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Original Article

Is biofilm production a prognostic marker in adults with candidaemia?

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ABSTRACT

Objectives: The role of biofilm production in the outcome of candidaemia remains under discussion. Current evidence relies on variable biofilm detection methods while evaluating distinct clinical end points. We aimed to determine the impact of biofilm production measured by metabolic activity (MA) and biomass (BM) on the prognosis of adults with candidaemia.

Methods: Retrospective cohort including 280 adults with candidaemia admitted from 2010 to 2016. BM was assessed using crystal violet binding stain and the XTT reduction assay was used to detect MA. Strains were classified as high and moderate-low biofilm producers according to published cut-offs. The primary outcome was overall mortality within 7 and 30 days. The secondary outcome was unfavourable prognosis defined as metastatic infection, admission to an intensive care unit due to the severity of candidaemia, or death within 30 days.

Results: High BM and high MA were detected in 90 (32.1%) and 114 (40.7%) of the 280 isolates, respectively. Comparison of high and moderate-low biofilm forming isolates revealed no correlation between biofilm production and 7-day mortality (BM high 15/90 (16.7%) versus moderate-low 24/190 (12.6%); MA high 12/114 (10.5%) versus moderate-low 27/166 (16.3%)), 30-day mortality (BM high 34/90 (37.8%) versus moderate-low 61/190 (32.1%); MA high 33/114 (28.9%) versus moderate-low 62/166 (37.3%)), or unfavourable prognosis (BM high 45/90 (50.0%) versus moderate-low 73/190 (38.4%); MA high 41/114 (36.0%) versus moderate-low 77/166 (46.4%)).

Conclusions: Biofilm production was not a predictor of mortality or of unfavourable prognosis in adults with candidaemia. **P. Muñoz, Clin Microbiol Infect 2018;▪:1**

Introduction

Candidaemia remains one of the leading causes of nosocomial bloodstream infections, with a significant impact on morbidity, mortality and hospital costs [1–6]. Prognosis is mainly associated with clinical aspects related to the host and therapeutic measures, such as early adequate antifungal treatment and appropriate infection source control [7–12]. However, until recently, less attention had been given to the virulence elements of *Candida* species.

Biofilm formation is commonly associated with *Candida* infections and is currently considered a potential contributing factor in pathogenesis [1,3–7,13–15]. Data regarding the clinical impact of *Candida* biofilm production are scarce and contradictory, owing to studies with a small number of patients, inclusion of only particular *Candida* species, and technical variability in biofilm detection methods [3,4,7,8,15–18].

We used two laboratory methods to evaluate the impact of *Candida* biofilm production on the prognosis of a large number of unselected patients with candidaemia.

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Materials and methods

Study design and population

We performed a retrospective, single-centre cohort study of all patients ≥18 years of age with a positive peripheral blood culture yielding a single *Candida* species, who presented clinical signs of infection and were admitted to a tertiary hospital from May 2010 to November 2016. A pre-established protocol with epidemiological and clinical data was completed for each patient based on medical, laboratory and pharmaceutical records. Charlson co-morbidity index and Pitt score were calculated accordingly. All patients discharged from hospital earlier than 30 days were checked for survival through clinical records containing either subsequent medical appointments or hospital admissions. None of the patients had to be excluded owing to missing information.

Candida isolates were classified as high or moderate-low biofilm producers in agreement with previously published cut-off points for each method [19]. The primary outcome was overall mortality within 7 and 30 days. The secondary outcome was unfavourable prognosis defined as metastatic complications (ocular candidiasis, thrombophlebitis, endocarditis or dissemination to other organs), admission to an intensive care unit owing to the severity of the candidaemia episode, or death within 30 days from the diagnosis.

Definitions

Primary candidaemia was defined as an infection with no apparent focus that did not match any other source criteria after adequate investigation. Catheter-related candidaemia was defined as growth of the same species both in peripheral blood cultures and semi-quantitative roll-plate culture of the catheter tip (≥15 CFU/plate). A urinary tract origin was established if a *Candida* species was isolated in the urine culture of a patient with a specific predisposing condition. An intra-abdominal origin required clinical and radiological evidence of invasive candidiasis or positive cultures with a *Candida* species obtained during surgery or needle aspiration. Other origins included those with an apparent focus that did not match any other criteria, or cases of unknown origin because of very early death, and therefore insufficient data to establish the source of the infection.

Antifungal therapy was considered inadequate if there was no antifungal prescription, lack of *in vitro* activity of the drug for the *Candida* isolated in blood, or inappropriate dose for the type of infection, patient's weight, and renal and liver function. Antifungal treatment with antibiofilm activity was defined when either echinocandins or lipid formulations of amphotericin B were prescribed for at least 72 consecutive hours. Infection source control was not limited to withdrawal of the central venous catheter (CVC), but rather depended on the origin of the candidaemia, including abscess drainage and other invasive procedures as needed. Episodes of primary candidaemia and those assigned as 'other sources', which did not require specific management, were classified as those with 'non-applicable source control', although the CVC was removed in most cases.

Microbiology and biofilm detection

Identification of *Candida* species relied on classic phenotypic methods combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Stored isolates were revived after incubation for 24 h at 35°C on Sabouraud dextrose agar plates before the study of biofilm formation according to a previously described procedure [19,20].

Biofilm was quantified using two different methods: biomass production measured by crystal violet staining according to a published protocol [19] and metabolic activity detected using the XTT reduction assay as in previous reports [19,21,22]. Biofilms were stratified as having either a high, moderate or low biomass, with proposed optical density at 540 nm cut-off values of <0.44, 0.44–1.17 and >1.17, respectively. They were also classified as high, moderate or low metabolic activity with optical density at 490 nm cut-off values of <0.097, 0.097–0.2 and >0.2 [19]. To avoid discrepancies in the number of subjects in each group, patients were compared as follows: high versus moderate-low biomass and high versus moderate-low metabolic activity.

Statistical analysis

Categorical variables were reported as percentages and compared using either Fisher's exact test or the χ^2 test, as appropriate. Continuous variables with a normal distribution were reported as mean ± standard deviation (SD) and compared using the *t*-test, whereas those with a non-normal distribution were reported as medians with ranges and compared using the Mann–Whitney *U*-test. Statistical significance was set at a two-tailed p-value <0.05. Variables derived from the univariate analysis with a p-value <0.1 and not correlated after testing for multicollinearity were included in the multivariate logistic regression model to determine predictors of unfavourable prognosis. The Hosmer–Lemeshow test was performed to establish goodness of fit, and the model's discriminatory performance was assessed based on the area under the ROC curve. The analysis was performed using SPSS V24 (SPSS Inc., Chicago, IL, USA).

Ethical approval

This study was approved by the institutional ethics committee (Comité Ético de Investigación Clínica del Hospital Gregorio Marañón (CEIC-A1), study code MICRO.HGUGM.2015-071). The need for informed consent was waived owing to the retrospective design of the study.

Results

Population

From May 2010 to November 2016, there were 280 consecutive first episodes of candidaemia with a single *Candida* species among all adults admitted to the hospital. The majority were male (181, 64.6%), with a median age of 70 years. The most frequent co-morbidity was solid organ malignancy (140, 50.0%), whereas the main risk factors for candidaemia were the use of antibiotics within the previous month (253, 90.4%) and the presence of a CVC at the onset of the candidaemia (201, 71.8%). No significant differences were found when comparing patients according to biofilm production, except for the group with a high metabolic activity, in which patients were slightly older (p 0.026). The patients' main epidemiological characteristics are summarized in the Supplementary material (Table S1).

Microbiology and biofilm detection

Over half of all candidaemia episodes were caused by non-*albicans* species (150, 53.6%), mostly *Candida parapsilosis*, which was responsible for 64 cases (22.9%). Isolates were classified according to biofilm production as follows: biomass quantification (high 90 (32.1%) and moderate-low 190 (67.9%)); metabolic activity (high 114 (40.7%) and moderate-low 166 (59.3%)). There was 55.7%

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