UNIVERSITA' DEGLI STUDI DI GENOVA

DIPARTIMENTO DI SCIENZE DELLA SALUTE (DISSAL)

DOTTORATO DI RICERCA IN METODOLOGIE INNOVATIVE APPLICATE A MALATTIE TRASMISSIBILI E CRONICO-DEGENERATIVE: EPIDEMIOLOGIA, STATISTICA, PREVENZIONE, MANAGEMENT E NURSING – CURRICULUM EPIDEMIOLOGIA, FISIOPATOLOGIA E GESTIONE DIAGNOSTICO-CLINICA DELLE MALATTIE INFETTIVE

(XXXII ciclo)

"Valutazione dei fattori di rischio per lo sviluppo di candidemia dopo interventi di cardiochirurgia maggiore"

"Risk factors for candidemia after open heart surgery"

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1. INTRODUCTION

The text of this section has been included in the following manuscripts, published as PhD student:

[1] *Daniele Roberto Giacobbe, Antonio Salsano, Silvia Corcione, et al. Current and emerging pharmacotherapy for the treatment of infections following open-heart surgery. Expert Opin Pharmacother 2019; 20:751-772.*

[2] *Matteo Bassetti, Daniele Roberto Giacobbe, Antonio Vena, et al. Diagnosis and treatment of candidemia in the intensive care unit. Semin Respir Crit Care Med 2019 40:524-539.*

1.1. Epidemiology of infections after open heart surgery

Open heart surgery is defined as any type of surgery performed on valves, arteries, or other heart structures, with the chest cut open. Despite its undoubted benefits, it exposes the patient to some potential complications, such as cardiac dysfunction, mechanical complications, dysrhythmias, bleeding, thrombosis, and infections [3-5]. Amongst others, the development of postoperative infections has been repeatedly associated with reduced survival, prolonged length of stay, and higher economic costs [6].

A variable proportion of 2-20% of patients undergoing open heart surgery suffers from infections in the postoperative period [7, 8]. One of the most common is ventilator-associated pneumonia (VAP), which develops in as much as 3-6% of cases [9-11]. The risk of VAP is generally associated with advanced age, chronic obstructive pulmonary disease (COPD), use of intra-operative inotropic agents, need for re-intervention or re-intubation, and extensive use of red blood cells transfusions during surgery [7, 12-14]. The most frequent causative agents of VAP after cardiac surgery are members of the order *Enterobacterales* (33-45%), *Pseudomonas aeruginosa* (20-29%), and *Staphylococcus aureus* (10-27%) [7, 8, 11].

Another frequent postoperative complication is surgical site infection (SSI) including sternal or thoracotomy wounds, graft wounds, and infections of access sites for cannulation. Superficial sternal wound infections (SSWI), which are limited to the skin, subcutaneous tissue, and pectoralis fascia, occur more frequently than deep sternal wound infections (DSWI) (4-10% vs. 1-3%, respectively), which reach the sternal bone and the mediastinum [15-17]. Sternal wound infections develop especially in subjects with diabetes mellitus, obesity, hyperlipidemia, advanced age, and of female gender [16, 18, 19]. The risk of DSWI, which is a much more severe complication than SSWI being often associated with systemic inflammatory response and reduced survival [20-22], is also increased in patients with prolonged cardiopulmonary bypass time, nasal colonization by S. aureus, emergency or prolonged intervention, and sternal instability due to osteoporosis, radiotherapy or immunosuppression [21]. Common causative agents of SSWI and DSWI include mainly Gram-positive (mostly *S. aureus* and coagulase-negative staphylococci), usually responsible for more than 70% of cases, and less frequently Gram-negative bacteria (mostly members of the order *Enterobacterales* and *P. aeruginosa*) [20, 22, 23]. As regards other types of surgical site infections, leg wound infection after saphenous vein harvesting occurs in 1 to 3.5% of cardiac surgery patients, while infection of access sites after peripheral cannulation, percutaneous or with cutdown and direct exposure of the vessels, is observed in 0-2% of cases [24, 25].

The risk of early-onset postoperative endocarditis after cardiac surgery is mainly linked to the valves replacement [26-28]. Early-onset postoperative endocarditis (i.e., developing within 1 year after valvular replacement) occurs in 0.5-2% of cardiac surgery patients and is mostly caused by *Staphylococcus aureus* or coagulative-negative staphylococci [29-31]. The proportion of patients with postoperative endocarditis might increase to 3-6% at 5 years from valvular replacement [32]. However, it should be noted that prosthetic valve endocarditis developing at least 1 year after valve replacement is less likely to be related to the cardiac surgical procedure,

and its etiology is more similar to that of endocarditis on native valves [32, 33].

Postoperative urinary tract infections (UTI) develop in 1-2% of patients after cardiac surgery, ad are mostly caused by members of the order *Enterobacterales* (70-90% of cases) [34, 35]. The most frequent risk factors for postoperative UTI are prolonged urinary catheterization, advanced age, female gender, and the presence of diabetes mellitus [34, 35].

Bloodstream infections (BSI) occur in 1-7% of cardiac surgery patients in the early postoperative period in intensive care unit (ICU) [14, 36, 37]. Causative agents seem to be equally divided between Gram-positive and Gram-negative bacteria, although the possibility of postoperative candidemia should also be taken into account in patients with classical risk factors (especially previous treatment with broad spectrum antibiotics) [14, 36-38].

1.2. Epidemiology and mortality of candidemia

Candidemia is the fourth most frequent healthcare-associated bloodstream infection, and the most frequent severe fungal infection developing in critically ill patients in ICU [39-42] . Up to 33-55% episodes of candidemia have been estimated to occur in ICU wards, with a cumulative incidence of 3.5-10 episodes per 1,000 ICU admissions, with an increasing trend overtime [40, 43-49]. The most frequent *Candida* species causing candidemia in ICU is represented by *C. albicans* (54-70%), followed by *C. glabrata* (13-15%) and *C. parapsilosis* (8-19%) [43, 45-47, 50].
In the EPIC II point-prevalence study conducted in 1265 ICU in 76 countries, mortality of candidemia was higher than those of bloodstream infections caused by Gram-positive and Gram-negative bacteria (43% vs. 25% and 29%, respectively) [45, 48]. Similar results were found in the observational, prospective, multicenter, EUROBACT study, conducted in 162 ICU in 24 countries, 28-day mortality of candidemia was 41% vs. 34% and 35% in

bloodstream infections caused by Gram-positive and Gram-negative bacteria, respectively [50, 51].
In light of the above epidemiological and mortality data, recognizing and appropriately treating patients with candidemia is considered an essential component of an optimized approach to ICU septic patients [52-54].

1.3. Diagnosis of candidemia

There are no specific symptoms of candidemia, with fever unresponsive to antibacterial therapy being the most common clinical presentation [55]. The use of laboratory tests for the diagnosis of candidemia is therefore fundamental and characteristically influenced by two therapeutic considerations.

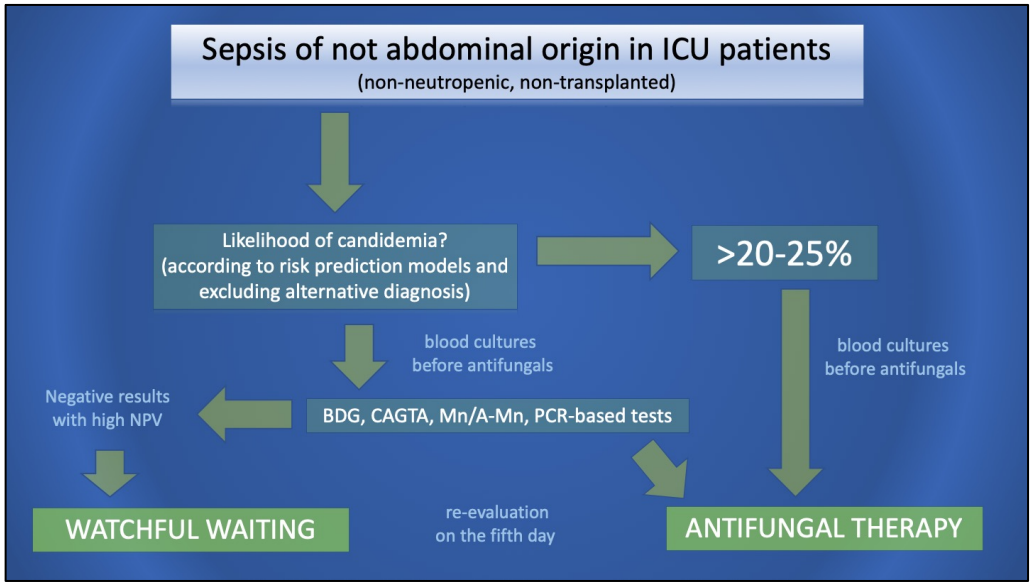
First, candidemia is a severe infection needing antifungal treatment. Although this may seem obvious nowadays, the need for antifungal therapy in candidemic patients had been debated long in the past, and eventually accepted only in the mid-seventies and early-nineties for neutropenic and non-neutropenic patients, respectively [56-60]. The reason for this behavioral change was that candidemic patients with mild symptoms and no evidence of hematogenous dissemination, previously considered at low risk and left untreated to avoid amphotericin B toxicity, were convincingly shown to have, conversely, an unacceptably high mortality without treatment [56, 57]. Second, candidemia should be treated promptly. Indeed, a delayed diagnosis– with consequent delayed therapy – has been associated with increased mortality in different studies [61-63].

The major diagnostic considerations stemming from these two therapeutic considerations are: (*i*) treat all patients with candidemia; (*ii*) make an early diagnosis. However, no currently available diagnostic test for candidemia has concomitantly 100% sensitivity and 100% specificity, and the turnaround time of the different test varies markedly. Consequently, different, complementary pieces of information may become available at different times. Therefore, diagnosis of candidemia is a complex task made

of both early and late assessments (e.g., at the onset of symptoms and after blood cultures results), in order to maximize the overall diagnostic performance and guarantee as much as possible both an early adequate therapy in patients with candidemia and the safe discontinuation of useless antifungals in those with no fungal infection.

A possible diagnostic work-flow to be adopted in ICU patients with suspected candidemia, based on the recent suggestions of a combined task force involving the systemic inflammation and sepsis and infection sections of the European Society of Intensive Care Medicine (ESICM) and the critically ill patients study group of European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [64], is shown in Figure 1, while a brief summary of the characteristics of laboratory tests for the diagnosis of candidemia is provided in Table 1.

Figure 1. Possible diagnostic algorithm in ICU patients with suspected candidemia according to the combined task force of the systemic inflammation and sepsis and infection sections of ESICM and the critically ill patients study group of ESCMID [64]



Modified from [64]. A-Mn, antimannan antibodies; BDG, (1,3)-β-D-glucan; CAGTA, *C. albicans* germ tube antigen; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; ESICM, European Society of Intensive Care Medicine, ICU, intensive care unit; Mn, mannan antigen; PCR, polymerase chain reaction.

Table 1. Main characteristics of different laboratory tests for the diagnosis of candidemia

Test	Characteristics
Blood cultures	Allow identification at species level and susceptibility testing Suboptimal sensitivity Long turnaround time (reduced with MALDI-TOF technology)
BDG	Rapid turnaround time High NPV Suboptimal specificity
Mn/A-Mn	Rapid turnaround time Variable performance across studies Reported low PPV
CAGTA	Rapid turnaround time Heterogeneous specificity Reported possible better performance in candidemia with deep-seated infection than without deep-seated infection
PCR-based methods	Rapid turnaround time Promising results of some newer methods Heterogeneity in the performance of first developed in-house and commercial methods Inability to detect all *Candida* species Usually expensive

A-Mn, antimannan antibodies; BDG, (1,3)-β-D-glucan; CAGTA, *C. albicans* germ tube antigen; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; Mn, mannan antigen; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

1.3.1. Blood cultures

Although remaining the diagnostic reference standard for candidemia, blood cultures are hampered by their suboptimal sensitivity, usually not higher than 63-83% [55, 65-69]. This suboptimal sensitivity does not reflect the inability of blood cultures to detect viable *Candida* species, but more likely other factors, such as an intermittent/transient release of viable yeasts in the bloodstream, or their absence in the captured volume of blood

[66, 68, 69]. Another critical limitation of blood cultures is their slow turnaround time (up to 48-72 h) [55, 65, 68]. Because of these limitations, blood cultures are not useful for early therapeutic decisions at the onset of symptoms (i.e., antifungal therapy yes vs. no), which are usually based on risk prediction models and/o rapid nonculture diagnostics.

Still, blood cultures remain essential within a comprehensive diagnostic approach, as they allow both identification of *Candida* at the species level and susceptibility testing [55, 70, 71]. Therefore, they should always be performed in the suspicion of candidemia, independent of the availability and results of noncultural diagnostics, possibly before treatment initiation to increase sensitivity [64, 65]. Of note, after a blood culture turns positive, time to identification (but currently still not to susceptibility testing, at least outside research laboratories [72-79]) may be shortened by the use of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technology (with >90% accuracy) [80, 81]. Huang and colleagues reported that the use of MALDI-TOF was able to reduce time to identification from 84 to 56 hours compared to conventional methods in a study involving 501 patients with bacteremia or candidemia [82].

1.3.2. Risk prediction models

In general, risk prediction models, which attempt to quantify the risk of a certain disease, can be used in two different ways: (*i*) before the development of the disease, mainly with prevention purposes; (*ii*) at the onset of the disease, for triggering dedicated diagnostic algorithms and/or guiding early therapeutic choices. In this latter situation, which is usually the case for candidemia, risk predictions models can be thought as an early component of the diagnostic process.

As such, being based on readily available clinical and possibly microbiological (colonization) information, their usually high negative predictive value (NPV) for candidemia allows to avoid, since the onset of the disease, useless fungal diagnostics and antifungal treatments in patients

unlikely to have candidemia (i.e., those with low scores according to risk prediction models) [64]. Conversely, since their positive predictive value (PPV) is very often modest, further diagnostics is indicated in patients deemed at risk of candidemia by prediction models. However, whether or not empirical antifungals should be administered in all patients at risk of candidemia according to prediction models (while waiting for the results of further diagnostics) is still a matter of debate [39, 83, 84]. A panel of experts has recently recommended to consider empirical antifungal therapy in ICU patients at risk of candidemia with septic shock and multi-organ failure (MOF) (strong recommendation, low quality of evidence) [64]. In addition, the panel has proposed an algorithm in which empirical antifungals are to be considered septic ICU patients with high probability of candidemia (>20-25% according to risk prediction models), independent of the presence of septic shock and MOF [64].

Most of the first proposed models were based on the presence of *Candida* colonization of non-sterile sites and/or on the intensity of *Candida* colonization (dependent of the number of colonized sites) [85-87]. Some subsequent prediction models are conversely based exclusively on clinical variables and patients' medical history, and not on colonization. For example, predictive rules for the development of invasive candidiasis (including not only candidemia but also deep-seated candidiasis) in surgical ICU patients have been developed by Paphitou and colleagues [88]. The highest risk of developing proven or probable invasive candidiasis (20%) was observed in patients with at least one among three possible predisposing factors (diabetes, total parenteral nutrition prior to ICU admission, or new onset haemodialysis) plus ICU stay longer than 4 days, use of broad-spectrum antibiotics, and no use of antifungal from day -7 to +3 with respect to ICU admission [88]. In another study conducted in cardiothoracic ICU patients, clinical variables that increased the risk of candidemia were ongoing mechanical ventilation ≥ 10 days, hospital-acquired bacterial infection, cardiopulmonary bypass time > 120 min, and diabetes mellitus [89]. The model showed a NPV of 90-100% [89]. According to the score proposed by Ostrosky-Zeichner and colleagues, and

based on a large cohort of 2890 ICU patients, the combination of antibiotic therapy and presence of a central venous catheter in the first 3 days of ICU stay, plus at least two among surgery, immunosuppression, pancreatitis, total parenteral nutrition, and steroid use was associated with a 10% risk of developing invasive candidiasis, with 97% NPV [90]. Guillemet and colleagues developed a score based on clinical variables for predicting the risk of candidemia in 2597 patients with severe sepsis or septic shock [91]. The independent predictors of candidemia included in the model were prior antibiotics within 30 days (+2 points), central venous catheter (+2 points), admission from a nursing home (+2 points), total parenteral nutrition (+2 points), admission from another hospital (+1 point), mechanical ventilation (+1 point), and lung as the presumed source of sepsis (- 6 points). The risk of candidemia was 1.2% for a cumulative score of – 6 points and 43% for a cumulative score of +8 points [91]. According to the Nebraska Medical Center rule, developed in a cohort of 352 ICU patients, a NPV of 99% for invasive candidiasis may be obtained by employing a model based on antibiotic therapy, central venous catheter, total parenteral nutrition, steroid therapy, abdominal surgery, and previous length of ICU stay [92]. The "Candida score", developed by León and colleagues in a cohort of 1699 ICU patients, is based on both clinical and microbiological information [93]. The independent predictors of invasive candidiasis included in the model were multifocal *Candida* colonization (+1 point), surgery on ICU admission (+1 point), severe sepsis (+2 points), and total parenteral nutrition (+1 point). A score of > 2.5 points was proposed as a cut-off for prompting empirical antifungal therapy based on a risk ratio of 7.35 [93]. Finally, Playford and colleagues by using two threshold scores identified three patient cohorts: those at high risk (score ≥ 6, 4.8% of total cohort, PPV: 11.7%), those at low risk (score ≤2, 43.1% of total cohort, PPV: 0.24%), and those at intermediate risk (score 3–5, 52.1% of total cohort, PPV: 1.46%) [53]. Most of prediction models have been internally or externally validated [89, 91-95].

1.3.3. Rapid tests based on antigen/antibody detection

The detection of fungal antigens or antifungal antibodies in blood may accelerate the diagnosis of candidemia, in turn anticipating administration of antifungals in those true cases who are not treated empirically [96].

1.3.3.1. (1,3)-β-D-glucan

The (1,3)-β-D-glucan (BDG) test is based on the detection of the polysaccharide BDG in serum [97, 98]. BDG is cell wall component of many pathogenic fungi, including *Candida* [97-99].

The nearly pan-fungal nature of BDG might appear as an important limitation for using it as a diagnostic tool for candidemia in the ICU. However, it should be noted that the other two most prevalent invasive fungal diseases (IFD) in ICU patients (and less frequent than candidemia) are invasive pulmonary aspergillosis and *Pneumocystis jirovecii* pneumonia, in which serum BDG may well be positive, but often accompanied by pulmonary radiological signs. Conversely, in septic ICU patients without lung involvement a positive serum BDG is usually indicative of candidemia rather than of other IFD.

In observational, prospective studies conducted in ICU patients at risk of candidemia, BDG showed high NPV (>95% in most studies), which thus makes candidemia unlikely when the test is negative [100-109]. It should nonetheless be noted that a few clinical experiences have suggested a possible reduced sensitivity of BDG for candidemia due to *C. parapsilosis* [101, 110, 111]. Therefore, some caution in discontinuing antifungals based on a negative BDG may be considered in centers with a high prevalence of candidemia due to *C. parapsilosis*, although there is also a need for large, prospective, confirmatory studies to definitely confirm this hypothesis. In contrast with this high NPV, the PPV is usually low (less than 20%) although it may increase with a second test as reported by Martín-Mazuelos and colleagues who found that BDG > 80 pg/mL in two consecutive measurements had a PPV of 35% [112].

A disadvantage of the BDG test reported by many authors is its suboptimal specificity due to multiple, possible causes of false positive results (e.g.,

haemodialysis, transfusions of blood and/or blood derivatives, treatment with immunoglobulins or albumin, bacteremia, treatment with ß-lactams, use of non-BDG-free laboratory equipment) [98, 113-125]. However, it is also true that the frequency of false positive results has likely been reduced in recent years, owing to the availability of modern dialysis membranes not releasing BDG, glucan-free laboratory material, surgical gauzes and blood products without or with a very few amount of BDG, and the evidence of a reduced number of false positive results in patients with bacteremia and/or treated with ß-lactams than previously suggested [126-131]. Furthermore, not all studies reporting a low BDG specificity were conducted in ICU patients deemed to be at risk of candidemia and with a consistent clinical picture (i.e., those in whom its PPV is maximized), but some also included other patients with a low likelihood of candidemia [107].

In an attempt to balance together advantages (early diagnosis) and disadvantages (false negative and false positive results) of using serum BDG in ICU patients at risk of candidemia, we conducted a post-hoc analysis of a prospective, observational study evaluating the diagnostic performance of serum BDG in 186 septic ICU patients with Candida Score ≥ 3 [107, 132]. We employed a desirability of outcome ranking (DOOR) method (i.e., to balance, on the basis of blood cultures results, the hypothetical benefits and harms of using a BDG-based strategy for deciding whether or not administer early pre-emptive antifungals vs. using an universal strategy based on the empirical administration to all patients at risk). According to the study results, the BDG-based strategy had a 67.8% probability (95% confidence intervals [CI] 67.3– 68.3) of prompting a "more desirable" therapeutic decision than the empirical strategy [132]. However, we recognize that several important issues, including arbitrariness in the definition of the ranked outcome and in the interpretation of results should be resolved before reliably using DOOR methods for this purpose [132, 133].

Randomized controlled trials (RCT) assessing the impact of BDG-based pre-emptive decisions (early treatment, discontinuation) have provided some

conflicting, or perhaps, still incomplete evidence. In the EMPIRICUS RCT, empirical and not pre-emptive therapy was evaluated, but some information regarding the possible usefulness of BDG testing can be garnered from the subgroup of patients with positive serum BDG. Indeed, fungal infection-free 28-day survival in ICU patients with severe sepsis and positive serum BDG (> 80 pg/ml) was higher in BDG-positive patients treated with empirical micafungin (58/91, 64%) than BDG-positive patients receiving placebo (47/84, 56%), with a trend towards a potentially beneficial effect (hazard ratio [HR] 1.41, 95% CI 0.85-2.23) [134]. Conversely, a similar trend was not observed when the endpoint was limited to 28-day mortality (with or without fungal infection) (HR 0.95, 95% CI 0.55-1.75) [134]. In an unblinded, single-center RCT, Rouzé and colleagues assessed the percentage of early discontinuation for reasons other than death in patients with risk factors for invasive candidiasis and receiving empirical antifungals for a consistent clinical presentation [135]. Patients were randomized in two groups: (*i*) biomarker strategy (discontinuation of empirical antifungals in case of negative BDG, mannan, and antimannan tests); *(ii*) routine strategy (14 days of therapy in patients improving after antifungal treatment according to the investigator's judgment). Early discontinuation of antifungals occurred more frequently in the biomarker strategy group (29/54, 54%) than in the routine strategy group (1/55, 2%) (odds ratio [OR] 63, 95% CI 8-486) [135]. No differences were detected in subsequent probable/proven IC, subsequent antifungal treatments, length of ICU stay, and mortality [135]. Other RCT evaluating the impact of BDG results on early therapeutic choices are ongoing or have been recently completed (NCT02734550, NCT03117439, NCT03090334, NCT03538912) [136]. Their results are awaited to ultimately, firmly delineate the impact of BDG results on pre-emptive therapeutic choices in ICU patients with suspected candidemia.

1.3.3.2. Mannan and antimannan

The polysaccharide mannan (Mn) is one of the major components of the *Candida* cell wall, and can be found in serum during candidemia or other forms of invasive candidiasis [137, 138]. Since the presence of circulating antimannan antibodies (A-Mn) may correlate with a reduction in circulating mannan antigens [139], the diagnostic performance of combined Mn/A-Mn testing was evaluated and deemed preferable to either Mn or A-Mn [138, 140, 141]. However, the PPV of the A-Mn component may be low due to previous *Candida* infections or *Candida* colonization [55, 142, 143], and also variable diagnostic performances of the combination Mn/A-Mn have been reported across different studies [144-150].

With regard to experiences restricted to ICU populations, in a retrospective case-control study of 43 ICU patients with candidemia and 67 controls, Mn/A-Mn testing showed 59% sensitivity and 65% specificity for the diagnosis of candidemia [105]. In another study among 233 ICU patients with severe abdominal conditions, 31 developed invasive candidiasis (11 candidemia; 20 intra-abdominal candidiasis) [146]. The diagnostic performances of Mn and A-Mn were evaluated separately. Mn showed 43% sensitivity, 67% specificity, 17% PPV, and 89% NPV, whereas A-Mn showed 26% sensitivity, 89% specificity, 27% PPV, and 89% NPV [146]. In the previously cited RCT conducted by Rouzé and colleagues, decisions regarding continuation or discontinuation of antifungals were based on a combination of BDG and Mn/A-Mn testing, but their separated impact was not evaluated [135]. In the discussion, the authors reported that the decision of continuing antifungals was only based on Mn/A-Mn in three cases [135].

1.3.3.3. Other antigen/antibody-based tests

The *C. albicans* germ tube antigen (CAGTA) test is able to detect specific antibodies for a fungal hyphal protein (namely, Hwp1), which is expressed by *Candida* spp. during biofilm formation and tissue invasion [151, 152]. Although the hyphal protein was initially found in *C. albicans* (hence the

name of the test), the CAGTA assay can be positive also in invasive candidiasis caused by other *Candida* species [55, 146, 153-155]. Experience in the use of CAGTA for invasive candidiasis is limited compared to BDG and Mn/A-Mn. According to the results of a recent meta-analysis of 7 studies [146, 152, 153, 155-158], the pooled sensitivity and specificity of CAGTA for the diagnosis of IC were 65% (95% CI 59-73) and 76% (95% CI 58-88) [159]. An important heterogeneity in specificity was detected [159]. Notably, in one study comparing the diagnostic performance of CAGTA in 29 patients with candidemia plus deep seated candidiasis vs. 21 patients with isolated candidemia, sensitivity was 69% and 5% in the former and in the latter, respectively [152].

Some tests for detecting *Candida* protein antigens have been hypothesized or developed, but their applicability in clinical practice remains low because of low sensitivity, possibly linked to rapid clearance, formation of immune complexes, and low serum concentrations [160-168]. Suboptimal performances and lack of standardization are also important limitations of tests based on the detection of the *Candida* sugar alcohol D-arabinitol in serum [161, 166, 169, 170].

1.3.3.4. Combination of available antigen/antibody tests

Some authors have tried to combine the use of available tests, in order to improve their usefulness in guiding pre-emptive therapeutic decisions. Martínez-Jiménez and colleagues evaluated the combined use of different, possible combinations of antigen/antibody markers (BDG, Mn, A-Mn, CAGTA) for differentiating candidemia (31 patients) from bacteremia (50 patients) [157]. The best combinations found by the authors were BDG plus CAGTA (97% sensitivity, 84% specificity, 79% PPV, 98% NPV) and Mn plus CAGTA (94% sensitivity, 86% specificity, 81% PPV, 96% NPV). Since the prevalence of candidemia in the study sample was quite high (38%), the authors also extrapolated their results to lower prevalences of candidemia (5-10%), showing a NPV of ~100% for both BDG plus CAGTA and Mn plus

CAGTA [157]. Subsequently, the same authors conducted a prospective study in which they measured BDG and CAGTA serum levels in 63 ICU and 37 non-ICU patients receiving empirical antifungals in the suspicion of invasive candidiasis, to evaluate the potential for using the BDG/CAGTA combination to guide safely discontinuation of antifungals when both the markers are negative [154]. In the overall study population, the NPV of the combination was 97%, reaching 100% in the subgroup of ICU patients [154]. Another experience regarding the combined use of BDG and CAGTA is that of León and colleagues, in which the combination (with the criterion for positivity being set to positivity of at least one of the two markers) showed 90% sensitivity, 42% specificity, 19% PPV, and 97% NPV for the diagnosis of invasive candidiasis in 233 ICU patients with severe abdominal conditions [146]. A lower discriminatory ability was observed for combinations involving Mn and/or A-Mn [146].

With the aim of reducing costs of combined testing, and also to explore combinations that may be available in a higher number of laboratories, we assessed the performance of serum BDG combined with the widely used serum procalcitonin (PCT) test for differentiating between candidemia and bacteremia in a retrospective cohort of 166 ICU patients (73 with candidemia and 93 with bacteremia) [103]. The rationale was based on the fact that serum PCT usually remains within the normal concentration range or is only slightly elevated in patients with candidemia, differently form bacteremia, during which high serum PCT concentrations are frequently measured [171-176]. Interestingly, while the NPV for candidemia observed by combining a positive BDG with low PCT levels (< 2 ng/ml) was similar to that of a positive BDG alone (95% vs 93%, respectively), the PPV of the combination was considerably higher than that of BDG alone (96% vs. 79%, respectively). Notably, PPV and NPV of PCT alone (66% and 84%, respectively) were markedly low than both those of BDG alone and those of the BDG/PCT combination [103].

1.3.4. Rapid tests based on polymerase chain reaction (PCR)

The possibility of rapidly identify *Candida* spp. in the blood or serum of patients with candidemia by means of PCR-based techniques has been extensively studied in the last decades, prompted by the inherent advantages of increased sensitivity compared with blood cultures, very rapid turnaround time, and rapid identification at species level [55, 151]. In a meta-analysis of 54 studies, pooled sensitivity and specificity for the diagnosis of invasive candidiasis (mainly candidemia) of PCR methods were 95% and 92%, respectively [177]. However, performance of both in-house and commercial PCR varied markedly across studies [177-181], and no test has been validated for the diagnosis of candidemia through dedicated, large, multicenter experiences.

Several studies have been recently published regarding the diagnostic performance of the T2Candida panel (T2 Biosystems, Lexington, MA, USA), which is FDA-cleared for the diagnosis of candidemia. The test is based on the mechanical lysis of cells, with subsequent amplification of DNA by means of PCR and target-specific primers (which enable the identification of the 5 most frequent *Candida* species). The amplified products are detected by measuring the agglomeration of amplicons-induced supermagnetic particles [182, 183]. FDA clearance was based on the results of the DIRECT study, conducted in 1801 hospitalized patients in whom blood cultures were ordered according to local standards of care [183]. The T2Candida panel demonstrated 91% sensitivity (95% CI 87-94) and 99% specificity (99%-100%). The median time to positive results (including species identification) and to negative results was 4.4 ± 1.0 hours and 4.2 ± 0.9 hours, respectively. A 99% NPV was estimated for a population with 10% prevalence of candidemia [183]. In a study conducted in 126 ICU patients at high risk of invasive candidiasis and with sepsis despite 3 days of broad-spectrum antibiotics, the sensitivity and specificity of the T2Candida panel for proven invasive candidiasis were 55% and 93% respectively, with 50% PPV and 93% NPV [184]. In another study conducted among 46 patients with severe sepsis or septic shock and multiple risk factors for candidemia, the T2Candida panel showed 100% sensitivity (95% CI 2.5-100), 92% specificity (95% CI 78-98), 25% PPV

(95% 1-81), and 100% NPV (95% CI 90-100) [185]. Of note, some authors have also suggested that a positive T2Candida test could be a potential marker of poor outcome in patients receiving empirical antifungal therapy for suspected invasive candidiasis [186]. In the future, it is likely that cumulative evidenced from different real-life experiences will allow to precisely delineate the positioning of the T2Candida panel within diagnostic algorithms, and to maximize its cost-effectiveness (also considering the local prevalence or *Candida* species not included in the panel) [187-189].

1.3.5. Susceptibility testing

Once *Candida* species responsible for candidemia are identified from blood cultures, detection of acquired resistance could be important for adjusting initially inadequate therapies and for allowing safe de-escalation to oral azole therapy whenever indicated by the patient's clinical conditions, although it should be noted that the guidelines of the Infectious Diseases Society of America (IDSA) recommend routine susceptibility testing for azole and echinocandin resistance in *C. glabrata*, while less value is attributed to routine susceptibility testing of other *Candida* species [190]. Some authors have nonetheless suggested that routine susceptibility testing of all *Candida* isolates from sterile sites could be important for registering resistance trends and for detecting the local emergence of resistance [75, 191]. In resource-limited settings, susceptibility testing of *Candida* species may be limited to breakthrough infections, treatment failures, or in presence of limited therapeutic options [75, 191].

Reference microbroth dilution methods suggested by the European Committee on Antimicrobial Susceptibility Testing [EUCAST] and the Clinical and Laboratory Standards Institute [CLSI], although excellent for detecting resistance, are not easy to implement in routine laboratory workflows, in which the most frequently used methods are commercial microbroth dilution tests, semiautomated broth dilution, and agar diffusion

[55]. In the future, further development and validation of MALDI-TOF-based detection of resistance could help reducing time to phenotypical susceptibility testing. As regards molecular methods, they may not be available in many laboratory, and have the limitations of identifying only already known determinants and of being of little use for detecting azole resistance, since involved genes may mutate at several locations [74, 192-195]. Nonetheless, they may be of help for rapidly detecting known determinants of echinocandin resistance [194, 196-201].

1.4. Treatment of candidemia

Because delayed treatment is associated with high morbidity and mortality [39, 61], many strategies have been implemented aiming to minimize the negative impact of candidemia in critically ill patients [54, 63, 202]. Apart from the prophylactic use of antifungal drugs for a few clinical scenarios [203-205], ICU physicians may adopt an empirical approach relying on signs and symptoms, fungal biomarkers and specific risk factors for invasive candidiasis in the absence of any identified pathogen [206]. Targeted therapy is based on microbiological evidence of an invasive candidiasis (e.g. a positive blood culture for *Candida* species) [207]. Moreover, once candidemia is diagnosed, an adequate source control of the infection (CVC removal, drainage, debridement) should be also performed as soon as possible [39, 62].

1.4.1. Antifungal agents

Over the past decade, there has been a considerable research in antifungal drugs against *Candida*. To date the antifungal drugs most commonly used for the treatment of candidemia are the echinocandins (caspofungin, micafungin, anidulafungin), azoles (fluconazole and voriconazole), and amphotericin B [208]. Doses of antifungals commonly used to treat candidemia are shown in table 2.

Table 2. Recommended adequate doses of antifungal drugs for empirical or targeted treatment of candidemia*

Drugs	Adequate dose	Comment
Caspofungin	70 mg loading dose followed by 50 mg daily	Recommended as first line therapy [64, 190, 209]
Anidulafungin	200 mg loading dose followed by 100 mg daily	
Micafungin	100 mg daily. No loading dose is required	
Fluconazole	12 mg/kg loading dose followed by 6 mg/kg daily	Recommended as an acceptable alternative to an echinocandin as initial therapy [64, 190, 209] Recommended for de-escalation therapy [64, 190, 209]
Voriconazole	3-4 mg/kg orally twice daily modified according to TDM	Recommended for de-escalation therapy [64, 190, 209]
L-AmB	3 mg/kg daily	Recommended as a reasonable alternative if there is intolerance, limited availability, or resistance to other antifungal agents [64, 190, 209]
ABLC	5 mg/kg daily	Not recommended
ABCD	3- 4 mg/kg daily	Not recommended

L-AmB Liposomal amphotericin B; ABLC Amphotericin B lipid complex; ABCD Amphotericin B colloidal dispersion

*Adequate doses refer to patients with normal renal and hepatic function and those with no drug-drug interactions.

Caspofungin, micafungin and anidulafungin are echinocandins for which only intravenous formulation is available. Echinocandins target the fungal cell wall and act by inhibiting BDG synthesis, showing fungicidal activity against most *Candida* species including biofilms forming and azole-resistant strains [210]. Intrinsic resistance to echinocandins is anecdotal but acquired resistance has been increasingly reported, especially in *C. glabrata* [211, 212]. In addition, echinocandins do not achieve therapeutically

effective concentrations in some tissues (e.g. eyes, central nervous system [CNS], urine) and their pharmacokinetic/pharmacodynamic (PK/PD) properties are poorly known for critically ill patients [213].

Echinocandins appear to be as effective as and better tolerated than amphotericin B formulations [214, 215] and, in two randomized trial, more effective than azoles [216, 217]. Particularly, in one of these trials including 245 patients with invasive candidiasis (89% of them with candidemia only) anidulafungin treatment resulted in superior combined clinical and microbiological response compared to fluconazole (at 2 weeks 65% vs 49%), although no differences were observed for 60-day mortality rates [217]. The use of echinocandins is further supported by a quantitative review of RCTs (1915 patients, 7 studies) showing that treatment with echinocandins led to decreased mortality [odds ratio (OR) 0.65; 95% confidence interval (CI) 0.45, 0.94] and increased treatment success (OR 2.33; 95% CI 1.27, 4.35) [218]. Moreover, a recent propensity score adjusted multivariable analysis of critically ill patients with proven candidemia showed that empirical therapy with echinocandins instead of fluconazole led to lower 30-day (OR 0.32; 95% CI 0.16, 0.66; p=0.002) and 90-day mortality (OR 0.50; 95% CI 0.27, 0.93; p=0.014) [219]. However, in a prospective study conducted in 29 hospitals in Spain with less severe patients (only 30% being in the ICU), empirical treatment with fluconazole was not associated with increased 30-day mortality compared with echinocandins in patients with candidemia [207]. There has been concern about the use of echinocandins as primary therapy against *C. parapsilosis* because of higher *in vitro* minimum inhibitory concentrations (MICs). A retrospective study on 307 episodes of *C. parapsilosis* candidemia demonstrated no difference in 30-day mortality between patients receiving an echinocandin as compared with fluconazole [220].

Because of their efficacy, tolerability, broader spectrum, fungicidal activity and fewer drug-drug interactions, echinocandins are currently recommended as first-line therapy in the treatment of invasive candidiasis in critically ill patients (see table 2) [64, 190, 209] and are also preferred in

non-critically ill patients with previous exposure to azoles and/or evidence of colonization with a *Candida* strain with reduced susceptibility to azoles.

Azoles (fluconazole and voriconazole) work by inhibiting the 14-alpha-demethylase enzyme which mediates the conversion of lanosterol to ergosterol in the fungus wall. This class is metabolized by P450 cytochromes, which can result in drug-drug interactions. Fluconazole is used in the treatment of candidemia as a de-escalation therapy with a significantly lower cost compared with the echinocandins. Fluconazole also remains a well-tolerated treatment of non-critically ill candidemic patients with no risk factors for azoles-resistant strains [209]. Other azoles such as posaconazole, itraconazole and isavuconazole are not approved for systemic *Candida* infections.

Amphotericin B is a polyene that acts binding to the ergosterol in the fungal membrane. Owing to its toxicity, amphotericin B deoxycholate has now been replaced by better-tolerated polyenes including liposomal amphotericin B (L-AmB), amphotericin B lipid complex (ABLC) and amphotericin B colloidal dispersion (ABCD). L-AmB is widely used and has favorable pharmacokinetics along with high intracellular penetration in the cerebral spinal fluid and in the eye. Both L-AmB and ABLC achieve therapeutically effective concentrations in the epithelial lung fluid of critically ill patients [221]. L-AmB is used as a first-line therapy for disseminated forms of *Candida* species infection, and as a second-line therapy for invasive candidiasis [213], especially when *C. glabrata* candidemia from urinary tract source is documented.

A few more antifungals are currently under investigation for the treatment of candidemia and invasive candidiasis, including new compounds belonging to known classes or molecules with novel mechanisms of action [222]. Rezafungin (previously CD101) is a novel long-acting echinocandin characterized by a spectrum of activity that is comparable to the other echinocandins but also a distinct safety PK/PD profile that enables high plasma drug exposure and extended interval dosing [223, 224]. *In vitro*, rezafungin has demonstrated potent activity against a broad range of *Candida* spp., including echinocandin- and azole-resistant strains [225], but

interlaboratory variation was observed thus warranting further investigation [226]. A multicentre, randomized, double-blind phase 2 trial evaluating the efficacy and safety of rezafungin once weekly compared with caspofungin in patients with candidemia has been recently finished (NCT023734682).

SCY-078 is a semisynthetic, triterpenoid, antifungal glucan synthase inhibitor, currently in development for the treatment of invasive and mucocutaneous fungal diseases [227]. SCY-078 has shown good bioavailability and has been studied as oral and intravenous formulations with once daily administration [227]. The drug is currently in phase 3 clinical development for the treatment of invasive fungal diseases.

1.4.2. Prophylaxis

The concept of prophylaxis, introduced almost 40 years ago, refers to the administration of antifungal drugs to patients with risk factors for invasive candidiasis without clinical signs or symptoms of infection [190, 202]. Although the benefits of antifungal prophylaxis are well established in neutropenic patients (e.g. haematological patients) or in solid organ transplant, especially in high-risk liver transplant patients [203-205], its utility in non-immunocompromised, critically ill patients with sepsis and no confirmed fungal infection is still controversial [84, 228] and is no currently recommended by the critically ill patients study group of ESCMID [64].

Over the last decade, several studies [229-232] have focused on the prevention of fungal infections in ICU patients administering echinocandins, azoles and oral nystatin. Despite the large number of publication, the quality of evidence still remains low in many studies, leading to uncertainty with regard to the reduction of mortality, reduction of invasive candidiasis, or the risks of fungal colonization [229]. Since the universal administration of antifungal prophylaxis remains an inefficient strategy that may increase subsequent azole-resistance or non-*albicans* candidemia [233, 234], it should be avoided in critically ill patients, and its use should be eventually restricted to selected ICU patients at highest risk

(>10%) of invasive candidiasis [190, 235] (surgical patients with anastomotic leakage after abdominal surgery or early re-intervention of the digestive tract).

1.4.3. Empirical approach

Although prompt initiation of appropriate antifungal therapy has been associated with a reduction in mortality [39, 61-63, 218], it is often delayed because of the low sensitivity of blood cultures, the time needed for blood cultures to turn positive and the possibility of negative blood cultures also in patients with proven disease. In order to overcome this problem, several studies have looked to identify strategies for initiating empirical treatment based on risk factors, positive culture collected from non-sterile sites (respiratory tract, urine), clinical scoring systems and surrogate markers of infection.

Previous studies also looked at prediction models to identify patients at highest risk for invasive candidiasis development. As discussed in the "Risk prediction models" paragraph, these studies are frequently based on risk scores (i.e., *Candida* score, *Candida* colonization index, Ostrosky score) with very low PPV [236, 237], that can lead to unnecessary antifungal treatment in a large number of patients. For example, in a prospective observational study performed in 36 ICUs, antifungal treatment was empirically administered according to *Candida* score to 180 out of 1,017 patients included in the study (17%), but only 5% of those really developed candidemia [238].

Surrogate markers that have been evaluated in critically ill patients include BDG, Mn/A-Mn, PCR testing and T2 Candida. BDG appears to be more sensitive than *Candida* colonization scores or indices, reaching a sensitivity of about 90% when performed twice weekly. On the other hand, PPV of the test is very low [106, 117, 239, 240] with a high percentage of false-positive results. According to its diagnostic performance, BDG seems to be more useful in excluding rather than diagnosing invasive candidiasis in the ICU setting [104, 154, 157]. Other studies analysed the role of Mn/A-Mn testing

[148, 241], real-time PCR [242], T2*Candida* [186] for implementing or discontinuing empirical antifungal therapy but recommendations for their clinical use cannot be made because of the lack of robust data in critically ill patients [64].

Limited clinical studies have evaluated the efficacy of empiric strategies. Three multicenter randomized clinical trials [84, 134, 237] evaluated empirical antifungal therapy for fungal infection suspicion in high risk patients. Neither study demonstrated a benefit with early antifungal therapy and no differences were observed in terms of resolution of fever, major adverse events and mortality. Recently, Timsit et al [134] compared the outcome of a 14-day empirical course of micafungin with placebo in a prospective randomized multicenter trial including 260 non-neutropenic critically ill patients with ICU-acquired sepsis, multiple Candida colonization and multi-organ failure. Although empirical use of micafungin was associated with a lower rate of new IFD diagnosis in comparison to placebo (4/128 patients [3%] vs 15/123 [12%]; p = .008), there were no differences between the two arms regarding death and invasive fungal disease-free at 28 days (hazard ratio, 1.35 [95% CI, 0.87-2.08]).

Despite these results, the fact is that the empirical approach remains a common practice both inside and outside ICU [186] and its role in high-risk patients still remains to be determined. In our opinion, further studies aimed to specify criteria for early initiate antifungal therapy in critically ill patients are needed.

Until such studies will be available, empiric antifungal therapy should be considered only in patients with septic shock and multi-organ failure who have more than one extra-digestive site (i.e. urine, mouth, throat, upper and lower respiratory tracts, skin folds, drains, operative site) with proven *Candida* species colonization [64].

Once empirical treatment is started, an echinocandin regimen should be preferred especially in hemodynamically unstable patients or those previously exposed to an azole, and in those colonized with azole-resistant *Candida* species [64]. Daily clinical revaluation should be performed, and treatment should be stopped earlier (within 4-5 days of antifungal

treatment) in patients who do not clinically improve or in those with no positive cultures or positive surrogate markers [104, 154]. Otherwise, a 14-days course of empirical therapy may be administered [243].

1.4.4. Targeted treatment

Regarding the treatment of proven infections, the last IDSA and European guidelines [64, 190, 209] recommend first-line treatment for *Candida* spp. infection with an echinocandin (e.g. caspofungin, anidulafungin or micafungin), rather than fluconazole. Evidences supporting this recommendation are mainly based on the increasing prevalence of fluconazole-resistant *Candida* spp. [202, 244, 245] and from previously described clinical trials in which echinocandins showed a significantly higher efficacy in comparison to azoles for the treatment of candidemia [216, 217]. Interestingly, when antifungal treatment was specifically assessed in the critically ill patients with septic shock due to candidemia, the administration of echinocandin was also associated with better survival in association with a prompt and adequate source control of the infection24. Despite growing evidence of the superiority of echinocandins, fluconazole still remains an acceptable alternative for candidemic patients who are not critically ill or at risk of fluconazole resistance. Moreover, fluconazole represents together with voriconazole the drugs of choice for de-escalation therapy according to disease severity and susceptibility testing results [64, 190, 209].

Regarding this issue, the optimal timing for de-escalating or switching to oral treatment in patients with candidemia has not been provided. In most trials, step-down therapy to azoles is permitted after 10 days of treatment. In a recent non-comparative trial, step-down to an oral azole was allowed after 5 days of intravenous treatment [243]. Although early de-escalation has no impact survival [246] and has been associated with a significant decrease in antifungal use [243], recent studies showed that only 20-40% of patients with fluconazole-susceptible strains have their treatment de-escalated from echinocandin to fluconazole in daily clinical practice.

As for duration of therapy, follow-up blood cultures should be performed every 24-48 hours until negativity and candidemia is usually treated for 14 days from the first negative blood culture. Treatment duration is prolonged in patients with evidence of deep-seated infections; thus, it is recommended to systematically perform a transoesophageal echocardiography and fundoscopy to all patients with a positive blood culture [64, 190, 209], irrespective of clinical signs or symptoms of metastatic infection or predisposing factors [247]. Once a deep-seated candidemia is diagnosed, the duration of treatment depends on the site of infection and on the quality of the source control.

1.4.5. Source control

Source control includes all measures to control invasive infection (i.e debridement, device removal, compartment decompression) and restore optimal function of the affected site [248]. An adequate source control of the infection has been shown to be a major determinant of outcome, more so than early adequate antifungal treatment [244, 249], and should never be considered as "covered" by the only antifungal therapy. Although CVC removal remains a controversial issue [54, 250], CVC withdrawal should be attempted in all patients with candidemia [190, 209]. Moreover, all surgical and radiological approach for obtaining an adequate source control of the infection must be systematically discussed, especially in patients with intra-abdominal infection [243] or those with a candidemia from urinary tract217. Importantly, physicians should always keep in mind that efficacy of source control is time-dependent [251, 252] and adequate procedures should therefore be performed as rapidly as possible especially in patients with septic shock [39].

2. RISK FACTORS FOR CANDIDEMIA AFTER OPEN HEART SURGERY

2.1. Background

Candida species rank fourth among the most frequent causative agents of healthcare-associated bloodstream infections, with a cumulative incidence of 3.5-10 episodes per 1,000 intensive care unit (ICU) admissions [39-49], and mortality higher than 40% in large cohorts of critically ill patients [45, 48, 50, 51].

Various risk factors for candidemia (e.g., administration of broad-spectrum antibiotics, prolonged length of hospital stay, presence of a central venous catheter) have been extensively characterized in the literature, and several scores have been built for helping clinicians to identify critically ill patients at risk of candidemia [88, 90, 92, 93]. Nonetheless, specific populations of critically ill patients may present additional, peculiar risk factors related to their medical or surgical reason for ICU admission [38, 88, 89, 253-255]. For example, the possible influence of cardiac surgery-related factors on the risk of postoperative candidemia has been previously addressed, although with non univocal results [38, 89].

To add to the literature and nurturing the discussion on this topic, we conducted a multicenter case-control study in seven hospitals in Italy. Our primary objective was to assess the predictors of development of candidemia after open heart surgery.

2.2. Material and methods

2.2.1. Study design and endpoints

The present observational, retrospective, case-control study was conducted in 8 Italian centers. The study period was from 1 January 2009 to 31 December 2016. All patients who developed candidemia during the study

period and during the ICU stay after open heart surgery were included as cases. Two controls without candidemia were matched to each case by the following criteria: (*i*) center; (*ii*) date of open heart surgery (±1 month); (*iii*) time at risk. For cases, time at risk was defined as the number of days elapsed from surgery to the onset of candidemia (i.e., the day when the first blood culture positive for *Candida* spp. was drawn). For controls, time at risk was defined as the number of days elapsed from surgery to hospital discharge or in-hospital death. To fulfill the matching criterion, the time at risk in controls had to be equal or longer than the time at risk in cases minus 5 days. Cases were included in the study only once, at the time of the first episode of candidemia after open heart surgery.

The primary study endpoint was development of candidemia. Crude mortality within 30 days after the onset of candidemia in cases was a secondary study endpoint. The study was approved by the ethical committee of the coordinating center (Ethical Committee of Liguria Region, registry number 320REG2017).

2.2.2. Data collection

The following baseline data (pre-operative and peri/intraoperative variables) were retrospectively collected from medical records and laboratory databases of the participating hospitals: age; gender; diabetes (defined as any preoperative diagnosis of diabetes mellitus requiring treatment); New York Heart Association (NYHA) class of heart failure; chronic kidney disease (defined as history of serum creatinine >200 mmol/L); chronic obstructive pulmonary disease (COPD, defined as long term use of bronchodilators or steroids for lung disease); history of immunosuppression (defined as one or more of the following: solid organ transplantation; malignancy; neutropenia [absolute neutrophil count <1000 cells/mm3]; HIV infection; chemotherapy within 45 days before surgery ;therapy with at least 10 mg of prednisone or its equivalent per day for >14 days prior to surgery); Charlson score [256]; peripheral vascular disease (defined as one or more of the following: carotid occlusion or >50% stenosis, claudication, amputation for arterial disease, previous or planned

intervention on the abdominal aorta, carotids or limb arteries); preoperative stroke (defined as any focal or global neurological syndrome caused by ischemia or hemorrhage not resolving within 24 h); previous acute myocardial infarction (within 3 months); left ventricular ejection fraction (LVEF); EuroSCORE II [257]; type of open heart surgery (categorized as isolated coronary artery bypass surgery, isolated valvular surgery, surgery of thoracic aorta, or other/combined procedures); preoperative mechanical ventilation; pacemaker implantation; cardiopulmonary bypass (CPB) time in minutes; aortic cross-clamp time in minutes; sequential organ failure assessment (SOFA) score at the time of surgery [258]; need for peri/intraoperative blood transfusions.

The following data were also collected over the duration of the time at risk for candidemia in both cases and controls (postoperative variables): presence of central venous catheter for >48 hours; receipt of total parenteral nutrition for >48 hours; hemodialysis therapy for >48 hours; administration of broad-spectrum antibiotics for >48 hours; Candida colonization (defined as isolation of Candida spp. from non-sterile sites in absence of signs and symptoms of infection); bacterial bloodstream infections (defined as isolation of bacteria from blood in presence of signs and symptoms of infections; at least two positive cultures were required for coagulase-negative staphylococci).

The following data were also collected for cases (candidemia-related variables): species of Candida isolated from blood; presence of septic shock at the time of candidemia (according to Sepsis-3 criteria [259]), removal of central venous catheter within 48 hours after the onset of candidemia, administration of antifungal therapy within 48 hours after the onset of candidemia.

2.2.3. Microbiology

Candida spp. were identified using the VITEK 2 automated system (bioMérieux, Marcy l'Etoile, France) or by MALDI-TOF mass spectrometry (bioMérieux, Marcy l'Etoile, France, or Bruker Daltonik, Bremen, Germany),

according to the standard laboratory diagnostic procedures adopted in the different participating centers.

2.2.4. Statistical analysis

The primary study analysis was the identification of factors associated with the development of candidemia after open heart surgery. To this aim, demographic and clinical variables were first tested for their association with the dependent variable (development of candidemia) in univariable conditional logistic regression models for matched pairs/sets, with strata composed by sets of single cases and their two matched controls [260]. Then, variables associated with the development of candidemia in univariable comparisons ($p < 0.05$) were included in an initial multivariable, conditional logistic regression model for matched pairs/sets, and further selected for the final multivariable model by means of a stepwise backward procedure. A secondary study analysis was the identification of factors associated with crude 30-day mortality in candidemia cases. To this aim, we employed univariable and multivariable comparisons as for the primary analysis, with the exception of using unconditional logistic regression models. The analyses were performed using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

2.3. Results

Overall, 222 patients were included in the study (74 cases and 148 controls). Strict application of matching criteria was possible for 56% of controls (83/148). Owing to the absence of other controls fulfilling all the three matching criteria, the remaining 44% of them (65/148) were selected as those with the nearest date of surgery (with respect to cases) outside the matching period (i.e., beyond ±1 month) but still fulfilling the center and time at risk matching criteria.

During the study period, 36,476 open-heart surgery procedures were performed in the participating centers. The cumulative incidence of postoperative candidemia over the study period was of 2.03 episodes per 1000 open-heart surgery patients. The median age of patients with

candidemia was 72 years (interquartile range [IQR] 64-78), and 55% were males. The median time of development of candidemia was of 23 days after surgery (IQR 14-36). Concomitant Candida endophthalmitis was diagnosed in 1% of cases (1/74). No concomitant Candida endocarditis was observed. Most candidemia episodes were due to C. albicans (48/74, 65%), followed by C. parapsilosis (10/74, 14%) and C. glabrata (7/74, 9%).

Table 1 shows the results of univariable and multivariable analyses of factors associated with the development of candidemia. In univariable analysis, NYHA class equal or greater than III, previous stroke, low LVEF, preoperative MV, higher EuroSCORE II score, preoperative mechanical ventilation, hemodialysis therapy, SOFA score at the time of surgery, previous therapy with cephalosporins, previous therapy with carbapenems, previous therapy with fluoroquinolones, and multifocal Candida colonization had a statistically significant association with the development of candidemia. In the final multivariable model, NYHA class equal or greater than III (odds ratio [OR] 23.81, 95% confidence intervals [CI] 5.73-98.95, $p < 0.001$), previous therapy with carbapenems (OR 8.87, 95% CI 2.57-30.67, $p = 0.001$), and previous therapy with fluoroquinolones (OR 5.73, 95% CI 1.61-20.41, $p = 0.007$) retained an independent association.

The 30-day crude mortality in patients with candidemia was 53% (39/74), whereas the crude in-hospital mortality of controls was 15% (22/148). The results of univariable and multivariable analyses of factors associated with 30-day mortality in patients with candidemia are shown in table 2. In univariable analysis, >5 peri/intraoperative blood transfusions, previous therapy with fluoroquinolones, and septic shock at the onset of candidemia were associated with increased 30-day mortality. Only septic shock, observed in as many as 36% of patients with candidemia, retained an independent association with the outcome in the final multivariable model (OR 5.64, 95% CI 1.91-16.63, $p = 0.002$).

Table 1. Univariable and multivariable analyses of factors associated with the development of candidemia after open heart surgery

			Univariable analysis		Multivariable analysis*	
Variable	**No. of cases (%)** 74 (100)	**No. of controls (%)** 148 (100)	**Odds ratio** (95% CI)	***P***	**Odds ratio** (95% CI)	***P***
Age in years, median (IQR)	72 (64-78)	72 (64-77)	1.00 (0.98-1.03)	0.932		
Male gender	41 (55)	99 (67)	0.64 (0.37-1.11)	0.113		
Diabetes mellitus	23 (31)	29 (20)	1.80 (0.96-3.39)	0.067		
NYHA class III/IV	53 (72)	40 (27)	6.26 (3.21-12.21)	<0.001	23.81 (5.73-98.95)	<0.001
Chronic kidney disease	29 (39)	42 (28)	1.63 (0.90-2.95)	0.105		
COPD	22 (30)	36 (24)	1.34 (0.70-2.56)	0.372		
History of immunosuppression	1 (1)	9 (6)	0.20 (0.02-1.64)	0.133		
Charlson score, median (IQR)	5 (3-7)	5 (3-6)	1.08 (0.95-1.23)	0.232		
Peripheral vascular disease	14 (19)	27 (18)	1.04 (0.52-2.10)	0.905		
Previous stroke	12 (16)	8 (5)	4.00 (1.38-11.57)	0.010	4.61 (0.68-31.28)	0.118
Previous IMA	13 (18)	31 (21)	0.83 (0.42-1.62)	0.577		
LVEF (%), median (IQR)	50 (37-55)	55 (45-55)	0.97 (0.95-1.00)	0.031	-	0.633

EuroSCORE II	6.61 (3.67-16.43)	3.51 (1.86-8.37)	1.06 (1.02-1.10)	0.001	-	0.177
Preoperative MV	16 (22)	9 (6)	3.56 (1.57-8.05)	0.002	-	0.275
Type of surgery				0.073		
Isolated coronary artery bypass surgery	6 (8)	22 (15)	(ref)			
Isolated valvular surgery	32 (43)	48 (32)	2.43 (0.88-6.71)			
Surgery of thoracic aorta	26 (35)	41 (28)	2.13 (0.78-5.82)			
Other/mixed procedures	10 (14)	37 (25)	0.93 (0.29-2.97)			
Pacemaker implantation	2 (3)	10 (7)	0.40 (0.09-1.83)	0.237		
CPB time (minute), median (IQR)	136 (98-208)	136 (92-197)	1.00 (1.00-1.00)	0.843		
Aortic cross-clamp time (minute), median (IQR)	75 (49-120)	87 (58-120)	1.00 (0.99-1.00)	0.127		
SOFA score at time of surgery, median (IQR)	4 (1-7)	3 (0-4)	1.19 (1.07-1.34)	0.002	1.20 (0.99-1.45)	0.058
Need for peri/intraoperative blood transfusion	61 (82)	124 (84)	0.85 (0.33-2.22)	0.854		
Need for >5 peri/intraoperative blood transfusions	47 (64)	86 (58)	1.56 (0.69-3.52)	0.288		
Central venous catheter >48 h	74 (100)	141 (95)	(model not converging)	-		
Total parenteral nutrition >48 h	42 (57)	86 (58)	0.94 (0.50-1.74)	0.833		
Hemodialysis >48 h	27 (37)	28 (19)	2.55 (1.32-4.91)	0.005	-	0.566
Previous therapy with cephalosporins >48 h	18 (24)	12 (8)	4.65 (1.81-11.94)	0.001	-	-

Previous therapy with carbapenems >48 h	52 (70)	51 (35)	4.49 (2.37-8.49)	<0.001	8.87 (2.57-30.67)	0.001
Previous therapy with fluoroquinolones >48 h	49 (66)	51 (35)	5.78 (2.64-12.65)	<0.001	5.73 (1.61-20.41)	0.007
Candida colonization	29 (39)	45 (30)	1.52 (0.83-2.80)	0.178		
Candida multifocal colonization (at least 2 sites)	19 (26)	14 (10)	2.95 (1.43-6.12)	0.004	-	0.723
Bacterial BSI**	23 (31)	38 (26)	1.30 (0.69-2.47)	0.415		

Results are presented as n (%) unless otherwise indicated. BSI, bloodstream infection; CI, confidence intervals; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; IMA, acute myocardial infarction; IQR, Interquartile range; LVEF, left ventricular ejection fraction; MV, mechanical ventilation; NYHA, New York Hearth Association.

* Odds ratio and 95% CI presented only for variable retained in the final multivariable model.

** Coagulase-negative staphylococci (n = 24); *Klebsiella* spp. (n = 8); *Staphylococcus aureus* (n = 6); *Enterobacter* spp. (n = 3); *Pseudomonas* spp. (n = 3); Enterococcus spp. (n = 2); other bacteria with lower frequencies (n = 15)

Table 2. Univariable and multivariable analyses of factors associated with 30-day mortality in patients with candidemia

			Univariable analysis		Multivariable analysis*	
Variable	**Non-survivors (%)** 39 (100)	**Survivors (%)** 35 (100)	**Odds ratio** (95% CI)	***P***	**Odds ratio** (95% CI)	***P***
Age in years, median (IQR)	75 (67-79)	68 (60-76)	1.03 (0.99-1.08)	0.129		
Male gender	21 (54)	20 (57)	0.88 (0.35-2.19)	0.776		
Diabetes mellitus	11 (28)	12 (34)	0.75 (0.28-2.02)	0.573		
NYHA class III/IV	29 (74)	24 (69)	1.33 (0.48-3.66)	0.582		
Chronic kidney disease	17 (44)	12 (34)	1.48 (0.58-3.80)	0.414		
COPD	12 (31)	10 (29)	1.11 (0.41-3.02)	0.836		
History of immunosuppression	1 (3)	0 (0)	(model not converging)	-		
Charlson score, median (IQR)	5 (3-7)	5 (2-6)	1.09 (0.90-1.32)	0.390		
Peripheral vascular disease	7 (18)	7 (20)	0.88 (0.27-2.80)	0.822		
Previous stroke	7 (18)	5 (14)	1.31 (0.38-4.59)	0.670		
Previous IMA	10 (26)	3 (9)	3.68 (0.92-14.69)	0.065		
LVEF (%), median (IQR)	50 (35-55)	48 (39-55)	1.00 (0.96-1.04)	0.984		

EuroSCORE II	13.57 (4.07-21.93)	4.57 (3.43-10.34)	1.05 (1.00-1.10)	0.052		
Preoperative MV	10 (26)	6 (17)	1.67 (0.54-5.19)	0.378		
Type of surgery				0.898		
Isolated coronary artery bypass surgery	4 (10)	2 (6)	(ref)			
Isolated valvular surgery	16 (41)	16 (46)	0.50 (0.08-3.13)			
Surgery of thoracic aorta	14 (36)	12 (34)	0.58 (0.09-3.76)			
Other/mixed procedures	5 (13)	5 (14)	0.50 (0.06-4.09)			
Pacemaker implantation	1 (3)	1 (3)	0.90 (0.05-14.86)	0.938		
CPB time (minute), median (IQR)	136 (98-198)	137 (93-213)	1.00 (1.00-1.00)	0.652		
Aortic cross-clamp time (minute), median (IQR)	73 (51-110)	89 (49-140)	1.00 (0.99-1.01)	0.603		
SOFA score at time of surgery, median (IQR)	4 (1-7)	3 (1-6)	1.02 (0.89-1.17)	0.749		
Need for peri/intraoperative blood transfusion	32 (82)	29 (83)	0.95 (0.29-3.14)	0.928		
Need for >5 peri/intraoperative blood transfusions	29 (74)	18 (51)	2.74 (1.03-7.28)	0.043	-	0.151
Central venous catheter >48 h	39 (100)	35 (100)	-	-		
Total parenteral nutrition >48 h	25 (64)	17 (49)	1.89 (0.75-4.80)	0.180		
Hemodialysis >48 h	16 (41)	11 (31)	1.52 (0.58-3.95)	0.393		
Previous therapy with cephalosporins >48 h	10 (26)	8 (23)	1.16 (0.40-3.38)	0.781		

Previous therapy with carbapenems >48 h	26 (67)	26 (74)	0.69 (0.25-1.90)	0.475		
Previous therapy with fluoroquinolones >48 h	30 (77)	19 (54)	2.81 (1.03-7.62)	0.043	-	0.115
Candida colonization	13 (33)	16 (46)	0.59 (0.23-1.52)	0.278		
Candida multifocal colonization (at least 2 sites)	9 (23)	10 (29)	0.75 (0.26-2.13)	0.590		
Bacterial BSI**	9 (23)	14 (40)	0.45 (0.17-1.23)	0.120		
Septic shock	21 (54)	6 (17)	5.64 (1.91-16.63)	0.002	5.64 (1.91-16.63)	0.002
Causative *Candida* species				0.184		
albicans	28 (74)	20 (59)	(ref)			
Non-*albicans****	10 (26)	14 (41)	0.51 (0.19-1.38)			
Early antifungal therapy (within 48 h*****)	13 (33)	16 (46)	0.59 (0.23-1.52)	0.278		
Early CVC removal (within 48 h*****)	13 (33)	14 (40)	0.75 (0.29-1.94)	0.552		

Results are presented as n (%) unless otherwise indicated. BSI, bloodstream infection; CI, confidence intervals; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; CVC, central venous catheter; IMA, acute myocardial infarction; IQR, Interquartile range; LVEF, left ventricular ejection fraction; MV, mechanical ventilation; NYHA, New York Hearth Association.

* Odds ratio and 95% CI presented only for variable retained in the final multivariable model.

** Coagulase-negative staphylococci (n = 8); *Klebsiella* spp. (n = 5); *Pseudomonas* spp. (n = 3); *Staphylococcus aureus* (n = 2); other bacteria with lower frequencies (n = 5)

*** 2 non-typed species not included in the comparison *albicans* vs. non-*albicans* species. Typed non-*albicans* species were as follows: *C. parapsilosis* (n = 10); C. *glabrata* (n = 7); *C. tropicalis* (n = 3); *C. krusei* (n = 2); *C. dubliniensis* (n = 1); C. *sake* (n = 1).

**** After the onset of candidemia (i.e., the day when the first positive blood culture for *Candida* spp. was draw

2.4. Discussion

In this retrospective, multicenter, case-control study, high NYHA class, previous therapy with carbapenems, and previous therapy with fluoroquinolones were associated with the development of candidemia after open heart surgery.

Risk factors for developing candidemia after open heart surgery have also been explored by other studies. Michalopoulos and colleagues conducted a single center, case-control study in 150 cardiac surgery patients (30 cases with postoperative candidemia and 120 controls without candidemia) [89]. Controls were matched to cases according to gender, body mass index, agents administered for general anesthesia and for postoperative sedation, type of employed cardioplegia, and CPB technique. Independent predictors of candidemia were MV >10 days, hospital-acquired bacterial infection and/or bacteremia, CPB time >120 min, and diabetes mellitus [89]. Subsequently, Pasero and colleagues assessed risk factors for candidemia in a cohort of patients admitted to a cardiac surgery ICU [38]. Among 349 patients, 26 developed candidemia. Independent predictors of candidemia were ICU length of stay >20 days, total parenteral nutrition, severe sepsis, and high simplified acute physiology score (SAPS II), whereas no association with development of candidemia was observed for CPB time [38].

A necessary premise that should be made before comparing the results of these two studies with those of our study is that the design of the three studies (case-control vs. cohort, type of matching criteria) was very different across the three experiences. This is not necessarily a disadvantage, since in our opinion, it allows to address the topic from different, complementary perspectives. For example, similar to Pasero and colleagues, we did not find an association between prolonged CPB and development of postoperative candidemia. However, it should be noted that CPB time was higher in patients with candidemia than in patients without candidemia in the study of Pasero and colleagues (mean 178 vs. 149 minutes, respectively), thus the absence of a statistically significant association could reflect the low power related to the small number of

patients with candidemia (n = 26) [38]. Conversely, in our study involving a higher number of patients with candidemia (n = 77), the absence of an association between CPB time and candidemia more likely reflect the fact that CPB time was truly similar in cases and controls (median 136 vs. 136 minutes). In this regard, we think that a fundamental aspect to be taken into account for interpreting these conflicting results is that in our study we matched for length of stay in ICU (more properly, for time at risk). Indeed, since prolonged length of stay (or possible proxies such a prolonged MV) is a well-recognized predictor of candidemia [38, 88, 89, 92, 261], we wanted to explicitly focus on a population of cardiac surgery patients with a prolonged length of stay, that is, on those patiens in whom the risk of candidemia is higher and has the major clinical implications (since the question whether or not administer empirical antifungals arises very more often for patients with prolonged stay than for patients discharged after a few days of ICU stay). Of note, in both our and Pasero's studies, candidemia mostly developed late during ICU stay (after a median stay of 20 and 23 days, respectively), and in the study of Michalopoulos and colleagues the length of ICU stay was considerably longer in cases than in controls (mean 27 vs. 2 days) [38, 89]. In our opinion, all these considerations suggest that: (*i*) further studies may be necessary to confirm whether prolonged CPB time could be a predictor for early candidemia after cardiac surgery; (*i*) prolonged CPB time may not be helpful for discriminating the risk of candidemia (absolutely or vs. that of bacterial BSI [262]) in cardiac surgery patients with prolonged ICU stay.

With regard to independent predictors, our results confirm that the previous administration of broad spectrum antibiotics is an important predictor of candidemia in cardiac surgery patients with prolonged postoperative ICU stay, in line with results of studies conducted in general ICU populations [88, 90-92]. In addition, we found a high baseline NYHA class to be an independent predictor of postoperative candidemia. This finding warrants further investigation, since this predisposing factor was not investigated in other studies assessing predictors of candidemia in cardiac surgery patients and because of the risk of spurious significance due

to multiple testing. However, it remains reasonable that a high NYHA class may represent a proxy for a higher burden of comorbidity, possibly and generally influencing the risk of postoperative infections.

Finally, as a secondary analysis, we also assessed the predictors of 30-day mortality in patients with postoperative candidemia. In this regard, the independent association we observed between septic shock and mortality further confirms the importance of the severity of clinical presentation in unfavorably influencing the outcome [49]. On the other hand, caution is needed before interpreting the absence of other independent predictors in our analysis as the absence of other associations that could be clinically relevant. For example, early antifungal therapy and early CVC removal have been previously indicated as important predictors of survival, and it is worth noting that a trend towards improved survival for these two factors was also appreciable in our univariable results, although not reaching statistically significance (possibly because of the reduced power of our secondary analysis).

This study has some important limitations. The most important are related to its retrospective nature, and mainly consisting in information biases (most importantly, we were ultimately unable to retrospectively collect sufficient data and/or adjustments for time at risk for some postoperative intensive care procedures [e.g., use of intra-aortic balloon pump] and some postoperative non-infectious complications [e.g., reoperations for bleeding] that may have influenced the risk of infection). Two other important limitations are the lack of long-term follow-up in survivors of candidemia 26 and the use of a single control group instead of two different control groups to separately assess (i) the predictors of candidemia vs. no infection and (ii) the predictors of candidemia vs. bacteremia. Finally, although increased with respect to previous studies, the power of our primary analysis remains somewhat suboptimal, thus we may have failed to detect other true associations that could be clinically relevant. Nonetheless, to our knowledge this is the largest cohort of candidemic cardiac surgery patients (n = 74) employed for assessing the risk of postoperative candidemia, and

it may add valuable information to the literature, complementary to that of previous studies with more limited sample sizes.

In conclusion, previous broad-spectrum antibiotic therapy and high NYHA class were independent predictors of candidemia in cardiac surgery patients with prolonged postoperative ICU stay, whereas no association between prolonged CPB time and candidemia was observed in the present case-control study. Further studies are needed to explore the possible role of CPB-related ischemia in influencing the risk of the few candidemia episodes occurring early after surgery.

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