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Candida-Antigen-Titer (CAG-Titer) for Detection and Early Treatment of Impending Candidemia

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Research Article

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ABSTRACT

Background: Candidemia is a life threatening infection with mortality over 20%.

Objective: Despite this there is little consensus on the use of serum-based diagnostics. At our institution we have used candida-antigen-titer (CAG titer) since 25 years for therapeutical decisions.

Methods: In this retrospective study correlation between CAG titer and mortality were evaluated on 945 burn intensive care patients admitted from January 1988 to December 2011. Inclusion criteria were age over 14 years, burn injury and intensive care treatment, no previous treatment outside our institution, at least one CAG titer measured and complete abbreviated burn severity index score (ABSI). Statistical evaluation was performed by uni- and multivariate analysis and the finale model using the SPSS program.

Results: Out of 945 patients 877 were included in the study. Mean age was 42.3 years, male patients were dominant (72.2%). Mean length of stay in the burn intensive care unit was 32.5 days and mean mortality rate was 22%. Increasing mortality was found with increasing CAG titers. This was concordant with increasing ABSI score and increasing total burned surface area (TBSA). Candida species most often detected was *candida albicans* and *parapsilosis*. The earliest detected antigens were *candida famata* and *tropicalis. Candida parapsilosis* was also detected first in the blood stream. Predicting variables for death were sex, multi-organ failure, age, total burned surface area, antibiotic use and CAG titer > 1:8.

Conclusion: Cut off points in candida titers beyond 1:4 in patients with infection not otherwise explainable offers the opportunity for early antimycotic therapy. Although the latex assay is not a perfect tool to diagnose candidemia in the very early stages, it is a useful armament in our hands to initiate treatment in burn intensive care patient population.

INTRODUCTION

Candidemia is a potential lethal infection, which, when causing sepsis, has a high mortality rate over 20%[1]. Even in the light of high mortality the use of serological biomarkers is still being discussed[2,3,4].

There are several test offered on the market to diagnose candida within the blood stream, such as Beta -(1,3)- D - Glucan (Fungitell®), Mannan Antigen (Platelia Candida Ag®), Anti - Mannan Antikörper (Platelia Candida Ab®), Candida species specific DNA (snPCR), Candida Antigen Titer (Cand-Tec ®)[3,5,6,7,8]. So far, there is no large study available using CAG titer for diagnosing mycosis in intensive care patients. At our intensive care burn unit CAG titers are being measured since 25 years in burn and

intensive care medicine for diagnosing candidemia mycosis[3]. However diagnosis of a mycosis caused by candida is challenging. Also, antimycotic therapy is costly and former used antimycotics had a high toxicity.

Several risk factors are described in literature for developing a candidosis: central venous catheters, high caloric parenteral nutrition, parenteral antibiotic therapy, topical antiseptic therapy that does not cover or covers only partially fungal infection e.g. Mafenidacetat 5%, or Sulfamylon, and necrotic skin.

A candidosis might result in the following clinical constellation before an antimycotic therapy is even started: the patient has fever and is getting circulation supporting drugs such as catecholamines; wound healing is slow or impeded; the patient receives via central vein catheter antibiotics however an improvement of the sepsis cannot be observed; an increased CAG titer points at the possibility of a candidosis.

The use of CAG titer and biomarkers are discussed controversially, because there is a lack of studies on this subject[4,9,10,11]. In addition the CAG titer is getting confused with other titer for candidosis. However the use of these other titers (e.g. candida - hemagglutinin (CHA), candida immunofluorescence (CIF) is questionable, because of these titers are positive only in the late phase of a candidosis[9,10,11]. Therefore we initiated a retrospective study to evaluate if knowledge on CAG-titer can be used for a rational therapy.

We hypothesized that early onset of antimycotic therapy can decrease mortality when a candidosis is suspected. Our hypothesis was based on several cases treated at our institution when antimycotic therapy resulted in a diminishing CAG antigen titer, paralleled by an improvement of the multi-organ failure (MOF).

In the current study we evaluated the use of the CAG titer fostering antimycotic therapy and if an antigen titer 1:8 and higher results in higher mortality, longer burn intensive care stay, and might be predictive for impending sepsis.

PATIENTS AND METHODS

The study complies with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The work presented here has been approved by the ethical committee of the University of Witten/Herdecke.

In the time from January 1988 to December 2011, CAG-titers (6124) were measured on 877 burn patients with thermic, electrical or chemical burns. Management had not changed significantly for these patients. Otherwise, during 1990 – 2006 some patients were treated with Nystatin for prophylaxis, however these patients were not included in the study. For evaluation several data were available. Especially data on concomitant diseases of every patient, process of the burn injury and other sustained injuries, calculated abbreviated burn severity index (ABSI), all microbiological data of every patient, all CAG titers of all patients and data on catecholamine use, respiratory therapy and FiO2, use of blood and blood products, antibiotic and antimycotic therapy. The ABSI score is a score for developed burn patients to estimate mortality[11]. Described mortality is the mortality of all the patients during and after burn intensive care treatment, patients who were discharged to other hospitals for further therapy were not included in the study. Inclusion criteria were: sustained burn injury and burn intensive care needed, age over 14 years, no previous treatment outside our institution, ABSI data, and at least one CAG titer measured.

In our hospital the CAG-titer is currently measured 2 x per week. When a patient shows increasing signs of infection in the laboratory (i.e. increasing white blood cell count, increasing C - reactive protein, increasing need of circulation supporting medication and increasing temperature) with MOF the titer is used for evaluation of the necessity of an antimycotic therapy:

A 1:2 titer is being ignored. If sepsis cannot be explained otherwise a titer of 1:4 may result in the use of antimycotics. Except for patients with pressure sores, who usually show titers of 1:4 to 1:8, a titer of 1:8 often results in the use of antimycotic therapy. In patients with titers of 1:16 and higher antimycotic therapy is always started. Antimycotics are chosen according to the antimycotic resistogram and by discussing on microbiology rounds, provided by the Department of Microbiology. In addition to antimycotic therapy dressings are changed more often and topical antifungals are applied such as Amphotericin B, or Betaisodona and all catheters are exchanged.

For statistical evaluation univariate (sex, gender, MOF, age, burned body surface, burn depth, escharotomy, fasciotomy, antibiotic use, co-morbidities, and intubation), Chi-square and Fischer Exact test, uni- and multivariate model analysis, finale model analysis and the Nagelkerke and Cox & Snell R-square tests were used. Differences were considered significant for a $p<0.05$. Statistical evaluation was done using the SPSS program version 14.0 (SPSS Chicago).

RESULTS

Out of 945 evaluated patients, a total of 877 patients were included in the study. 68 patients were excluded because of missing data or incomplete data. Basic data of all patients are demonstrated in **Table 1**. Interestingly the male patient population was dominant. Median total burned surface area (TBSA) was relatively low in the patient population. Most often *candida albicans*

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and *candida parapsilosis* antigens were found in the entire patient population. We observed an increasing mortality with higher CAG titers, the highest mortality being 62.5 % at a CAG titer of 1:16. This finding was concordant with higher TBSA and a higher ABSI score **(Table 2)**. When comparing the titers measured the last before the patient was discharged mortality was found proportional to TBSA with the highest mortality being observed in the group with a 1:8 CAG titer **(Table 3)**. The lowest mortality was found in patients with no CAG titer shown and in patients with a CAG titer of 1:2 **(Tables 2,3)**. Most patients received antimycotic treatment with Fluconazol, resulting in the lowest observed mortality rate (39.7%). Voriconazol and Amphotericin B had the highest (80%) and second highest (58.1%) mortality rates in the groups of antimycotics given **(Table 4)**. Of the various species of candida, *candida famata* antigen was detected the earliest (1 day), followed by candida tropicalis antigen (6, 7 days), **(Table 5)**. *Candida parapsilosis* itself was detected first within blood cultures (8 days) **(Figure 1)**, whereas *candida guillermomdi* was detected last (31 days) **(Table 6)**.

Table 1."Basic data of patient population."

Mean TBSA (%), (range)	27,6 (2 - 99)
Mean age (years) (range)	42,3 (3 - 96)
Femalen (%)	244 (27,8)
Male n (%)	633 (72,2)
Inhalation injury n (%)	370 (42,4)
Intubation n (%)	442 (50,4)
Mean intensive care length of stay (days), (range)	32,5 (15 - 180)
Meanventilatordays (range)	19,0 (1 - 180)
Necrectomy (n=583) mean number of operations	3,3
Escharotomy (n=241) mean number of operations	4,4
Antibiotic treatment n (%)	693 (79,0)
Mortality n (%)	193 (22,0)

Table 2. "Evaluation of CAG titers, Maximum of measured CAG titers, mean age in years."

CAGtiter		TBSA (%)	Mean age	ABSI	Mortality (%)
neg	357	18,6	39,4	4,4	7,6
1:2	175	25,5	42,1	5,6	16,6
1:4	148	34,1	43,2	6,8	25,0
1:8	121	37,4	47,9	7,8	45,5
1:16	56	47,9	44,4	8,5	62,5
1:32	11	44,3	49,8	8,5	54,6
1:64	2	51,3	62,5	6	50,0

Table 3. "Antimycotic treatment given."

Antimycotic	n (Pt)	TBSA (%)	ABSI	Mortality (%)
Fluconazol	194	41,6	8,3	39,7
Ampho B	62	49,0	9,4	58,1
Flucytosin	34	43,5	8,8	43,5
Voriconazol	20	43,3	7,1	80,0
Ambisome	7	55,7	9,9	71,4
Caspofungin	5	65,5	9	100
Anidulafungin	3	65,3	10	66,7

Table 4. "Evaluation of CAG titers: first time CAG titer detection for different species of Candida(Day f) and first time serological detection of Candida species in blood (Day b)."

Species	n	Day f	n	Day b
albicans	231	11,3	12	28,1
parapsilosis	32	21,3	2	8
tropicalis	18	6,7	0	
guillermomdi	5	7	2	31
kefyr	1	19	9	
glabrata	24	15,0	1	13
krusei	5	25,2	0	
pseudotropicalis	1	7	0	
famata	1	1	0	

55.18% of the patients were intubated and 80.77% of all patients had received antibiotic treatment before first signs candidemia were proven. Mortality was 22.54% because of multi organ failure; however, 28.1% did die but not because of multi organ failure but of pneumonia from associated inhalation injury, and total survival rate was 49.38% **(Table 7)**.

Univariate analysis showed significant differences for sex ($p < 0.094$), MOF ($p < 0.001$), age ($p < 0.001$), burned body

surface ($p < 0.001$), CAG titer 1:8 ($p < 0.022$) and antibiotic use ($p < 0.006$). Multivariate analysis confirmed these results, the finale model analysis showed that CAG titer 1:8 or higher as an independent predicting variable for death, with an odds ratio of 1.77, a significance of $p < 0.021$ and an R^2 (Nagelkerke) of 0.602. Interestingly, no effect was found for intubation, escharotomy or fasciotomy or comorbidities.

Table 5. "Evaluation of CAG titers: first time CAG titer detection for different species of Candida (Day f) and first time serological detection of Candida species in blood (Day b)."

Species	n	Day f	n	Day b
albicans	231	11,3	12	28,1
parapsilosis	32	21,3	2	8
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guillermomdi	5	7	2	31
kefyr	1	19	9	
glabrata	24	15,0	1	13
krusei	5	25,2	0	
pseudotropicalis	1	7	0	
famata	1	1	0	

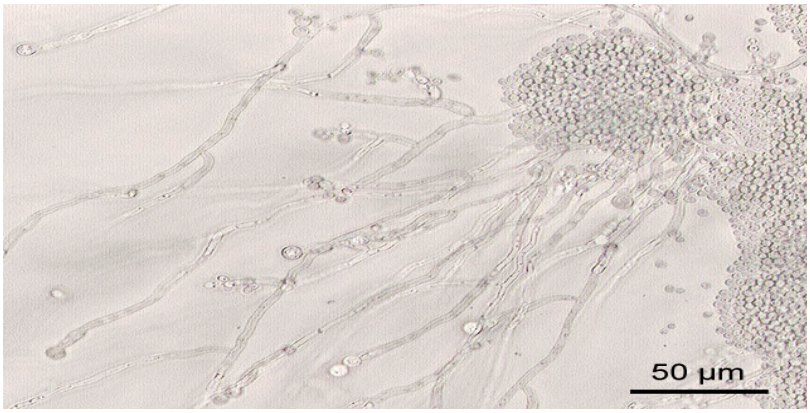

Figure 1. "Candida parapsilosis pseudohyphae and spores detected in blood culture."

Table 6. "First time serological detection of Candida species in blood."

Species	n	Day
albicans	12	28,1
parapsilosis	2	8
tropicalis	0	
guillermomdi	2	31
kefyr	9	
glabrata	1	13
krusei	0	
pseudotropicalis	0	
famata	0	

Table 7. "Percentages of antibiotic treatment, intubation and mortality because of multi organ failure."

	Antibiotic treatment	Intubation	Multi organ failure death
True	80,8%	55,2%	22,5%
False	19,2%	44,8%	28,1%

DISCUSSION

In literature there is evidence of an increase of invasive fungal infections, i.e. also an increase in candidemia[13,14,15]. Reasons quoted are an increase in immuno-compromized patients, also induced by immuno-compromizing medication. There is an increased risk for opportunistic candidemia found in patients on the surgical intensive care units[16]. Candidemia causes 10% of all nosocomial acquired septicemias, thereby being statistically the fourth most causing microorganism[17]. Mortality for candidemia is being quoted as high as 10%[18,19]. Sweeney at al. have shown, that candidemia is associated with decreased immuno-function of the host, especially impaired polymorphonuclear lymphocyte function[20].

Therefore there is an increased need for early and reliable diagnosis of candidemia to start early and efficient therapy[21]. There are several diagnostic kits on the market. In our institution we routinely use the Latex agglutination test. This Cand-Tec assay® (Ramco Laboratories Inc., Houston, Tex.) uses latex beads coated with anti-Candida polyclonal antibody[22]. The circulating

mannan antigen is detected by using the Pastorex Candida assay (Diagnostics Pasteur, Marnes-la-Coquette, France), in which latex beads are coated with the anti-mannan monoclonal antibody[23]. In addition there are the beta-1,3-glucan test (Fungitell®, the Mannan antigen test (Platelia Candida Ag®), the Anti Mannanantbody test (Platelia Candida Ab®), and the Candida species specific DNA test (snPCR). In a previous study Mitsutake et al evaluated sensitivity and specificity of the Mannan antigen test, beta-1,3-gliucan test, latex-agglutination test and the enolase antigen test. Each of the tests had its drawbacks, so to increase sensitivity and specificity the combination of two tests were recommended[3]. However, Mitsutake et al had a relatively small patient sample of 39 patients. In our study 877 patients were included.

The detection of candida antibodies is of limited value. A false-positive test in healthy individuals may result from exposition from the intestinal flora or other sites. A positive test does therefore not distinguish between past and present infections. If blood samples are collected in early stages of an infection a humeral response might not have been developed and the test will show in a false-negative result. Other false-negative tests might be the consequence of immuno - compromised patients unable to develop an effective antibody response[3]. Burn patients are immuno - compromised. Therefore this assay might not be suitable for burn intensive care patients. In a consequence we have used the Latex agglutination test.

There have been reports of improved test for candida antigens[24,25]. These test are mainly based on the detection of mannan antigen, the test dominating the markets today is the Pastorex Candida assay[3]. Although sensitivity and specificity of this assay are high, it has, however, a drawback as it does not react with mannan antibodies from *candida krusei*[22]. Mitsutake et al have shown in their study, that when using the Pastorex Candida assay the mannan antigen was not detected in the majority of their patient population with positive blood cultures[3]. The first Candida species detected were *candida famata* and *candida tropicalis*.

A proportionally increasing mortality was found with increasing TBSA and candidemia in our patient population. However the amount of burned surface and thereby proportional reduced immunological response might have contributed to a higher infection rate with candida, consequently leading to a higher mortality rate. This is supported by recent literature[20,26]. A higher TBSA was also concordant with a higher intubation rate. However, a higher TBSA was often associated with a higher rate of inhalation injury, thereby leading to a higher intubation rate. Additionally, antibiotic therapy is theorised as being a factor influencing favourably the beginning of candidemia, thereby increasing mortality[26,27,28]. 80% of our patients had received antibiotic treatment before candidemia was proven. The aim of this study, however, was not to show influence of antibiotic treatment on candidemia. Therefore, this question remains to be answered in a further study.

Although Bang et al. have shown in their study that multi-organ-failure (MOF) was cause of death in 60 – 85% polymicrobial sepsis, only 22.54% of patient in study our suffering from candidemia died of MOF, 28.1% died of different causes, most often from pneumonia from associated inhalation injuries[29]. 49%, however, survived in our study. Gareyet al. and Labelle et al. have shown that initiation of antimycotic treatment in time is essential in reducing mortality[30,31]. In our study we have observed after early onset of antimycotic treatment at candida titers of 1:4 lower death rate from MOF than described in literature. A titer of 1:8 proved to be a statistically independent variable for death, using the finale model analysis for our patient population.

The cut off points in candida titers beyond 1:4 in patients with infection not otherwise explainable offered us the opportunity to react early at its first time of onset and initiate antimycotic therapy. The Latex agglutination test is described not to be ideal for detecting candidemia at very early stages. However, it is a useful additional tool in our hands to initiate antimycotic therapy and treat candidemia infections. We recommend starting an antimycotic therapy at a CAG titer 1:8.

REFERENCES

1. Kollef M, et al. Septic shock attributed to Candida infection: importance of empiric therapy and source control. Clin Infect Dis. 2012;54:1739-1746.
2. Pontón J. Usefulness of biological markers in the diagnosis of invasive candidiasis. BMC Infect Dis. 2007;7:103.
3. Mitsutake K, et al. Enolase antigen, mannan antigen, Cand-Tec antigen, and beta-glucan in patients with candidemia. J ClinMicrobiol. 1996;34:1918-1921.
4. Marchetti O, et al. European Conference on Infections in Leukemia (ECIL) Laboratory Working Groups. ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients. Bone Marrow Transplant. 2012;47:846-854.
5. Nguyen MH, et al. Performance of Candida real-time polymerase chain reaction, β-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. Clin Infect Dis. 2012;54:1240-1248.
6. Lefort A, et al. French Mycosis Study Group. Diagnosis, management and outcome of Candida endocarditis. ClinMicrobiol Infect. 2012;18:E99-E109.
7. Alam FF, et al. Comparative evaluation of (1, 3)-beta-D-glucan, mannan and anti-mannan antibodies, and Candida species-specific snPCR in patients with candidemia. BMC Infect Dis. 2007;7:103.
8. Mitsutake K, et al. Enolase antigen, mannan antigen, Cand-Tec antigen, and beta-glucan in patients with candidemia. ClinMicrobiol. 1996;34:1918-1921.

9. Rüchel R. Diagnosis of invasive mycoses in severely immunosuppressed patients. Ann Hematol. 1993;67:1-11.

10. Reiss E and Morrison CJ. Nonculture methods for diagnosis of disseminated candidiasis. ClinMicrobiol Rev. 1993 Oct;6:311-323.

11. Walsh TJ, et al. Infections due to Trichosporon species: new concepts in mycology, pathogenesis, diagnosis and treatment. Curr Top Med Mycol. 1993;5:79-113.

12. Tobiasen J, et al. The abbreviated burn severity index. Ann Emerg Med. 1982;11:260–262.

13. Martin GS, et al. The epidemiology of sepsis in the United States from 1979 through 2000. N Eng J Med. 2003;348:1546-1554.

14. Pfaller M, et al. Invasive fungal pathogens: current epidemiological trends. ClinInfec Dis. 2006; 43:S3–S14.

15. Trick WE,et al. National Nosocomial Infections Surveillance System Hospitals. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. Clin Infect Dis. 2002;35:627-630.

16. Lichtenstern C, et al. Update: invasive Pilzinfektionen. Diagnose und Therapie in der operativen Intensivmedizin. Anästhesist. 2010;59:30–52.

17. Wisplinghoff H, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309-317.

18. Kuse ER, et al. Micafungin Invasive Candidiasis Working Group. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. Lancet. 2007;369:1519-1527.

19. Mora-Duarte J, et al. Caspofungin Invasive Candidiasis Study Group. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med. 2002;347:2020-2029.

20. Sweeney JF, et al. Elevated candida antigen titers are associated with neutrophil dysfunction after injury. ClinDiagn Lab Immunol. 1994;1:111 –114.

21. Cortes JA, et al. Diagnostic methods in candidemia: a systematic review of literature with meta-analysis. Rev Chil Infect. 2011;28:423-428.

22. Fung JC, et al. Candida detection system (CAND-TEC) to differentiate between Candida albicans colonization and disease. J. Clin. Microbiol. 1986;24:542–547.

23. Herent P, et al. Retrospective evaluation of two latex agglutination tests for detection of circulating antigens during invasive candidiasis. J. Clin. Microbiol. 1992;30:2158–2164.

24. Buckley HR, et al. Immunodiagnosis of invasive fungal infection. J. Med. Vet. Mycol. 1992;30(Suppl.):249–260.

25. Kohno S. Serological diagnosis of deep-seated mycosis. Asian Med. J 1991;34:460–466.

26. Bassetti M, et al. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. PLoS One. 2011;6:e24198.

27. Holzknecht BJ, et al. Decreasing candidaemia rate in abdominal surgery patients after introduction of fluconazole prophylaxis*. ClinMicrobiol Infect. 2011;17:1372-1380.

28. De Luca C, et al. Candidemia: species involved, virulence factors and antimycotic susceptibility. New Microbiol. 2012;35:459-468.

29. Bang RL, et al. Septicaemia after burn injury: a comparative study. Burns. 2002;28:746-751.

30. Garey KW, et al. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin Infect Dis. 2006;43:25-31.

31. Labelle AJ, et al. Treatment-related risk factors for hospital mortality in Candida bloodstream infections. Crit Care Med. 2008;36:2967-2972.