Early diagnosis of systemic candidal infection

J Antimicrob Chemother 1993; 31: 809–812

Over the last 30 years Candida albicans has emerged as a major cause of nosocomial infection (Edwards, 1991). Recent studies indicated that the mortality for systemic candidosis ranges from 38–59% (Wey et al., 1988; Komshian et al., 1989). This has led to intensive research not only for new antifungal agents but also for better serodiagnostic tests, on the premise that earlier diagnosis and treatment should reduce mortality. Ideally a serological test should not only make an accurate diagnosis early on in the course of infection, but also help in monitoring the response to treatment and in determining the duration of therapy. The latter is particularly important when treatment regimens are either toxic or expensive which is especially pertinent to the management of systemic candidosis.

Systemic candidosis may affect single or multiple organs following haematogenous spread. With the exception of infections of the digestive, respiratory or urogenital tracts, systemic candidosis is usually disseminated, although infection of one organ may dominate the clinical picture, as for example with candida endocarditis or meningitis. More commonly the patient presents with fever unresponsive to broad-spectrum antibiotics. Blood cultures are unreliable in confirming the diagnosis being negative in 56% of necropsy-proven disseminated Candida infections (de Repentigny & Reiss, 1984). Lysis centrifugation leads to earlier and more frequent recovery of Candida spp. with recovery rates up to 79% in cancer patients (Bille et al., 1983; Walsh et al., 1991a). Recovery rates may also be improved by venting the culture bottles, using biphasic media or brain-heart infusion broth.

Confirmation of the diagnosis of systemic candidosis requires multiple consecutive blood cultures to be positive (to exclude transient candidaemia) or the demonstration of candida in an otherwise sterile site, by culture or histology. Culture of biopsied tissue from cases of hepatosplenic candidosis are usually negative so that histological confirmation is necessary (Walsh, Lee & Pizzo, 1991b). In systemic candidosis cultures of suspicious skin lesions often yields Candida spp. Recent developments in diagnostic imaging have made it possible to detect very small lesions in the liver and spleen of patients with hepatosplenic candidosis. Such patients usually have negative blood cultures at the time of diagnosis. Likewise almost a quarter of patients with candida endocarditis have persistently negative blood cultures (Odds, 1988).

In clinical practice, any blood culture positive for candida in a high risk patient should prompt treatment when the clinical features are compatible with systemic candidosis. In addition to confirming the diagnosis by repeat blood cultures, serological tests should be considered as an aid to confirming the diagnosis and monitoring response to treatment. Serological testing is particularly valuable in establishing the diagnosis in the pyrexial, high-risk patient with negative blood cultures. The early serological tests were antibody assays which have been well reviewed by de Repentigny & Reiss (1984). These tests lacked sensitivity and specificity although immunoblotting proved useful in dissecting the antibody response to individual cytoplasmic antigens (Greenfield & Jones, 1981; Matthews, Burnie & Tabaqchali, 1984; Strockbine et al., 1984). When multiple sequential serum samples were examined from patients with proven systemic candidosis, those with a poor prognosis either had no antibody detectable or had antibody titres which fell as their condition deteriorated. In contrast patients either recovering from systemic infection, or exhibiting extensive colonization in the absence of infection, produced good antibody responses (Matthews et al., 1984; Matthews, Burnie & Tabaqchali, 1987). Thus the detection of anti-candida antibodies was useful prognostically but not diagnostically since antibody titres were highest in those individuals least likely to succumb to systemic infection. Patients with AIDS who suffer from recurrent superficial...
candidal infections frequently have detectable antibody to *Candida* spp. but remarkably rarely develop systemic candidosis (Matthews *et al.*, 1988c). Patients with candida endocarditis differ from other patients with systemic candidosis in that they have high anti-candida titres and little free circulating antigen (personal observation).

Subsequently assays were developed directed at the detection of circulating candidal metabolites such as arabinitol (Wong *et al.*, 1982; Gold *et al.*, 1983). This approach requires quantification by gas liquid chromatography, after extraction and derivatization of each sample, and correction against serum creatinine. A recent prospective study (Walsh *et al.*, 1992) in 103 patients (1132 sera examined) gave elevated arabinitol/creatinine ratios in 77% of patients with invasive candidosis and 83% with fungaemia. Only 55% were positive before fungaemia was detected. In 82% of patients the level correlated with therapeutic response.

Currently the most popular serological tests for candidosis are based on the detection of circulating antigens. Reverse passive latex agglutination assays detecting antigens of unknown identity have been widely used with varying success. One of these is commercially available as the Cand-tec kit from Ramco Laboratories (Gentry *et al.*, 1983). Such tests seem to yield a better bimodal distribution between colonized and systemically infected patients when applied to post-operative patients rather than neutropenic patients (Burnie, 1991). An advantage of these quantitative antigen assays is that the titre can be used to monitor the response to therapy. In those patients who recover there is an initial rise in titre when therapy is started which then falls rapidly, while in those patients who succumb antigen titres remain persistently elevated (Matthews & Burnie, 1988a). This initial rise in antigen titre may be the result of release of candidal antigens from cell lysis in response to treatment.

Mannan has been widely investigated as a marker of invasive infection but it circulates at low concentrations and is cleared rapidly from the serum necessitating repeat serum sampling to improve sensitivity (Jones, 1980). It exists in the form of immune-complexes which must be dissociated by hydrolysis and heat (Weiner & Coates-Stephen, 1979). We now know that two immunodominant antigens, the 48–52 kD enolase antigen (Mason, Brandt & Buckley, 1989; Franklyn *et al.*, 1990) and the 47 kD hsp 90 antigen (Matthews & Burnie, 1989) circulate in systemic candidosis. These are biochemically and immunologically distinct. Both have been used as a basis for serodiagnostic tests. A double-sandwich liposomal immunoassay for candida enolase was applied prospectively to serial sera collected from 170 high risk patients by Walsh *et al.* (1991a). Among the 24 patients who developed invasive candidosis, enolase was detectable in 85% with deep tissue infection and 64% with fungaemia. Specificity was high (96%) but sensitivity rather low (54%) Six of the 24 patients (25%) had no detectable antigenaemia. The enolase assay was briefly commercialized by Becton-Dickinson, (Directigen; Becton-Dickinson, Sparks, MA, USA); but subsequently withdrawn.

A dot immunobinding assay using antibody affinity—purified against the 47 kD antigen was positive in 83% of patients with systemic *C. albicans* infections (Matthews & Burnie, 1988b). This dropped to 76% for species other than *C. albicans* and was less sensitive in neutropenic (77% positive) than non-neutropenic (87% positive) patients. The initial serum collected when the diagnosis was first considered, was positive in just 50%. The antigenic determinants among candidal hsp 90 have now been mapped by the Pepscan technique, identifying both conserved and species-specific epitopes (Matthews, Burnie & Lee, 1991). Antibody probes against the latter should provide a sensitive and specific serological test since large amounts of hsp 90 and its breakdown products circulate in systemically infected patients (Matthews *et al.*, 1987).

The application of the polymerase chain reaction (PCR) to the diagnosis of candidosis has produced some encouraging initial results, on small numbers of clinical specimens (sputa, urines, blood, wound), through amplification of the fungus-specific C14-demethylase gene (Buchman *et al.*, 1990), although the identity of the PCR product was not confirmed by hybridization. Kan & Bennett (1992), applied PCR, amplifying the actin gene, to sera from eight patients who were blood culture positive for *Candida* spp. Six of the patients were positive by PCR and the specificity of the product was confirmed by hybridization. Two false-negatives is perhaps surprising for a test, capable of detecting 25 fg DNA. A means of circumventing the potential problem of amplifying highly conserved genes, is the selection of primers from a species-specific part of an otherwise conserved gene. This approach has been applied to the hsp 90 gene of *C. albicans* in a prospective study of clinical specimens.
In conclusion, assays for circulating candidal antigens currently offer the best laboratory diagnosis over and above blood cultures. While the sensitivity of these tests could be better, particularly early on in the infection, their specificity is high. These assays can distinguish colonized from systemically infected patients; they are more sensitive than blood cultures alone and they provide a means of monitoring response to therapy. They are particularly useful in high-risk ill patients with no positive cultures or with positive cultures of unknown significance. Serum for testing should be taken before treatment starts or very soon after when antigen titres are likely to be at their highest. Thereafter follow-up sera should be taken every couple of days to confirm that the titre is falling—if it does not this indicates that treatment is suboptimal and a poor prognosis likely. Which assay system is selected might depend on the patient population being examined; the Cand-tec kit for post-operative cases and if available the enolase assay before treatment starts or very soon after when antigen titres are likely to be at their highest. Therefore unlike antigen assays, it may not be possible to determine a cut-off point above which a 'positive' result is considered significant.

RUTH C. MATTHEWS
Department of Medical Microbiology,
University of Manchester,
Oxford Road,
Manchester M13 9PT, UK

References


American Society for Microbiology, Washington, DC.


Matthews, R. C. & Burnie, J. P. (1989). Cloning of a DNA sequence encoding a major fragment of the (Crampin & Matthews, 1993). Overall, PCR was more sensitive than routine culture (37% positive compared to 23% respectively) but it did give some false-negatives and was sensitive to just 50 pg DNA (5 pg after Southern blotting against a radiolabelled probe). If PCR is to be useful in the early diagnosis of systemic candidosis it must be more sensitive than blood culture and not give false positives in patients who are merely colonized. The latter may be a problem if a sensitive PCR assay is developed, if quantification of the PCR is not achievable. Therefore unlike antigen assays, it may not be possible to determine a cut-off point above which a 'positive' result is considered significant.
Leading article

Cancer Treatment Reviews appears in a new format and with a new editorial team. A journal devoted to important advances in the field of cancer treatment, the broad variety of articles have always been a unique feature of the journal. Reviews now appear under new section headings for easy access to information; everyone working in oncology should always find topics of major interest to them. Cancer Treatment Reviews provides an especially valuable forum for physicians and oncologists by publishing the opinions of leading experts on recent developments in the therapy of specific cancers and in related basic sciences.

**Research Areas**
- All aspects of treatment including:
  - Surgery
  - Radiotherapy
  - Chemotherapy
  - Immunotherapy
  - Combined modality approaches

**A Selection of Recent and Forthcoming Papers**

**Controversy**

**Tumour Review**
Chemotherapy of primary brain tumours, S. Grotta.

**Anti-Tumour Treatments**
Fractionation of radiotherapy, H. Bertelskink.

**New Drugs**
Taxol and taxotere, M.M. Rubart.

**Complications of Treatment**
Strategies for prevention of anthracycline cardiotoxicity, J.R. Bassett & M.D. Green.

**General and Supportive Care**

**The Laboratory-Clinic Interface**
Potential for protease inhibition in cancer treatment, D. Dziewulski & A. Howitt.

**Required Reading from Academic Press**

Cancer Treatment Reviews ISSN: 0305-7372
Publication: Volume 19, 1993, Quarterly
Subscription: Institutional Rate, £68.00 (U.K. /Europe) /US.$119.00 (Elsewhere)
* Personal Rate, £34.00 (U.K. /Europe) /$68.00 Elsewhere
Please add VAT at your local rate or add your VAT number here ______________
☐ Please enter my subscription for Volume 19, 1993
☐ Please send me a sample copy / "Instructions to Authors"
☐ I enclose a cheque/Eurocheque for ____________________________
☐ I wish to pay by credit card. Amount ____________________________
Diners Club/American Express/Visa/Barclaycard/MasterCard (delete as appropriate)
Card Number: ____________________________ Expiry Date: ______________
Signature ____________________________ Name (Capitals) ____________________________
(Two signatures is essential, especially when paying by credit card)
Address ____________________________
Date ______________

*Personal Rates are available only on orders placed with the publisher and paid for out of personal funds.*
Once-a-day
Rocephin®
IV-IM

Once-daily administration

Plasma levels exceeding the MIC99 for most susceptible pathogens for 24 hours

Broad spectrum bactericidal activity

Proven efficacy and established safety profile

Cost-effectiveness and convenience

The World’s Best Selling Injectable Antibiotic

References

Brief Prescribing Information
Indications Pneumonia, sepsis, meningitis, bone, skin and soft tissue infections, infections in neutropenic patients, gonorrhoea, per-operative prophylaxis of infections associated with surgery. Treatment may be started before the results of susceptibility tests are known. Dosage and Administration Rocephin should be administered by deep intramuscular injection, slow intravenous injection, or as a slow intravenous infusion after reconstitution of the solution. Adults and children 12 years and over: Standard dosage - 1g once daily. Severe infections - 2-4g normally once daily. Duration of therapy varies according to course of disease. Gonorrhoea - single dose of 250mg i.m. Per-operative prophylaxis - usually single dose of 1g, colorectal surgery 2g in conjunction with a suitable agent against anaerobic bacteria. Children under 12 years: Standard dosage - 20-50mg/kg once daily. Severe infections - maximum 80mg/kg once daily. Dosage of 50mg/kg or over should be given by slow intravenous infusion over at least 30 minutes. Renal and hepatic impairment: In the absence of hepatic impairment dose reduction is required only in severe renal failure (creatinine clearance <10ml/min), when the daily dose should be 2g or less. No dose reduction is required in liver damage provided renal function is intact. In severe renal impairment accompanied by hepatic insufficiency the plasma concentration should be determined at regular intervals and dosage adjusted. Serum concentrations should be monitored in dialysis. Contra-indications, Warnings etc.: Cephalosporin hypersensitivity. Premature infants. Full term infants during first six weeks of life. Safety in pregnancy has not been established. Precautions: Severe renal failure should not be exceeded. Caution in patients with a history of hypersensitivity (especially anaphylactic reaction) to penicillins or other non-cephalosporin beta-lactam antibiotics. Anaphylactic shock requires immediate countermeasures. Severe renal impairment accompanied by hepatic insufficiency (see Dosage). Side-effects and Adverse Reactions Gastro-intestinal side-effects including loose stools, diarrhoea, nausea, vomiting, stomatitis and glossitis. Cutaneous reactions including maculopapular rash, pruritus, urticaria, oedema and exanthema multiforme. Haematological reactions including anaemia (all grades), leucopenia, neutropenia, thrombocytopenia, eosinophilia, agranulocytosis, positive Coombs’ test and prolongation of prothrombin time. Regular blood counts should be carried out during treatment. Other reactions include headache, dizziness, drug fever and transient elevations in liver function tests. Rarely: hypoglycaemia, rigors, haematuria, anaphylaxis and bronchospasm. Very rarely precipitation of ceftriaxone calcium salt in urine in patients on higher than recommended dose. Reversible precipitates of calcium ceftriaxone have been described in gallbladder sonograms. In symptomatic cases (which are rare), conservative non-surgical management is recommended. Superinfections with yeasts, fungi or other resistant organisms. Rare instances of pseudomembranous colitis. Injection site pain and local phlebitis. Legal Category POM. POM: Presentations and Basic NHS Cost 250mg vials i.m. and i.v. (containing 250mg ceftriaxone) - £30.00 1g vials i.m. and i.v. (containing 1g ceftriaxone) - £72.00 2g vials for infusion (containing 2g ceftriaxone) - £24.00. Product Licence Numbers PL 0031/0169 (250mg vials) PL 0031/0171 (1g vials) PL 0031/0172 (2g vials)
Product Licence Holder Roche Products Limited, PO Box 8, Welwyn Garden City, Hertfordshire. ALTER DAY Full prescribing information is available on request.