *In vitro* detection limits of ≤10 cfu/mL

Commercially available methods for direct pathogen detection in the whole blood

Assay	Technology	Pathogen panel	Detection limit (cfu/ml)	Sensitivity (%)	Specificity (%)	Turnaround time (h)
T2Candida® test (T2Biosystems inc)	PCR + magnetic resonance technology	5 Candida species	1	100	98	3
SeptiFast (Roche Diagnostics, Germany)	Real-time PCR	25 Species/genus targets of bacteria and fungi	3–30	68–75	86–92	6
SepsiTest (Molzym, Germany)	PCR + sequencing	345 Bacteria + fungi	20–460	86–87	83–85	8–12
Vyoo® (SIRS, Germany)	PCR + electrophoresis	34 Bacteria, 6 fungi targets + 5 resistance markers	5–100	60	70–75	7
Magicplex™ (Seegene, Korea)	Real-time PCR	85 Bacteria 6 fungi + 3 resistance markers	–	65	92	6

Technology behind novel diagnostic methods for fungal infections



Arvanitis et al., Clin Microbiol Rev 2014

T2*Candida* Panel powered by T2 Magnetic Resonance (T2MR)

T2MR combines proven magnetic resonance with innovative nanotechnology to accurately identify *Candida* pathogens in whole blood faster and easier than blood culture-based diagnostics.
T2Candida is cleared by the US FDA and EMA for the diagnosis of candidemia.

Pfaller et al., Future Microbiol 2016

"T2MR-biosensing to detect cells and nucleic acids within complex matrices such as blood, without the need for isolating organisms"

Two multicenter studies demonstrate the clinical and performance advantages of the T2Dx® Instrument (which runs the T2Candida® Panel) over blood culture:

- DIRECT trial (*Mylonakis et al., 2015*)
- DIRECT2 trial (*Clancy et al., 2018*)

Multicenter DIRECT trial (*Mylonakis et al., Clin infect Dis 2015*)

T2Candida was devised to not amplify freely circulating, non-cell-associated DNA. Using primers for rDNA ITS2 (internal transcribed spacer region 2), results are reported as positive or negative for *C. albicans/C. tropicalis*, *C. glabrata/C. krusei*, and *C. parapsilosis.*

>1500 control patients with *Candida*-negative blood cultures
6 patients with *Candida*-positive blood cultures
250 contrived blood samples spiked with *C. albicans*/C. *tropicalis*, *C. glabrata*/*C. krusei*, and *C. parapsilosis*

T2Candida identified 98.1% of patients as noncandidemic, with a mean time to negative result of 4.2 ± 0.9 hours. The overall sensitivity per patient (excluding invalid results) was found to be 91.1%, with a mean time to positive result of 4.4 ± 1.0 hours.

The limit of detection was 1–3 cfu/mL (1 cfu/mL for *C. tropicalis* and *C. krusei*, 2 cfu/mL for *C. albicans* and *C. glabrata*, and 3 cfu/mL for *C. parapsilosis*).

Follow-up, multicenter DIRECT2 trial (*Clancy et al., Clin Infect Dis 2018*)

In continuing studies of the T2Candida system for detecting candidemia, adult patients with a positive diagnostic blood culture (BC) were included. As expected, some of the patients were receiving antifungal therapy by the time repeat blood samples were collected.

The clinical sensitivity for the five *Candida* species identified by T2Candida was found to be 89% (excluding invalid results) in 36 of 152 patients, who were identified as having a positive diagnostic BC for *Candida* species and then resampled (within 1 to 6 days) for both BCs and T2Candida testing.

Among the study patients, T2Candida tests were more likely to remain positive than concomitantly collected BCs (45% [69/152] and 24% [36/152]; $P < .0001$).

Interestingly, higher positivity for T2Candida was found among the patients who were already receiving antifungal therapy. This suggests that the system can reveal the presence of nonviable or drug-inhibited *Candida* organisms if the patient is on antifungal therapy.

"Assuming sensitivity of 90% and specificity of 98%, anticipated positive and negative predictive values (PPVs/NPVs) of T2Candida can be calculated" *(Clancy and Nguyen, J Antimicrob Chemother 2018)*

Prevalence	Representative patient	90% Sensitivity/ 98% specificity	
		PPV	NPV
0.4%	Any hospitalized patient in whom a blood culture is collected.[7]	15%	>99.9%
1%	Patient admitted to critical care unit.[19,20]	31%	99.9%
2%	Patient with febrile neutropenia, baseline rate of candidaemia prior to empirical antifungal treatment.[21–24]	47%	99.8%
3%	Patient with sepsis, shock or >3–7 day stay in critical care unit.[20,25–27]	67%	99.7%
10%	Patient at increased risk of candidaemia based on clinical prediction models.[4,28,29]	82%	99%
20%	Neutropenic bone marrow transplant recipient or leukaemia patient not receiving antifungal prophylaxis.[30–33]	92%	98%

Thus, "T2Candida performance characteristics enable clinicians to assign clinical settings in which T2Candida is most likely to be useful in guiding antifungal treatment decisions". Indeed, "PPVs may exceed a threshold that justifies antifungal treatment, while corresponding NPVs render active candidemia extremely unlikely".

What are the limitations of T2Candida?

Yielding no organisms that can be subsequently tested for species identification and antifungal susceptibility ("the system does detect three *Candida* groups based on their typical antifungal susceptibility patterns")

Only detecting the 5 most common *Candida* species ("these target species account for >95% of cases of candidemia"… but "additional *Candida* species, such as the multidrug-resistant *C. auris*, are not identified by T2Candida")

"Therefore, T2Candida must serve as a supplement to existing blood culture systems" (*Hawley HB, Clin Infect Dis, 2018*). However, the limit of detection for the T2Candida panel is as low as 1 cfu/mL of whole blood, which is superior to that reported for PCR assays.

"T2MR-biosensing to detect cells and nucleic acids within complex matrices such as blood, without the need for isolating organisms"

Moving beyond the diagnostic performance of T2Candida, new US (*Patch et al., 2018; Mylonakis et al., 2018*) and EU studies (*Muñoz et al., 2018a; 2018b*) begin to frame the T2Candida Panel in the context of early therapy, improving outcomes, and antimicrobial stewardship.

Impact of rapid, culture-independent diagnosis of candidaemia and invasive candidiasis in a community health system

M.E. Patch, E. Weisz, A. Cubillos, S.J. Estrada and M.A. Pfaller (*JAC 2018; Suppl 4: iv27–30*)

A study conducted at Lee Health in Florida found:

- **The T2Candida Panel enables patients to receive targeted therapy 28 hours faster:** Patients suspected of a *Candida* infection who had a positive T2Candida rapid diagnostic test result received targeted therapy nearly 6 times faster (i.e., only 6 hours compared to conventional practices that took 34 hours; *P* = .0147).

- **Improved stewardship and pharmacy savings:** Due to the more targeted use of antifungal drugs, the average duration of antifungal therapy with T2Candida testing was reduced by 4 days and therapy was discontinued after a single dose or avoided altogether in 58.4% (101/173) of patients, with no adverse impact on patient mortality. The authors estimate savings in antifungal costs after T2Candida testing was introduced at an average of $280 per patient tested.

T2Candida MR as a predictor of outcome in patients with suspected invasive candidiasis starting empirical antifungal treatment: a prospective pilot study (*Muñoz et al., JAC 2018; Suppl 4: iv6–12*)

T2MR contributes to the very early diagnosis of complicated candidaemia. A prospective study (*Muñoz et al., JAC 2018; Suppl 4: iv13–19*)

Again, two studies from Gregorio Marañón Hospital in Madrid, Spain (T2MadRid study group) reported findings about the ability of the T2Candida Panel to predict patient outcomes:

- **Strong indicator of complications:** Positive T2Candida Panel results identified patients who had an over 30x increased likelihood of developing health complications, including mortality.
- **Strong indicator of poor outcomes:** The T2Candida Panel is a better predictor of patients at risk of having poor outcomes than existing diagnostic tests. A negative T2Candida Panel result may significantly shorten the duration of antifungal therapy for 67% of patients.
- **Improved distinction between complicated and uncomplicated infection:** The T2Candida Panel distinguishes between complicated and uncomplicated infection better than available diagnostic tests.

Efficacy of T2 magnetic resonance assay in monitoring candidemia after initiation of antifungal therapy: the serial therapeutic and antifungal monitoring protocol (STAMP) trial (*Mylonakis et al., JCM 2018; 56*)

Finally, a multicenter prospective clinical trial, designated the STAMP trial, showed that:

- Thirteen of 31 patients (41.9%) who completed the trial had at least 1 positive surveillance T2MR and/or a blood culture result, and only 7/23 (30.4%) T2MR results had an accompanying positive blood culture.
- Based on the log rank test, the authors found a statistically significant improvement in posttreatment surveillance using the T2MR assay compared to blood culture (P = .004), 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.

In conclusion, T2MR assay outperformed blood cultures in monitoring the clearance of *Candida* spp. in candidemic patients receiving antifungal therapy. This could be at least partially explained by the fact that T2MR results are not suppressed by antifungal agents.

Molecular tests for diagnosing intra-abdominal candidiasis (IAC)

Clancy and Nguyen, JCM 2018

Test	Method	Study groups (n)	Sensitivity (%)	Specificity (%)	Author, year
PCR	Candida Real-time PCR Panel[a]	IAC (n = 34) vs. at-risk ICU patients (n = 73)	88[b]	70	Nguyen et al., 2012
	Multiplex Candida Real-time PCR[c]	IAC or urologic candidiasis (n = 11) vs. at-risk ICU patients and healthy controls (n = 76)	91	97	Fortún et al., 2014
	Multiplex Candida Real-time PCR[c]	IAC (n = 20) vs. at-risk ICU patients (n = 202)	86[d]	33[d]	León et al., 2018
T2Candida			No data		

[a] Viracor Eurofins, Lee's Summit, MO. The Candida Real-time PCR Panel detects *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*.
[b] Sensitivity of blood cultures for IAC: 17%.
[c] Mycology Service of the Spanish National Microbiology Center and Ramon y Cajal Hospital, Madrid, Spain. The Multiplex Candida Real-time PCR Panel detects *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. guilliermondii.*
[d] PCR was not performed in all patients. Results were positive in 12/14 patients with IAC, and negative in 57/85 at-risk critical care patients and healthy controls.

Risk stratification and the predictive value of PCR assay for IAC

Clancy and Nguyen, JCM 2018

Prevalence of disease (%)	Representative patient	PCR					
		León *et al.*		Nguyen *et al.*		Fortún *et al.*	
		PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)
5	- Low-to-moderate risk peritoneal dialysis patient with peritonitis	6	97.7	13	98.9	59	99
10	- Patient with emergent surgery for intra-abdominal infection (IAI) - Patient with colonic perforation	12	95.2	24	97.7	76	98.3
20	- Patient with high-risk severe acute or necrotizing pancreatitis - Patient with small bowel perforation - Patient with emergent surgery for nosocomial IAI	24	89.9	41	94.9	88	97.5
30	- Patient who undergone high-risk GI/hepatobiliary surgery - Patient with a biliary leak - Patient with a gastric/duodenal perforation	35	83.7	55	91.6	93	93.8

PPVs and NPVs within more darkly shaded boxes signify patients in whom non-culture testing may have greatest clinical utility, assuming that antifungal treatment is justified at a threshold likelihood of IC of ≥~15–30%. For these patients, a positive result is anticipated to move the likelihood of IAC from below the threshold to above the threshold. At the same time, negative tests should assure that the likelihood of IAC is less than the threshold.

CONCLUSIONS (I part)

While we herald the era of culture-independent diagnostics in medical mycology, it is estimated that within 5–10 years molecular diagnostics will have an important complementary role to culture-based methods in the diagnosis of fungal infections.

As stated nearly 50 years ago by Dr. Raymond C. Bartlett (*Am J Clin Pathol, 1974*), more attention has to be paid to the "clinical relevance in medical microbiology", because laboratory costs may increase at a rate higher that of hospital costs.

New molecular diagnostic systems, such as T2Candida, show great potential for early directed interventions. However, the cost-effectiveness of these systems will depend upon the pre-test probability of invasive candidiasis in individual hospitals as well as high-risk groups of patients in those hospitals.

CONCLUSIONS (II part)

More data will be necessary to understand the performance of a molecular test in a given clinical context, particularly for the diagnosis of culture-negative, deep-seated candidiasis.

Also, the questions whether T2Candida or another molecular assay will be able to reduce mortality, shorten hospital days, or limit the emergence of antifungal resistance associated with invasive candidiasis need urgent answers.

Meanwhile, it will be necessary that centres adopting molecular diagnostics report their experiences in order for the community to learn how to use the tests in a most effective and efficient way. If possible, by profiting from the expertise of diagnostic stewardship teams.

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Thanks!!

Any Questions?

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… and special thanks to

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