Antibodies to *Candida albicans* germ tubes in two intensive care patients with invasive candidiasis

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Summary

Two cases of invasive candidiasis in intensive care patients are presented to illustrate the usefulness of detection of antibodies to *Candida albicans* germ tubes in the diagnosis of invasive candidiasis and in monitoring the efficacy of the antifungal treatment.

Key words

Candida albicans, ICU, Antibody detection, Germ tube antigens

Anticuerpos anti-micelio de *Candida albicans* en dos pacientes decuidados intensivos con candidiasis invasora

Resumen

Se describen dos casos de candidiasis invasora en pacientes de una Unidad de Cuidados Intensivos en los que se muestra la utilidad de los anticuerpos frente a la fase micelial de *Candida albicans* en el diagnóstico de la candidiasis invasora y en el seguimiento del tratamiento antifúngico.

Palabras clave

Candida albicans, UCI, Detección de anticuerpos, Antígenos de fase micelial

The incidence of systemic *Candida* infections in patients in intensive care units (ICUs) has increased in recent years as a result of a combination of factors [1]. The greater number of immunocompromised patients, the more frequent use of surgery, instrumentation and broad-spectrum antibiotics are particularly important [2].

The diagnosis of *Candida* infections may be difficult, as the clinical presentation is variable and nonspecific. Although candidemia is generally used as an indicator of antifungal therapy, unfortunately systemic infections can occur when blood cultures are negative [3]. Definitive diagnosis is often not made until late in the course of infection and is associated with significant morbidity and mortality in ICU patients [4]. A number of approaches of non-culture methods have been investigated in order to obtain an early diagnosis of invasive candidiasis [5].

These techniques include detection of ß (1,3) D-glucan [6], D-arabinitol [7], enolase [8], DNA [9-10], antigens and antibodies [11]. Nevertheless, each technique has limitations and none has found widespread clinical use.

The clinical usefulness of antibody detection in invasive candidiasis has been limited by problems of specificity and sensitivity [11]. The antibodies to defined antigens of *Candida albicans* blastopores (CAB), essentially cell-wall mannan, have been shown to be ubiquitous in human sera [3,12]. Antibodies to CAB were elevated in patients with invasive candidiasis when measured in serially drawn sera, but this response could not be clearly distinguished from that observed in patients who were not actively infected with *Candida* [13]. However, in recent years encouraging results have been obtained with the detection of antibodies to *C. albicans* germ tubes (CAGT), even in immunocompromised patients with invasive candidiasis [14-15]. In addition to its diagnostic value, detection of antibodies to CAGT has been claimed to be useful to monitorize the efficacy of the antifungal therapy [16]. We report two cases of invasive candidiasis in critical patients in whom serially drawn sera were studied for detecting antibodies to CAB and CAGT.

Patient 1. A 37-year-old man was admitted to the hospital because of multiple trauma after a car accident. On admission, the patient was confused. Physical examination revealed thoracic and abdominal contusion, several rib fractures and rhabdomyolisis. A cranial computerised tomography (CT) scan showed cerebral oedema. Respiratory failure developed on the third hospital day. Endotracheal intubation was inserted and assisted ventilation begun. A tracheal aspirate specimen yielded

Figure 1. A. Main clinical and microbiological data of patient 1.
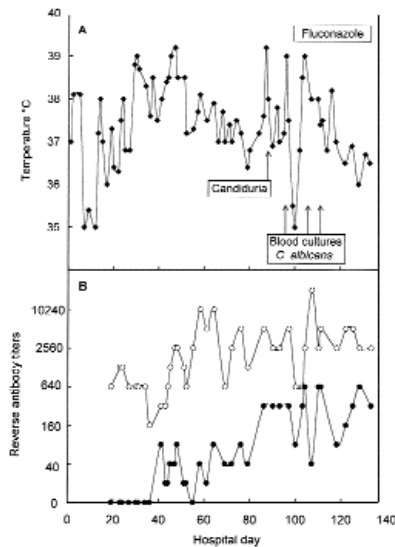B. Kinetics of antibodies to CAGT (●) and CAB (o).

Figure 2. A. Main clinical and microbiological data of patient 2.
B. Kinetics of antibodies to CAGT (●) and CAB (o).

Morganella morganii. On the fifth hospital day hypotension and anuric renal failure required the administration of vasopressor agents and continuous hemofiltration. On the 17th hospital day a radiograph of the chest showed areas of consolidation in both lower fields. A protected sampling brush revealed >1000 c.f.u./ml of *Acinetobacter baumannii* and imipenem and amikacin therapy was begun. On the 23rd hospital day bacteremia by *Enterococcus faecalis* occurred. An abdominal CT scan showed no abnormalities. Ampicillin was added and amikacin discontinued. The patient became more stable and continuous hemofiltration and vasopresssor agents were discontinued on days 38 and 42 respectively. The weaning of the ventilator was difficult because of altered consciousness of the patient. Urine samples were obtained for culture twice a week, but all the specimens were negative until the 82nd hospital day, when *Pseudomonas aeruginosa* was yielded and ciprofloxacin therapy was started. On 88th hospital day fever and hypotension developed requiring fluid expansion and high doses of vasopressor agents. A blood culture was negative, but *C. albicans* was isolated in urine (Figure 1). On 97th hospital day temperature rose to 40°C and shock developed. It became necessary to administer maximal doses of pressor agents and continuous hemofiltration was reinstaurated. Blood cultures obtained on 97th hospital day as well as on 105th and 108th days yielded *C. albicans*. Intravenous catheters were replaced and fluconazole therapy was given intravenously for four weeks at a dose of 400 mg/day. A transthoracic cardiac ultrasonography did not reveal endocarditis. As the patient's condition became more stable, dopamine and dobutamine were discontinued. The patient was discharged to an hospitalization room since his temperature did not exceed 38°C.

Serum samples were drawn at least twice a week and whenever any blood culture was obtained since the second week of hospital admission until the discharge from the ICU. Antibodies to CAB and CAGT were detected as previously reported [14,17]. The patient showed a high titer of antibodies to CAB from the first sample (1/1280) and experienced a moderate increase at the time the candidemia was documented (1/5120 to 1/20480) (Figure 1). On the other hand, antibodies to CAGT were
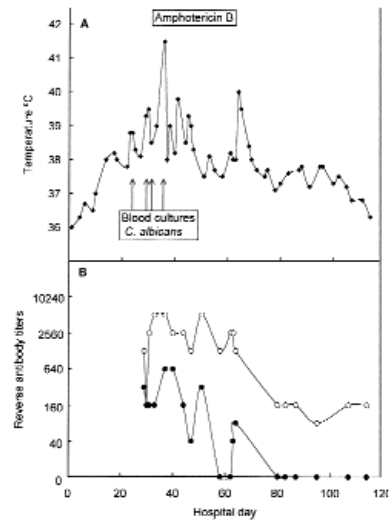
not detected during the first month, when bacterial pneumonia and bacteremia developed. Along the second month of the hospital stay, low titers of antibodies to CAGT (1/20 to 1/80) were detected and an increase in the titers of antibodies to CAGT (≥1/160) was shown at the time the first episode of fever, hypotension and funguria by *C. albicans* was documented. The titers of antibodies to CAGT remained high when new episodes of fever, hypotension and candidemia were documented.

Patient 2. A 24-year woman with Crohn´s disease was admitted to the hospital because of elective surgery after recurrent episodes of diarrhea during the last year. After colectomy and ileorectal anastomosis, metronidazole and gentamicin were administrated. On the 13th hospital day fever occurred and piperacillin-tazobactam therapy was instaurated. On the following days, fever continued and progressive dyspnea developed, being admitted in ICU on the 21st hospital day where the patient was intubated by respiratory failure. Radiographs of the chest showed diffuse bilateral air-space opacities. Severe hypotension developed requiring dopamine and norepinephrine. Blood, urine and bronchial aspirate specimens were drawn and empiric therapy with vancomycin, imipenem and amikacin was instaurated. The temperature rose to 40°C on each of the next six days, and laparotomy with permanent ileostomy was performed. Blood cultures obtained on admission in UCI as those obtained six days earlier yielded *C. albicans* (Figure 2). Therapy with amphotericin B was administered from the 32nd until 61st hospital day to complete a cumulative dose of 1.3 g. Temperature did not decline under 38°C until 12 days after antifungal therapy was begun. The patient remained in UCI for three months because of polyneuropathy of critical patient. An *A. baumannii* bacteremia on 75th hospital day was treated with imipenem and amikacin. The patient was discharged to an hospitalization room after a long stay (86 days) in UCI.

Serum samples were drawn since her admission in UCI until her discharge. The first sample showed high titers of antibodies to CAB and CAGT (Figure 2). Although antibodies to CAB remained high after the antifungal therapy, the antibodies to CAGT declined progres-

sively and were undetectable four weeks after antifungal therapy with amphotericin B was begun.

DISCUSSION

Patients admitted in ICUs are at high risk for nosocomial infections [18]. Serious *Candida* infection can present as generalised sepsis in critical patients and it can be indistinguishable from sepsis with gram-negative or gram-positive bacteria [2]. For treatment to be successful it must be instituted promptly and, on occasions, empirically. Establishing the diagnosis of invasive candidiasis is difficult because the extent to which candidemia detects deeply invasive candidiasis remains limited [3]. As a result, new diagnostic techniques have been developed. Although the clinical usefulness of antibodies in invasive candidiasis has been controversial, detection of antibodies to CAGT may be a good marker of *Candida* systemic infection [13,15].

Antibodies to CAGT are induced against type I and II antigens, which are primarily expressed on the germ tube cell wall surface [19-21]. Since yeast-to-mycelial conversion occurs during tissue invasion [22,23], antibodies produced to antigens expressed only in the mycelial phase might be diagnostically useful in invasive candidiasis [24]. Detection of antibodies to CAGT may be useful in both the diagnosis and therapeutic monitoring of patients with invasive candidiasis. Patient 1 is an example of its diagnostic usefulness. In this patient antibodies to CAGT were not detected during the first month of hospitalization. During the second month antibodies to CAGT were detected at low titers, but titers of 1/160 were detected on 88th hospital day when the patient had an episode of fever and hypotension with negative blood culture. Antibodies to CAGT remained at titers ≥1/160 when new episodes of candidemia appeared.

In contrast to candidemia that arises from an intravascular focus, episodes of candiduria-associated candidemia can be low-grade and of short duration, and therefore it is not uncommon to obtain negative blood culture [25,26]. Particularly interesting was the detection of antibodies to CAGT at titers 1/160 nine days earlier than *C. albicans* was yielded in blood culture, suggesting that invasive candidiasis could be present when the episode of fever with candiduria and negative blood culture occurred.

Patient 2 is an example of the role of sequential detection of antibodies to CAGT in monitoring the efficacy of antifungal therapy in invasive candidiasis. In this patient, titers of antibodies to CAGT declined with amphotericin B therapy and eventually disappeared, four weeks after beginning antifungal therapy. These results are in agreement with previous observations by our group and confirm that monitoring of titers of antibodies to CAGT can be useful to evaluate the response to antifungal therapy [15,27].

In contrast to detection of antibodies to CAGT, antibodies to CAB, which are mainly detected against mannan, are found in most human sera, and therefore, are less useful to make the diagnosis of invasive candidiasis [3,28]. As it is shown in patient 1, antibodies to CAB were detected since the first serum sample, at titers 1/1280, and remained at high titers along the first two months, when various bacterial infections but no fungal infection occurred. Although a slight increase in titers was observed when *C. albicans* was isolated in blood culture, it is difficult to establish a cutoff to discriminate patients with invasive candidiasis from those without candidal infection, since patients without invasive candidiasis may also have high titers of antibodies to CAB. On the other hand, although antifungal therapy was associated with a progressive decrease in the titers as is shown in patient 2, antibodies to CAB remained at titers 1/160 three months after therapy with amphotericin B was begun.

In conclusion, the cases presented in this paper provide evidence of the usefulness of detection of antibodies to CAGT in the diagnosis and therapeutic monitoring of invasive candidiasis in intensive care patients. Although a titer ≥1/160 is highly suggestive of invasive infection by *C. albicans*, much information can be gained from the sequential detection of antibodies to CAGT in patients at risk for developing invasive candidiasis.

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