Intensive Care Med (2019) 45:834–843
https://doi.org/10.1007/s00134-019-05622-0

ORIGINAL

Impact of bronchial colonization with *Candida* spp. on the risk of bacterial ventilator-associated pneumonia in the ICU: the FUNGIBACT prospective cohort study

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Abstract

Introduction: Respiratory tract *Candida* spp. colonization is associated with more frequent bacterial ventilator-associated pneumonia (VAP). However, this colonization could be causally related to VAP or simply reflect the immune paralysis associated with multiple organ failure.

Objective: To prospectively evaluate the relationship between *Candida* spp. colonization and bacterial VAP in mechanically ventilated patients with multiple organ failure.

Inclusion: Patients receiving mechanical ventilation for > 4 days and presenting multiple organ failure were included. Tracheal colonization with *Candida* spp. was evaluated at inclusion (day 0, D0) and every 4 days until extubation. Quantitative proximal and tracheal cultures were performed at each VAP episode. Monocyte human leukocyte antigen-DR isotype (mHLA-DR) expression and the ratio of polymononuclear leukocytes to lymphocytes were used to evaluate immunoparalysis at D0 and D7. The relationship between fungal colonization and VAP was modelled using cause-specific models for repeated events with adjustment for time-dependent confounders and immune factors.

Results: A total of 213 patients, with a median age of 64, simplified acute physiology score II (SAPS II) score 55 and sequential organ failure assessment (SOFA) score 10, mainly admitted for medical reasons ($n = 197$, 92%), were enrolled in 2012–2015. The median ICU stay was 24 days and the mortality rate was 32% (69 cases). Median mHLA-DR was 5916 Ab-bound/cell [3863–8934]; median lymphocyte count, 0.9Giga/L [0.6–1.3]; neutrophil-to-lymphocyte ratio, 10.9 [6.5–19.7]. Overall, 146 cases (68.5%) had tracheal colonization with *Candida* spp. An episode of VAP occurred (either for the first or only time) in 62 (29.1%) cases 5.5 days (median) after D0; a second episode occurred in 12 (5.6%) cases, 15.5 days (median) after D0. After adjustment, bronchial colonization with *Candida* was not associated with VAP [adjusted cause-specific hazard ratio = 0.98 (0.59–1.65), $p = 0.95$].

Conclusion: In patients with mechanical ventilation for more than 4 days and multiple organ failure, bronchial colonization with *Candida* spp. was not associated with VAP, even after adjustment for immune function.

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Keywords: Sepsis, *Candida*, Immunoparalysis, Ventilator-associated pneumonia, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

Introduction

Candida spp. colonization in bronchial samples is common in mechanically ventilated ICU patients. It occurs in about 30% of patients receiving mechanical ventilation (MV) for more than 48 h and in 50% of those with a clinical suspicion of ventilator-associated pneumonia (VAP) [1]. Recovery of *Candida* spp. from the respiratory tract is associated with increased duration of MV and of ICU and hospital stay, and with a worse outcome [2–5].

Other than in deeply immunosuppressed subjects, who may develop true fungal pneumonia, a lower respiratory tract yielding *Candida* spp. isolates should not usually be considered a marker of lung infection [6–10].

However, several clinical data suggest that *Candida* spp. airway colonization may play a significant role in the development of bacterial pneumonia. In a cohort study, Azoulay et al. found that *Candida* spp. bronchial colonization increased the risk of *Pseudomonas aeruginosa* pneumonia [11]. A single-center retrospective case–control study has shown that antifungal therapy administered to patients with *Candida* spp. airway colonization could prevent *P. aeruginosa* VAP [12].

However, another observational trial that compared colonized patients who received aerosolized amphotericin B with other colonized patients failed to identify any impact on VAP risk, even though the treatment produced a significant decrease in the bronchial colonization [13].

In addition, in a randomized, double-blind, placebo-controlled multicenter study, an empirical anti-fungal strategy in patients with a clinical diagnosis of VAP and *Candida* spp. airway colonization failed to improve either inflammatory reaction or patient outcome [14]. Moreover, empirical therapy with micafungin did not have any impact on the risk of VAP [10] in a recent randomized, double-blind, placebo-controlled study in multiple colonized non-immunocompromised mechanically ventilated patients with multiple organ failure.

The discrepancies observed between these studies could be related, in part, to the contribution of the host immune response. Cellular immunity is of major importance for effective clearance of fungi and bacteria.

Critical illness can induce a state of profound immune dysfunction with severe monocyte and lymphocyte dysfunction [15]. Reduced expression of the human leukocyte antigen-DR isotype (HLA-DR) on monocytes is a reliable biomarker for assessing the immune status of critically ill patients [16].

Take-home message

In a prospective study of mechanically ventilated patients, bronchial *candida* colonization was frequent and did not impact the risk of ventilator-associated pneumonia, even after adjustment for immune status

The temporal relationship between airway *Candida* spp. colonization and subsequent bacterial VAP is quite complex. Both immunoparalysis and *Candida* spp. airway colonization could promote bacterial VAP depending on their reciprocal interactions. However, *Candida* spp. airway colonization and VAP onset can also be independent and, for instance, both due to prior antibiotic exposure or to profound immunoparalysis, as observed in patients with a severe acute illness and prolonged ICU length of stay (LOS) [17, 18].

In view of these considerations, we conducted a prospective study to assess the role of *Candida* spp. airway colonization in the development of subsequent late-onset bacterial VAP in patients mechanically ventilated for more than 4 days and presenting with multiple organ failure.

Materials and methods

We designed a prospective study conducted between 2012 and 2015 in two French medical ICUs (The University Hospitals of Grenoble and Clermont-Ferrand).

Ethics and funding

The study was sponsored by the University of Grenoble and received grant funding from the French Ministry of Health [ethics committee: ("Comité de Protection des Personnes") CPP South East IV 11-055 August 30th 2011—No. ID RCB 2011-A00767-34; www.clinicaltrials.gov: NCT01770015].

Written informed consent was obtained from all participants or their proxies. In situations of impaired decision-making capacity and absence of surrogates, informed consent was obtained later on from the patient (in compliance with French laws).

Data collected

Adult patients receiving invasive MV for more than 4 days and with at least one additional organ dysfunction were considered for enrolment. Severely

immunocompromised patients (i.e. patients with neutropenia, solid organ transplant and bone marrow transplant recipients, patients receiving corticosteroid therapy consisting of > 2 mg/kg of methylprednisolone or equivalent) and patients on prior antifungal treatment were excluded. Day 0 (D0) was defined as the day of inclusion. Daily monitoring was performed in all patients for signs and symptoms of infection, organ dysfunction and other ICU-acquired complications. Blood samples were drawn at D0 and every 7 days thereafter until ICU discharge, to be used to measure HLA-DR expression on monocytes, as previously described [15, 19], and the neutrophil-to-lymphocyte ratio [20, 21], in order to assess immune function. Endotracheal aspirates were also obtained at D0 and every 7 days thereafter to identify *Candida* spp. airway colonization. Candida sp. identification was performed as previously described [22]. Briefly, specimens were inoculated onto CAN2 chromogenic isolation plates and/or into Sabouraud chloramphenicol tubes (bioMérieux, Lyon, France) and incubated for 3–6 days at 35 °C. The following rapid tests were used for identification: rapid assimilation or agglutination tests (Glabrata RTT, Bichro-Latex Albicans and Krusei-Color; Fumouze Diagnostics, Levallois-Perret, France) and api-ID32C (bioMérieux, Lyon, France).

The primary outcome was the occurrence of late-onset VAP. VAP was defined by the presence of clinical signs and symptoms of VAP, and either a broncho-alveolar lavage (BAL) culture yielding at least 10^4 cfu/ml or a quantitative tracheal aspirate culture yielding at least 10^5 cfu/ml. All episodes of VAP were taken into account. Late-onset *P. aeruginosa* and *S. aureus* VAP were secondary outcomes (Fig. E1). The VAP prevention policy was similar between ICUs and is described elsewhere [23]. Subglottic aspiration was not used as routine practice.

Statistical analysis

A sample size of 550 patients was originally planned in order to detect a 20–33.3% increase in the incidence of late-onset bacterial VAP with an odds ratio of 2, on the basis of an expected 25% *Candida* airway colonization rate. After inclusion of 100 patients, the observed airway *Candida* colonization rate was higher than 45%, and so the sample size was revised and decreased to 230 patients to maintain an acceptable power of 80% with the same hypothesis.

In the statistical analysis, ICU discharge or death and late-onset VAP were considered as competing events until D60. Models for competing events were therefore used to assess the association between *Candida* airway colonization and subsequent late-onset VAP and expressed as cause-specific hazard ratio (CSHR). Models were adjusted for the risk factors of late-onset VAP (see Table E1) defined by the univariate models, and for neutrophil-to-lymphocyte ratio and monocyte HLA-DR (mHLA-DR). *Candida* airway colonization and impaired immune function were fitted as time-dependent covariables. The direct effect of *Candida* airway colonization on the risk of VAP was estimated by CSHR. We used SAS 9.4 (Sas Institute, NC, USA) and R 3.0.2 (Vienna, Austria) for all the statistical analyses.

Results

The study involved 230 patients. Informed consent was withheld by 17 of them. Therefore, 213 patients were included in the analyses (Fig. 1). Their characteristics are shown in Table 1. The patients were mainly males, admitted for medical reasons, with high simplified acute physiology score II (SAPS II) and sequential organ failure assessment (SOFA) scores, prolonged ICU LOS, and high ICU- and 60-day mortality.

At inclusion, the population was immunoparalyzed, as demonstrated by the low median level of mHLA-DR, the high percentage of patients with mHLA-DR levels of less than 8000 monoclonal antibodies per cell (AB/c), the low median lymphocyte counts and the high median neutrophil-to-lymphocyte ratio (Fig. 2). *Candida* spp. airway colonization was observed in 146 patients (98 at D0 and 48 during the follow-up period; Table 2).

Of the 213 patients, 62 (29.1%) developed at least one episode of VAP (Table 1). This first or only episode of VAP occurred at a median of 5.5 days after D0, while in 12 patients (5.6%), a second episode occurred at a median of 15.5 days after D0. *Candida* spp. airway colonization was present at D0 in 27 of the 62 patients with VAP. VAP was due to *Staphylococcus aureus* in 13 patients, including 8 with *Candida* airway colonization at D0, and due to *P. aeruginosa* in 17 patients, including 5 with *Candida* airway colonization at D0 (Table 2 and Fig. E2).

The distribution of *Candida* species isolated from the lower respiratory tract samples and the distribution of micro-organisms causative of VAP are shown in Table 1 and 2. *C. albicans* accounted for 71% of *Candida* airway colonization at D0. This species was recovered from the lower respiratory tract prior to VAP in 26/34 (76%) patients, and it was also recovered in 71/103 (69%) patients who did not develop subsequent VAP.

In the univariate analysis, the cumulative risk of the first episode of late-onset VAP did not differ between patients with or without bronchial *Candida* colonization at D0 (Fig. 3). In the multivariate cause-specific models, after adjustment for VAP risk factors (Table E1) and time-dependent co-factors, *Candida* airway colonization had no impact on the CSHR of late-onset VAP, even after adjustment for immunoparalysis status (Table 3). In sensitivity analysis, using the same models, *Candida* airway

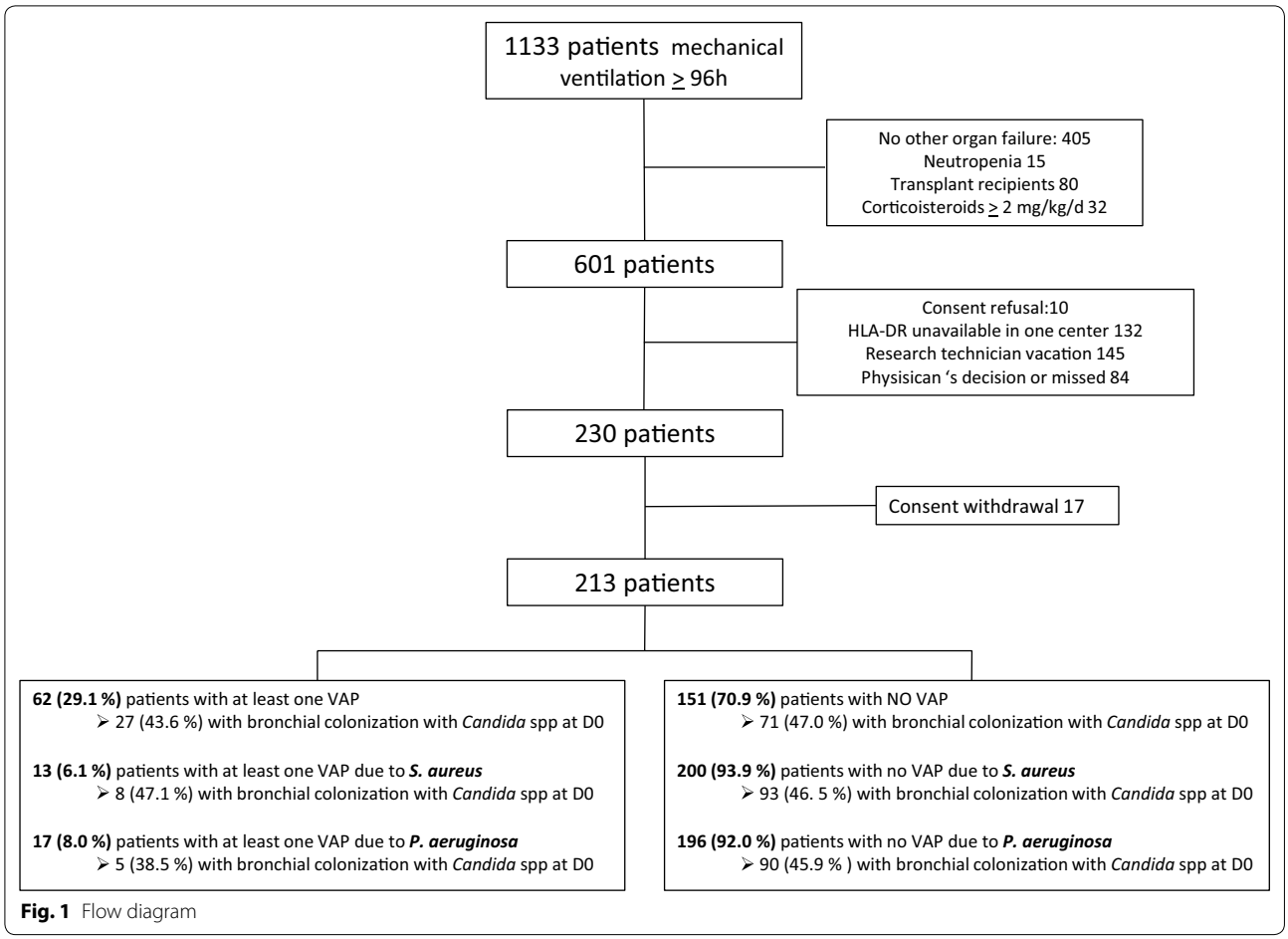

Fig. 1 Flow diagram

colonization had no impact on the CSHR of late-onset *P. aeruginosa* VAP (Table E2) or *Staphylococcus aureus* VAP (Table E3).

Discussion

In a prospective, two-center cohort study of long-term mechanically ventilated patients, bronchial colonization with *Candida* spp. was not found to be a risk factor either for bacterial VAP or for death. These findings remained similar after adjustment for host immune status reflected by HLA-DR and ratio of neutrophils to lymphocytes.

The immune response, both innate and adaptive, is important for controlling *Candida* spp. colonization and infection [24]. Blood monocytes and tissue macrophages recognize pathogen-associated molecular patterns like fungal cell wall components. Antigen presentation and subsequent cytokine secretion lead to T-helper lymphocyte activation and a secondary adaptive immune response [25]. The expression of cell surface markers such as mHLA-DR reflects the activation of monocytes and immune function. Accordingly, low mHLA-DR expression has been described as a robust marker of immune dysfunction [26] and of outcome following septic shock or trauma. Furthermore, mHLA-DR expression lower than 8000 AB/c has been associated with an increased risk of hospital-acquired infection following septic shock [15, 26] or multiple trauma [27]. Apoptotic cell death represents the major mechanism triggering sepsis-induced lymphocyte anergy/dysfunctions. After sepsis and severe trauma, immunoparalysis has been shown to be associated with a marked decrease in circulating lymphocyte numbers, in correlation with the development of nosocomial infections [21, 28] and altered patient prognosis.

Therefore, markers of immune paralysis should be taken into account to evaluate the possible link between *Candida* spp. colonization or infection and nosocomial infections such as VAP.

Findings on the interactions between *Candida* spp. and bacterial pathogens are controversial. More than 10 years ago, in a prospective cohort study, we reported that *Candida* spp. airway colonization was an independent risk factor for the development of *P. aeruginosa* VAP (9 vs. 4.8% in non-colonized patients, $p = 0.048$) [29]. Similarly, a single-center retrospective case-control study showed

Table 1 Characteristics of the patients enrolled

	All patients (*n* = 213)	Patients without *Candida* airway colonization (*n* = 67)	Patients with *Candida* airway colonization at inclusion (*n* = 98)	Patients with *Candida* airway colonization during the follow-up (*n* = 48)
Males (*n*, %)	144 (68)	46 (68.7)	66 (67.3)	32 (66.7)
Age, years (median [IQR])	64 [55; 72]	65.1 [57.9; 72.4]	64.6 [55.4; 74.1]	63.5 [51.1; 69.3]
Chronic disease (medical history) (*n*, %)	139 (65.3)	42 (62.7)	65 (66.3)	32 (66.7)
Hepatic chronic disease (*n*, %)	22 (10.3)	6 (9)	13 (13.3)	3 (6.3)
Cardiac chronic disease (*n*, %)	56 (26.3)	15 (22.4)	28 (28.6)	13 (27.1)
Respiratory chronic disease (*n*, %)	34 (16)	12 (17.9)	14 (14.3)	8 (16.7)
Renal chronic disease (*n*, %)	14 (6.6)	6 (9)	7 (7.1)	1 (2.1)
Immunodepression[a] (*n*, %)	22 (10.3)	9 (13.4)	6 (6.1)	7 (14.6)
Medical admission (*n*, %)	197 (92)	64 (95.5)	88 (89.8)	45 (93.8)
Admission SAPS II score (median [IQR])	55 [43; 66]	56 [45; 68]	54.5 [43; 66]	54.5 [38; 66]
Cause of admission				
Shock or multiple organ failure (*n*, %)	73 (34.3)	24 (35.8)	32 (32.7)	17 (35.4)
Coma (*n*, %)	30 (14.1)	9 (13.4)	14 (14.3)	7 (14.6)
Respiratory distress (*n*, %)	91 (42.7)	30 (44.8)	43 (43.9)	18 (37.5)
Other (*n*, %)	19 (8.9)	4 (6)	9 (9.2)	6 (12.5)
Sepsis on admission (*n*, %)	97 (45.5)	25 (37.3)	47 (48)	25 (52.1)
SOFA score	10 [7, 12]	10 [7, 13]	10 [7, 12]	9 [7, 12]
Procalcitonin at day 0 (µg/L)	1.2 [0.2; 10.3]	0.9 [0.2; 10.9]	1.3 [0.2; 9.7]	1.3 [0.2; 12.8]
mHLA-DR, mAb per cell at day 0 (median [IQR])	5916 [3863; 8934]	5850 [3772; 8804]	6141 [3667; 9467]	5995 [4513; 8219]
mHLA-DR < 8000 mAb per cell at day 0 (*n*, %)	135 (68)	45 (71.4)	61 (65.6)	29 (67.4)
Lymphocyte count, Giga/L at day 0 (median [IQR])	0.9 [0.6; 1.3]	1 [0.5; 1.4]	0.8 [0.6; 1.2]	1 [0.7; 1.6]
Neutrophil-to-lymphocyte ratio at day 0 (median [IQR])	10.9 [6.5; 197]	9.2 [6.4; 17.6]	11.9 [7.4; 22.9]	9.3 [6.1; 15.4]
First episode of late-onset VAP (*n*, %)	62 (29)	19 (28.4)	27 (27.6)	16 (33.3)
P. aeruginosa (*n*, %)	15 (24)	3 (16)	7 (26)	5 (31)
S. aureus (*n*, %)	13 (21)	4 (21)	5 (19)	4 (25)
Other gram-negative bacteria (*n*, %)	36 (58)	11 (58)	16 (59)	9 (56)
Other bacteria (*n*, %)	9 (15)	3 (16)	4 (15)	2 (13)
Second episode of late-onset VAP (*n*, %)	12 (6)	4 (6)	5 (5.1)	3 (6.3)
P. aeruginosa (*n*, %)	3 (25)	1 (25)	1 (20)	1 (33)
S. aureus (*n*, %)	1 (8)	0	0	1 (33)
Other gram-negative bacteria (*n*, %)	6 (50)	2 (50)	3 (60)	1 (33)
Other bacteria (*n*, %)	3 (25)	1 (25)	2 (40)	0
ICU length of stay, days (median [IQR])	24 [17; 36]	23 [15; 37]	23 [16; 33]	29.5 [20.5; 44]
ICU mortality (*n*, %)	69 (32)	28 (41.8)	28 (28.6)	13 (27.1)
Day 60 mortality (*n*, %)	83 (39)	31 (46.3)	35 (35.7)	17 (35.4)

SAPS II simplified acute physiology score II, *SOFA* sequential organ failure assessment, *VAP* ventilator-associated pneumonia, *mHLA-DR* monocyte human leukocyte antigen-DR isotype

[a] Referred to HIV patients, cancer patients without neutropenia, and patients who received steroids < 2 mg/kg/day

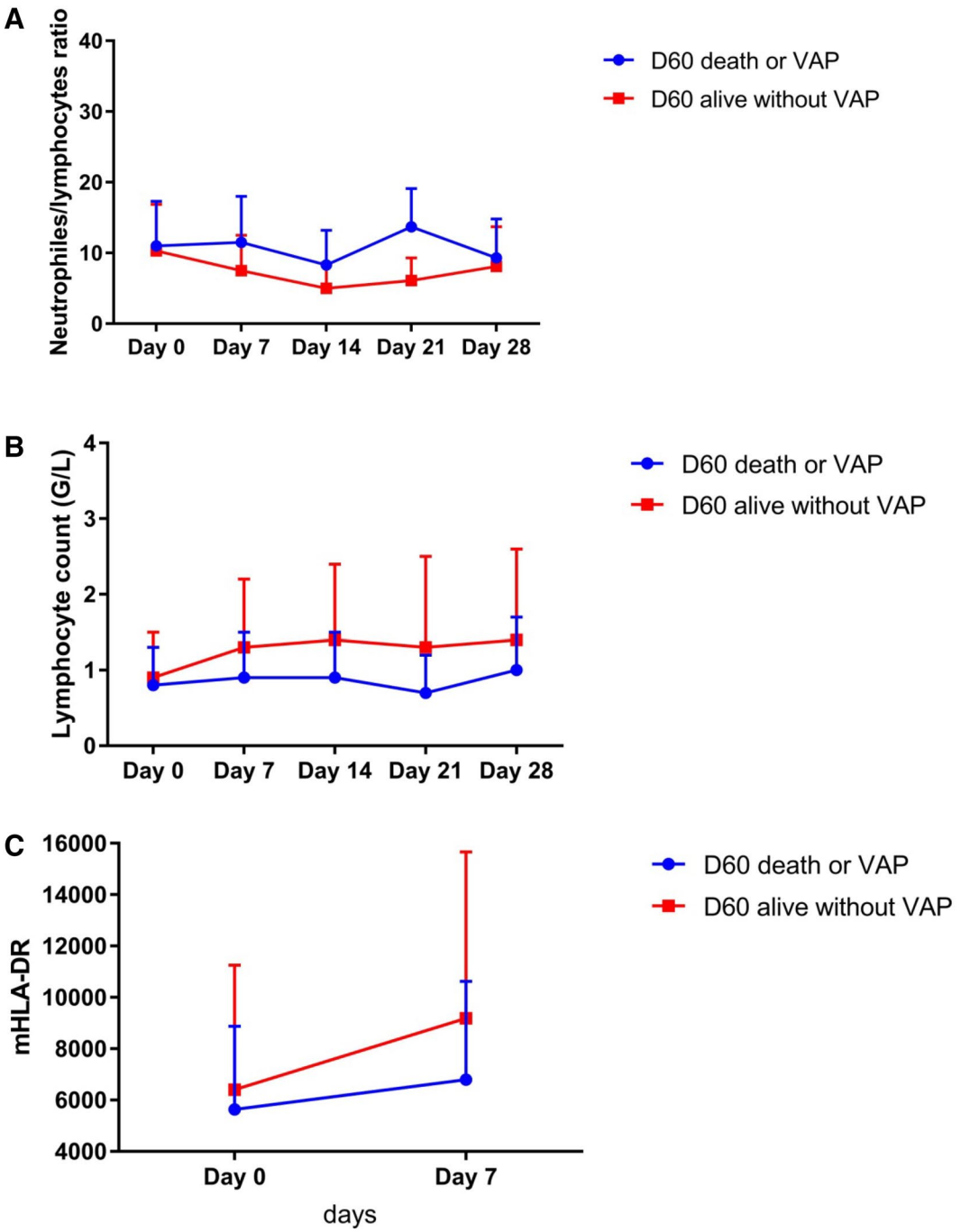

Fig. 2 Evolution of the ratio of neutrophils to lymphocytes (**a**), lymphocyte count (**b**) and mHLA-DR (**c**). *NB* none of the differences was statistically significant, *VAP* ventilator-associated pneumonia

that antifungal therapy administered to patients with *Candida* airway colonization could prevent *P. aeruginosa* VAP [12]. More recently, *Candida* airway colonization was found to be independently associated with *Acinetobacter baumannii* VAP [30]. In an experimental murine model, the beta-glucan component of the *Candida* spp. cell wall has been shown to stimulate the release of inflammatory markers and cause alveolar macrophage and neutrophil dysfunction [31]. The interactions between bacterial and fungal species could be greater

Table 2 Summary of the rates of *Candida* spp. colonization at inclusion and during the follow-up

Rates of colonization	Colonization at Day 0 ($n=98$)	Colonization in subjects without VAP ($n=103$)	Colonization before the first episode of VAP ($n=34$)	Colonization after the first episode of VAP ($n=9$)	Total colonization ($n=146$)
Candida spp.	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
C. *albicans*	70 (71.4)	71 (68.9)	26 (76.5)	6 (66.7)	103 (70.5)
C. glabrata	2 (2)	4 (3.9)	0	2 (22.2)	6 (4.1)
C. kefyr	2 (2)	2 (1.9)	1 (2.9)	0	3 (2.1)
C. krusei	3 (3.1)	3 (2.9)	0	0	3 (2.1)
C. lusitaniae	3 (3.1)	3 (2.9)	0	0	3 (2.1)
C. parapsilosis	8 (8.2)	8 (7.8)	2 (5.9)	1 (11.1)	11 (7.5)
C. tropicalis	5 (5.1)	5 (4.9)	3 (8.8)	0	8 (5.5)
Other	5 (5.1)	7 (7)	2 (5.9)	0	9 (6.2)

VAP ventilator-associated pneumonia

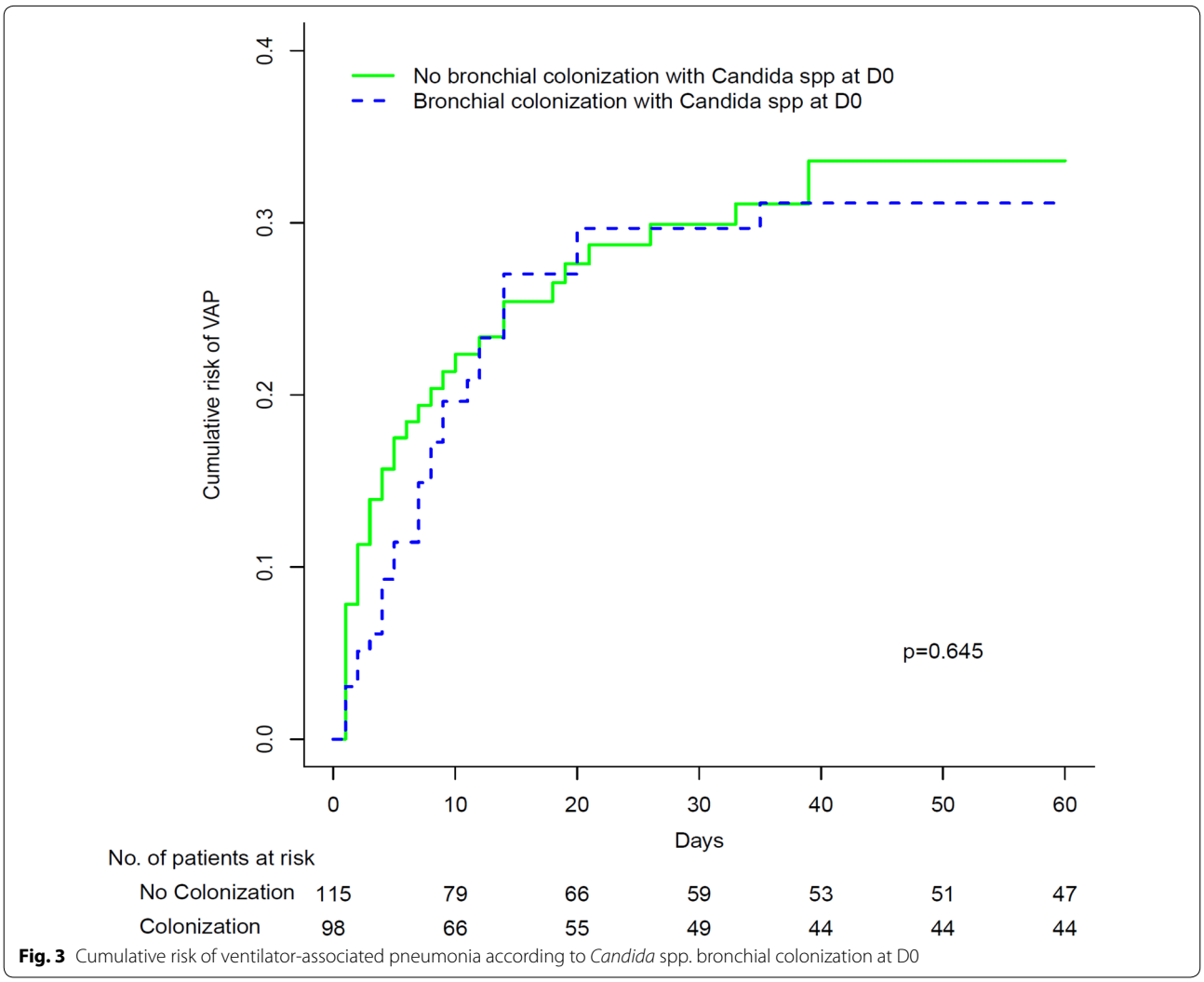

Fig. 3 Cumulative risk of ventilator-associated pneumonia according to *Candida* spp. bronchial colonization at D0

Table 3 Results of the final steps of the cause-specific models

Parameter	VAP				ICU discharge			
	CSHR	95% CI		*p* value	CSHR	95% CI		*p* value
Model 1 adjusted for chronic respiratory diseases, case mix, creatinine, Procalcitonin, use of broad-spectrum β-lactams								
Candida airway colonization (time-dependent)	0.97	0.60	1.60	0.91	1.41	0.93	2.14	0.11
Model 2 adjusted for chronic respiratory diseases, case mix, creatinine, Procalcitonin, use of broad-spectrum β-lactams and markers of immune paralysis								
Candida airway colonization (time-dependent)	0.98	0.60	1.60	0.91	1.45	0.93	2.14	0.11
mHLA-DR ≤ 8000 (time-dependent)	1.06	0.60	1.87	0.85	0.81	0.52	1.27	0.35
Lymphocytes Giga/l > 1 (time-dependent)	0.80	0.44	1.43	0.45	0.76	0.48	1.20	0.24
Neutrophils–lymphocyte ratio > 10 (time-dependent)	0.64	0.35	1.14	0.13	0.93	0.59	1.46	0.75

VAP ventilator-associated pneumonia, *CSHR* cause-specific hazard ratio, *mHLA-DR* monocyte human leukocyte antigen-DR isotype

than previously considered. Both species can sense and respond to the diverse diffusible signaling molecules produced in the niches where they coexist. The interaction between fungi and bacteria could lead to increased toxin production and increased host damage and inflammation [32].

Secondly, intratracheal instillation of live *Candida* spp. in rats can trigger the host inflammatory response, alter the lung innate immune response, and favor the development of experimental *P. aeruginosa*, *S. aureus* and *Escherichia coli* pneumonia [33]. On the contrary, in a murine model, Ader et al. reported that animals colonized by direct tracheal inoculation of live *Candida* spp. with a protocol designed to obtain *Candida* spp. colonization without epithelial injury were protected against *P. aeruginosa* pneumonia [34].

Most in vitro studies suggested that the interaction between *Candida* spp. and *P. aeruginosa* is likely to be antagonistic [34, 35]. *C. albicans* and *P. aeruginosa* are able to interact either directly, by contact, toxin-induced killing, or by the inhibition of virulence factors [32].

In addition, in a cohort study, the use of nebulized amphotericin B in mechanically ventilated patients with *Candida* spp. airway colonization had no impact on the incidence rate of VAP or on ICU mortality despite increasing the rate of *Candida* spp. decolonization [13]. Similarly, micafungin therapy of patients with multiple *Candida* spp. colonization, multiple organ failure and new sepsis of unknown etiology did not result in a decrease of VAP incidence as compared to placebo [10].

This study has some limitations. The local immune system is important for pulmonary clearance of microorganisms, but was not assessed. Indeed, the local presence of granulocyte/macrophage colony-stimulating factor in the lung apparently decreases endotoxin tolerance and may partly protect the lung from local immune suppression [36] and direct *Candida*-related insult [6]. Second, we recruited/enrolled a population of very severely ill patients with profound immunoparalysis. Whether our results can be extrapolated to less severely ill patients is therefore questionable. Third, the small number of cases of *Staphylococcus aureus* VAP and *P. aeruginosa* VAP jeopardizes the reliability of the sensitivity analyses.

Conclusion

In a prospective study, *Candida* spp. airway colonization was not associated with an increase in the risk of VAP even when immune status was taken into account.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-019-05622-0) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical standards

The study was sponsored by the University of Grenoble and received grant funding from the French Ministry of Health (ethics committee: ("Comité de Protection des Personnes") CPP South East IV 11-055 August 30th 2011—No. ID RCB 2011-A00767-34; www.clinicaltrials.gov: NCT01770015).

Informed consent
Written informed consent was obtained from all participants or their proxies. In situations of impaired decision-making capacity and absence of surrogates, informed consent was obtained later on from the patient (in compliance with French laws).

Publisher's Note

Received: 28 December 2018 Accepted: 13 April 2019
Published online: 24 April 2019

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