
3rd European Conference on Infections in Leukemia
Non-Invasive Diagnostic Procedures for Yeast Infections
Małgorzata Mikulska, Thierry Calandra, Maurizio Sanguinetti, Daniel Poulain and Claudio Viscoli (leader)
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ELN European LeukemiaNet
EBMT
EORTC European Organisation for Research and Treatment of Cancer
ICHS

Non-invasive diagnosis of *Cryptococcus* infections

Background

- **Tests available:**
  - **Latex agglutination (LAT)**
  - **Enzyme immunoassay (EIA)**
- **Standard for diagnosing cryptococcosis**
- **False positive results of LAT (0-0.4%):**
  - **Rheumatoid factor**
  - **Infections with other fungi (e.x. *Trichosporon)***
- **Protease enzyme treatment significantly increases the specificity**
- **Reported sensitivity and specificity:**
  - **LAT 93%-100% & 93%-98%**
  - **EIA 85%-99% & 97%**

Materials and methods

- **Systematic analysis of available literature since 01/01/1998 + bibliographies screened**
- **Key words: cryptococcosis, antigen, Cryptococcus, diagnosis, cryptococcal.**
- **Excluded:**
  - **Animal or *in vitro* studies**
  - **Articles in languages other than English**
  - **Case reports and studies with < 10 patients**
  - **Articles with no mention of antigen as a diagnostic methods**
  - **Reviews**

The articles were divided into three clinical categories:

1. **Disseminated cryptococcosis (with or without meningitis)**
2. **Cryptococcal meningitis (CM)**
3. **Pulmonary cryptococcosis**

Limitations of this literature review

1. Various tests used (LAT and EIA)
2. Some studies - no mention of the test used
3. Reference method for diagnosis included antigen (as in EORTC criteria)
4. Patients divided in two groups, based on the HIV status

Results 1

- **30 articles**
- **High concordance between all the tests used (LAT and EIA)**

Babady et al. (2009); Saha et al. (2008)

- **Thus, no distinction between the type of test used was made in the review**

Results 2 – Sensitivity of serum antigen in cryptococcosis

7 studies, mostly retrospective (6/7), single centre (4/7). Patients: 33-306. Diagnosis based on culture, histology, India ink and antigen (4/7).

Underlying condition	No. positive by serum antigen (%)	No. positive by blood culture (%)
AIDS	**95% (394/415)**	**43% (136/318)**
HIV negative	**77% (286/371)**	**40% (61/52)**
Total	**87% (680/786)**	**42% (197/470)**

Antinori, Galimberti et al. 2001; Chuang, Ho et al. 2008; Dromer, Mathoulin-Pelissier et al. 2007; Husain, Wagener et al. 2001; Jongwutiwes, Sungkanuparph et al. 2008; Pappas, Perfect et al. 2001; Vilchez, Shapiro et al. 2003.

Results 3 – Sensitivity of CSF & serum antigen in meningitis

13 studies, mostly retrospective (10/13), single centre (8/13).
Patients: 10-2753.

Host condition	No. pos by CSF Ag (%)	No. pos by CSF culture (%)	No. pos by CSF India Ink (%)	No. pos by serum Ag (%)
Solid organ transplant	**99% (110/111)**	89% (150/169)	67% (50/75)	94% (73/78)
All HIV negative (SOT included)	**97% (369/380)**	89% (390/436)	61% (203/332)	89% (189/212)
AIDS	**96% (1897/1966)**	97% (3036/3114)	94% (2902/3078)	97% (142/146)
TOTAL	**97% (2266/2346)**	**97% (3426/3550)**	**91% (3105/3410)**	**92% (331/358)**

Antinori, Galimberti et al. 2001; Antinori, Radice et al. 2005; Capoor, Nair et al. 2007; Dromer, Mathoulin-Pelissier et al. 2007; Husain, Wagener et al. 2001; Imwidthaya and Poungvarin 2000; Jenney, Pandithage et al. 2004; Jongwutiwes, Sungkanuparph et al. 2008; McCarthy, Morgan et al. 2006; Pappas, Perfect et al. 2001; Pasqualotto, Bittencourt Severo et al. 2004; Singh, Dromer et al. 2008; Singh, Lortholary et al. 2008.

Results 3a – Specificity of CSF antigen in cryptococcal meningitis

- Unable to assess specificity in the aforementioned studies

- A single report of 12 false positive results of CSF antigenemia (low titres) in 12 cancer patients without CM

Kontoyiannis 2003

Results 4 – Sensitivity of serum antigen in pulmonary cryptococcosis

8 studies, retrospective. Diagnosis made by culture, histology or antigen (7/8).

Host condition	No. positive by serum Ag, %
Immunocompetent	37% (13/35)
Immunocompromised	76% (60/79)
Disseminated	95% (52/55)
Isolated pulmonary	56% (81/146)
Total	**62% (200/322)**

Aberg, Mundy et al. 1999; Baddley, Perfect et al. 2008; Chang, Tzao et al. 2006; Hung, Tsai et al. 2008; Nadrous, Antonios et al. 2003; Pappas, Perfect et al. 2001; Singh, Alexander et al. 2008; Vilchez, Shapiro et al. 2003.

Although the performance of antigen testing in BAL fluid has not been validated, several authors report its usefulness in diagnosing pulmonary cryptococcosis (Kralovic and Rhodes 1998).

Use of cryptococcal serum or CSF antigen for outcome prognosis and follow-up

- Possible? Accurate? Reliable?

Results 5 - use of baseline serum or CSF antigen titres for prognosis

- Initial high titres (≥1:1024) demonstrate a high burden of yeasts in the host and poor host immunity

  Mandell & Bennet 2005; Chayakulkeeree and Perfect 2006

- High titres seem to be associated with severe disease, relapse, mycological failure or mortality, particularly in HIV-positive subjects

  Graybill, Sobel et al. 2000; Lortholary, Poizat et al. 2006; Dromer, Mathoulin-Pelissier et al. 2007; Hung, Tsai et al. 2008

- Other authors did not confirm the association between the high titre and a poor prognosis

  Pasqualotto, Bittencourt Severo et al. 2004; Singh, Lortholary et al. 2008

Results 6 - Use of serum or CSF antigen titres to assess response to treatment

Most patients who responded to treatment had decreasing antigen titres

Goodman, Kaufman et al. 1971; Pappas, Perfect et al. 2001;Lu, Zhou et al. 2005; Chang, Tzao et al. 2006

However:

1. **Accuracy of titres can vary among tests**
2. **Kinetics of antigen elimination remains unclear**
3. **Positive antigen test results may persist for years despite a favourable clinical outcome (negative serum antigen after 2 years - only 35%)**
4. **Patients may experience an increase in serum Ag without failing**
5. **Despite a decrease in CSF or serum Ag titres a relapse can occur or a post-mortem exam can document the presence of disseminated cryptococcosis**

Aberg, Watson et al. 2000; Antinori, Galimberti et al. 2001; Antinori, Radice et al. 2005; Chang, Tzao et al. 2006; Chayakulkeeree and Perfect 2006; Imwidthaya and Poungvarin 2000; Lortholary, Poizat et al. 2006; Mandell & Bennet 2005

Grading system used

Category, grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for or against use
B	Moderate evidence to support a recommendation for or against use
C	Poor evidence to support a recommendation
Quality of evidence	
I	Evidence from ≥1 properly randomized, controlled trial
II	Evidence from ≥1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); from multiple time-series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

Recommendations

Use of serum antigen to diagnose disseminated cryptococcosis*	A II
Use of CSF antigen to diagnose cryptococcal meningitis	A II
Use of serum antigen to diagnose pulmonary cryptococcosis	B III
Use of baseline antigen titres for prognosis	B III
Use of serum or CSF antigen kinetics (titres) to assess response to treatment	C III

* Higher sensitivity in HIV-positive than HIV-negative patients

Non-invasive diagnosis of *Candida* infections

Antigen and antibody testing

Background

- Need for diagnostic techniques better than culture
- Numerous different tests are reported:
  - Thermolabile antigen (Cand-Tec)
  - Immunoanalysis assay (Unimedi *Candida* monotest)
  - D-arabinitol
  - Enolase
  - Enzyme immunoassay (EIA) for manann antigen and anti-mannan antibodies

Material and Methods

- PubMed search for articles since 01/01/1998
- MeSH terms: Candida, candidiasis, candidemia, antigen, antibody, diagnosis, mannan, ELISA, Platelia
- 556 results retrieved and screened
- Excluded:
  - Animal or in vitro studies
  - Languages other than English
  - Case reports and studies with < 10 patients
  - Reviews
- Bibliographies screened for any other pertinent articles
- Literature regarding ELISA (Platelia Bio-Rad) for mannan, antimannan and combined mannan/antimannan was reviewed

Aim

- Analyse sensitivity and specificity for:
  - Different clinical presentations (candidemia and hepatosplenic candidiasis)
  - Different patient populations
  - Different *Candida* species

Limitations of this literature review

- 14 studies for EIA (Platelia)
- Publication bias
- Retrospective studies mostly
- Heterogeneous populations (ICU & surgery, haematological malignancies)
- Sensitivities and specificities calculated sometimes per patient and sometimes per sample
- Different cut-off values
- Positive result defined as a single sample or 2 samples positive
- Control groups not included in all the studies and very heterogeneous (ranging from healthy subjects to patients at high risk for candidemia but with negative blood cultures)

Results 1
Description of 14 studies

- **Mannan antigen (Ag) testing - performed in 14 studies**
- **Anti-mannan antibodies (Ab) - 10**
- **Sensitivity evaluated in 14, specificity in 11**
- **No. of case patients included: from 7 to 105**
- **Study populations:**
  - **Haematological malignancies only – 4**
  - **Mostly ICU & surgery - 10 (1 neonatal ICU)**
  - **No HSCT recipients**
- **Forms of invasive candidiasis:**
  - **Mostly candidemia**
  - **Hepatosplenic candidiasis (2 studies)**

Lunel Diagn Microbiol Infect Dis 2009; Ellis J Med Microbiol 2009; Sendid Clin Vaccine Immunol 2008; Oliveri Clin Microbiol Infect 2008; Alam BMC Infect Dis 2007; Fujita J Med Microbiol 2006; Prella Diagn Microbiol Infect Dis 2005; White J Clin Microbiol 2005; Sendid J Clin Microbiol 2004; Sendid J Clin Microbiol 2003; Sendid J Med Microbiol 2002; Persat Mycoses 2002; Yera Eur J Clin Microbiol Infect Dis 2001; Sendid J Clin Microbiol 1999.

Results 2
Sensitivity, median (range)

	Antigen	Antibody	Ag/Ab
Per patient	60% (31-100)	60% (46-100)	89% (75-100)
Per sample	53% (17-100)	60% (39-100)	83% (75-100)

Lunel Diagn Microbiol Infect Dis 2009; Ellis J Med Microbiol 2009; Sendid Clin Vaccine Immunol 2008; Oliveri Clin Microbiol Infect 2008; Alam BMC Infect Dis 2007; Fujita J Med Microbiol 2006; Prella Diagn Microbiol Infect Dis 2005; White J Clin Microbiol 2005; Sendid J Clin Microbiol 2004; Sendid J Clin Microbiol 2003; Sendid J Med Microbiol 2002; Persat Mycoses 2002; Yera Eur J Clin Microbiol Infect Dis 2001; Sendid J Clin Microbiol 1999.

Results 3
Specificity, median (range)

	Antigen	Antibody	Ag/Ab
Per patient	96% (65-100)	90% (38-100)	88% (21-98)
Per sample	96% (65-100)	91% (38-100)	88% (21-98)

- **11 studies**
- **Specificity was repeatedly high both for Ag & Ab, except for the prospective study by Ellis et al. that included 12 haematological patients and where specificity of combined Ag/Ab was only 21%, but cut-off for Ab was lower than in other studies.**

Lunel Diagn Microbiol Infect Dis 2009; Ellis J Med Microbiol 2009; Oliveri Clin Microbiol Infect 2008; Alam BMC Infect Dis 2007; Fujita J Med Microbiol 2006; Prella Diagn Microbiol Infect Dis 2005; White J Clin Microbiol 2005; Sendid J Clin Microbiol 2004; Sendid J Clin Microbiol 2003; Persat Mycoses 2002; Sendid J Clin Microbiol 1999.

Results 4
Timing of Ag positivity vs culture

- Serum antigen positivity significantly preceded the positive blood culture result
- In one study 73% of patients had one test positive before the blood culture results

Lunel, Mennink-Kersten et al. 2009; Oliveri, Trovato et al. 2008;
Prella, Bille et al. 2005; Sendid, Poirot et al. 2002;
Year, Sendid et al. 2001.

Results 5
Different *Candida* species

Platelia Candida Ag test is based on the use of a monoclonal antibody EB-CA1, which recognizes a mannopentose epitope of *C. albicans*. This epitope has also been found at high levels in *C. glabrata* and *C. tropicalis*, but at lower levels in *C. krusei*, *C. kefyr* and *C. parapsilosis.*

Sendid, Poirot et al. 2002; Jacquinot et al. 1998; Rimek et al. 2003

Consistently with in vitro research, the sensitivity of Ag & Ab was the highest for *C. albicans* and the lowest for *C. parapsilosis* or *krusei.*

	Ag	Ab	Ag & Ab
C. albicans	62%	67%	100%
C. glabrata	58%	83%	83%
C. tropicalis	70%	60%	80%
C. parapsilosis	30%	10%	40%
C. krusei	25%	38%	50%

Results 6

Invasive candidiasis other than candidemia

Hepatosplenic candidiasis

- Prella et al. - usefulness of Ag/Ab serum testing, allowing the diagnosis before neutrophil recovery in 78% of patients (18/21 (86%) subjects with hepatosplenic lesions had positive Ag or/and Ab at a median of 16 days before radiological detection of lesions).
- Ellis et al. - 7/12 case-patients with invasive candidiasis had the hepatosplenic form diagnosed with Ag and Ab.

Meningitis

- Interestingly, Verduyn Lunel et al. reported 5 patients with Candida meningitis, in whom 4/5 CSF tested positive for mannan.

Verduyn Lunel, Voss et al. 2004

Recommendations

The use of combined Ag/Ab is preferred over Ag or Ab only for diagnosing invasive Candida infection*	B II
The combined Ag/Ab testing is useful for supporting the diagnosis of candidemia	C II
The combined Ag/Ab testing is useful for diagnosing hepatosplenic candidiasis	B III

*Studies included a majority of ICU/surgery patients.