23/04/2026 22:06

Open Forum Infectious Diseases

MAJOR ARTICLE

Comparison of COVID-19 vs non-COVID-19 patients without hematological malignancies treated for invasive pulmonary aspergillosis: a 5-year retrospective cohort study with propensity-based adjustment

Sara Volpi [1*], Shaniko Kaleci[2], Erica Franceschini[1], Samuele Cantergiani[1], Gabriella Orlando[1], Adriana Cervo[1], Andrea Bedini[1], Stefania Casolari[1], Sara Esperti[1], Davide Chemello[1], Maddalena Albertini[1], Laura Cancian[3], Paola Buonadonna[3], Jacopo Baldi[3], Roberto Tonelli[4], Stefano Busani[5], Lucia Serio[5], Lucio Brugioni[6], Antonello Pietrangelo[7], Gabriele Melegari[5], Giovanni Pinelli[8], Claudia Venturelli[9], Irene Venturelli[9], Massimo Girardis[5], Mario Sarti[9], Cristina Mussini[1], Marianna Meschiari[1#]

[1]Infectious Disease Department, University Hospital of Modena, University of Modena and Reggio Emilia - Modena (Italy); [2]Clinical and Experimental Medicine, University of Modena and Reggio Emilia - Modena (Italy); [3]Department of Pharmaceutical Sciences, University of Modena and Reggio Emilia - Modena (Italy); [4] Respiratory Diseases Unit, Department of Medical and Surgical Sciences, University Hospital of Modena, University of Modena and Reggio Emilia - Modena (Italy); [5]Department of Anesthesia and Intensive Care Medicine, University Hospital of Modena, University of Modena and Reggio Emilia - Modena (Italy); [6]Internal Medicine

[*]**Corresponding author:** Sara Volpi, M.D., Infectious Disease Department, University of Modena and Reggio Emilia, Largo del Pozzo 71, 41124, Modena, Italy; Phone 0594225830, fax 0594223709, email saravolpi@outlook.com

[#]**Alternate corresponding author:** Marianna Meschiari, M.D., Infectious Disease Department, University of Modena and Reggio Emilia, Largo del Pozzo 71, 41124, Modena, Italy; Phone 0594225830, fax 0594223709, email mariannameschiari1209@gmail.com

DOI: 10.1093/ofid/ofaf159

Department, University Hospital of Modena, University of Modena and Reggio Emilia - Modena (Italy); [7]Department of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia - Modena (Italy); [8]Division of Internal and Emergency Medicine, University Hospital of Modena - Modena (Italy); [9]Clinical Microbiology Laboratory, University of Modena and Reggio Emilia - Modena (Italy)

Background: Our aim was to compare epidemiological, clinical and treatment characteristics, and outcomes between patients diagnosed with COVID-19-associated pulmonary aspergillosis (CAPA) and Putative Invasive Pulmonary Aspergillosis (PIPA), without hematological malignancies.

Methods: Retrospective, monocentric comparative observational cohort study, including non-hematological patients treated for invasive pulmonary aspergillosis (IPA) between 2018-2022. Primary study end points were risk factors for 30-day mortality and clinical failure. To account for the imbalance in antifungal treatment allocation, propensity score (PS) weighting approach was adopted.

Results: 209 patients were included, 93 (44.5%) CAPA and 116 (55.5%) PIPA: 144 (68.9%) admitted to Intensive Care Unit. PIPA had higher Charlson Comorbidity Index value (5.8 ±2.6 [0-14]), higher prevalence of Chronic Obstructive Pulmonary Disease (30.7%), solid cancer (36.8%), liver cirrhosis (12.3%) and concomitant immunosuppressive therapies (26.1%). CAPA received more invasive mechanical ventilation (IMV) (70.5%) and corticosteroids (90.1%), had more frequently positive galactomannan on bronchoalveolar lavage (BAL-GM) (80.5%) and higher length of hospital stay (LOS) (62.7 ±52.1 [8-276]) and ICU stay (36 ±30.7 [2-168]). No differences in clinical cure and mortality were observed between groups. In multivariable analysis, isavuconazole was the only independent factor for clinical cure, reported also in the PSM analysis (OR 0.41, 95%CI 0.16-1.03, p=0.059). Positive serum-GM was independently associated with 30-day mortality (1.78, 95%CI 1.02-3.10, p=0.042).

Conclusions: CAPA patients have less comorbidities and higher fungal burden compared to PIPA, but clinical outcomes are similar between groups. Independent predictor for better clinical cure was isavuconazole and for 30-day mortality was positive serum-GM.

Keywords. CAPA; COVID-19; aspergillosis; immunocompromised; critical care.

BACKGROUND

The European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) definitions for Invasive Pulmonary Aspergillosis (IPA) specify the presence of commonly accepted host factors predisposing to aspergillosis: neutropenia, hematologic malignant diseases, bone marrow or solid organ transplantation, and Graft Versus Host Disease – GVHD[1].

Since the development of the original guidelines, additional host factors, such as severe inherited immunodeficiencies and low CD4 count, have been included to the revised guidelines in 2020[2]. New populations at risk for IPA have also been recognized among the scientific community, including immunocompetent patients[3]. Definition of Putative Invasive Pulmonary Aspergillosis (PIPA) was developed by Blot et al. in 2012 and then modified by Schauwvlieghe et al in 2018 to classify patients at risk of IPA in intensive care units (ICU)[4,5]. However, this definition has also been applied in studies assessing patients treated in non-intensive settings[6].

A strong association between IPA and SARS-CoV2 severe pneumonia has been demonstrated, with an incidence rate of 0.7%-7.7%[7], defined as COVID-19 Associated Pulmonary Aspergillosis (CAPA)[8]. Compared to IPA in immunocompromised patients, CAPA is clinically more challenging to manage due to an unusual clinical and radiological presentation and a more frequent observation in older patients[9,10]. In the post-pandemic scenario, a more detailed microbiological, clinical and therapeutic characterization among all hospitalized patients at risk for IPA, not only those critically-ill, is essential. This characterization can assist in the development of new definitions for medical wards and elderly populations.

Studies directly comparing PIPA and CAPA in non-hematological patients admitted to ICU and medical and surgical wards are required.

During the COVID-19 pandemic, the newly introduced antifungal agent isavuconazole, was quickly adopted for patients with IPA. However, the tolerability and efficacy profile of isavuconazole compared to voriconazole is undefined, despite the post-hoc analysis from the VITAL and SECURE trials[11]. Data are particularly missing for elderly patients.

The aim of this study was to compare demographic, clinical, microbiological, radiological and treatment characteristics, 30- and 90- day mortality, clinical cure and IPA infection relapse between patients with CAPA vs PIPA. Risk factors for 30-day mortality and clinical failure were also assessed. Finally, a subanalysis of comparative outcomes was performed in selected IPA patients treated with isavuconazole or voriconazole as primary regimen, without any switch to a second line treatment.

METHODS

Study design and clinical definitions

We conducted a retrospective, observational monocentric cohort study to include consecutive adult patients with an infectious disease consultation and treatment for IPA, in ICU, medical and surgical wards at the University Hospital of Modena, between January 2018 and December 2022.

Patients with an IPA diagnosis were screened. Cases were retrospectively reclassified according to the currently available definitions. Patients with concomitant or recent COVID-19 ($\leq$ 1month)

and diagnosed according to the Koehler et al. definition of "probable", "possible" or "proven" IPA, were grouped as CAPA.[8] Patients without a diagnosis of SARS-CoV2 infection (≤ 1month), and identified with "putative" or "proven" IPA (according to the modified-AspICU algorithm of Schauwvlieghe et al)[4] and/or "probable", "possible" or "proven" IPA (according to the EORTC/MSG definition)[2] were classified as PIPA. SARS-CoV2 infection was defined as a positive RT-PCR or antigenic test on respiratory specimen.

Criteria specified the exclusion of patients (i) affected by other forms of aspergillosis (non-IPA); (ii) with any type of hematological neoplasia; (iii) who received antifungal agents as prophylaxis; (iv) who received antifungal agents for other concomitant fungal infections.

The study was approved by the ethics committee Comitato Etico Indipendente Area Vasta Emilia Nord (n. 624/2023/OSS/AOUMO).

Data collection

For each patient, demographic and clinical data at time of diagnosis were collected, particularly age, sex, year and ward of diagnosis (medical, surgical or ICU), invasive mechanical ventilation (IMV), co-morbidities and Charlson Comorbidity Index (CCI), use of steroids or other immunosuppressive therapies. Symptoms related to aspergillosis and sequential organ failure assessment (SOFA) score were also obtained. Radiological patterns were derived from written reports and reviewing images, when necessary. For antifungal treatment, we collected data about the molecule used (voriconazole, isavuconazole and liposomal amphotericin B, L-Amb), as first regime or as a possible second line treatment, reason of switch, treatment duration and associated adverse events (AEs).

Microbiological analyses

For aspergillosis identification, all respiratory samples were evaluated at microscopic examination, to detect the presence of fungal hyphae or spores. Culture examinations were always performed and, when positive, *Aspergillus* species was identified through MALDI-TOF when feasible, and/or with the help of macroscopic and microscopic examination of the colonies. Susceptibility to voriconazole, isavuconazole and L-Amb was determined with standardized broth microdilution methods (using the Sensititre Yeast One ITAMYUCC test, Thermo Scientific). Minimum inhibitory concentration (MIC)–was interpreted according to guidelines of the Clinical and Laboratory Standards Institute (CLSI), using the break point established only for voriconazole for *A. fumigatus* and the epidemiological cut off (E.C.V.) for other *Aspergillus* species[12,13].

Galactomannan (GM) was detected through enzyme-linked immunosorbent assay (ELISA; PlateliaTM *Aspergillus*, BioRad, Marnes-La-Coquette, France) and, for diagnostic purposes, we considered a positivity cut-off of 0.5 optical density index (ODI) on serum and of 1.0 ODI on respiratory samples. For 1,3-β-D-glucan (BDG) on serum, we used Fungitell assay (Associates of Cape Cod, East Falmouth, MA, USA) with a positivity cut-off of 80 pg/mL.

DNA extraction for PCR and RT-PCR analyses was performed on an ELITe InGenius automated platform using the *Aspergillus* spp. ELITe MGB kit (Elitgroup, Puteaux, France). DNA was extracted from a 1mL volume of bronchoalveolar lavage (BAL) or bronchoaspirate (BAS) fluid and was eluted in a 200µL saline solution prior to DNA amplification in the same platform. The ELITe MGB kit for RT-PCR was CE-in-vitro diagnostic (CE-IVD) validated on a diverse range of sample types. The target region was the ribosomal DNA18S (rDNA18S) and the human B-globin gene was used as an internal standard. The fungal DNA copy number was expressed as copies/mL in relation to a rDNA18s standard curve.

Outcomes

Primary outcomes were defined as follows: 30-day mortality was calculated from start of therapy (SOT), clinical cure was considered as a substantial improvement in symptoms and/or radiological patterns at the end of treatment (EOT). Secondary outcomes were: 90-day mortality from SOT, infection relapse (defined as recurrence of IPA ≤90 days from EOT in patients who previously experienced clinical cure), length of hospital stay (LOS) and length of ICU stay.

The subgroup analysis included selected IPA patients treated with isavuconazole or voriconazole (as primary regimen without any switch to a second line treatment) with 30- and 90- day mortality, clinical cure, IPA infection relapse and AEs.

Statistical analysis

A descriptive analysis of all collected variables was conducted; categorical variables were presented as numbers and percentages, and continuous variables were reported as mean (± standard deviation) if normally distributed, or median (interquartile range) if not normally distributed. Univariate analysis assessed differences between groups and risk factors. Chi-square or Fisher's exact tests were used for categorical variables, and T- or Mann-Whitney U-tests were used for continuous variables. Statistical significance was defined as a p-value <0.05.

Multivariate analysis models using logistic- and Cox-regression models were performed; significant and clinically relevant variables were included. Results were presented as Odds Ratios (OR) or Hazard Ratios (HR) with 95% confidence intervals. Propensity score (PS) matching (PSM) accounted for potential confounding in antifungal treatment allocation. The PSs were calculated using a logistic regression model including relevant covariates. The PSs were then used to perform 1:1 matching between patients treated with voriconazole and those treated with isavuconazole, using the nearest neighbor method (caliper = 0.2 SD from the logit of the PS). Balance between PSM groups was assessed using standardized mean differences, <0.1 indicated adequate balance, minimizing potential confounding effects.

Kaplan-Meier survival curves were generated to compare 30-day survival from the SOT between groups and subgroups, and to compare clinical failure for subgroups. Survival distribution comparisons were performed using the log-rank test. Statistical analysis was performed using

STATA® software version 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.).

RESULTS

Between January 2018 and December 2022, 209 patients were included: 93 CAPA (44.5%) and 116 PIPA (55.5%). IPA diagnosis was mostly made in ICU (70%) and 30% in medical wards. In the whole population, clinical cure was 46.6%, 30- and 90-day mortality were respectively 37.8% and 50.2%. Finally, 2.5% of patients experienced infection relapse.

Comparison between CAPA and PIPA groups

The main demographic, clinical, microbiological and radiological characteristics, 30- and 90- day mortality, clinical cure, IPA infection relapse of CAPA and PIPA patients are provided in Table 1.

Most cases of IPA (170/209 cases, 81.3%) were diagnosed after 2020 (93 as CAPA and 77 as PIPA). Incidence of CAPA increased from 0.19/1000-patient days in 2020 to 0.25/1000-patient days in 2021 and decreased to 0.08/1000-patient days in 2022. Incidence of PIPA increased after 2020, reaching and incidence of 0.19/1000-patient days in 2022 (Supplementary, Figure S1). All CAPA cases were classified as "probable". PIPA classifications were different according to the definitions applied: the modified-AspICU algorithm was quite sensitive since 67.2% of cases were classified as "putative" and only 23.3% did not meet the diagnostic criteria; 70.7% of cases were "non-classifiable" with the EORTC/MSG definition, only 17.2% were "probable" and 9.5% "possible". Three patients had "proven" IPA according to both models (Supplementary, Table S1).

Compared to PIPA, more CAPA patients were diagnosed in ICU (89.3% vs 52.6%, p<0.001) and, consequently, underwent IMV more often (70.5% vs 35.3%, p<0.001). Patients with CAPA received more steroids and tocilizumab during LOS (respectively, 90.1% vs 52.6%, p<0.001; and 62.3% vs 0.8%, p<0.001). Conversely, more PIPA patients were diagnosed in medical wards (46.6% vs 10.8%, p<0.001), they had higher CCI (3.9 ±2.3 [0-10] vs. 5.8 ±2.6 [0-14], p<0.001) and suffered more from Chronic Obstructive Pulmonary Disease (COPD) (19.3% vs. 30.7%, p=0.004), solid cancer (14.6% vs. 36.8%, p<0.001) and liver cirrhosis (1.1% vs. 12.3%, p=0.002). They were also more frequently co-infected with *P. jirovecii* (1.2% vs. 23.0%, p<0.001) and treated more often with non-corticosteroid immunosuppressive therapies (10.1% vs. 26.1%, p=0.004).

Symptoms did not differ between the groups, except for "recurrent fever", which was more common in PIPA (3.2% vs. 12.9%, p=0.013). Radiological patterns of "ground glass" and "consolidation" were more often present among CAPA patients (80.3% vs. 48.2%, p<0.001, and 91.4% vs. 77.7%, p=0.012), and "tree in bud" pattern among PIPA patients (2.5% vs. 20.5%, p<0.001).

Almost half (47.4%) of the patients had a positive culture for *Aspergillus* species on at least one respiratory sample: the prevalent species was *A. fumigatus* (62.8%), followed by *A. flavus* (18.6%), *A. terreus* (6.2%), *A. niger* (5.3%) and *A. nidulans* and *A. oriyzae* (together 1.8%). On average, all species were susceptible to antifungal agents, except for *A. oryzae* which showed higher MIC for L-Amb and one strain of *A. fumigatus* which was resistant to voriconazole and L-Amb (Supplementary, Table S2).

When performed, BAL-GM was positive in 72.8% of cases (n=126/173), with a mean value of 4.57±2.16 (1.04-16.8); positivity was more frequently observed among CAPA patients (80.5% vs 66.7%, p=0.042), with similar mean values in PIPA and CAPA groups. There was a trend towards higher mean values of serum-GM among CAPA patients (2.14±2.13 [0.51-10.5] vs 1.53±1.42 [0.53-5.85], p=0.056).

PCR for *Aspergillus* was performed on at least one respiratory sample in 72.7% (n=152/209) of patients: on BAL in 131 patients (62.7%) and on BAS in 24 (11.5%). For three patients, both PCR on BAL and PCR on BAS were available. PCR on respiratory sample was positive in 110/152 patients (72.4%), on BAL in 93/131 patients (71%) and on BAS in 18/24 patients (75%). There was a trend towards higher mean value of PCR among CAPA patients compared to PIPA, both on BAL (139579.1 ±417435.7 [134-1645414] vs 66100.3 ±237680.3 [132-1352255], p=0.395) and BAS (358896 ±874340.8 [154-2143634] vs 238552.9 ±695922.6 [128-2094166], p=0.771) samples. Significantly longer mean LOS (62.7 ±52.1 [8-276] days vs. 49.9 ±38.6 [8-189] days, p=0.044) and ICU stays (36 ±30.7 [2-168] days vs. 24.8 ±26.8 [1-138], p=0.034) were registered among CAPA patients. No other differences in clinical outcomes, including clinical cure and overall mortality, were observed among the groups (Figure 1a).

Characteristics of antifungal treatments among all patients

Overall, voriconazole was the preferred first line regimen, prescribed in 76% of the patients and significantly more frequently prescribed for CAPA (87.1% vs 68.1%, p=0.001). No significant differences were observed for prescriptions of isavuconazole between the groups. More PIPA patients were treated with L-Amb (15.5% vs 0%, p<0.001) (Table 1).

Of the patients treated with voriconazole as first line regimen, 20/160 (12.5%) discontinued it (12 switched to isavuconazole); of those treated with L-Amb 10/18 (55.6%) changed to a second line therapy; and of those treated with isavuconazole, only 3/31 (9.7%) switched to another regimen following clinician consideration (particularly, for cost restrictions). Overall, drug-related AEs were registered in 17.7% (n=3/18) of patients with first line treatment with L-Amb (which was the less tolerated regimen), 7.8% (n=12/160) of patients with voriconazole, and no patients with isavuconazole (Supplementary, Table S3).

Risk factors associated with clinical failure

Among all patients, 109 patients (53.4%) experienced clinical failure. Significant risk factors were CCI (OR 1.21, 95%CI 1.08-1.36, p=0.001), diabetes (OR 2.17, 95% CI 0.13-4.17, p=0.020), IMV (OR 1.72, 95% CI 0.98-3.01, p=0.058), SOFA score (OR 1.14, 95% CI 1.01-1.28, p=0.028), positive serum BDG (OR 2.41, 95% CI 1.34-4.35, p=0.003) and treatment with voriconazole (OR 1.83, 95% CI 1.02-3.32, p=0.043) (Table 2 and Supplementary, Table S4). Independent risk factors were CCI (1.30 [1.13-1.49], p<0.001) and IMV (2.21 [1.15-4.25], p=0.018), but only the latter was confirmed after the PSM adjusted analysis (OR 2.24 [1.16-4.31], p=0.016). A trend towards the use of isavuconazole and clinical cure was observed at multivariate analysis (OR 0.41, 95% CI 0.16-1.02, p=0.058), and at PSM adjusted analysis (OR 0.41, 95% CI 0.16-1.03, p=0.059) (Table 3).

Risk factors for overall 30-day mortality

Overall, patients who died within 30 days from SOT had higher CCI (5.7 ±2.8 [0-14] vs 4.6 ±2.4 [0-11], p=0.003) and SOFA scores (6.1 ±4.1 [1-17] vs 4.3 ±2.9 [0-15], p=0.009), had more often diabetes (37.3% vs 20.3%, p=0.008), underwent IMV for frequently (61.3% vs 44.2%, p=0.018), had more frequent serum-GM positivity (31% vs 16.1%, p=0.016) and higher copies of *Aspergillus* (detected by PCR) on BAS (848154.4 ±1160159 [128-2143634] vs 5958 ±9255.9 [237-28971], p=0.033) compared to survivors (Table 2).

Risk factors for 30-day mortality were higher CCI (HR 1.14, 95% CI 1.05-1.24, p=0.002), diabetes (HR 2.07, 95% CI 1.29-3.30, p=0.002), liver cirrhosis (HR 2.02, 95% CI 1.01-4.06), IMV (HR 1.66, 95% CI 1.04-2.63, p=0.033), SOFA score (HR 1.09, 95% CI 1.02-1.19, p=0.016) and positive serum-GM (HR 1.79, 95% CI 1.09-2.97, p=0.023) (Supplementary, Table S4). Independent risk factors were IMV (HR 3.96, 05% CI 2.24-6.96, p<0.001) and positive serum-GM (HR 1.76, 95%CI 1.01-3.07, p=0.046). After PSM adjustment for age, IMV and CCI, serum-GM positivity was the only independent risk factor identified (HR 1.78, 95%CI 1.02-3.10, p=0.042) (Table 4).

Subgroup analysis

The subgroup comparison between characteristics of patients treated with voriconazole or isavuconazole only is reported in Supplementary, Table S5. Isavuconazole was more often prescribed for cardiopathic patients. Mean total days of treatment with voriconazole and isavuconazole were similar (29.6 ±35.5 [4-264] and 27.9 ±31.7[1-89], p=0.907). Isavuconazole was associated with higher clinical cure (67.9% vs 41.6%, p=0.011) and a trend towards less infection relapse (0% vs 1.5%, p=0.520) and lower 30- and 90-day mortality (28.6% vs 40.7%, p=0-288 and 39.3% vs 52.9%, p= 0.190; Figure 1b and Supplementary, Table S6). The association between voriconazole use and clinical failure is shown in Figure 2.

DISCUSSION

Our study represents a large cohort of Invasive Pulmonary Aspergillosis in patient without hematological malignancies including more than two hundred patients, half of which were CAPA and 30% were diagnosed outside the ICU. Our study reports that CAPA and PIPA patients present with different comorbidities and underlying conditions and selected mycological characteristics, but outcomes are similar. Main risk factors for 30-day mortality was serum-GM positivity and for clinical failure was IMV. A trend towards the improved clinical cure with the use of isavuconazole was observed. Additionally, subgroup analysis of patients initially treated with azole agents as the primary regimen, who did not experience any changes to their antifungal therapy during the treatment period, demonstrated that isavuconazole was associated with a higher clinical cure rate compared to voriconazole.

The characteristics of the patients included allowed us to outline the main epidemiological, clinical and prognostic differences of these two distinct diseases: PIPA and CAPA. While IPA was extensively investigated among critically-ill and hematological patients in literature, little is known about its clinical features in non-ICU and immunocompetent patients.

Incidence of PIPA steadily increased through years, doubling incidence rates in 2022 compared to 2020, while CAPA incidence peaked during 2021 to reduce afterwards. We believe that the increase in PIPA incidence is the results of several factors that have contributed to enhancing clinicians' knowledge and awareness of IPA.

First of all, in recent years, the definition of IPA has been updated or newly developed for different population. In 2018 the modified-AspICU was elaborated[4], the EORTC/MSG definitions were updated in 2019[2], IAPA (Influenza Associated Pulmonary Aspergillosis) was best defined in 2020[14] and, at the end of the same year, CAPA definition was firstly published[8]. More recently, the FUNDICU consensus document introduced new definitions for IPA in non-neutropenic adult ICU patients, a highly heterogeneous population with a broad range of baseline comorbidities and predisposing conditions[15]. However, the fact that a significant percentage of PIPA cases in our study could not be classified according to the EORTC/MSG or modified-AspICU definitions highlights that the current algorithms still have limitations and need further refinement to make them more broadly applicable, as already reported in literature[16,17].

A second factor contributing to the increase in PIPA incidence is the heightened focus and interest in IPA following the COVID-19 pandemic and the establishment of the CAPA definition.

Finally, inclusion of new biomarkers such as *Aspergillus* PCR in diagnostic definition (not as exclusive mycological criteria), could have enhanced efficiency in IPA diagnosis, despite its performance in non-neutropenic, critically-ill patients is still debated[15,18–20].

Aspergillus PCR has been available at our center since 2017 and it has been performed on respiratory samples in considerable percentage of patients included in our study, with high

positivity rate both on BAL and BAS. Although not statistically significant, the average PCR value tended to be higher in CAPA patients compared to PIPA patients, in a concordant manner on both BAL and BAS. Moreover, positivity rate of *Aspergillus* PCR is also in line with positivity rate results of BAL-GM, which is already a well-recognized diagnostic criterion for IPA[21,22]. Therefore, we could suggest that *Aspergillus* PCR could be useful in diagnosing IPA, even from non-bronchoscopic lavage samples when BAL cannot be performed. Further analyses are needed to confirm this assumption.

Beyond the average PCR value, CAPA patients also had higher positivity of BAL-GM and mean serum-GM values compared to PIPA. These findings suggest a possible greater fungal disease load compared to PIPA patients[23], favored by COVID-19 damage on respiratory epithelium, immune response dysregulation, cytokine storm and hyperinflammation, as previously reported[24]. On the contrary, typical radiological features and symptoms (such as cavitation, nodules, or halo sign, but also hemoptysis and chest pain) are less common in non-neutropenic patients, especially when there is an overlap with other infections[25]. Data in literature reports typical radiologic findings in 28.8% of PIPA patients [6] and in 17.6% of CAPA patients[24].

Another difference in mycological markers between the two groups was the higher positivity rate and mean vale of BDG among PIPA patients compared to CAPA. While BDG is primarily used for its negative predictive value, elevated levels can serve in increasing suspicion of a fungal infection that warrants further investigations, particularly in the absence of known confounding factors (such as beta-lactam therapy, surgery or other recognized influences)[26,27]. This finding could be especially relevant for patients admitted to non-ICU wards (such as PIPA patients in our study), where deep respiratory samples (BAL or BAS) may not be readily available.

Our analysis highlights, in line with literature[3,28–30], that patient with PIPA had more comorbidities than CAPA: higher CCI, more COPD, solid cancer and liver cirrhosis. This result seems to suggest that in CAPA group, COVID-19 itself and concurrent COVID-19 treatments (corticosteroids and immunomodulators) are the predominant predisposing factors for aspergillosis development. More recently, Feys et al. demonstrated that ICU admission in the vaccination era was independently associated with CAPA development, suggesting the need to consider antifungal prophylaxis in critically-ill patients with COVID-19 requiring mechanical ventilation and high doses of corticosteroids[10].

In this study, 30- and 90-day mortality were similar to those previously reported in literature. Cornillet et al. compared mortality rates among non-neutropenic and neutropenic patients, reporting rates of 89% vs 60%. They hypothesized that this difference was related to a lower level of suspicion and monitoring for aspergillosis in non-neutropenic patients, leading to poorer management and a delay in the initiation of therapy.[31] Additionally, Meersseman et al. observed a mortality of 91% among ICU patients without malignancies.[32] However, while Feys et al. found higher 90-day mortality rates for CAPA patients comparing to those without CAPA (48% vs 21%)[10], in our study no differences in mortality were observed between the CAPA and PIPA

groups. This difference may be explained by all diagnoses in our study being approved by an infectious disease consultant, who limited misdiagnosis or inclusion of colonized patients. Misdiagnosis or inclusion of colonized patients may occur more frequently associated with PIPA due to a lack of validated diagnostic definitions in non-neutropenic patients. Another reason of this difference may be the independent role of patients' management and therapies. Indeed, the Feys et al. study cohort only included patients who underwent IMV, whilst only 70% of patients included in our study with CAPA underwent IMV. Furthermore, Feys et al. used the EORTC/MSG host criteria for patient selection, which may have led to the inclusion of some hematological more fragile patients, excluded from our cohort. Finally, Feys et al. found that azoles were the preferred drugs used, but antifungal therapies were not distinguished in detail.[10]

Another important and novel finding of our study is the confirmation of the independent prognostic role of serum-GM in predicting 30-day mortality, even in non-hematological patients[33].

Importantly, isavuconazole in our cohort tended to be associated with better clinical cure than voriconazole, also when adjusted for age, comorbidities and severity of clinical presentation. Randomized prospective clinical comparative trials are lacking, but recent evidence from several retrospective studies, reported similar efficacy and improved safety between isavuconazole and voriconazole.[34–36] The most marked difference in clinical failure between isavuconazole and voriconazole was observed during the first month of treatment (as shown in Kaplan-Meier curve), highlighting the importance of an early appropriate management.

Moreover, in line with SECURE trial, adverse events were less in patients treated with isavuconazole. In the post-hoc analysis of the VITAL and SECURE trials[11], considering patients treated with isavuconazole, authors concluded that AEs were more frequent and more severe in the ≥65-year-old subgroup than in the <65 years subgroup, and that outcomes were basically worst in the older patient group (higher mortality and lower overall, clinical, microbiological and radiological response). However, the post-hoc analyses also noted a more favorable safety profile for isavuconazole vs. voriconazole, also in the subgroup of younger patients, due to less frequency of hepatotoxicity[11]. In our study, the overall mean age and of the patients treated with isavuconazole was higher than that reported in other studies suggesting a good safety profile in elderly patients. It is important to note that, at our university hospital, we began performing therapeutic drug monitoring (TDM) not only for voriconazole but also for isavuconazole in selected patients during the last year of the study. Unfortunetely, we were unable to retrieve the TDM results for this study as they were performed at another hospital and were not available in the electronic medical records. Although the evidence remains uncertain,[37] we believe that the absence of AEs in our population could serve a proof of concept for the necessity of TDM for isavuconazole, at least for elderly patients to prevent toxicity.

Finally, a trend towards isavuconazole with lower mortality and IPA infection relapse was observed. Considering the good tolerability and improved outcomes associated with isavuconazole observed in our study, this drug could be considered the optimal therapeutic choice for elderly

patients and those hospitalized with multiple comorbidities. Due to the monocentric retrospective nature of the study, these results need to be confirmed in future randomized controlled trials (RCT) studies.

Our study has some limitations: i) a relatively small sample size for sub-analyses of age and treatment groups and analyzed risk factors for clinical failure and 30-days mortality; ii) unmatched analyses susceptible to confounding factors. However, to limit the indication biases propensity score weighting approach was adopted accounting for the imbalance in antifungal treatment allocation; iii) criteria to diagnose probable aspergillosis in non-hematological patients have not been extensively validated. To overcome this general limitation, we enrolled only patients treated in agreement with the hospital's infectious disease consultant.

In conclusion, we show that IPA has a different presentation in COVID-19 comparing to non-COVID-19 patients, with a higher grade of fungal burden in the CAPA group, which does not translate into any differences in outcomes. Treatment with isavuconazole seems to be associated with better clinical cure and optimal safety profile, but due to the retrospective nature our data need to be confirmed by further prospective multicenter studies. Finally, serum-GM confirmed to be a predictor of mortality even in non-hematological patients.

Supplementary data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors would like to thank Johanna Chester for her assistance in revising the content and clarity of the manuscript.

Financial support. No external funding was received for the present study.

Potential conflicts of interest. All the authors report no potential conflicts of interest.

References

1. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) C. *Clin Infect Dis*. 2008;46(12):1813-1821. doi:10.1086/588660
2. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis*. 2020;71(6):1367-1376. doi:10.1093/cid/ciz1008

3. Kluge S, Strauß R, Kochanek M, Weigand MA, Rohde H, Lahmer T. Aspergillosis: Emerging risk groups in critically ill patients. *Med Mycol*. 2021;60(1):1-13. doi:10.1093/mmy/myab064
4. Schauwvlieghe AFAD, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med*. 2018;6(10):782-792. doi:10.1016/S2213-2600(18)30274-1
5. Blot SI, Taccone FS, Van den Abeele A-M, et al. A Clinical Algorithm to Diagnose Invasive Pulmonary Aspergillosis in Critically Ill Patients. *Am J Respir Crit Care Med*. 2012;186(1):56-64. doi:10.1164/rccm.201111-1978OC
6. Corcione S, Lupia T, Raviolo S, et al. Putative invasive pulmonary aspergillosis within medical wards and intensive care units: a 4-year retrospective, observational, single-centre study. *Intern Emerg Med*. 2021;16(6):1619-1627. doi:10.1007/s11739-021-02705-z
7. Egger M, Bussini L, Hoenigl M, Bartoletti M. Prevalence of COVID-19-Associated Pulmonary Aspergillosis: Critical Review and Conclusions. *J Fungi*. 2022;8(4):1-9. doi:10.3390/jof8040390
8. Koehler P, Bassetti M, Chakrabarti A, et al. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. *Lancet Infect Dis*. 2021;21(6):e149-e162. doi:10.1016/S1473-3099(20)30847-1
9. Bassetti M, Azoulay E, Kullberg B-J, et al. EORTC/MSGERC Definitions of Invasive Fungal Diseases: Summary of Activities of the Intensive Care Unit Working Group. *Clin Infect Dis*. 2021;72(Supplement_2):S121-S127. doi:10.1093/cid/ciaa1751
10. Feys S, Lagrou K, Lauwers HM, et al. High Burden of COVID-19-Associated Pulmonary Aspergillosis in Severely Immunocompromised Patients Requiring Mechanical Ventilation. *Clin Infect Dis*. 2024;78(2):361-370. doi:10.1093/cid/ciad546
11. Hamed K, Engelhardt M, Kovanda LL, Huang JJ, Yan J, Aram JA. Post-hoc analysis of the safety and efficacy of isavuconazole in older patients with invasive fungal disease from the VITAL and SECURE studies. *Sci Rep*. 2023;13(1):1-11. doi:10.1038/s41598-023-31788-1
12. Clinical and Laboratory Standards Institute. *Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi. CLSI Supplement M61*.; 2020.
13. Clinical and Laboratory Standards Institute. *Epidemiological Cutoff Values for Antifungal Susceptibility Testing. CLSI Supplement M57S*.; 2022.
14. Verweij PE, Rijnders BJA, Brüggemann RJM, et al. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. *Intensive Care Med*. 2020;46(8):1524-1535. doi:10.1007/s00134-020-06091-6
15. Bassetti M, Giacobbe DR, Agvald-Ohman C, et al. Invasive Fungal Diseases in Adult Patients in Intensive Care Unit (FUNDICU): 2024 consensus definitions from ESGCIP, EFISG, ESICM, ECMM, MSGERC, ISAC, and ISHAM. *Intensive Care Med*. 2024;50(4):502-515. doi:10.1007/s00134-024-07341-7
16. Hatzl S, Geiger C, Kriegl L, et al. Performance of Diagnostic Algorithms in Patients With Invasive Pulmonary Aspergillosis. *Clin Infect Dis*. Published online December 20, 2024:1-8. doi:10.1093/cid/ciae633
17. Liu R ting, Chen Y, Li S, et al. A Comparison of Diagnostic Criteria for Invasive Pulmonary Aspergillosis in Critically Ill Patients. *Infect Dis Ther*. 2023;12(6):1641-1653. doi:10.1007/s40121-023-00818-w

18. Feys S, Carvalho A, Clancy CJ, et al. Influenza-associated and COVID-19-associated pulmonary aspergillosis in critically ill patients. *Lancet Respir Med*. 2024;12(9):728-742. doi:10.1016/S2213-2600(24)00151-6
19. Avni T, Levy I, Sprecher H, Yahav D, Leibovici L, Paul M. Diagnostic Accuracy of PCR Alone Compared to Galactomannan in Bronchoalveolar Lavage Fluid for Diagnosis of Invasive Pulmonary Aspergillosis: a Systematic Review. *J Clin Microbiol*. 2012;50(11):3652-3658. doi:10.1128/JCM.00942-12
20. Mikulska M, Furfaro E, Dettori S, et al. Aspergillus-PCR in bronchoalveolar lavage - diagnostic accuracy for invasive pulmonary aspergillosis in critically ill patients. *Mycoses*. 2022;65(4):411-418. doi:10.1111/myc.13428
21. Heng SC, Morrissey O, Chen SCA, et al. Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematology patients: A systematic review and meta-analysis. *Crit Rev Microbiol*. 2015;41(1):124-134. doi:10.3109/1040841X.2013.804033
22. Meersseman W, Lagrou K, Maertens J, et al. Galactomannan in Bronchoalveolar Lavage Fluid. *Am J Respir Crit Care Med*. 2008;177(1):27-34. doi:10.1164/rccm.200704-606OC
23. Verdaguer V, Walsh TJ, Hope W, Cortez KJ. Galactomannan antigen detection in the diagnosis of invasive aspergillosis. *Expert Rev Mol Diagn*. 2007;7(1):21-32. doi:10.1586/14737159.7.1.21
24. Tsotsolis S, Kotoulas SC, Lavrentieva A. Invasive Pulmonary Aspergillosis in Coronavirus Disease 2019 Patients Lights and Shadows in the Current Landscape. *Adv Respir Med*. 2023;91(3):185-202. doi:10.3390/arm91030016
25. Jenks JD, Nam HH, Hoenigl M. Invasive aspergillosis in critically ill patients: Review of definitions and diagnostic approaches. *Mycoses*. 2021;64(9):1002-1014. doi:10.1111/myc.13274
26. Zhang L, Guo Z, Xie S, et al. The performance of galactomannan in combination with 1,3-β-D-glucan or aspergillus-lateral flow device for the diagnosis of invasive aspergillosis: Evidences from 13 studies. *Diagn Microbiol Infect Dis*. 2019;93(1):44-53. doi:10.1016/j.diagmicrobio.2018.08.005
27. Talento AF, Dunne K, Joyce EA, et al. A prospective study of fungal biomarkers to improve management of invasive fungal diseases in a mixed specialty critical care unit. *J Crit Care*. 2017;40:119-127. doi:10.1016/j.jcrc.2017.03.025
28. Bulpa P, Duplaquet F, Dimopoulos G, Vogelaers D, Blot S. Invasive Pulmonary Aspergillosis in Chronic Obstructive Pulmonary Disease Exacerbations. *Semin Respir Crit Care Med*. 2020;41(6):851-861. doi:10.1055/s-0040-1702210
29. Lahmer T, Peçanha-Pietrobom PM, Schmid RM, Colombo AL. Invasive fungal infections in acute and chronic liver impairment: A systematic review. *Mycoses*. 2022;65(2):140-151. doi:10.1111/myc.13403
30. Bassetti M, Bouza E. Invasive mould infections in the ICU setting: Complexities and solutions. *J Antimicrob Chemother*. 2017;72:i39-i47. doi:10.1093/jac/dkx032
31. Cornillet A, Camus C, Nimubona S, et al. Comparison of Epidemiological, Clinical, and Biological Features of Invasive Aspergillosis in Neutropenic and Nonneutropenic Patients: A 6-Year Survey. *Clin Infect Dis*. 2006;43(5):577-584. doi:10.1086/505870
32. Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Wijngaerdert E. Invasive aspergillosis in critically ill patients without malignancy. *Am J Respir Crit Care Med*. 2004;170(6):621-625. doi:10.1164/rccm.200401-093OC

33. Fisher CE, Stevens AM, Leisenring W, Pergam SA, Boeckh M, Hohl TM. The serum galactomannan index predicts mortality in hematopoietic stem cell transplant recipients with invasive aspergillosis. *Clin Infect Dis*. 2013;57(7):1001-1004. doi:10.1093/cid/cit393
34. Van Matre ET, Evans SL, Mueller SW, MacLaren R, Fish DN, Kiser TH. Comparative evaluation of isavuconazonium sulfate, voriconazole, and posaconazole for the management of invasive fungal infections in an academic medical center. *Ann Clin Microbiol Antimicrob*. 2019;18(1):1-7. doi:10.1186/s12941-019-0311-3
35. Maertens JA, Raad II, Marr KA, et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by Aspergillus and other filamentous fungi (SECURE): a phase 3, randomised-controlled, non-inferiority trial. *Lancet*. 2016;387(10020):760-769. doi:10.1016/S0140-6736(15)01159-9
36. Herbrecht R, Kuessner D, Pooley N, Posthumus J, Escrig C. Systematic review and network meta-analysis of clinical outcomes associated with isavuconazole versus relevant comparators for patients with invasive aspergillosis. *Curr Med Res Opin*. 2018;34(12):2187-2195. doi:10.1080/03007995.2018.1502659
37. Höhl R, Bertram R, Kinzig M, et al. Isavuconazole therapeutic drug monitoring in critically ill ICU patients: A monocentric retrospective analysis. *Mycoses*. 2022;65(7):747-752. doi:10.1111/myc.13469

Table 1. Comparison of demographic and baseline characteristics, treatment and outcomes between CAPA and PIPA

		Total n=209	CAPA, n= 93 (44.5)	PIPA, n = 116 (55.5)	p-value
Age		67.3 ±12.3(23-94)	67.4 ±10.4(34-91)	67.2 ±13.6(23-94)	0.910
Gender, M		137 (65.5)	70 (75.3)	67 (57.8)	**0.008**
Ward of diagnosis	ICU	144 (68.9)	83 (89.3)	61 (52.6)	**<0.001**
	Medical	64 (30.6)	10 (10.8)	54 (46.6)	**<0.001**
	Surgical	1 (0.5)	0 (0)	1 (0.8)	0.369
Year of diagnosis	2018	15 (7.2)	0 (0)	15(12.9)	**0.001**
	2019	20 (9.6)	0 (0)	20 (17.2)	**<0.001**
	2020	46 (22.0)	32 (34.4)	14 (12.1)	**<0.001**
	2021	81 (38.8)	46 (49.5)	35 (30.2)	**0.004**
	2022	47 (22.5)	15 (16.1)	32 (27.6)	**0.049**
Time from admission to IPA, mean ±SD (range) days		20.7 ±18.9(1-129)	21.7 ±18.2(2-95)	19.8 ±19.6(1-129)	0.468
Comorbidities					
CCI, mean ±SD (range)		5 ±2.62 (0-14)	3.9 ±2.3 (0-10)	5.8 ±2.6 (0-14)	**<0.001**
Heart disease		58/203 (28.6)	26 (29.2)	32 (28.1)	0.858
Hypertension		127/203 (62.6)	58 (65.9)	69 (60)	0.389
Vasculopathy		43/203 (21.2)	17 (19.1)	26 (22.8)	0.521
Neurologic disease		25/203 (12.3)	8 (8.9)	17 (14.9)	0.203
Diabetes		54/203 (26.6)	27 (30.3)	27 (23.7)	0.287
Dyslipidemia		57/203 (28.1)	28 (31.5)	29 (25.4)	0.343
Renal failure		27/203 (13.3)	12 (13.3)	15 (13.3)	0.990
COPD		47/203 (23.2)	12 (13.5)	35 (30.7)	**0.004**
Other pneumopathy		32/203 (15.7)	10 (11.1)	22 (19.3)	0.110
Previous tuberculosis		6/203 (3)	1 (1.1)	5 (4.4)	0.173
Solid cancer		55/203 (27.1)	13 (14.6)	42 (36.8)	**<0.001**
HIV		2/203 (1)	0 (0)	2 (1.8)	0.209

Solid organ transplant		18/203 (8.9)	4 (4.5)	14 (12.3)	0.053
Liver cirrhosis		15/203 (7.4)	1 (1.1)	14 (12.3)	**0.002**
Other liver disease		19/203 (9.4)	6 (6.7)	13 (11.4)	0.258
Autoimmune disease		22/203 (10.8)	8 (8.9)	14 (12.3)	0.454
Active smoker		33/200 (16.5)	12 (13.5)	21 (18.9)	0.447
Concomitant Influenza		3/209 (1.44)	1 (1.1)	2 (1.7)	0.695
Extrinsic factors					
IMV		103/204 (50.5)	62 (70.5)	41 (35.3)	**<0.001**
Steroids		143/207 (69.1)	82 (90.1)	61 (52.6)	**<0.001**
Steroids duration >3 weeks		59/207 (28.5)	25 (29.4)	35 (31.5)	0.750
Tocilizumab		54/203 (26.6)	53 (62.3)	1 (0.8)	**<0.001**
Other Immunosuppressive therapies		39/204 (19.1)	9 (10.1)	30 (26.1)	**0.004**
Active Chemotherapy		18/203 (8.9)	6 (6.8)	12 (10.4)	0.369
Concomitant antibiotic therapy		173/199 (86.9)	78 (90.7)	95 (84.1)	0.169
Co-infection on respiratory sample					
CMV		48/198 (24.2)	22 (25.6)	26 (23.2)	0.700
P. jirovecii		27/198 (13.6)	1 (1.2)	26 (23.0)	**<0.001**
P. aeruginosa		39/200 (19.5)	21 (24.4)	18 (15.8)	0.127
Clinical presentation					
SOFA, mean ±SD (range)		4.9 ±3.5 (0-17)	4.9 ±3.1 (1-17)	4.9 ±3.9 (0-17)	0.893
Septic shock		33/196 (16.8)	14 (16.9)	19 (16.8)	0.992
Hemoptysis		7 (3.4)	1 (1.1)	6 (5.2)	0.102
Worsening respiratory insufficiency		168 (80.4)	80 (86.0)	88(75.9)	0.066
Dispnoea		144 (68.9)	62 (66.7)	82 (70.7)	0.532
Persistent fever		19 (9.1)	6 (6.5)	13 (11.2)	0.235
Recurrent fever		18 (8.6)	3 (3.2)	15 (12.9)	**0.013**
Chest pain		3 (1.4)	2 (2.2)	1 (0.9)	0.436
Pleural friction rub		1 (0.5)	1 (1.1)	0 (0)	0.263
Tracheobronchial pseudomembranes/ulcerations		3 (1.4)	2 (2.2)	1 (0.9)	0.436
CT-scan		193/209 (92.3)	81/93 (87.1)	112/116 (96.6)	
Cavitation		28 (14.5)	11 (13.6)	17 (15.2)	0.756
Ground glass		119 (61.7)	65 (80.3)	54 (48.2)	**<0.001**
Consolidation		161 (83.4)	74 (91.4)	87 (77.7)	**0.012**
Nodules		62 (32.1)	21 (25.9)	41 (36.6)	0.117
Tree in bud		25 (12.9)	2 (2.5)	23 (20.5)	**<0.001**
Halo sign		6 (3.1)	3 (3.7)	3 (2.7)	0.686
Air crescent sign		0 (0)	0 (0)	0 (0)	.
Microbiology					
Culture positive on respiratory sample (at least one)		99/209 (47.4)	43 (46.2)	56 (48.3)	0.769
Microscopy on BAL		15/185 (8.1)	5 (6.2)	10 (9.6)	0.395
BAL GM	Positive BAL GM (>1.0)	126/173 (72.8)	62 (80.5)	64 (66.7)	**0.042**
	BAL GM mean value	4.57 ±2.16 (1.04-16.8)	4.76 ±2.41 (1.08-16.8)	4.39 ±1.87 (1.04-7.29)	0.341
PCR positive on respiratory sample (at least one)		110/152 (72.4)	41 (68.3)	69 (75.0)	0.369
PCR on BAL	Positive BAL PCR	93/131 (71)	36 (70.6)	57 (71.3)	0.935
	Mean value BAL PCR	97235.4 ±324942.4 (132-1645414)	139579.1 ±417435.7(134-1645414)	66100.3 ±237680.3 (132-1352255)	0.395
PCR on BAS	Positive BAS PCR	18/24 (75)	6 (60.0)	12 (85.7)	0.151

	Mean value BAS PCR	286690.1 ±743974 (128-2143634)	358896 ±874340.8 (154-2143634)	238552.9 ±695922.6 (128-2094166)	0.771
Serum GM	Positive serum GM (>0.5)	41/189 (21.7)	19 (21.4)	22 (22)	0.914
	Serum GM mean value	2.14 ±2.13 (0.51-10.5)	2.82 ±2.58(0.51-10.50)	1.53 ±1.42 (0.53-5.85)	**0.056**
BDG	Positive BDG (>80.0)	93/188 (49.5)	33 (36.7)	60 (61.2)	**0.001**
	Mean value BDG	423.8 ±355.4 (84.3-1802.9)	308.7 ±225.1 (86-787)	487.2 ±397.4 (84.3-1802.9)	**0.019**
Histology		3/13 (23.1)	0 (0)	3 (42.9)	0.067
Therapy					
First line, also with switch	Voriconazole	160 (76.6)	81 (87.1)	79 (68.1)	**0.001**
	Isavuconazole	31 (14.8)	12 (12.9)	19 (16.4)	0.482
	L-Amb	18 (8.6)	0 (0)	18 (15.5)	**<0.001**
Total days of therapy, mean ±SD (range)		34.5 ±43.9(4-413)	32.4 ±49.7 (4-413)	36.3 ±38.5 (4-264)	0.533
Outcomes					
LOS, mean ±SD (range) days		55.6 ±45.6(8-276)	62.7 ±52.1 (8-276)	49.9 ±38.6 (8-189)	**0.044**
Length ICU stay, mean ±SD (range) days		31.1 ±29.5 (1-168)	36 ±30.7(2-168)	24.8 ±26.8 (1-138)	**0.034**
Clinical cure		95/204 (46.6)	45/91 (49.5)	50 (44.3)	0.459
Relapse		5/204 (2.5)	2 (2.2)	3 (2.7)	0.834
30-day mortality		79/209 (37.8)	35 (37.6)	44 (37.9)	0.965
90-day mortality		105/209 (50.2)	42 (45.2)	63 (54.3)	0.189

Abbreviations: M, male; ICU, Intensive Care Unit; LOS, Length of hospital Stay; CCI, Charslon Comorbidities Index; COPD, Chronic Obstructive Pulmonary Disease; IMV, Invasive Mechanical Ventilation; SOFA, Sequential Organ Failure Assessment; GM, galactomannan; BDG, beta-D-glucan; BAS, bronchoaspirate; BAL, bronchoalveolar lavage

Table 2. Comparison of characteristics of patients who experienced clinical failure and 30-day mortality or not

		Clinical failure			30-day mortality		
		Yes (109/209, 53.4%)	**No (95/209, 46.6%)**	**p-value**	**Yes (79/209, 37.8%)**	**No (130/209, 62.2%)**	**p-value**
Age		68.6 ±10.4(41-90)	66.0 ±13.9 (23-94)	0.132	68.8 ±10.0(41-90)	66.8 ±13.4 (23-94)	0.167
Gender, M		77 (70.6)	56 (58.9)	0.080	56 (70.9)	81 (62.3)	0.206
Ward of diagnosis	ICU	77 (70.6)	64 (67.4)	0.614	59 (74.7)	85 (65.4)	0.159
	Medical	32 (29.4)	31 (32.6)	0.614	20 (25.3)	44 (33.9)	0.195
	Surgical	0 (0)	0 (0)	.	0 (0)	1 (0.8)	0.435
Year of diagnosis	2018	6 (5.5)	7 (7.4)	0.383	5 (6.3)	10 (7.7)	0.346
	2019	16 (14.7)	4 (4.2)	**0.012**	12 (15.2)	8 (6.2)	**0.031**
	2020	25 (22.9)	19 (20.0)	0.611	20 (25.3)	26 (20.0)	0.368
	2021	36 (33.0)	44 (46.3)	**0.052**	23 (29.1)	58 (44.6)	**0.026**
	2022	26 (23.8)	21 (22.1)	0.767	19 (24.1)	28 (21.5)	0.673

Time from admission to IPA, mean ±SD (range) days	19.7 ±16.7 (1-198)	21.8 ±21.6(1-129)	0.453	19.9 ±17.5 (1-98)	21.1 ±19.8 (1-129)	0.649
Comorbidities						
CCI, mean ±SD (range)	5.6 ±0.3(0-14)	4.4 ±0.2 0-11)	**<0.001**	5.7 ±2.8 (0-14)	4.6 ±2.4 (0-11)	**0.003**
Heart disease	30 (28.9)	27 (28.4)	0.947	21 (28)	37 (28.9)	0.890
Hypertension	69 (65.7)	56 (59.6)	0.371	47 (61.8)	80 (62.9)	0.870
Vasculopathy	21 (20.2)	21 (22.1)	0.741	16 (21.3)	27 (21.1)	0.968
Neurologic disease	16 (15.4)	9 (9.5)	0.209	9 (12)	16 (12.5)	0.917
Diabetes	35 (33.7)	18 (18.9)	**0.019**	28 (37.3)	26 (20.3)	**0.008**
Dyslipidemia	30 (28.9)	27 (28.4)	0.947	25 (33.3)	32 (25.0)	0.202
Renal failure	12 (11.5)	15 (15.8)	0.382	10 (13.3)	17 (13.3)	0.992
COPD	24 (23.1)	22 (23.2)	0.989	15 (20)	32 (25.0)	0.415
Other pneumopathy	16 (15.2)	14 (14.7)	0.921	13 (17.1)	19 (14.8)	0.668
Previous tuberculosis	2 (1.9)	4 (4.2)	0.346	1 (1.3)	5 (3.9)	0.296
Solid cancer	31 (29.8)	22 (23.2)	0.289	24 (32)	31 (24.2)	0.229
HIV	1 (0.9)	1 (1.1)	0.949	1 (1.3)	1 (0.8)	0.701
Solid organ transplant	8 (7.7)	10 (10.5)	0.486	5 (6.7)	13 (10.2)	0.399
Liver cirrhosis	10 (9.5)	4 (4.2)	0.141	9 (11.8)	6 (4.7)	0.058
Other liver disease	11 (10.6)	8 (8.4)	0.605	8 (10.7)	11 (8.6)	0.625
Autoimmune disease	11 (10.6)	10 (10.5)	0.991	8 (10.7)	14 (10.9)	0.952
Active smoker	18 (17.7)	13 (13.8)	0.417	10 (13.7)	23 (18.1)	0.692
Concomitant COVID-19	46 (42.2)	45 (47.4)	0.459	35 (44.3)	58 (44.6)	0.965
Concomitant Influenza	1 (0.9)	2 (2.1)	0.482	1 (1.3)	2 (1.5)	0.872
Extrinsic factors						
IMV	60 (57.7)	42 (44.2)	**0.057**	46 (61.3)	57 (44.2)	**0.018**
Steroids	71 (66.4)	70 (73.7)	0.257	53 (68.8)	90 (69.2)	0.952
Steroids duration >3 weeks	28 (28.3)	32 (34.4)	0.360	19 (27.1)	41 (32.8)	0.378
Other Immunosuppressive therapies	18 (17.3)	21 (22.1)	0.394	14 (18.7)	25 (19.4)	0.901
Tocilizumab	22 (21.6)	32 (34.0)	0.051	36 (28.8)	18 (23.7)	0.428
Active Chemotherapy	11 (10.7)	7 (7.4)	0.418	6 (8.0)	12 (9.4)	0.739
Concomitant antibiotic therapy	93 (88.6)	76 (84.4)	0.398	67 (88.2)	106 (86.2)	0.687
Co-infection on respiratory sample						
CMV	25 (24.0)	22 (24.2)	0.982	20 (26.7)	28 (22.8)	0.534
P. jirovecii	15 (14.4)	12 (13.3)	0.827	13 (17.3)	14 (11.4)	0.237
P. aeruginosa	23 (21.7)	14 (15.6)	0.273	16 (20.8)	23 (18.7)	0.718
Clinical presentation						
SOFA, mean ±SD (range)	5.8 ±4.2 (0-17)	4.2 ±2.4 (0.11)	**0.022**	6.1 ±4.1 (1-17)	4.3 ±2.9(0-15)	**0.009**
Septic shock	18 (18.6)	15 (15.9)	0.635	10 (14.3)	23 (18.3)	0.477
Hemoptysis	3 (2.8)	2 (2.1)	0.766	2 (2.5)	5 (3.9)	0.609
Worsening respiratory insufficiency	90 (82.6)	75 (78.9)	0.512	68 (86.1)	100 (76.9)	0.106
Dispnoea	77 (70.6)	63 (66.3)	0.506	56 (70.9)	88 (67.7)	0.629
Persistent fever	7 (6.4)	12 (12.6)	0.128	7 (8.9)	12 (9.2)	0.928
Recurrent fever	8 (7.3)	10 (10.5)	0.423	4 (5.1)	14 (10.8)	0.154
Chest pain	0 (0)	3 (3.2)	0.062	0 (0)	3 (2.3)	0.174
Pleural friction rub	0 (0)	1 (1.1)	0.283	0 (0)	1 (0.8)	0.435
Tracheobronchial pseudomembranes/ulcerations	1 (0.9)	2 (2.1)	0.482	1 (1.3)	2 (1.55)	0.872
CT-scan						
Cavitation	13 (13.4)	15 (16.5)	0.553	9 (13.2)	19 (15.2)	0.711
Ground glass	60 (61.9)	57 (62.6)	0.912	42 (61.8)	77 (61.6)	0.982

Consolidation		78 (80.4)	79 (86.8)	0.237	55 (80.9)	106 (84.8)	0.485
Nodules		31 (31.9)	31 (34.1)	0.759	20 (29.4)	42 (33.6)	0.552
Tree in bud		15 (15.5)	10 (10.9)	0.367	7 (10.3)	18 (14.4)	0.417
Halo sign		3 (3.1)	3 (3.3)	0.937	3 (4.4)	3 (2.4)	0.442
Air crescent sign		0 (0)	0 (0)	.	0 (0)	0 (0)	.
Microbiology							
Culture positive on respiratory sample (at least one)		48/109 (44.0)	47/95 (49.5)	0.437	39/77 (50.6)	59/130 (45.4)	0.461
Microscopy on BAL		5/96 (5.2)	8/84 (9.5)	0.264	4/69 (5.8)	11/115 (9.5)	0.374
BAL-GM	Positive BAL-GM (>1.0)	65/91 (71.4)	59/78 (75.6)	0.537	44/65 (67.7)	82/108 (75.9)	0.238
	BAL-GM mean value	4.28 ±1.77(1.04-7.29)	4.85 ±2.50(1.12-16.8)	0.145	4.2 ±1.6(1.0-6.8)	3.5 ±2,6 (1,1-7,3)	0.177
Positive PCR on respiratory sample (at least one)		51/74 (68.9)	58/77 (75.3)	0.453	32/48 (66.7)	78/104 (75.0)	0.286
PCR on BAL	Positive PCR on BAL	43/63 (68.2)	49/66 (74.2)	0.452	29/44 (65.9)	64/87 (73.6)	0.362
	Mean value PCR on BAL	86103.8 ±282715.3(132-1387200)	105420 ±356771.4(134-1645414)	0.823	117116.3 ±330424.2(262-1387200)	88507 ±326258.3 (132-1645414)	0.759
PCR on BAS	Positive PCR on BAS	9/12 (75.0)	9/12 (75.0)	1.000	6/7 (85.7)	12/17 (70.6)	0.437
	Mean value PCR on BAS	534177.6 ±978248.1(128-2143634)	3847.3 ±5419(237-15235)	0.177	848154.4 ±1160159 (128-2143634)	5958 ±9255.9 (237-28971)	**0.033**
Serum-GM	Positive serum-GM (>0.5)	26/99 (26.3)	14/87 (16.1)	0.092	22/71 (31)	19/118 (16.1)	**0.016**
	Serum-GM mean value	2.02 ±1.78(0.56-5.85)	2.37 ±2.72 (0.51-10.5)	0.631	2.2 ±1.8(0.6-5.8)	2.0 ±2.4(0.5-10.5)	0.749
BDG	Positive BDG (>80.0)	59/99 (59.6)	33/87 (37.9)	**0.003**	42/72 (58.3)	51/116 (43.9)	0.055
	Mean value BDG	405.4 ±325.9 (84.3-1623)	466.7 ±405.8 (95.6-1802.9)	0.431	423.6 ±344.7(96.5-1623)	424.0 ±367.2(84.3-1802.9)	0.995
Histology		3/8 (37.5)	0/0 (0.0)	0.118	1/4 (25)	2/9 (22.2)	0.913
Diagnosis							
CAPA		46 (42.2)	45 (47.4)	0.459	35 (44.3)	58 (44.6)	0.965
PIPA		63 (57.8)	50 (52.6)	0.459	44 (55.7)	72 (55.4)	0.965
Therapy							
First line	Voriconazole	89 (81.7)	67 (70.5)	0.062	64 (81)	96 (73.9)	0.236
	Isavuconazole	11 (10.1)	20 (21.1)	**0.030**	9 (11.4)	22 (16.9)	0.275
	L-Amb	9 (8.3)	8 (8.4)	0.966	6 (7.6)	12 (9.2)	0.683
Voriconazole (only treatment, without switch)		80 (73.4)	57 (60.0)	**0.042**	57 (72.2)	83 (63.8)	0.216
Isavuconazole (only treatment, without switch)		9 (8.3)	19 (20.0)	**0.015**	8 (10.1)	20 (15.4)	0.279
Total days of therapy		19.7 ±18.3 (4-114)	51.0 ±56.9(4-413)	<0.001	11.5 ±5.8 (4-28)	48.4±50.6 (4-413)	**<0.001**
Outcomes							
LOS, mean ±SD (range) days		40.9 ±27.9 (8-172)	74.0 ±55.5(11-276)	**<0.001**	31.8 ±18.8 (8-107)	70.6 ±50.7 (8-276)	**<0.001**

Length ICU stay, mean ±SD (range) days	27.3 ±25.9 (1-68)	35.4 ±32.7 (1-138)	0.125	20.9 ±13.9 (1-70)	38.2 ±34.9 (1-168)	**0.001**

Abbreviations: M, male; ICU, Intensive Care Unit; LOS, Length of hospital Stay; CCI, Charslon Comorbidities Index; COPD, Chronic Obstructive Pulmonary Disease; IMV, Invasive Mechanical Ventilation; SOFA, Sequential Organ Failure Assessment; GM, galactomannan; BDG, beta-D-glucan; BAS, bronchoaspirate; BAL, bronchoalveolar lavage

Table 3. Unadjusted and adjusted Odds Ratio of risk factors for clinical failure

	Unadjusted OR (95% CI)	p-value	Adjusted* OR (95% CI)	p-value
Age	1.01 (0.97-1.03)	0.650	1.01 (0.98-1.04)	0.531
CCI	1.30 (1.13-1.49)	**<0.001**	0.98 (0.45-2.19)	0.979
IMV	2.21 (1.15-4.25)	**0.018**	2.24 (1.16-4.31)	**0.016**
Isavuconazole (only treatment, without switch)	0.41 (0.16-1.02)	**0.058**	0.40 (0.16-1.03)	**0.059**

*OR adjusted for: CCI and ICU admission

Abbreviations: CCI, Charslon Comorbidities Index; IMV, Invasive Mechanical Ventilation; ICU, intensive care unit

Table 4. Multivariate analysis of risk factors for 30-day mortality with unadjusted and adjusted Hazard Ratio

	Unadjusted HR (95% CI)	p-value	Adjusted HR* (95%CI)	p-value
Age	1.02 (0.99-1.04)	0.196	1.02 (0.99-1.08)	0.194
Gender, M	1.46 (0.81-2.61)	0.200	1.51 (0.84-2.70)	0.171
LOS	0.95 (0.93-0.97)	**<0.001**	0.95 (0.93-0.97)	**<0.001**
CCI	1.04 (0.93-1.17)	0.466	1.52 (0.50-4.64)	0.458
IMV	3.96 (2.24-6.98)	**<0.001**	22.24 (0.14-3581.20)	0.232
Liver cirrhosis	1.93 (0.78-4.78)	0.156	1.86 (0.74-4.65)	0.184
Positive serum-GM	1.76 (1.01-3.07)	**0.046**	1.78 (1.02-3.10)	**0.042**

*Adjusted for: age, IMV, CCI

Abbreviations: M, male; LOS, length of hospital stay; CCI, Charslon Comorbidities Index; IMV, Invasive Mechanical Ventilation; GM, galactomannan

Figure 1. Kaplan-Meier survival curves after 30 days from the start of therapy of CAPA vs PIPA (1a) and treatment with isavuconazole vs voriconazole (for patients who did not switch to a second line regimen) (1b)

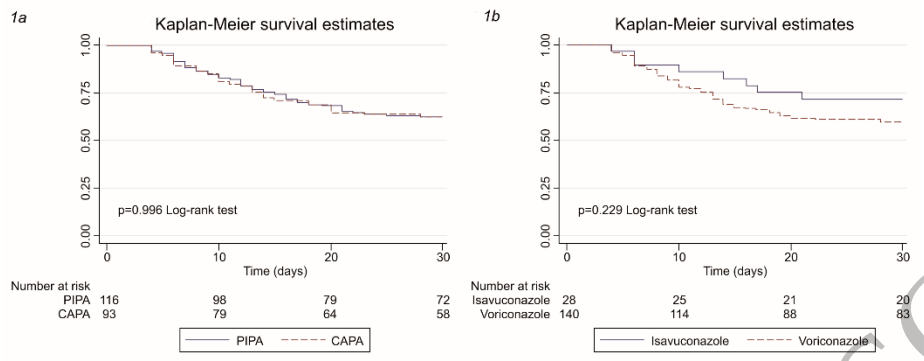

Figure 2. Kaplan-Meier curve of occurrence of clinical failure between patients treated only with voriconazole or isavuconazole (without switching to a second line regimen)