Intensive Care Med (2014) 40:1313–1322
DOI 10.1007/s00134-014-3352-2

ORIGINAL

Martin Albert
David Williamson
John Muscedere
Francois Lauzier
Coleman Rotstein
Salmaan Kanji
Xuran Jiang
Mark Hall
Daren Heyland

Candida in the respiratory tract secretions of critically ill patients and the impact of antifungal treatment: a randomized placebo controlled pilot trial (CANTREAT study)

Received: 29 January 2014
Accepted: 22 May 2014
Published online: 1 July 2014

On behalf of the Canadian Critical Care Trials Group.

www.clinicaltrials.gov, number: NCT00934934

A related editorial can be found at doi: 10.1007/s00134-014-3364-y.

Take-home message This study describes the first placebo-controlled, randomized trial looking at the effect of an empiric antifungal therapy in patients with Candida spp. in their respiratory tract secretions. The results provide an insightful description of the inflammatory response and clinical outcome associated with an antifungal treatment in these patients.

Electronic supplementary material The online version of this article (doi: 10.1007/s00134-014-3352-2) contains supplementary material, which is available to authorized users.

M. Albert (✉)
Département de Médecine, Centre de recherche de l'Hôpital du Sacré-Coeur de Montréal, Université de Montréal, 5400 Gouin Ouest, Montreal, Canada
e-mail: m.albert@umontreal.ca
Tel.: 514-338-2050

D. Williamson
Département de Pharmacie, Centre de recherche de l'Hôpital du Sacré-Coeur de Montréal, Université de Montréal, Montreal, Canada

J. Muscedere · D. Heyland
Kingston General Hospital, Kingston, ON, Canada

F. Lauzier
Départements de médecine et d'anesthésiologie, Division de soins intensifs adultes, Centre de recherche du Centre Hospitalier affilié Universitaire de Québec, Université Laval, Quebec, Canada

C. Rotstein
Division of Infectious Diseases, Department of Medicine, University Health Network, University of Toronto, Toronto General Hospital, Toronto, Canada

S. Kanji
The Ottawa Hospital Research Institute, Ottawa, Canada

X. Jiang · D. Heyland
Clinical Evaluation Research Unit, Queen's University, Kingston General Hospital, Kingston, ON, Canada

M. Hall
Department of Pediatrics, Critical Care Medicine, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH, USA

Abstract *Purpose:* *Candida* spp. are frequently recovered from endotracheal secretions in critically ill patients suspected of having ventilator-associated pneumonia. Observational studies reported an association with worse clinical outcomes but the effect of antifungal therapy in these patients remains unclear. We designed this pilot study to assess the feasibility of a larger trial and to evaluate inflammatory profiles and clinical outcomes in these patients. *Methods:* We conducted a double-blind, placebo-controlled, multicenter pilot randomized trial of antifungal therapy in critically ill patients with a clinical suspicion of ventilator-associated pneumonia with positive airway secretion specimens for *Candida* spp. We also included an observational group without *Candida* spp. in their airway secretions. We measured recruitment rate, inflammatory and innate immune function profiles over time, and clinical outcomes. *Results:* We recruited 60 patients into the randomized trial and 29 patients into the observational study. Markers of inflammation and all clinical outcomes were comparable between placebo and antifungal treatment group at baseline and over time. At baseline, plasma TNF-α levels were higher in patients with VAP and *Candida* compared to the observational group (mean ± SD) (21.8 ± 23.1 versus 12.4 ± 9.3 pg/ml, $p = 0.02$) and these patients had lower innate immune function as evidenced by reduced whole blood ex vivo LPS-induced TNF-α production capacity (854.8 ± 855.2 versus 1,559.4 ± 1,290.6 pg/ml, $p = 0.01$). *Conclusions:* This study does not provide evidence to support a larger trial examining the efficacy of empiric antifungal treatment in patients with a clinical suspicion of ventilator-

associated pneumonia and *Candida* in the endotracheal secretions. The presence of *Candida* in the lung may be associated with persistent inflammation and immunosuppression.

Keywords Nosocomial infection · Pneumonia · Intensive care · *Candida* infection · Antifungal therapy

Abbreviations

APACHE	Acute physiology and chronic health evaluation
ICU	Intensive care unit
MODS	Multiple organ dysfunction score
MV	Mechanical ventilation
ROC	Receiver operating characteristics
RT	Respiratory tract
SOFA	Sequential organ failure assessment
VAP	Ventilator-associated pneumonia

Introduction

Ventilator-associated pneumonia (VAP) is associated with significant morbidity and mortality [1]. In as many as 54.6 % of patients with clinically suspected VAP (csVAP), cultures remain negative [2–4]. These patients exhibit longer duration of mechanical ventilation (MV), intensive care unit (ICU) stay, and increased ICU and hospital mortality compared to patients with an identified bacterial pathogen [5].

Amongst culture-negative VAP, *Candida* spp. are commonly recovered from the respiratory tract (RT) secretions [6]. Although *Candida* spp. have not historically been considered to be pathogenic, recent studies have challenged this dogma [7]. *Candida* spp. may have an important role in the host inflammatory response [8]. An association between the presence of *Candida* spp. in the RT and increased inflammatory cytokine levels has been reported [9]. This increased inflammatory response could be related to beta-glucan, a cellular membrane component of *Candida* spp. [10]. Studies also demonstrated that *Candida* spp. in the RT is associated with increased *Pseudomonas* spp. superinfection [3, 11], selection of multidrug-resistant bacteria [12], and increased morbidity [13]. Recently, we demonstrated an association between *Candida* spp. isolated only from the RT secretions and hospital mortality [13]. This relationship could also be explained by increased *Candida* colonization in the lungs of the sickest patients with the longest ICU stays, greater antibacterial exposure, and highest mortality risk. Although the literature suggests a plausible pathogenic role for *Candida* spp., differentiating between an association and a causative relationship remains challenging.

To define the pathogenic role of *Candida* spp. when isolated in patients with csVAP, we conducted a multicenter trial exploring the hypothesis that presence of *Candida* spp. in RT secretions may explain the excess morbidity and mortality. The primary objective of this pilot trial was to investigate the feasibility of a larger trial evaluating an antifungal strategy to reduce morbidity. The secondary objectives were to investigate the effect of the antifungal therapy on inflammatory markers and clinical outcomes.

Methods

We conducted a prospective, double-blind, multicenter, randomized, placebo-controlled pilot trial of critically ill patients with csVAP and *Candida* in their RT secretions (Clinicaltrials.gov NCT00934934). The primary outcome was feasibility as judged by enrolment rate. Secondary outcomes included changes to innate immune responsiveness as measured by whole blood ex vivo LPS-induced TNF-α production capacity and serum levels of procalcitonin (PCT), C-reactive protein (CRP), and interleukins-1B, 6, 8, and 10 (IL-1B, IL-6, IL-8 and IL-10). Additional outcomes included organ function; ICU and hospital length of stay; acquired infection; acquired resistance to antifungal therapy; duration of MV; ICU, 28-day post-randomization, and hospital survival. Written informed consent was obtained from all patients or legal representatives before enrolment. The local research ethics board approved the study.

Non-immunocompromised adult patients admitted to ICU for at least 96 h who developed a csVAP after 48 h of MV were considered for enrolment (see Electronic Supplementary Material for detailed pneumonia definition). To be included, patients had to grow *Candida* spp. from RT secretion cultures (bronchoalveolar lavage or endotracheal aspirate) collected within 24 h of suspicion of infection. We excluded patients with *Candida* spp. in any other sites (see Electronic Supplementary Material for complete exclusion criteria). To better understand the pathophysiologic role of *Candida* spp., an observational group of ICU patients with csVAP was recruited using the same inclusion criteria except for the absence of *Candida* from the RT secretions.

Study patients were randomized using a web-based system to receive antifungals or matching placebo. Following enrolment, study intervention was started as soon as possible. Anidulafungin or matching placebo was initiated as a 200-mg intravenous dose followed by 100 mg daily for at least 72 h. Study medication was de-escalated in a blinded manner by the local research pharmacist to fluconazole or matching placebo when the *Candida* spp. were sensitive to fluconazole. If not, anidulafungin or a suitable alternative was prescribed on the basis of

susceptibility results. Study intervention was continued for a total of 14 days. Patients were followed daily for the ICU stay or until 28 days after enrolment, whichever came first. All patients were managed according to the Canadian VAP guidelines [14, 15]. Adjudication of all csVAP was also performed.

We collected age, sex, ICU admission diagnosis, chronic health diseases, APACHE II score, and SOFA scores at admission and randomization. We calculated daily SOFA scores and measured duration of MV, ICU and hospital length of stays, and mortality. Culture results and antifungal and antibiotic sensitivity on all sampled cultures were collected. Observation of a moderate to large amount of yeast on the gram stain led to culture and identification of the *Candida* spp. (see Electronic Supplementary Material for susceptibility methodology) [16]. No surveillance cultures were requested as per protocol. Critical care physicians were allowed subsequent cultures on the basis of clinical information. We collected all culture results requested until ICU discharge (or death).

We drew blood samples at baseline and days 3, 8, and 14 to measure immune function and inflammatory profiles. Innate immune function was measured by quantitation of the capacity of subjects' whole blood samples to produce TNF-α upon ex vivo stimulation with LPS (see Electronic Supplementary Material for methodology) [17–19]. Plasma from unstimulated whole blood was collected at each sampling point and stored at −80 °C for batch analysis of TNF-α, IL-1-beta, IL-6, IL-8, and IL-10. Plasma PCT, CRP, beta-glucan, and intestinal fatty acid binding protein (iFAPB) were measured from these sampling points as well (see Electronic Supplementary Material for methodology).

Statistical analysis

A sample size of 120 patients was planned on the basis of the plasma levels observed from 21 patients colonized with *Candida* in previous work and had the power to detect relatively large differences in CRP (90 % power to detect a 29 % decrease of CRP), procalcitonin (90 % power to detect a 55 % decrease of PCT), and interleukin 6 (90 % power to detect a 52 % decrease of IL-6) at a two sided $\alpha = 0.05$ assuming a log-normal distribution. All randomized patients were included in the intention-to-treat analysis of the primary and secondary endpoints except that one patient without *Candida* mistakenly randomized to the intervention arm was moved to the observation group prior to initiating treatment. A convenience multicentric sample of 40 patients was planned for the observational group. Categorical variables were described as counts and percentages and compared by Chi square tests. Continuous data were reported as mean ± standard deviation or median with interquartile range and compared between groups by the Wilcoxon–Mann–Whitney test or Kruskal–Wallis test. The raw means and standard errors of the biomarkers were plotted by group over the first 14 days. The differences between groups in the mean baseline and mean change over time were tested by a linear mixed effect model with a random patient-specific intercept. All analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary NC.) except the longitudinal plots of biomarkers which were drawn using R 2.9. This analysis is considered exploratory and hypothesis-generating so no correction was made for multiplicity of tests.

Results

A total of 133 ICU patients were screened for eligibility between August 2010 and July 2012 in five participating centers in Canada (Fig. 1). Seventy-three patients were excluded mainly because they did not meet the inclusion criteria or refused consent. We enrolled 29 patients in the placebo group, 31 patients in the antifungal strategy group and 29 patients in the observational group. We obtained an overall enrolment rate per month of 0.6 patients per site for the randomized trial. Consequently, recruitment was halted prematurely despite efforts to optimize enrolment because of difficulty in recruiting patients and diminishing study resources. Many patients were excluded because their ICU length of stay was expected to be less than 72 h ($n = 13$) or they had positive cultures with *Candida* spp. from other sites than respiratory ($n = 18$). Eleven of 82 (13.4 %) eligible patients refused informed consent.

In all three groups, patients were predominantly males with a mean APACHE II score 22.3 and a mean SOFA score of 3.8 (Table 1). The most common reasons for ICU admission were respiratory, traumatic injury, gastrointestinal, and neurological diseases. Baseline characteristics were similar among the three groups. Patients in the intervention group received anidulafungin for a mean of 5.9 ± 3.0 days and 77.4 % were sequentially transferred to fluconazole for an additional 7.3 ± 5.3 days. Patients received a mean fluconazole dose of 391 mg/day. The mean total duration of antifungal therapy was 11.5 ± 5.6 days. Patients developed their csVAP 7.8 ± 8.0 days and 7.2 ± 6.8 days (mean ± SD) after ICU admission in the placebo group and intervention group, respectively. On the basis of cultures, seven patients from the intervention group only received anidulafungin. Although no patients received anidulafungin in the placebo arm, three patients received some fluconazole after randomization as ordered by treating physicians.

Most of the randomization samples were endotracheal aspirates (only three BAL and one protected brush). Quantitative results of these endotracheal aspirate cultures were reported only in four patients but all were above 15 cfu/ml. *Candida albicans* was identified from 48.3 and 35.5 % of the respiratory specimens from the placebo and

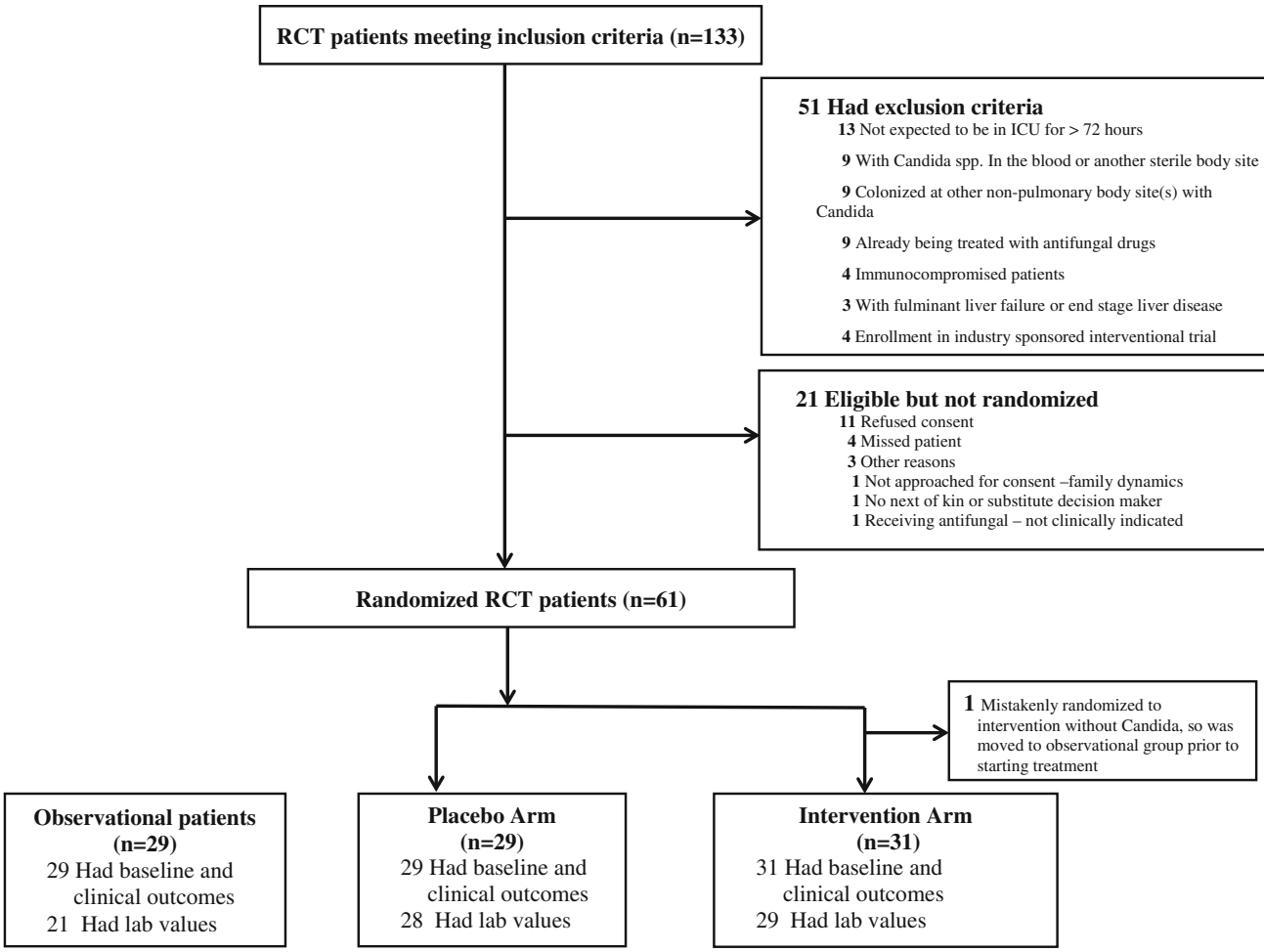

Fig. 1 Patient flow diagram

the intervention group, respectively (Electronic Supplementary Material Table 1). *C. glabrata* was the second most commonly isolated (12.9 % in the intervention arm versus 17.2 % in the placebo arm) followed by *C. parapsilosis* (9.7 % in the intervention arm and 0 % in the placebo arm). Pathogenic bacteria grew from the enrolment cultures of seven and eight patients from the placebo and interventional groups, respectively. *Pseudomonas* sp. was retrieved from the respiratory secretion cultures sent after 72 h in one patient from each group. *Candida* spp. were cultured from three RT specimens obtained after randomization (Table 2). For the entire population of randomized patients, initial csVAP was adjudicated ultimately to VAP as the most likely diagnosis in 33 of the 60 patients.

Inflammatory profiles were comparable between placebo and antifungal groups at baseline and over their ICU stays (Electronic Supplementary Material Table 2). Both antifungal and placebo patients had increased levels of cytokines including pro-inflammatory (TNF-α, IL-6) and anti-inflammatory mediators (IL-10). CRP was also elevated at baseline in both antifungal (145.8 ± 149.2 mg/l) and placebo patients (120.1 ± 65.8 mg/l). Beta-glucan plasma levels were elevated and remained relatively high during the first 14 days after randomization (139.7 ± 232.5 pg/ml for the antifungal group and 148.3 ± 212.2 pg/ml for the placebo group at day 14). IL-6, CRP, and PCT plasma levels over time and the LPS-induced TNF-α production capacity of each group were not significantly different (Fig. 2).

Hospital mortality was similar in the placebo and intervention arms (24.1 versus 22.6 %, $p = 0.90$) (Table 2). No significant differences in ICU and hospital length of stay were observed. Maximum SOFA scores (5.9 ± 3.3 versus 5.9 ± 3.6, $p = 0.95$) and median (IQR) MV-free days [8.0 (6.0–11.0) versus 9.0 (4.0–10.0); $p = 0.91$] were similar in the placebo and the interventional arm, respectively.

When comparing the randomized trial to the observational group, we found plasma TNF-α levels were higher at baseline in patients with RT isolation of *Candida* compared to the observational group (21.8 ± 23.1 pg/ml versus 12.4 ± 9.3 pg/ml, $p = 0.02$) and these

Table 1 Patient characteristics

Patient characteristics	Placebo	Intervention	Observational
n	29	31	29
Age	63.0 ± 13.8	57.6 ± 17.1	55.8 ± 17.8
Sex			
Male	23 (79.3 %)	21 (67.7 %)	19 (65.5 %)
Female	6 (20.7 %)	10 (32.3 %)	9 (31.0 %)
APACHE II score	23.0 ± 7.4	22.9 ± 7.6	20.9 ± 8.2
Total number of comorbidities	1.6 ± 1.7	1.6 ± 1.6	1.7 ± 1.7
Charlson comorbidity index	1.1 ± 1.3	1.0 ± 1.4	0.8 ± 1.1
Functional comorbidity index	0.9 ± 1.1	0.8 ± 1.1	1.1 ± 1.2
Baseline SOFA score	3.8 ± 2.2	3.8 ± 2.8	3.8 ± 2.6
Admission category			
Medical	23 (79.3 %)	22 (71.0 %)	16 (55.2 %)
Surgical elective/surgical emergency	6 (20.7 %)	9 (29.0 %)	12 (41.4 %)
Ethnicity			
White	28 (96.6 %)	29 (93.5 %)	28 (96.6 %)
Aboriginal	1 (3.4 %)	2 (6.5 %)	0 (0.0 %)
Primary ICU diagnosis			
Cardiovascular/vascular	2 (6.9 %)	3 (9.7 %)	5 (17.2 %)
Respiratory	9 (31.0 %)	6 (19.4 %)	2 (6.9 %)
Gastrointestinal	3 (10.3 %)	2 (6.5 %)	1 (3.4 %)
Neurologic	3 (10.3 %)	3 (9.7 %)	4 (13.8 %)
Sepsis	2 (6.9 %)	5 (16.1 %)	1 (3.4 %)
Trauma	3 (10.3 %)	3 (9.7 %)	3 (10.3 %)
Metabolic	1 (3.4 %)	0 (0.0 %)	0 (0.0 %)
Postoperative vascular/cardiovascular	5 (17.2 %)	9 (29.0 %)	12 (41.4 %)
Number of days in hospital before ICU admission	0.3 [0.0–2.2]	0.1 [0.0–0.3]	0.3 [0.0–0.9]

All results are presented *n* (%), or mean ± SD or median [Q1–Q3]
APACHE II acute physiological and chronic health assessment, *SOFA* sequential organ failure assessment, ICU intensive care unit

Table 2 Clinical outcomes

Patient characteristics	Placebo arm	Intervention arm	*p* values (placebo versus intervention)	Observational
n	29	31		29
Maximum SOFA	5.9 ± 3.3	5.9 ± 3.6	0.95	6.1 ± 3.4
Delta SOFA	2.1 ± 2.0	2.1 ± 2.0	0.97	2.3 ± 1.9
MV-free days within the first 28 days	8.0 [6.0–11.0]	9.0 [4.0–10.0]	0.91	4.5 [2.0–10.0]
ICU-free days within the first 28 days	14.0 [0.0–17.0]	4.0 [0.0–17.0]	0.22	6.0 [0.0–19.0]
Antibiotics-free days within the first 28 days	16.0 [12.0–20.0]	10.0 [0.0–20.0]	0.32	15.0 [5.0–22.0]
ICU LOS (survivors)	11.5 [8.0–20.0]	13.0 [8.0–28.0]	0.35	11.0 [4.0–22.0]
Hospital LOS (survivors)	29.0 [17.0–38.0]	28.0 [18.0–46.0]	0.90	29.5 [16.0–53.0]
MV duration (survivors)	5.5 [2.0–13.0]	7.0 [4.0–19.0]	0.06	8.5 [1.0–18.0]
ICU acquired infection (>72 h from randomization)	13 (44.8 %)	11 (35.5 %)	0.46	12 (41.4 %)
28-day mortality	6 (20.7 %)	7 (22.6 %)	0.86	5 (17.2 %)
Hospital mortality	7 (24.1 %)	7 (22.6 %)	0.89	6 (20.7 %)
Mortality at day 90	7 (24.1 %)	10 (32.3 %)	0.49	6 (20.7 %)

All results are presented as *n* (%), or mean ± SD or median [Q1–Q3]
SOFA sequential organ failure assessment, *MV* mechanical ventilation, *ICU* intensive care unit, *LOS* length of stay

patients had a lower TNF-α response to the LPS stimulation test (854.8 ± 855.2 versus 1,559.4 ± 1,290.6 pg/ml; $p = 0.01$) (Table 3). No other differences were found in the inflammatory profiles of these groups. We did not observe any difference in beta-glucan levels with both groups having increased plasma levels of beta-glucan.

Discussion

Candida is frequently encountered in RT secretions of patients with csVAP. It has been accepted that the isolation of *Candida* represents colonization and this normal commensal organism plays no pathogenic role in non-

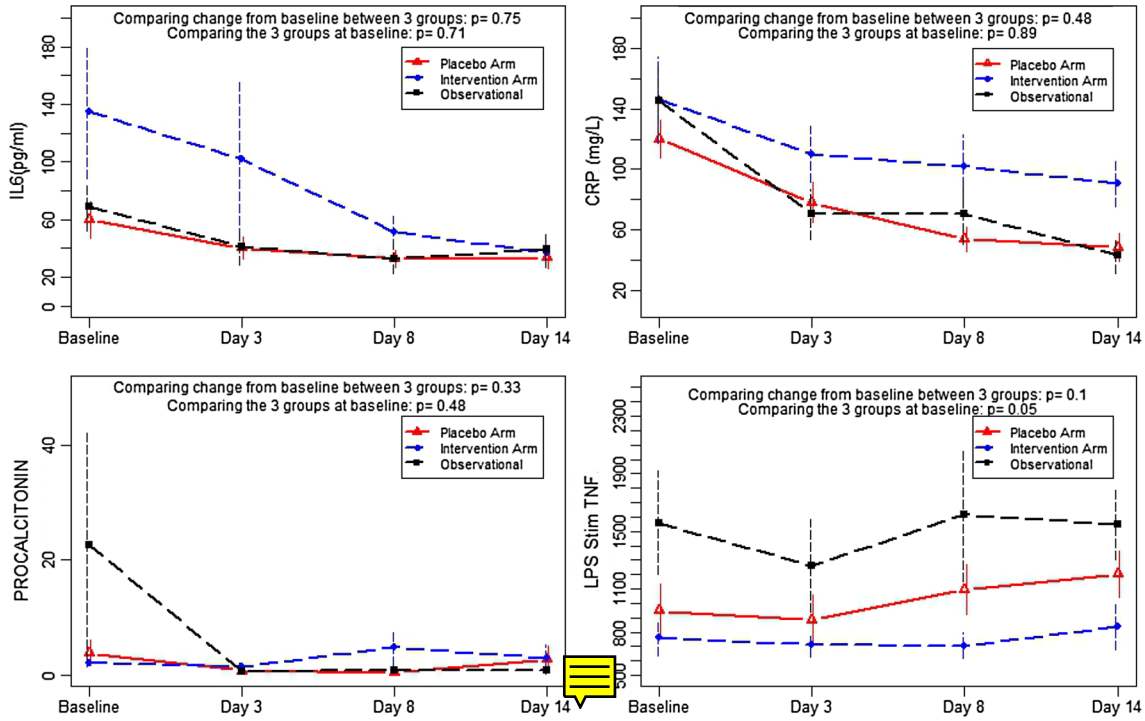

Fig. 2 Inflammatory profiles over time. All results are presented mean ± SE. Raw means with standard error bars. *P* values compare the mean baseline and mean change from baseline between groups over time by a linear mixed effect model. Normal reference ranges: (1) High-sensitivity CRP (hsCRP), median 0.14 mg/dl with an upper 97th percentile of 1.1 mg/dl; (2) procalcitonin (PCT), less than 0.05 ng/ml for healthy people, 0.05–0.5 ng/ml for localized infections, 0.5–2 ng/ml for systemic infections, and greater than 2 ng/ml for severe systemic infections (sepsis); (3) levels of interleukin-6 (IL6) are generally undetectable in the plasma of healthy individuals; (4) LPS-stimulated TNFα, in our patient populations, healthy subjects typically have values around 1,000 pg/ml. Values less than 600 pg/ml are associated with increased risk of subsequent secondary infection and values less than 250 pg/ml are associated with increased risk of death

Table 3 Baseline laboratory according to *Candida* status

	VAP with *Candida*	VAP without *Candida*	*p* values
Baseline	*n* = 56	*n* = 21	
TNF (pg/ml)	21.8 ± 23.1 (0.0–155.0)	12.4 ± 9.3 (0.0–39.9)	0.02
IL-6 (pg/ml)	97.5 ± 183.0 (0.0–1,210.0)	69.0 ± 74.9 (0.0–295.0)	1.00
IL-10 (pg/ml)	9.7 ± 25.8 (0.0–132.0)	3.0 ± 10.3 (0.0–44.6)	0.26
IL-1B (pg/ml)	1.4 ± 7.1 (0.0–49.9)	0.0 ± 0.0 (0.0–0.0)	0.29
IL-8 (pg/ml)	39.2 ± 49.2 (0.0–242.0)	29.1 ± 40.8 (0.0–158.0)	0.10
CRP (mg/l)	133.0 ± 115.0 (28.8–756.0)	145.7 ± 104.0 (13.3–424.8)	0.63
PCT (ng/ml)	3.0 ± 8.8 (0.0–60.9)	22.5 ± 89.4 (0.0–401.6)	0.73
IFABP (pg/ml)	891.8 ± 2,568.2 (0.0–19,084.4)	607.3 ± 845.4 (0.0–3,937.8)	0.98
Beta-glucan (pg/ml)	116.0 ± 171.0 (0.0–539.8)	129.1 ± 190.5 (0.0–558.7)	0.59
LPS stim TNF (pg/ml)	854.8 ± 855.2 (14.0–3,795.0)	1,559.4 ± 1,290.6 (147.0–4,844.0)	0.01

All results are presented mean ± SD (min–max). Normal reference ranges: (1) Levels of TNF-α, IL-6, IL-10, IL-8, and IL-1beta are generally undetectable in the plasma of healthy individuals; (2) high-sensitivity CRP (hsCRP), median 0.14 mg/dl with an upper 97th percentile of 1.1 mg/dl; (3) procalcitonin (PCT), less than 0.05 ng/ml for healthy people, 0.05–0.5 ng/ml for localized infections, 0.5–2 ng/ml for systemic infections, and greater than 2 ng/ml for severe systemic infections (sepsis); (4) beta-glucan, a positive test result (implying fungal disease) is greater than 80 pg/ml; (5) intestinal fatty acid binding protein (iFABP), in healthy individuals, the plasma iFABP level is undetectable (<47 pg/ml) with higher values indicating some degree of intestinal cell death; (6) LPS-stimulated TNF-α, in our patient populations, healthy subjects typically have values around 1,000 pg/ml. Values less than 600 pg/ml are associated with increased risk of subsequent secondary infection and values greater than 250 pg/ml are associated with increased risk of death

neutropenic patients with VAP. In contrast to this belief, we have previously demonstrated that *Candida* spp. in the RT secretions may impact on the hospital morbidity and mortality of patients with VAP. We postulated that the addition of an antifungal agent to such patients would prove beneficial [10, 20, 21]. Unfortunately, we had to discontinue the study before attaining our target enrolment because of slow recruitment rates. However, we observed that *Candida* spp. is associated with an increased inflammatory profile and did not detect any improvement in clinical and inflammatory outcomes, although our study was underpowered to exclude any positive signal. Consequently, a larger clinical trial looking at the effect of an antifungal treatment is unlikely to be feasible.

In humans, *Candida* spp. are commensal organisms found on the skin, as well as gastrointestinal, genitourinary, and respiratory tracts. In critically ill patients, perturbations of normal flora under the influence of factors including broad-spectrum antibiotics and relative immunosuppression can promote proliferation of *Candida* spp. [10, 20, 21]. The beta-glucan component of the cell wall of *Candida* spp. has been shown to trigger immune responses [10, 20, 21]. Beta-glucans stimulate the liberation of inflammatory markers such as IL-1 and TNF-α and engender reactive oxygen intermediates [22–25]. Recently, circulating plasma levels of beta-glucans have been demonstrated to help discriminate between *Candida* colonization and invasive infection [26]. Roux et al. observed a 60 % decrease in reactive oxygen species production by alveolar macrophages in rats colonized with *C. albicans*, suggesting an effect on innate immune response [27]. In ICU patients on MV, RT colonization with *Candida* spp. is associated with greater tracheal IL-8 and IL-6 levels than patients colonized with bacteria [27]. We have shown that the presence of *Candida* spp. in the endotracheal secretions of VAP patients is associated with increased inflammatory markers (CRP, IL-6, and PCT) and worse clinical outcome [9, 28]. The increased inflammatory marker levels were similar to the levels reached in patients with positive bacterial cultures and with negative culture results for bacteria and were statistically increased compared to a control group [9]. The hypothesis that emerged is that the *Candida* spp. in the airways are potentially directly responsible for the observed worse clinical outcomes.

The results of this randomized study do not support this hypothesis. We observed that csVAP with *Candida* has baseline increases in TNF-α plasma levels and decreased ex vivo LPS-induced TNF-α production capacity, suggesting an inflammatory state with decreased innate immune response. This concept of high levels of plasma cytokines with concurrent reduction in leukocyte cytokine production capacity has been described in the settings of sepsis and multiple organ failure and may represent ongoing tissue injury in the face of immune suppression [29, 30]. It remains unclear if these patients were immunosuppressed prior to colonization with *Candida* or as a result of *Candida* colonization. We did not, however, observe any significant modification of their inflammatory and innate immune function profiles with the use of antifungal therapy. It is noteworthy that plasma levels of beta-glucan and iFABP were high in subjects with and without positive lower airway culture for *Candida*. This suggests the possibility of alternative sources of *Candida* (e.g., from the intestine) in the experimental groups despite the absence of positive cultures from other sites. Alternatively, false positive results for beta-glucan have been described in numerous situations including dialysis with cellulose membranes, concomitant use of some antibiotics, bacteremia due to several gram-positive organisms, gauze in surgical dressings, use of albumin products and coagulation factors manufactured using cellulose depth filters, and contamination by extensive manipulation [31]. Some of these conditions could also explain elevated beta-glucan levels. *Candida* spp. may also have complex repercussions on lung inflammatory and infectious processes as well as interactions with systemic inflammation. In a mouse model receiving a single or combined intratracheal administration of *C. albicans* and *Pseudomonas aeruginosa*, a significant decrease in lung endothelial permeability and bronchiole inflammation was observed with prior *C. albicans* colonization [32]. Mortality rate was also unchanged by prior *C. albicans* colonization in this model. Moreover, two human studies did not show any lung tissue invasion in non-neutropenic patients with positive respiratory tract secretions growing *Candida* spp. [33, 34]. Recently, nebulized amphotericin B to decrease *Candida* airway colonization in MV ICU patients showed lowers rates of colonization (86 versus 62 %) but a trend towards increased VAP rate (6.5 versus 5.5 VAP per 1,000 ICU days, $p = 0.64$), an increased ICU length of stay (23 versus 14 days, $p = 0.004$), and no mortality difference [35]. Consequently, our present study and recent literature suggest the alternative paradigm that *Candida* spp. grow opportunistically in relation to the relative immunosuppression found in ICU patients with an increased inflammatory profile.

The ability of *Candida* spp. to form biofilms is also an important factor in their pathogenesis [36]. Some recent studies support a potential facilitating role of RT colonization with *Candida* spp. on subsequent *Pseudomonas* superinfection [11, 32, 37, 38]. A retrospective study found that MV patients colonized with *Candida* spp. in the airways were at increased risk of *P. aeruginosa* VAP [38]. Antifungal therapy reduced the risk of *P. aeruginosa* lung infection in such patients [11]. However, we could not corroborate such an association as only 3 (5 %) of the randomized patients developed subsequent positive cultures for *Pseudomonas* spp.

Our study's strengths include the use of a placebo and double-blinding, the multicenter design, and a comprehensive clinical and inflammatory outcome assessment.

We included patients with different ICU admission conditions improving the external validity of our results. There are also significant limitations to our findings. Firstly, the sample size was powered for feasibility; interpretation of inflammatory and clinical outcome must be done cautiously. However, we did not observe any significant trend in clinical and surrogate inflammatory outcomes. Secondly, patients colonized with *Candida* in other sites were excluded, limiting extrapolation of our results to patients with higher organism burdens. Thirdly, when comparing the randomized patients to patients in the observational component of the study, observed differences could be due to other factors (confounding variables) than the presence or absence of *Candida* sp. However, we think that this observational component was essential to help to better define the role of the *Candida* spp. in the RT. By comparing a group of patients with a suspicion of ventilator-associated pneumonia with the same inclusion criteria except for the presence of *Candida* spp., we aimed to have a better understanding of the differences in the inflammatory state and evaluate the innate immune function. The comparison was obviously exploratory given the non-randomized aspect of the observational group. When comparing patients in the randomized trial to those in the observational study, we found increased levels of inflammation in patients with RT isolation of *Candida* and decreased innate immune response. These results suggest that the presence of *Candida* spp. in the context of a suspicion of VAP is probably the consequence of a relatively significant level of persistent inflammation that leads to relative immunosuppression. Lastly, the *Candida* cultures were qualitative and were not quantified in most patients. However, the two involved laboratories had strict protocols regarding the reporting of yeast in the cultures from endotracheal specimens. To pursue the culture and identification of the *Candida* spp., the microbiology technicians had to observe a moderate to important amount of yeast on the gram stain. Small amounts of yeast on the gram stain did not lead to the report of the *Candida* spp. Consequently, only patients with a significant amount of *Candida* spp. were included as demonstrated by the available quantitative culture results. Nevertheless, it is impossible to exclude that greater quantities of *Candida* organisms or a specific threshold of organisms is needed to influence the level of systemic inflammation.

Finally, we observed that feasibility of a larger phase 3 trial evaluating the impact of antifungal treatment on isolated pulmonary *Candida* colonization is undoubtedly compromised. Although *Candida* spp. airway colonization is frequently encountered, recruitment was challenging. Potential explanations include restrictive inclusion criteria especially regarding the limiting randomization time frame, non-pulmonary sites colonization with *Candida* spp., treating physicians' decision to use an antifungal therapy for various reasons. Difficulty in obtaining informed consent prior to randomization was also substantial, mostly related to relatives' refusal and probably also related to the restrictive time frame.

Conclusion

In this randomized study evaluating an empirical antifungal strategy in patients with csVAP and *Candida* colonization, we did not observe any clinical or laboratory signal supporting a potential treatment benefit. The presence of *Candida* in the lung could be associated with persistent inflammation and immunosuppression rather than representing true infection requiring treatment. We also identified challenges in pursuing larger clinical trials in these patients. Although our pilot study was underpowered, our data does not provide any indication to support the use of antifungal treatment in patients with VAP and *Candida* in the endotracheal secretions.

Acknowledgments We would like to thank Shariq Haider, Marc Perreault, Andrew Day, Susan Richardson, Valéry Lavergne, and Marie-Soleil Delisle for their participation in the realization of this study. Sources of support: Physicians' Services Incorporated Foundation and Pfizer Inc.

Conflicts of interest No potential conflict of interest to declare.

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