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Is neutropenia still the main risk factor for invasive aspergillosis? A contemporary university hospital retrospective cohort of invasive aspergillosis in neutropenic and non-neutropenic patients

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Abstract

Introduction In times of mold active prophylaxis, invasive aspergillosis (IA) epidemiology is evolving. Presentation in non-neutropenic may differ from neutropenic.

We investigated the cases of IA in our center with a focus on differences between neutropenic and non-neutropenic, and analyzed the impact of cryptic and non-fumigatus *Aspergillus* species.

Methods Retrospective observational study including all adult patients admitted to the Puerta de Hierro-Majadahonda Hospital between January 2018 and April 2024 with IA.

Results 112 IA were identified. Only 11 (9.8%) had neutropenia as risk factor for IA. Most frequent risk factors were corticosteroids (77.2%), SOT (46.5%), SARS-CoV2 (29.7%) and CMV replication (28.7%). 89.3% were pulmonary IA with 6 cases (5.4%) of disseminated infection. *A. fumigatus* was the most frequent species 48 (51.6%). 13 cases (14%) were caused by cryptic *Aspergillus* spp. Non-neutropenic patients, compared to neutropenic patients, were more likely to have positive fungal cultures (83.2% versus 54.5%, p = 0.023[NS]), and not to present a halo sign (7.4% versus 45.5%, p = 0.003 [NS]). In addition, in non-neutropenic patients, compared to neutropenic patients, there was a trend towards a greater probability of positive GM from BAL (81.3% versus 66.7%, p = 0.304) and a trend towards a lower probability of positive serum GM (25.7% versus 45.5%, p = 0.137). 41/112 (36.6%) cases presented breakthrough IFI and in 51.2%, (21/41 cases), the isolate was resistant to the prior antifungal. One presented *A. fumigatus* with the TR34-L98H mutation.

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Conclusion Risk factors different than neutropenia are currently the most common in IA. The clinical presentation in non-neutropenic patients differs from neutropenic. Resistance to antifungals is emerging especially in breakthrough IA.

Keywords Invasive aspergillosis, Non-neutropenics, Antifungal resistance

Introduction

Despite the advances in diagnosis and treatment, invasive aspergillosis (IA) continues to convey a high morbidity and mortality in immunocompromised patients [1–4]. The severity of the condition depends on the patient's immune status [3]. Classically, neutropenia has been considered the most important risk factor associated to IA. However, in recent years IA is increasingly described in non-neutropenic patients without the typical risk factors [1, 4]. Not only solid organ transplantation recipients (especially lung transplantation), or patients taking prolonged corticosteroid therapy, but new hosts such as those receiving targeted therapies like tyrosine kinase inhibitors, or suffering from respiratory virus infections (mainly SARS-CoV-2 or influenza), have been associated with a higher risk of developing IA [1, 5].

In non-neutropenic patients, the diagnosis of IA is hampered by non-specific symptoms, the difficulty of differentiating between colonization and infection, and the uncertainty about the yield of diagnostic tests in this population [6–8]. Several studies found a higher mortality due to IA in non-neutropenic patients compared to neutropenic patients, related to the delay in the diagnosis in the former [6, 9].

In spite of *A. fumigatus* being the most frequently isolated microorganism in IA, non-fumigatus *Aspergillus* species as well as cryptic species are emerging, and, due to their increased resistance to antifungals, should be considered relevant [2, 10]. Likewise, the rise in the number of patients exposed to the azoles has contributed to azole resistance with a consequent increase in mortality due to the difficulty of administering an appropriate antifungal [5].

In view of this paradigm shift, we intended to investigate the cases of invasive aspergillosis in our center with a focus on differences between neutropenic and non-neutropenic hosts, and to analyze the impact of emerging cryptic and non-fumigatus Aspergillus species.

Patients and methods

Design, study period and subjects

Our institution is a 613-bed tertiary-care university hospital in Madrid, Spain. The hospital has a Hematology department with an active hematopoietic stem cell transplantation (HSCT) program, which includes allogeneic SCT (including haploidentical and cord transplantation) and CAR-T therapy, Medical Oncology and Radiotherapy departments and a solid organ transplantation (SOT) program (liver, kidney, heart and lung), in addition to several medical and surgical Intensive Care Units (ICU).

We carried out a retrospective observational study including all adult patients admitted to the Puerta de Hierro-Majadahonda Hospital between January 2018 and April 2024 diagnosed with IA according to the criteria of the European Organization for Research and Treatment of Cancer (EORTC) and Mycosis Study Group (MSC) [11], the European Confederation of Medical Mycology and International Society for Human & Animal Mycology (ECMM/ISHAM) [12] or the BULPA and ICU criteria [13], depending on the characteristics of the host.

Clinical antifungal susceptibility profiles were determined using the EUCAST E.Def version 9.4, 2022.

Data collection

The infectious diseases team at our center evaluates patients admitted to different medical and surgical units presenting with infectious diseases to provide support and optimize their management. patients with a diagnosis of IA were selected through these consultations. An additional way to detect patients with a diagnosis of IA was with the collaboration of the Pharmacy Department, that in the context of antifungal stewardship program usually surveys prescriptions for restricted drugs (Amphotericin B, Isavuconazole or Voriconazole), that are subsequently reviewed by infectious disease specialists.

Epidemiological, clinical (including risk factors for aspergillosis, immunosuppressants and targeted therapies, aspergillosis site), microbiological (including *Aspergillus* species, antifungal MICs, antifungal treatment), and imaging data were extracted from electronic medical records (System SELENE, Cerner Iberia, S.L.U., Madrid, Spain) using a standardized data collection form. All data were included by a group of clinicians and subsequently reviewed and verified by two experienced physicians.

Definitions

IFI was considered **proven, probable/putative or possible** according to the revised EORTC/MSG classification, BULPA criteria or ICU criteria.

We considered **neutropenia** the determination of < 500 neutrophils/mm3 at the time of diagnosis of aspergillosis or during the previous month, and **lymphopenia**, the

determination of < 1000 lymphocytes/mm3 at the time of diagnosis or in the previous month.

Steroid exposure has been described as equivalent to receipt of prednisone 20 mg daily for 4 weeks or higher.

Regarding **CMV infection**, the cut-off point was detection of > 35 IU/mL in plasma.

During the study period, there were changes in the methods used for the determination of fungal biomarkers, which motivated changes in the respective cut-off thresholds. Regarding galactomannan (GM) cut-off level in serum, the test used by our laboratory until November 2019 was Platelia™ (Bio-Rad, Hercules, California, USA), with a cut-off point of 0.5 ng/ml. Since then, it was changed to the Virclia monotest® (Vircell, Granada, España) test with a cut-off point of 0.2 µg/mL. Similarly, for GM cut-off level in bronchoalveolar lavage (BAL) the Platelia™ cut-off point was 1 ng/ml, and since then on, due to the change to Virclia monotest® Vircell test, the cut-off point of GM in BAL was moved to 0.5 µg/mL.

Regarding β-D-glucan, the test used until February 2020 was Fungitell™, with a cut-off point of 80 pg/ml. Since then, the Fujifilm Wako™ BDG assay has been used with the manufacturer´s recommended cut-off point of 11 pg/ml.

Antifungal prophylaxis is summarized in Supplementary Material 1.

Data analysis

Quantitative variables were expressed as means and standard deviations (SD) and/or medians and interquartile ranges (IQR), and qualitative variables were expressed as frequencies and proportions. Characteristics of patients with and without neutropenia were compared by means of Chi square test for categorical variables (or Fisher exact test when necessary) and Student t-test (or Mann–Whitney's U when necessary) for quantitative variables. The patient characteristics Tables 1, 6 only show p-values for variables that are clinically significant (potentially clinically associated to IA). The remaining variables presented in the table are considered descriptive, and, as such, a p-value is not provided (in order not to increment type I error due to the high number of variables included). The Bonferroni correction has been applied to the variables with a p-value, the recalculated significance level in this study being 0.001, both for the analysis of the general characteristics and the analysis of the subset of hematological patients. All statistical analyses were performed with SPSS version 25 software (SPSS INC., Chicago, Illinois, USA).

Ethics

The study was approved by the Institutional Review Board (CEIm) at Hospital Universitario Puerta de Hierro (Majadahonda) (PI 156/24), and a waiver for informed consent was granted. The study complied with the provisions in European Union (EU) and Spanish legislation on data protection and the Declaration of Helsinki.

Results

During the study period, 112 patients with proven or probable/putative IA were identified.

Characteristics of patients with IA

The detailed characteristics of patients with IA are available in Table 1.

Only 11 patients (9.8%) were neutropenic at the time of IA diagnosis or in the previous month. Prevalence of IA among neutropenic and non-neutropenic patients evolved along time (Fig. 1), with a sharp increase in non-neutropenic cases during COVID first waves, and a general predominance of non-neutropenic hosts in the whole series, with a trend to a decrease in neutropenic cases.

Main underlying conditions were previous corticosteroid use (71.4%), chronic lung disease (42.9%), lung transplantation (31.3%), SARS-CoV2 infection (26.8%), CMV infection (25.9%), chronic kidney disease 21.4%, hematological diseases (19.6%) and oncological disease 9 (8%). All patients with CMV reactivation had, in addition, another underlying disease (17 COVID, 3 HSCT, 10 SOT, 5 hematological malignancies, 1 HIV AIDS stage with IA at diagnosis and one with severe exacerbated pneumoconiosis with admission to the intensive care unit).

The majority of IA cases 88 (78.6%) were considered probable/putative. The most commonly involved organ was the lung in 100 (89.3%) patients. Six cases (5.4%) presented disseminated infection.

28 patients (25%) had a ^{18}F-FDG-PET-TC performed in addition to conventional imaging (53.6% staging, 14.3% monitoring and 32.1% due to persistent fever).

Serum galactomannan (GM) was performed in 81 patients, with a 28.4% positivity rate. 78 patients (71.4%) underwent bronchoscopy. Among 41 GM tests performed in BAL, 32 (78%) were positive.

In 90 (80.4%) cases a positive *Aspergillus* culture was available. The most frequent species was *A. fumigatus* sensu stricto (53 [56.9%]). Other species were: *A. terreus* (7 [7.5%]), *A. flavus* (6 [6.5%]), and *A. niger* (4 [4.3%]). As many as 13/93 (14%) isolates were identified as cryptic species, including among Fumigati: *Neosartorya udagawae* (1), *A. lentulus* (8), *A. fumigatiaffinis* (1); among Terrei: *A. hortai* (2); among Nidulans: *A. spinulosporus* (1). Evolution over the years of cryptic species IA is shown in Fig. 2.

The distribution of resistance is summarized in Tables 2, 3. Resistance to Voriconazole and Isavuconazole was found only in 2 (3.8%) *A. fumigatus* sensu stricto

Table 1 Characteristics of patients with IA

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (Significance level p < 0.001)
Number of patients	112	101 (90.2%)	11 (9.8%)	
Demographic data				
Sex:				
Male	78 (69.6%)	71/101 (70.3%)	7/11 (63.6%)	
Female	34 (30.4%)	30/101 (29.7%)	4/11 (36.4%)	
Age (median [IQR])		64 (58–70.5)	68 (58–75)	
Hematologic malignancy	22 (19.6%)	12/101 (11.9%)	11/11 (100%)	**0.0001**
HSCT	7 (6.3%)	5/101 (4.9%)	2/11 (18.2%)	0.140
Chronic lung disease	48 (42.9%)	46/101 (45.5%)	2/11 (18.2%)	0.112
COPD	11 (9.8%)	10/101 (9.9%)	1/11 (9.1%)	0.999
Chronic kidney disease	24 (21.4%)	23/101 (22.8%)	1/11 (9.1%)	
Dialysis	5 (4.5%)	5/101 (4.9%)	0 (0%)	
Liver disease	6 (5.4%)	6/101 (5.9%)	0 (0%)	
Cirrhosis	3 (2.7%)	2/101 (1.9%)	1/11 (9.1%)	
Heart disease	16 (14.3%)	16/101 (15.8%)	0 (0%)	
Gastrointestinal disease	7 (6.3%)	6/101 (5.9%)	1/11 (9.1%)	
Neurologic disease	7 (6.3%)	6/101 (5.9%)	1/11 (9.1%)	
Post-surgical	1 (0.9%)	0 (0%)	1/11 (9.1%)	
Other	14 (12.5%)	12/101 (11.9%)	2/11 (18.2%)	
Solid cancer	9 (8%)	8/101 (7.9%)	1/11 (9.1%)	0.999
Lung cancer	6 (5.4%)	5/101 (4.9%)	1/11 (9.1%)	
Lung methastasis	2 (1.8%)	2/101 (1.9%)	0 (0%)	
Pulmonary radiotherapy	4 (3.6%)	4/101 (3.9%)	0 (0%)	
Immunotherapy	3 (2.7%)	3/101 (2.9%)	0 (0%)	
SARS-CoV2 (last month)	30 (26.8%)	30/101 (29.7%)	0 (0%)	0.035
Influenza (last month)	4 (3.6%)	4/101 (3.9%)	0 (0%)	0.999
CMV reactivation	29 (25.9%)	29/101 (28.7%)	0 (0%)	0.037
Lymphopenia (< 1000)	49 (43.8%)	42/101 (41.6%)	7/11 (63.6%)	0.206
Chemotherapy	15 (13.4%)	6/101 (5.9%)	9/11 (81.8%)	**0.0001**
Previous steroids	80 (71.4%)	78/101 (77.2%)	2/11 (18.2%)	**0.0001**
SOT	48 (42.9%)	47/101 (46.5%)	1(2.1%)	0.023
Liver transplant	3 (2.7%)	2/101 (1.9%)	1/11 (9.1%)	
Kidney transplant	8 (7.1%)	7/101 (6.9%)	1/11 (9.1%)	
Heart transplant	3 (2.7%)	3/101 (2.9%)	0 (0%)	
Lung transplant	35 (31.3%)	35/101 (34.7%)	0 (0%)	0.016
Immunosuppressants	74 (67%)	65/101 (64.4%)	10/11 (90.9%)	0.097
Tacrolimus	47 (42%)	44/101 (43.6%)	3/11 (27.3%)	
Cyclosporin	4 (3.6%)	4/101 (3.9%)	0 (0%)	
Mycophenolate	40 (35.7%)	39/101 (38.6%)	1/11 (9.1%)	
Everolimus	6 (5.4%)	6/101 (5.9%)	0 (0%)	
Sirolimus	1 (0.9%)	1/101 (0.9%)	0 (0%)	
Others	3 (2.7%)	3/101 (2.9%)	0 (0%)	
Targeted therapy:	10 (8.9%)	8/101 (7.9%)	2/11 (18.2%)	0.255
Ibrutinib	1 (0.9%)	1/101 (0.9%)	0 (0%)	
Venetoclax	2 (1.8%)	1/101 (0.9%)	1/11 (9.1%)	
Rituximab	2 (1.8%)	1/101 (0.9%)	1/11 (9.1%)	
Other	6 (5.4%)	5/101 (4.9%)	1/11 (9.1%)	
Tocilizumab	23 (20.5%)	23/101 (22.8%)	0 (0%)	

Table 1 (continued)

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (Significance level p < 0.001)
HIV/AIDS	1 (0.9%)	1/101 (0.9%)	0 (0%)	0.999
Other causes of immunosuppression	10 (8.9%)	10/101 (9.9%)	0 (0%)	
IFI data				
IFI category				0.999
Proven	13 (11.6%)	12/101 (11.9%)	1/11 (9.1%)	
Probable/putative	88 (78.6%)	79/101 (78.2%)	9/11 (81.8%)	
Possible	11 (9.8%)	10/101 (9.9%)	1/11 (9.1%)	
Presentation of the IFI				0.999
Disseminated	6 (5.4%)	6/101 (5.9%)	0 (0%)	
Localized	106 (94.6%)	95/101 (94.1%)	11/11 (100%)	
Site of involvement:				
Endocarditis	1 (0.9%)	1/101 (0.9%)	0 (0%)	
Pulmonary	100 (89.3%)	89/101 (88.1%)	11/11 (100%)	
Skin-soft tisssue	4 (3.6%)	4/101 (3.9%)	0 (0%)	
Sinusal	1 (0.9%)	1/101 (0.9%)	0 (0%)	
Cerebral	2 (1.8%)	2/101 (1.9%)	0 (0%)	
Osteoarticular	1 (0.9%)	1/101 (0.9%)	0 (0%)	
Surgical wound	2 (1.8%)	2/101 (1.9%)	0 (0%)	
Tracheobronchitis	8 (7.1%)	8/101 (7.9%)	0 (0%)	
Others (suture, empyema, endophthalmitis…)	10 (8.9%)	10/101 (9.9%)	0 (0%)	
Reason for admission: IFI	14 (12.5%)	13/101 (12.9%)	1/11 (9.1%)	
Microbiology				
Patients with at least one positive culture	90 (80.4%)	84/101 (83.2%)	6/11 (54.5%)	0.023
Aspergillus section (including cryptic species)				
Fumigati	61/93 (65.6%)	56/86 (65.1%)	5/7 (71.4%)	0.350
Terrei	9/93 (9.7%)	9/86 (10.5%)	0 (0%)	
Nidulantes	2/93 (2.2%)	2/86 (2.3%)	0 (0%)	
Flavi	6/93 (6.5%)	6/86 (7%)	0 (0%)	
Nigri	4/93 (4.3%)	3/86 (3.5%)	1 (14.3%)	
Mixed	8/93 (8.6%)	8/86 (9.3%)	0/7 (0%)	
Spp	3/93 (3.2%)	2 (2.3%)	1/7 (14.3%)	
Cryptic species: *A. lentulus, A. hortai, A. spinilosporus, A. fumigatiaffinis, Neosartorya udagawae*	13/93 (14%)	11/86 (12.8%)	2/7 (28.6%)	0.252
Beta-D-glucan + serum (total n = 14)	4/14 (28.6%)	4/13 (30.8%)	0/1 (0%)	0.999
Galactomannan + serum (total n = 81)	23/81 (28.4%)	18/71 (25.7%)	5/11 (45.5%)	0.137
Bronchoscopy performed	78 (71.4%)	67/101 (66.3%)	11/11 (100%)	
Fungal stain BAL				
KOH	15/78 (19.2%)	11/13(84.6%)	4/4 (100%)	
Calcofluor white	2/78 (2.6%)	2/13 (15.4%)	0 (0%)	
Not performed	61/78 (54.5%)			
Fungal stain in BAL (total = 17)				
Septate hyphae	4/17(23.5%)	4/13(30.8%)	0 (0%)	
Negative	13/17 (76.5%)	9/13 (69.2%)	4/4(100%)	
Galactomanan + BAL (total = 41)	32/41 (78%)	26/32 (81.3%)	6/9 (66.7%)	0.304
PCR Aspergillus BAL (n = 22)				
Positives	13/22 (59.1%)	8/13 (61.5%)	5/9 (55.5%)	0.999
PCR Mucor BAL (n = 4)				
Positives	0/4 (0%)	0/2 (0%)	0/2 (0%)	–

Table 1 (continued)

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (Significance level p < 0.001)
PCR Fusarium BAL (n = 4)				
Positives	0/4 (0%)	0/2 (0%)	0/2 (0%)	–
PCR Scedosporium BAL (n = 4)				
Positives	0/4 (0%)	0/2 (0%)	0/2 (0%)	–
PCR panfungal BAL (n = 17)				
Positives	5/17 (29.4%)	4/10 (40%)	1/7 (14.3%)	0.304
Precipitins (IgG Aspergillus) (n = 13)				
Positive	1/13 (7.7%)	1/13 (7.7%)	0 (0%)	–
Biopsy performed	19/112 (17%)	18/101 (17.8%)	1/11 (9.1%)	
Biopsy positive	9/19 (47.4%)	8/18 (44.4%)	1/1 (100%)	
Imaging tests				
CNS CT scan performed	15 (13.4%)	12/101 (11.9%)	3/11 (27.3%)	
*Fungal involvement	3/14 (14.3%)	3/12 (1.9%)	0/3 (0%)	
Chest CT scan performed	105 (93.8%)	94/101 (93.1%)	11/11 (100%)	
Pulmonary nodules	49/105 (46.7%)	41/94 (43.6%)	8/11 (72.7%)	0.108
Halo sign	12/105 (11.4%)	7/94 (7.4%)	5/11 (45.5%)	0.003
Cavitation- crescent	23/105 (21.9%)	20/94 (21.3%)	3/11 (27.3%)	0.702
Tree in bud	13/105 (12.4%)	13/94(13.8%)	0/11 (0%)	0.352
Ground glass opacities	53/105 (50.5%)	44/94 (46.8%)	9/11 (81.8%)	0.052
Consolidation	39/105 (37.1%)	33/94 (35.1%)	6/11 (54.5%)	
Interstitial infiltrates	33/105 (31.4%)	30/94 (31.9%)	3/11 (27.3%)	
Pleural effusion	35/105 (33.3%)	31/94 (32.9%)	4/11 (36.4%)	
Sinusal CT scan performed	2 (1.8%)	1/101 (0.9%)	1/11 (9.1%)	
*Fungal involvement	1 (50%)	0/1 (0%)	1/1 (100%)	
PET-CT performed	28 (25%)	22/101 (21.8%)	6/11 (54.5%)	0.027
Indication PET-CT				
Fever	9/28 (32.1%)	6/22 (27.3%)	3/6 (50%)	
Staging	15/28 (53.6%)	12/22 (54.5%)	3/6 (50%)	
Monitoring	4/28 (14.3%)	4/22 (18.2%)	0	
Antifungal therapy				
Antifungal prophylaxis: breakthrough IFI	41 (36.6%)	34/101 (33.7%)	7/11 (63.6%)	0.664
Prior AF prophylaxis:				
Fluconazole	7/41 (17.1%)	4/34 (11.8%)	3/7 (42.9%)	
Posaconazole	3/41 (7.3%)	0 (0%)	3/7 (42.9%)	
Voriconazole	1/41 (2.4%)	1/34 (2.9%)	0 (0%)	
Isavuconazole	1/41 (2.4%)	1/34 (2.9%)	0 (0%)	
Micafungin	2/41 (4.9%)	2/34 (5.9%)	0 (0%)	
Anidulafungin	2/41 (4.9%)	1/34 (2.9%)	1/7 (14.3%)	
L-Amphotericin B (nebulized)	33/41 (80.5%)	33/34 (97.1%)	0 (0%)	
Resistant to prior AF	21/41 (51.2%)	17/34 (50%)	4/7 (57.1%)	0.686
Empirical AF therapy				
Posaconazole	3 (2.7%)	2/101 (1.9%)	1/11 (9.1%)	
Voriconazole	37 (33%)	34/101 (33.7%)	3/11 (27.3%)	
Isavuconazole	60 (53.6%)	58/101 (57.4%)	2/11 (18.2%)	
Caspufungin	2 (1.8%)	2/101 (1.9%)	0	
Micafungin	15 (13.4%)	15/101 (14.9%)	0	
Anidulafungin	8 (7.1%)	8/101 (7.9%)	0	
L-Amphotericin B (intravenous)	12 (10.7%)	6/101 (5.9%)	6/11 (54.5%)	

Table 1 (continued)

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (Significance level p < 0.001)
Resistant to empiric AF	11 (9.8%)	8/101 (7.9%)	3/11 (27.3%)	0.070
Targeted AF therapy				
Posaconazole	2 (1.8%)	1/101 (0.9%)	1/11 (9.1%)	
Voriconazole	25 (22.3%)	20/101 (19.8%)	5/11 (45.5%)	
Isavuconazole	63 (56.3%)	60/101 (59.4%)	3/11 (27.3%)	
Caspufungin	2 (1.8%)	1/101 (0.9%)	1/11 (9.1%)	
Micafungin	11 (9.8%)	11/101 (10.9%)	0 (0%)	
Anidulafungin	13 (11.6%)	10/101 (9.9%)	3/11 (27.3%)	
L-Amphotericin B (intravenous)	25 (22.3%)	18/101 (17.8%)	7/11 (63.6%)	
Duration of therapy Median (IQR)	44 (20–122)	48 (21–161)	40 (15–53)	
Evolution				
Coinfection (global)	77 (68.8%)	72/101 (65.5%)	5/11 (45.5%)	0.079
Virus coinfection	36/77 (46.8%)	35/72 (48.6%)	1/5 (20%)	0.101
Bacterial coinfection	54/77 (70.1%)	49/72 (68.1%)	5/5 (100%)	
Micobacterial coinfection	3/77 (3.9%)	3/72 (4.2%)	0 (0%)	
ICU due to IFI	5 (4.5%)	3/101 (2.9%)	2/11 (18.2%)	
Source Control	9 (8%)	9/101 (8.9%)	0 (0%)	
Reduction of immunosuppressants	26 (23.2%)	24/101 (23.8%)	2/11 (18.2%)	
Outcome				
Graft loss if SOT	5/48 (10.4%)	4/47 (8.5%)	1/1 (100%)	0.104
Delay chemotherapy or HSCT	8 (7.1%)	3/101 (2.9%)	5/11 (45.5%)	0.637
Hospital death	38 (33.9%)	33/101 (32.7%)	5/11 (45.5%)	0.685
Discharged under AF	55 (49.1%)	51/101 (50.5%)	4/11 (36.4%)	0.685
Evaluated by ID	63 (56.3%)	52/101 (51.5%)	11/11 (100%)	

Significant values are in bold

IQR interquartile range, *HSCT* hematopoietic stem cell transplantation, *COPD* chronic obstructive pulmonary disease, *CMV* Cytomegalovirus, *SOT* solid organ transplant, *HIV* human immunodeficiency virus, *IFI* invasive fungal infection, *BAL* bronchoalveolar lavage, *GM* galactomannan, *CNS* central nervous system, *CT* computed tomography, *PET-TC* positron emission tomography-computed tomography, *AF* antifungal, *ICU* Intensive Care Unit, *ID* Infectious disease specialist

*Significance level after applying Bonferroni correction

isolates, and 1 (1.9%) isolate was resistant to Posaconazole, Itraconazole and Amphotericin B. One of these presented the TR34-L98H mutation.

Among the cryptic species of the Fumigati family, resistance was higher with 10 isolates with Voriconazole MIC ≥ 2, 8 with Isavuconazole MIC >2 and 7 Amphotericin B MIC ≥ 2.

Breakthrough IFI was detected in 41/112 (36.6%) cases and in more than half of them (21/41 cases, 51.2%), the isolate was resistant to the prior antifungal.

The most frequently used empirical therapies were Isavuconazole (60 [53.6%]) and Voriconazole (37 [33%]). Resistance to empirical therapy was observed in 11/112 (9.8%) patients. The most common targeted antifungals were Isavuconazole (63 [56.3%]), Voriconazole (25 [22.3%]) and L-Amphotericin B (25 [22.3%]).

Among breakthrough IA, the most common empirical antifungal therapy was Voriconazole in 4 patients (8.9%), Isavuconazole in 35 patients (77.8%) and L- Amphotericin B in 6 patients (13.3%). Out of them, 7 (15.6%) cases were resistant to the empirical antifungal.

Among SOT, 5 recipients (4.5%) lost the graft as a consequence of IA. IA motivated a delay of chemotherapy or HSCT in 8 patients (7.1%). In-hospital mortality rate was 33.9% (38/112).

Differences between neutropenic and non-neutropenic patients

Differences between neutropenic and non-neutropenic patients were found regarding underlying diseases,

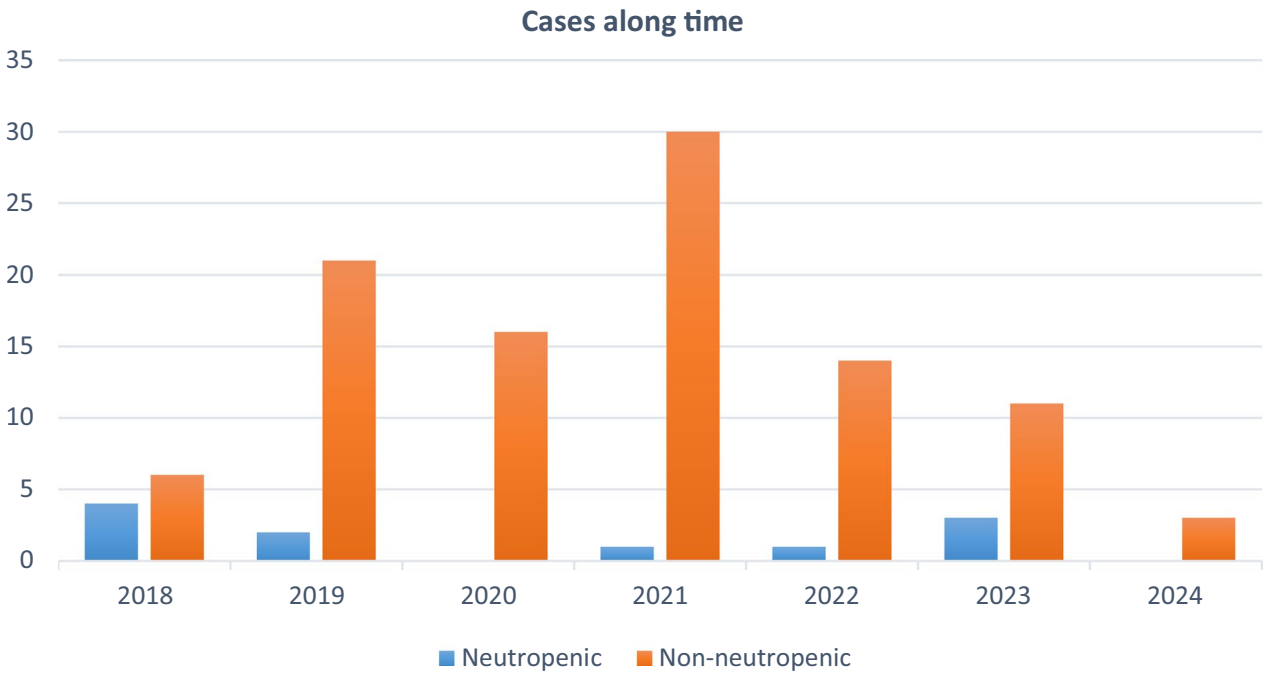

Fig. 1 Evolution of cases of invasive aspergillosis along the study period

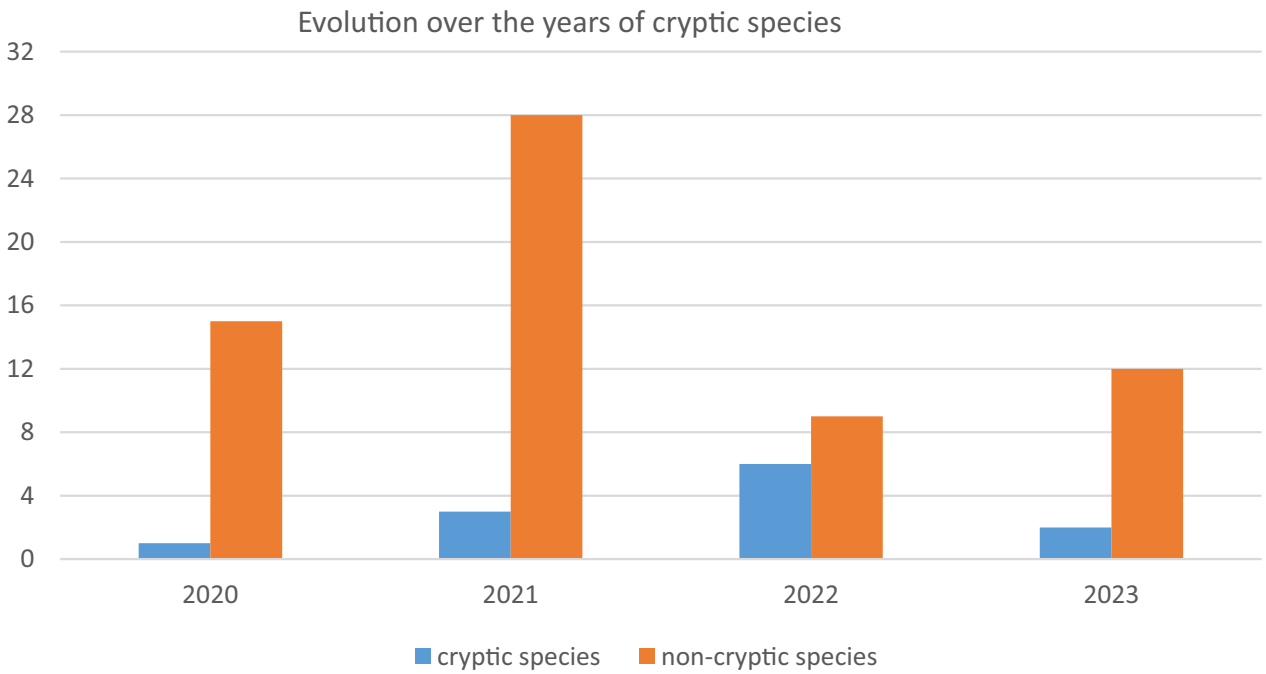

Fig. 2 Evolution over the years of cryptic species

Table 2 Resistance in Fumigati section

MIC (mg/L)	*A. fumigatus* sensu stricto* (n = 53)	Cryptic species (10)
Itraconazole ≥ 2	1 (1.9%)	6 (60%)
Voriconazole ≥ 2	2 (3.8%)	10 (100%)
Posaconazole > 0.25	1 (1.9%)	1 (10%)
Isavuconazole > 2	2 (3.8%)	8 (80%)
Amphotericin B ≥ 2	1 (1.9%)	7 (70%)

*One of these isolates presented the TR34_L98H mutation

clinical and radiological presentation, microbiological data and antifungal therapy (Fig. 3).

Underlying diseases

The most common underlying disease in non-neutropenic was SOT, especially lung transplant in 35/48 (34.7%; p-value 0.016), whereas in neutropenic, it was hematological disease in all patients (100%; p-value 0.0001), and receipt of chemotherapy in 9 cases (81.8%; p-value 0.0001).

Previous corticoid use was detected more frequently in non-neutropenic, 78 (77.2%), as compared to 2 (18.2%) among neutropenic (p-value 0.0001).

Regarding infections that are known to constitute a risk factor for aspergillosis, in non-neutropenic, 30 cases of SARS-CoV2 infection (29.7%; p-value 0.035), 4 of influenza (3.9%) and 29 CMV reactivations (28.7%; p-value 0.037%) were diagnosed in the previous month, in contrast with 0 cases in neutropenic patients. We detected 9 cases of IA associated with solid tumors, 6 (66.7%) of them were lung cancer and the other 3 were metastatic cancer. The most common presentation was pulmonary aspergillosis (77.8%), with cavitated nodules being the most common finding. 2 of the patients had previous cavities related to the oncological pathology.

Clinical presentation and microbiological data

The most widespread form of presentation was pulmonary aspergillosis in both groups, in 88.1% of non-neutropenic and in 100% of neutropenic, but non-neutropenic presented other sites of involvement as well.

Six cases of disseminated infection were diagnosed in non-neutropenic patients (5.9%). The characteristics of disseminated aspergillosis are shown in Table 4. Disseminated cases presented mainly in SOT recipients, although one case occurred in a patient with COVID-19 and significant immunosuppression and another had a probable endovascular source. Two of them survived, in keeping with a reduction of immunosuppression, that in the case of the kidney transplant conveyed the loss of the graft.

Among the included patients with IA, ground glass opacities (81.8% [9/11] vs 46.8% [44/94]; p-value 0.052) and lung nodules (72.7% [8/11] vs 43.6% [41/94]; p-value 0.108) with halo sign (45.5% [5/11] vs 7.4% [7/94]; p-value 0.003[NS]) were more common in patients with neutropenia than in those without neutropenia. In non-neutropenic, there was a wider variety of chest CT findings.

Positive cultures were detected more often in non-neutropenic (85 [84.2%]), as compared to neutropenic (6 [54.5%]); more information about positive cultures is shown in Table 5. In both groups, the most frequently isolated species was *A. fumigatus*, and there were no significant differences in the distribution of *Aspergillus* species, although cryptic species were more common in neutropenic (28.6% versus 12.8%, p-value 0.252), and some species were found only in non-neutropenic (*A. flavus, A. terreus, A. nidulans*…).

Serum GM was positive more frequently in the neutropenic group, 5 (45.5%) versus 18 (25.7%) non-neutropenic (p-value 0.137). Among the 4 IAPA cases, only 3 had GM tested in serum, with 33.3% positivity compared to 40% GM positivity in 30 CAPA cases. All neutropenic patients underwent bronchoscopy (100%) detecting a positive GM in BAL in 6 cases (66.7%) compared to 26 (81.3%) positive GM in BAL in non-neutropenic (p-value 0.304).

Neutropenic patients were receiving previous antifungals more often, and consequently presented breakthrough IFI in a greater proportion: 7 (63.6%) neutropenic compared to 34 (33.7%) non-neutropenic. The most commonly used prophylaxes in neutropenic were

Table 3 MIC of the rest of the isolated *Aspergillus* species

MIC (mg/L)	*A. flavus* (n = 6)	*A. terreus* (n = 7)	*A. niger* (n = 4)	*A. nidulans* (n = 1)	Cryptic species (n = 13)	*Aspergillus* spp (n = 3)
Itraconazole ≥ 2	0	0	0	1	7	0
Voriconazole ≥ 2	3	3	0	1	11	0
Posaconazole > 0.25	1	1	0	1	2	0
Isavuconazole > 2 (For *A. nidulans* > 0.25)	0	1	0	1	9	0
Amphotericin B ≥ 2	5	5	0	1	11	0

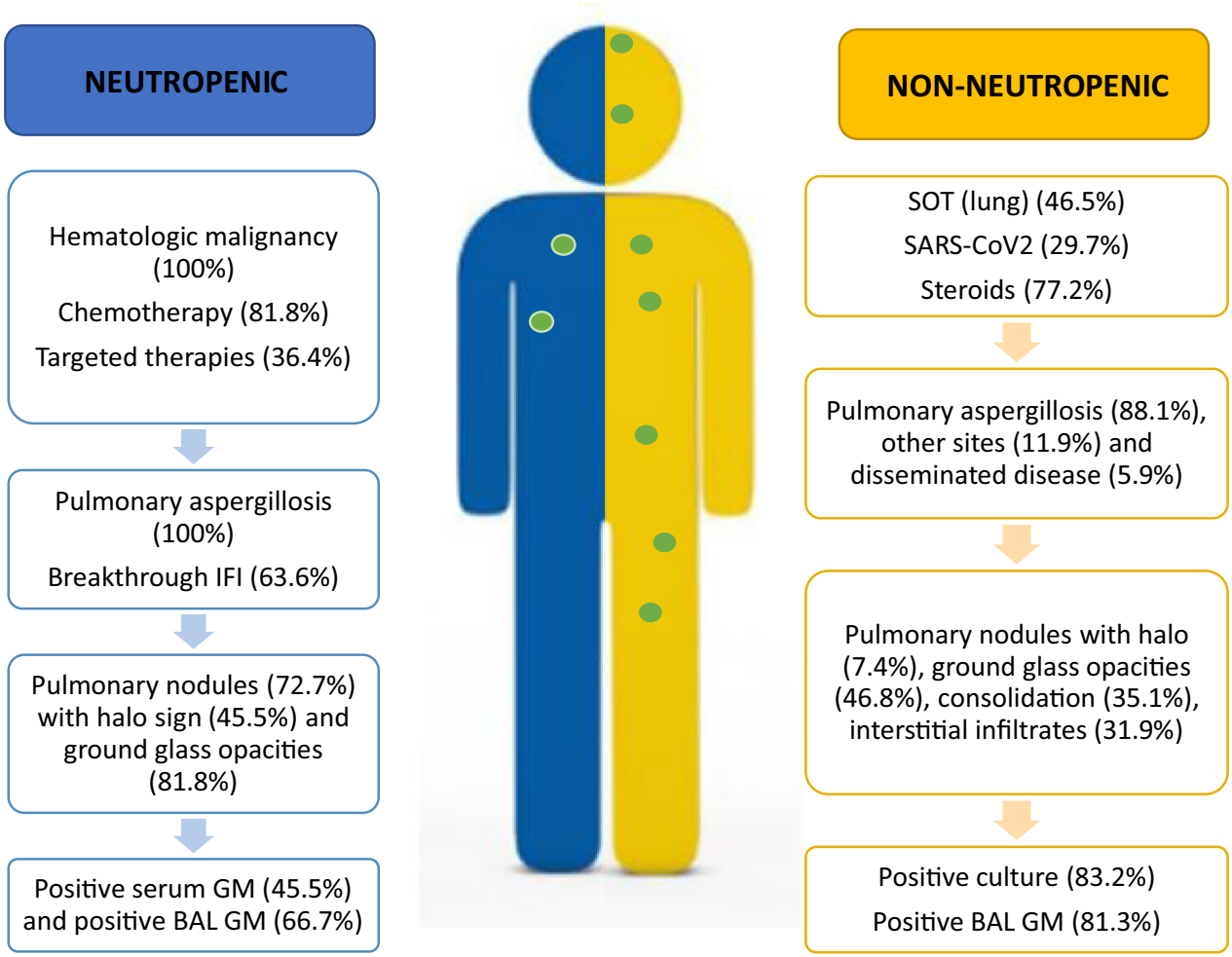

Fig. 3 Differences between neutropenic and non-neutropenic patients

Fluconazole or Posaconazole, 3 each (42.9%), while in non-neutropenic it was nebulized L-Amphotericin B in 33 (97.1%). In 4 (57.1%) neutropenic and 17 (50%) non-neutropenic patients the *Aspergillus* species that caused the breakthrough infection was resistant to the antifungal used for prophylaxis.

In neutropenic cases, the most common used antifungal was intravenous L-Amphotericin B, both empirically (54.5% [6/11] vs 5.9% [6/101]) and as targeted treatment (63.6% [7/11] vs 17.8% [18/101]). On the contrary, in non-neutropenic patients, Isavuconazole was the most widespread option both empirically (57.4% [58/101] vs 18.2% [2/11]) and as targeted therapy (59.4% [60/101] vs 27.3% [3/11]), respectively.

There were no significant differences in mortality, although it was higher among neutropenic patients (45.5% [5/11] versus 32.7% [33/101]) in non-neutropenic (p-value 0.685).

Characteristics of patients with hematological disease

The characteristics of patients with IA and hematological disease are summarized in Table 6.

Almost half of the 22 patients with underlying hematological disease had neutropenia, 10 patients (45.5%), whereas among the 7 (31.8%) patients that had undergone a HSCT, only 2 (20%) were neutropenic. Among patients with IA and hematologic malignancies, chemotherapy was more common in patients with neutropenia than in those without neutropenia (90% [9/10] vs 25% [3/12], p-value 0.004[NS]) while the previous use of steroids (75% [9/12] vs 10% [1/10]; p-value 0.004[NS]) was more frequent in non-neutropenic. 8/22 patients (36.4%) were under new targeted therapies (Ibrutinib, Venetoclax, Dasatinib, bispecific antibodies).

IA presented as persistent fever in 100% neutropenic individuals versus only in 6 (50%) non-neutropenic (p-value 0.009[NS]), while respiratory failure was the most common presentation among non-neutropenic

Table 4 Characteristics of disseminated invasive aspergillosis

	Underlying disease	Reason for admission at IFI diagnosis	IFI site of involvement	*Aspergillus* species	Prior AF (breakthrough IFI)	Concomitant coinfection	Outcome
Patient 1	Bilateral lung transplant. Second bilateral lung transplant one year later	Surgical wound dehiscence and infection	Surgical wound, eye, sternum and mediastinum	*A. terreus*	Nebulized AmphoB	No	Dead
Patient 2	Acute respiratory distress syndrome secondary to SARS-Cov2 infection	SARS-CoV2	Lung and eye	*A. fumigatus*	No	Several infections during admission (*R. ornithinolytica* nosocomial pneumonia, *E. faecalis* bacteremia. Empyema due to *E. faecium*. Cystitis due to *P. aeruginosa*	Dead
Patient 3	Cardiogenic shock due to acute myocardial infarction, need for ventricular assistance	Cardiogenic shock due to acute myocardial infarction, ventricular assistance	Lung, mediastinum and surgical wound infection and ventricular assistance infection	*A. fumigatus*	No	No	Transfer to referral hospital
Patient 4	Lung transplant	Acute rejection	Lung, brain, skin and pulmonary vein	*A. fumigatus*	Nebulized AmphoB	*Pseudomonas aeruginosa* pneumonia	Dead
Patient 5	Kidney transplant	Solid mass in left leg	Lung and muscle	*A. fumigatus*	No	No	Alive, lost graft, suppressive chronic AF
Patient 6	Acute promyelocytic leukemia	Induction chemotherapy	Lung, brain and spine	*A. terreus*	Posaconazole	*K. pneumoniae* pneumonia	Alive without AF

IFI Invasive fungal infection, *AF* antifungal

Table 5 Number of samples with positive culture in the complete cohort

Microbiology	Total	Non-neutropenic	Neutropenic
Patients with any positive culture	90 (80.4%)	84/101 (83.2%)	6/11 (54.5%)
Total positive culture sample			
BAL	30/90 (33.3%)	26/84 (30.9%)	4/6 (66.7%)
BAS	61/90 (67.8%)	58/84 (69%)	3/6 (50%)
TBB	3/90 (3.3%)	3/84 (3.6%)	0/6 (0%)
Sputum	21/90 (23.3%)	21/84 (25%)	0/6 (0%)
Pleural fluid	2/90 (2.2%)	2/84 (2.4%)	0/6 (0%)
Other (vegetation, sinus, collection, vitreous humor, bone …)	10/90 (11.1%)	9/84 (10.7%)	1/6 (16.7%)

BAL bronchoalveolar lavage, *BAS* bronchoalveolar aspirated, *TBB* transbronchial biopsy

(10 [83.3%]) as compared to neutropenic (5 [50%]) (p-value 0.172).

Pulmonary nodules were the most common imaging finding in both groups (9 [75%] non-neutropenic and 8 [80%] neutropenic), but the halo sign was more prevalent among neutropenic (5) as compared to non-neutropenic (1) (50% vs 8.3%, p-value 0.056).

Breakthrough IFI was seen mainly in neutropenic (6 [60%]) as compared to non-neutropenic (2 [16.7%]) (p-value 0.074).

Discussion

The results of the present study support previous findings that encounter a number of underlying conditions that could act as risk factors for IA in non-immunocompromised patients. Neutropenia has been known for decades as the main risk factor for developing IA, but in recent years other factors unrelated to neutropenia have been recognized as playing an increasingly important role in the development of IA. In our registry, only 10% of patients presented neutropenia at IA diagnosis. In non-neutropenic patients, main underlying conditions in IA were the use of corticosteroids, viral co-infections such as SARS-CoV2 or CMV, chronic lung diseases and SOT, mainly lung transplantation.

IA associated with viral infections is an emerging disease, which conveys a high morbidity and mortality. Influenza-associated aspergillosis (IAPA) and SARS-CoV2 associated aspergillosis (CAPA) are two well-described entities that occur mainly in severely ill patients [14–16]. During the COVID waves, we observed a sharp increase in IA cases, mainly due to CAPA cases. The epithelium damaged by the viral infection facilitates the adhesion of conidia, favors invasion and hampers the elimination of fungi [16]. Although they share certain characteristics, there are some relevant differences between IAPA and CAPA regarding diagnosis and outcome [16]. IAPA presents earlier after ICU admission than CAPA, which has a later presentation. Due to the epithelial damage caused by viral infections, in these cases, the typical IA presentation is tracheobronchitis, also described in lung SOT, which is more common in IAPA than in CAPA. Another interesting difference is that IAPA has a greater capacity to produce angioinvasion, and consequently, serum GM is positive more often compared to cases of CAPA where this is uncommon, facilitating the diagnosis in critically ill patients who are not always suitable to undergo invasive techniques. [14, 15]. In spite of this, in our registry, positivity of serum galactomannan was similar in IAPA and CAPA, probably due to the scarcity of IAPA cases.

The association of CMV replication is well known to favor the dysregulation of T lymphocytes and is considered a risk factor for IA in patients with SOT, however, recent studies have also shown that non-SOT critically ill patients with SARS-CoV2 infection and CMV replication are at greater risk of developing IA [17]. In our series, 17 (56.7%) the patients with CAPA presented CMV replication. In this scenario, CMV could also be considered a marker of immunosuppression.

The risk of IA in hematological malignancies is clearly recognized, but patients with solid tumors also have an increased risk of IA, especially those with other comorbidities, who receive steroids, chemotherapy or lung radiotherapy [18]. Previous studies, such as of the one by Peghin et al., have verified the relationship between the presence of previous cavitated lung lesions secondary to lung cancer or lung metastases with the development of subacute aspergillosis on these lesions [19]. In the same line, among our cases of IA in patients with solid tumors, the most common presentation was cavitated pulmonary nodules, with at least 2 of the patients presenting previous pulmonary cavities related to the oncological pathology that subsequently facilitated the development of IA.

Even among the group of 22 patients with hematological malignancies, the majority, 55%, did not present neutropenia. This is in line with other recent epidemiological

Table 6 Invasive aspergillosis in hematological patients

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (significance value p < 0.001)
Number of patients	22	12/22 (54.5%)	10/22 (45.5%)	
Demographic data				
Sex:				
Male	16 (72.7%)	10/12 (83.3%)	6/10 (60%)	
Female	6 (27.3%)	2/12 (16.7%)	4/10 (40%)	
HSCT	7 (31.8%)	5/12 (41.7%)	2/10 (20%)	0.381
GVHD (only HSCT)	3/7 (42.9%)	3/7 (42.9%)	0 (0%)	0.429
Lung disease	6 (27.3%)	4/12 (33.3%)	2/10 (20%)	0.646
COPD	2 (9.1%)	1/12 (8.3%)	1/10 (10%)	0.999
Kidney disease	3 (13.6%)	3/12 (25%)	0 (0%)	
Dialysis	0 (0%)	0 (0%)	0 (0%)	
Liver disease	0 (0%)	0 (0%)	0 (0%)	
Heart disease	1 (4.5%)	1/12 (8.3%)	0 (0%)	
COVID (last month)	3 (13.6%)	3/12 (25%)	0 (0%)	0.221
Influenza (last month)	0 (0%)	0 (0%)	0 (0%)	–
Prior letermovir	0 (0%)	0 (0%)	0 (0%)	–
CMV reactivation	4 (18.2%)	4/12 (33.3%)	0 (0%)	0.999
Lymphopenia (< 1000)	11 (50%)	4/12 (33.3%)	7/10 (70%)	0.198
Quimiotherapy	12 (54.5%)	3/12 (25%)	9/10 (90%)	0.004
Previous steroids	10 (45.5%)	9/12 (75%)	1/10 (10%)	0.004
Targeted therapy	8 (36.4%)	6/12 (50%)	2/10 (20%)	0.204
Ibrutinib	1 (4.5%)	1/12 (8.3%)	0 (0%)	
Venetoclax	2 (9.1%)	1/12 (8.3%)	1/10 (10%)	
Rituximab	2 (9.1%)	2/12 (16.7%)	0 (0%)	
Other	4 (18.2%)	3/12 (25%)	1/10 (10%)	
Tocilizumab	3 (13.6%)	3/12 (25%)	0 (0%)	
IFI data				
IFI category				
Proven	3 (13.6%)	2/12 (16.7%)	1/10 (10%)	0.560
Probable	18 (81.8%)	9/12 (75%)	9/10 (90%)	
Possible	1 (4.5%)	1/12 (8.3%)	0 (0%)	
Presentation of the IFI				
Disseminated	1 (4.5%)	1/12 (8.3%)	0 (0%)	0.999
Localized	21 (95.5%)	11 (91.7%)	10/10 (100%)	
Site of involvement				
Pulmonary	22 (100%)	12/12 (100%)	10/10 (100%)	
Cerebral	1 (4.5%)	1/12 (8.3%)	0 (0%)	
Others	0 (0%)	0 (0%)	0 (0%)	
Microbiology				
Patients with any positive culture	10 (45.5%)	5/12 (41.7%)	5/10 (50%)	
Aspergillus species isolates (total n = 13, cultures or PCR)				
A. fumigatus sensu stricto	8/13 (61.5%)	5/7 (71.4%)	3/6 (50%)	0.444
A. flavus	1/13 (7.7%)	1/7 (14.3%)	0 (0%)	
A. niger	1/13 (7.7%)	0 (0%)	1/6 (16.7%)	
A. terreus	1/13 (7.7%)	1/7 (14.3%)	0 (0%)	
A. nidulans	0 (0%)	0 (0%)	0 (0%)	
Aspergillus sp	1/13 (7.7%)	0 (0%)	1/6 (16.7%)	

Table 6 (continued)

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (significance value p < 0.001)
Mixed	0 (0%)	0 (0%)	0 (0%)	
Cryptic species: *Neosartorya udagawae*	1/13 (7.7%)	0 (0%)	1/6 (17.5%)	
Beta-D-glucan + serum (total n = 6)	1/6 (16.7%)	1/1 (100%)	0 (0%)	0.236
Galactomannan + serum (total n = 22)	7 (31.8%)	2/22 (9.1%)	5/10 (50%)	0.172
Bronchoscopy performed	20 (90.9%)	10/12 (83.3%)	10/10 (100%)	
Galactomannan + BAL (total n = 19)	14/19 (73.7%%)	8/10 (80%)	6/9 (66.7%)	0.476
PCR Aspergillus BAL (total n = 15): Positives	8/15 (53.3%)	3/6 (50%)	5/9 (55.6%)	0.162
PCR Mucor BAL (total n = 4): Positives	0 (0%)	0 (0%)	0 (0%)	–
PCR Fusarium BAL (total n = 4): Positives	0 (0%)	0 (0%)	0 (0%)	–
PCR Scedosporium BAL (total n = 4): Positives	0 (0%)	0 (0%)	0 (0%)	–
PCR panfungal BAL (performed n = 12): Positives	4/12 (33.3%)	3/5 (60%)	1/7 (14.3%)	0.120
Biopsy performed	4 (18.2%)	3/12 (25%)	1/10 (10%)	
Biopsy positive	3/4 (75%)	2/3 (66.7%)	1/1 (100%)	
Clinical presentation				
Persistent fever	16 (72.7%)	6/12 (50%)	10/10 (100%)	0.009
Cough	9 (40.9%)	5/12 (41.7%)	4/10 (40%)	0.937
Thoracic pain	3 (13.6%)	1/12 (8.3%)	2/10 (20%)	0.571
Dyspnea	4 (18.2%)	3/12 (25%)	1/10 (10%)	0.594
Respiratory failure	15 (68.2%)	10/12 (83.3%)	5/10 (50%)	0.172
Imaging tests				
Chest CT scan performed	22 (100%)	12/12 (100%)	10/10 (100%)	0.999
Pulmonary nodules	17 (77.3%)	9/12 (75%)	8/10 (80%)	0.999
Halo sign	6 (27.3%)	1/12 (8.3%)	5/10 (50%)	0.056
Cavitation- crescent sign	3 (13.6%)	0 (0%)	3/10 (30%)	0.078
Tree in bud	1 (4.5%)	1/12 (8.3%)	0 (0%)	
Ground glass opacities	18 (81.8%)	9/12 (75%)	9/10 (90%)	
Consolidation	12 (54.5%)	6/12 (50%)	6/10 (60%)	
Interstitial infiltrates	8 (36.4%)	5/12 (41.7%)	3/10 (30%)	
Pleural effusion	11 (50%)	7/12 (58.3%)	4/10 (40%)	
PET-CT performed	9 (40.9%)	3/12 (25%)	6/10 (60%)	
Indication PET-CT				
Fever	3/9 (33.3%)	0 (0%)	3/6 (50%)	
Staging	6/9 (66.7%)	3/3 (100%)	3/6 (50%)	
Antifungal therapy				
Antifungal prophylaxis: breakthrough IFI	8 (36.4%)	2/12 (16.7%)	6/10 (60%)	0.074
Prior AF prophylaxis:				
Fluconazole	4/8 (50%)	1/2 (50%)	3/6 (50%)	
Posaconazole	3/8 (37.5%)	0 (0%)	3/6 (50%)	
Amphotericin (nebulized)	1/8 (12.5%)	1/2 (50%)	0 (0%)	
Resistant to prior AF	5/8 (62.5%)	1/2 (50%)	4/6 (66.7%)	0.135
Empirical AF therapy				
Posaconazole	1 (4.5%)	0 (0%)	1/10 (10%)	
Voriconazole	4 (18.2%)	1/12 (8.3%)	3/10 (30%)	
Isavuconazole	11 (50%)	10/12 (83.3%)	1/10 (10%)	
Caspofungin	1 (4.5%)	1/12 (8.3%)	0 (0%)	
L-Amphotericin (intravenous)	6 (27.3%)	0 (0%)	6/10 (60%)	
Resistant to empiric AF	4 (18.2%)	1/12 (8.3%)	3/10 (30%)	0.285

Table 6 (continued)

	Total	Non-neutropenic	Neutropenic (NF < 500)	p-value* (significance value p < 0.001)
Definitive AF therapy				
Posaconazole	1 (4.5%)	0 (0%)	1/10 (10%)	
Voriconazole	4 (18.2%)	0 (0%)	4/10 (40%)	
Isavuconazole	14 (63.6%)	11/12 (91.7%)	3/10 (30%)	
Caspufungin	1 (4.5%)	0 (0%)	1/10 (10%)	
Micafungin	1 (4.5%)	1/12 (8.3%)	0 (0%)	
Anidulafungin	4 (18.2%)	2/12 (16.7%)	2/10 (20%)	
L-Amphotericin (intravenous)	6 (27.3%)	0 (0%)	6/10 (60%)	
Duration of therapy: Median (IQR)		29 (14–67.5)	40 (15–53)	
Evolution				
Coinfection	15 (68.2%)	11/12 (91.7%)	4/10 (40%)	0.020
Virus coinfection	4/15 (26.7%)	4/11 (36.4%)	0 (0%)	0.096
Bacterial coinfection	12/15 (80%)	8/11 (72.7%)	4/4 (100%)	
Mycobacterial coinfection	0 (0%)	0 (0%)	0 (0%)	
ICU due to IFI	1 (4.5%)	0 (0%)	1/10 (10%)	
Outcome				
Delay of chemotherapy or HSCT	7 (31.8%)	2/12 (16.7%)	5/10 (50%)	0.999
In-hospital death	10 (45.5%)	6/12 (50%)	4/10 (40%)	0.716

HSCT hematopoietic stem cell transplantation, *GVHD* Graft versus host disease, *COPD* chronic obstructive pulmonary disease, *CMV* Cytomegalovirus, *IFI* invasive fungal infection, *BAL* bronchoalveolar lavage, *CT* computed tomography, *PET-TC* positron emission tomography-computed tomography, *AF* antifungal, *ICU* Intensive Care Unit

*Significance level after applying Bonferroni correction

studies that suggest that in times of mold active prophylaxis, neutropenia is not anymore the most frequent risk factor for IA [20, 21].

A recently identified group of patients at risk for IA are those treated with new targeted therapies such as Ibrutinib, Venetoclax, Dasatinib or bispecific antibodies [11, 22]. In the present study, only a small proportion (8.9%) of the cases were receiving such treatments, however they represent 36.4% of Hematology patients, illustrating that even among them, the risk factors for IA have changed.

The presentation of IA is influenced by the number of neutrophils, that marks the predominant pattern since these are the cells responsible for containing the angioinvasion of conidia [23]. The presence or not of neutropenia and its degree determines the predominance of the phase of broncho-invasion or angioinvasion, which in turn condition different patterns of clinical presentation [24]. The more severe the neutropenia, the faster and easier the angioinvasion occurs, while in non-neutropenic patients the broncho-invasion phase is the most important. These phases will condition the differences both in clinical presentation and in the radiology and microbiological results. In neutropenic patients, the appearance of pulmonary nodules with the halo sign is characteristic, which reflects angioinvasion and tissue necrosis, while in non-neutropenic patients the symptoms are more subacute and nonspecific, and the radiological findings are more varied with the presence of micronodules, tree in bud, ground glass… Due to angioinvasion, neutropenic patients present positive serum GM more frequently than non-neutropenic patients, while the latter, having a more significant broncho-invasive phase, will more frequently present positive GM in BAL and positive cultures of respiratory samples [23, 25, 26].

Based in these differences, the most appropriate tests for diagnosis might be different depending on the immune condition of the host. Microbiological diagnosis is essential for a correct treatment. In addition to culture, other techniques such as GM, especially in those without anti-mold prophylaxis, or PCRs, are known to be useful in the diagnosis of neutropenic patients [22]. On the contrary, the performance of fungal biomarkers in non-neutropenic patients is still not well known, although current data indicate that they are less sensitive than in neutropenic patients. In recent years, the usefulness of these techniques for the diagnosis of pulmonary IA in non-neutropenic has been further explored; several studies support the diagnostic value of GM in BAL in this group while its yield in serum remains modest, with the

exception of influenza-associated cases, as aforementioned [15, 27, 28]. On the contrary, neutropenic patients rarely present positive cultures [23]. In accordance, in our study, the diagnosis in non-neutropenic patients was obtained mainly from positive cultures, (around 85%), and positive GM was more common in BAL than in serum (81% versus 25%). In neutropenic patients, the diagnosis was established by a combination of biomarkers, PCRs and cultures. As expected, the percentage of positive GM in serum was higher than in non-neutropenic subjects. These differences between neutropenic and non-neutropenic patients were even more marked when we exclusively analyzed patients with hematological malignancies.

In our series, we differentiated two different patterns of clinical presentation depending on whether the patient was or not neutropenic (Fig. 3). Even if 100% of neutropenic patients presented pulmonary aspergillosis, in non-neutropenic the presentation was more varied with involvement of other locations including 6 cases of disseminated disease. The delay in diagnosis and effective treatment of this entity in patients considered low risk until a few years ago is a potential explanation for dissemination. Furthermore, in non-neutropenic patients symptoms tend to be more subtle and the findings in imaging tests non-specific and variable, as it occurred in our patients, making early diagnosis even more difficult [6, 8, 20]. Based on these data, it seems necessary to rule out dissemination of IA, especially in non-neutropenic patients. In this sense, several studies carried out by our group on the usefulness of ^{18}F-FDG-PET-CT in IFI and FN, suggest that ^{18}F-FDG-PET-CT can be useful for the diagnosis of occult or silent IA lesions [29, 30]. For more information about the contribution of the ^{18}F-FDG-PET-TC in IFI in this series, please review a previous article by our group that underlines the usefulness of this technique for diagnosis, staging and monitoring of IFI [29]. We are currently carrying out a prospective multicenter study on the usefulness of ^{18}F-FDG-PET-CT in IFI that we hope will shed light in this regard [31] (Clinical trials gov identifier NCT05688592; Study Registration Dates: First Submitted 2022-12-20; First Submitted that Met QC Criteria 2023-01-15; First Posted 2023-01-18).

Considering these diagnostic and clinical differences between IA presentation in neutropenic and non-neutropenic patients, we propose a diagnostic algorithm for IA according to the presence or absence of neutropenia in Fig. 4.

Another relevant issue is resistance to antifungal drugs. When considering resistance to antifungals in *Aspergillus* spp, it is important to distinguish between *A. fumigatus* sensu stricto as compared to the cryptic species of *A. fumigatus* or species of non-fumigatus *Aspergillus*. The former may present acquired resistance to antifungals, while the latter present higher rates of intrinsic resistance to antifungals. The resistance profile may be quite different, with a particularly higher incidence of resistance in cryptic species [2, 10]. However, in recent years, concern about azole-resistant *A. fumigatus* sensu stricto is increasing [3, 10, 32]. IA caused by azole-resistant strains has been associated with a higher mortality rate [3, 5]. Rapid recognition of resistant strains is paramount to establish a timely appropriate treatment [3, 5]. Some records showed a prevalence around 5% of azole-resistant *A. fumigatus* sensu stricto [32, 33]. In addition, IA caused by non-fumigatus *Aspergillus* and cryptic species is emerging as well.

Among *A. fumigatus* sensu stricto, the most commonly reported mutation is the TR34-L98H which confers resistance to triazoles; resistance in other *Aspergillus* spp is not as well characterized [3, 32, 33]. This mutation has been widely recognized in other countries, mainly Denmark and the Netherlands [34, 35], but not in Spain. A recent epidemiological study carried out by Monzó et al. at the Hospital Clinic in Barcelona, found a significant increase in cases of azole-resistant *A. fumigatus* due to the TR34-L98H mutation in Spain, and a prevalence of almost 10% of cryptic species [31]. In our registry, only one patient presented this mutation, but, on the contrary, there were 14% of cases caused by cryptic species, with 30.8% of resistance to the azoles and 84.7% of resistance to amphotericin B, and 30.8% resistance to both classes. These differences may be due to the geographical differences between Madrid and Barcelona and point out the need for surveillance of resistance both at the local and the national level. The delay in the availability of the antifungigram with the consequent delay in starting the appropriate antifungal therapy is an everyday concern. The early identification of the species with the MALDI-TOF technique is of help to make an approximation of the susceptibility and to adjust the treatment accordingly. Rapid resistance detection techniques such as PCR that detect the presence or absence of the most common mutations associated to azole resistance in *A. fumigatus* are showing a clinical impact [32].

Mold active prophylaxis in high risk patients may condition resistance with development of breakthrough IFI [36]. In our study, overall, we found that almost 20% of aspergillosis were resistant to prior prophylactic antifungal and 10% resistant to empirical therapy. We encountered this profile mainly in patients with lung transplantation who usually receive prophylaxis with nebulized Amphotericin B, favoring the emergence of resistant species different from *A. fumigatus*. Another subset with an increased risk of resistance in the present series were hematological patients receiving

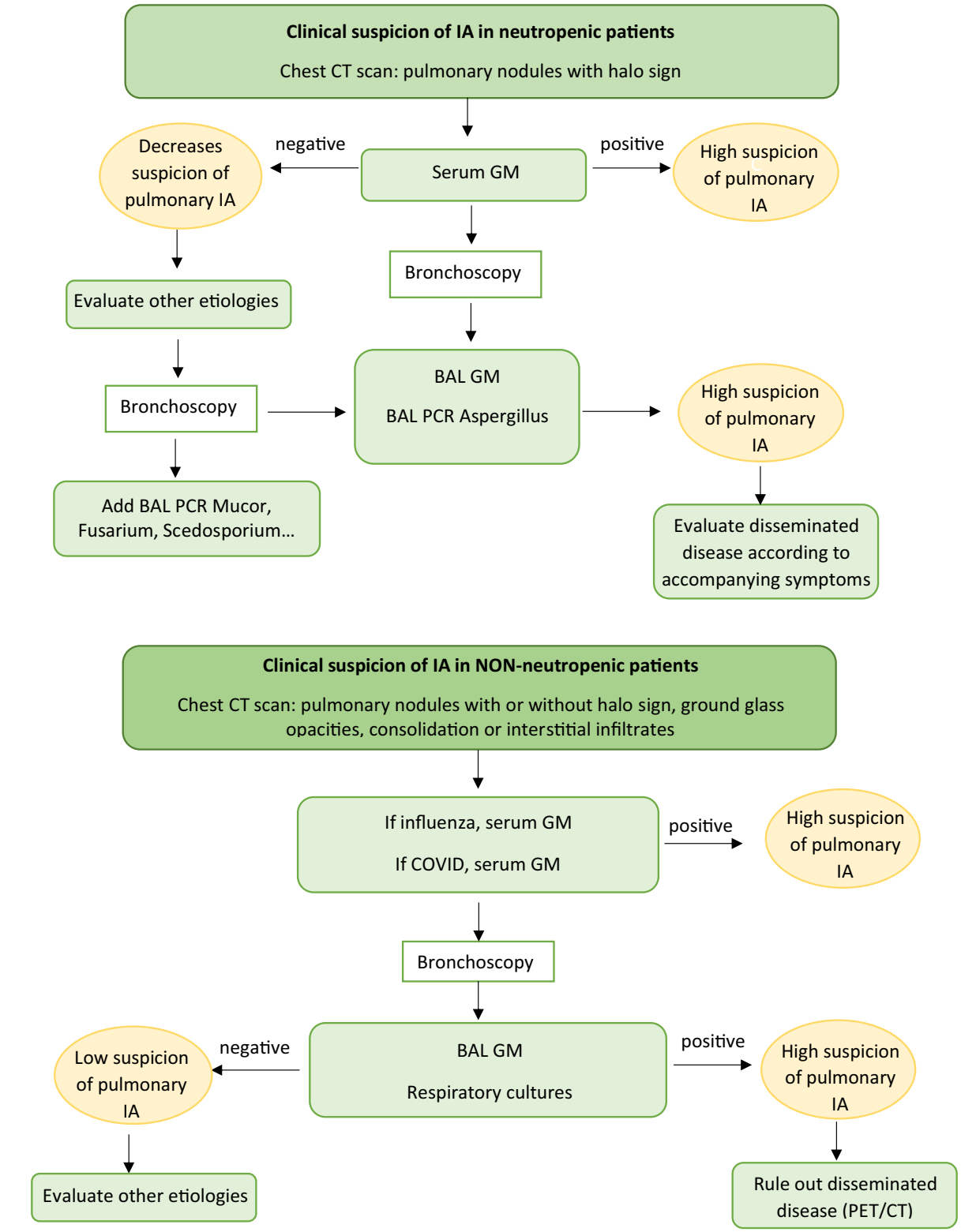

Fig. 4 Algorithm of IA diagnosis in neutropenic versus non-neutropenic patients

posaconazole prophylaxis. *A. fumigatus* sensu stricto was the etiological agent in 51% of these patients, but the other half of the IA were caused by different *Aspergillus* species, including 14% cryptic species, with higher antifungal resistance profiles. We relate the emergence of these more resistant species and the increase in resistance to azoles to the use of antifungal prophylaxis, the environment and the increase in the number of patients at risk of IA [2, 5, 32].

In spite of new drugs such as Isavuconazole which facilitate adherence, and new diagnostic techniques such as Aspergillus PCR that advance the diagnosis, IA mortality remains unacceptably high.

Conclusion

The underlying conditions and clinical presentation of patients with IA are evolving. Risk factors other than neutropenia have increased and the new profile of patients with IA must be taken into account in order to optimize the diagnostic process and adjust a timely appropriate treatment. IA resistant to antifungals is increasingly present and we must strive to improve the diagnostic techniques and treatments given the higher mortality it conveys.

Abbreviations

IA	Invasive aspergillosis
HSCT	Hematopoietic stem cell transplantation
CAR-T	Chimeric Antigen Receptor T-Cell
SOT	Solid organ transplant
ICU	Intensive Care Unit
EORTC	European Organization for Research and Treatment of Cancer
MSC	Mycosis Study Group
ECMM/ISHAM	European Confederation of Medical Mycology and International Society for Human & Animal Mycology
SD	Standard deviations
IQR	Interquartile range
BAS	Bronchoalveolar aspirate
BAL	Bronchoalveolar lavage
GM	Galactomannan
IFI	Invasive fungal infection
AF	Antifungal
IAPA	Influenza associated pulmonary aspergillosis
CAPA	COVID-19 associated pulmonary aspergillosis
NS	Non-significant

Supplementary Information

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Supplementary Information

Additional file 1.

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Author contributions

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Availability of data and materials

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

The study has been approved by the Ethical Research Committee of the Puerta de Hierro-Majadahonda Hospital (PI 156/24).

Competing interests

The authors declare no competing interests.

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