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COVID-19–associated pulmonary aspergillosis: a prospective single-center dual case series

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Abstract

Background: COVID-19–associated pulmonary aspergillosis (CAPA) has emerged as an invasive fungal disease, often affecting previously immunocompetent, mechanically ventilated, intensive care unit (ICU) patients. Incidence rates of 3.8%–33.3% have been reported depending on the geographic area, with high (47%) mortality.

Objectives: Here, we describe a single-centre prospective case series with CAPA cases from both the first (March-May, *n* = 5/33) and second (mid-September through mid-December, *n* = 8/33) COVID-19 wave at a 500-bed teaching hospital in the Netherlands.

Patients/Methods: In the first COVID-19 wave, a total of 265 SARS-CoV-2 PCR-positive patients were admitted to our hospital of whom 33 needed intubation and mechanical ventilation. In the second wave, 508 SARS-CoV-2 PCR-positive patients were admitted of whom 33 needed mechanical ventilation. Data were prospectively collected.

Results: We found a significant decrease in COVID-19 patients needing mechanical ventilation in the ICU in the second wave ($p < .01$). From these patients, however, a higher percentage were diagnosed with CAPA (24.2% vs 15.2%), although not significant ($p = .36$). All CAPA patients encountered in the second wave received dexamethasone. Mortality between both groups was similarly high (40%–50%). Moreover, we found environmental TR_{34}/L98H azole-resistant *Aspergillus fumigatus* isolates in two separate patients.

Conclusions: In this series, 19.7% (*n* = 13/66) of mechanically ventilated SARS-CoV-2 patients were diagnosed with CAPA. In addition, we found a significant reduction in COVID-19 patients needing mechanical ventilation on the ICU in the second wave. Numbers are too small to determine whether there is a true difference in CAPA incidence in mechanically ventilated patients between the two waves, and whether it could be attributed to dexamethasone SARS-CoV-2 therapy.

KEYWORDS
Aspergillus fumigatus, CAPA, dexamethasone, ICU, pulmonary aspergillosis, TR_{34}L98H

1 | INTRODUCTION

COVID-19–associated pulmonary aspergillosis (CAPA) is a recently described disease entity being mainly reported in the Intensive Care Unit (ICU), also affecting immunocompetent patients. Recently published small case series describe an overall high incidence of CAPA in SARS-CoV-2-positive patients admitted to the ICU with acute respiratory distress syndrome requiring mechanical ventilation. The United Kingdom,[1,2] The Netherlands,[3,4] Belgium,[5] Germany,[6] Italy[7] and France[8,9] report incidences of 12.3%–33.3%. In Denmark,[10] a 25% (*n* = 2/8) incidence of invasive aspergillosis in COVID-19 patients undergoing extracorporeal membrane oxygenation (ECMO) was described, totalling 7.4% when adding the non-ECMO ICU population (*n* = 19). China,[11] Mexico[12] and Switzerland[13] report incidences of 8%, 9.7% and 3.8% in mechanically ventilated patients, respectively. In addition, Spain[14] and Pakistan[15] reported CAPA incidences of 3.3% and 21.7% in single studies, respectively, although patient data describing the use of mechanical ventilation were incomplete. Altogether, CAPA is associated with a high mortality of approximately 50% in these series, underscoring the importance of global awareness and early diagnosis.

The above-mentioned percentages of CAPA are alarming, keeping in mind that novel and mixed existing definitions[16–19] are used in diagnosing cases, often not confirmed by histopathology. The higher percentages are comparable with observations made in influenza, which is an independent risk factor for invasive pulmonary aspergillosis in the ICU setting.[20,21] However, clinically and mechanistically, influenza-associated pulmonary aspergillosis (IAPA) and CAPA are clearly distinct clinical entities.[16,22] More published data are needed to delineate the true incidence of CAPA in the ICU setting. Fortunately, the recently published CAPA 2020 ECMM/ISHAM consensus criteria[23] should provide clinical guidance and uniformity in classifying patients. Here, we report prospective findings from CAPA patients admitted to the ICU of a 500-bed teaching hospital in the Netherlands during the first and second waves of the COVID-19 pandemic during the year 2020.

2 | PATIENTS AND METHODS

In the first COVID-19 wave during a 2-month-period from March until May 1 2020, a total of 265 SARS-CoV-2 PCR-positive patients were admitted to our hospital of whom 33 needed intubation and mechanical ventilation. In the second wave, a 3-month-period from mid-September through mid-December 2020, 508 SARS-CoV-2 PCR-positive patients were admitted of whom 33 needed mechanical ventilation due to respiratory insufficiency. Data were prospectively collected in a study named 'Clinical course and prognostic factors for COVID-19', approved March 2020 by the Canisius Wilhelmina hospital medical ethics committee CWZ-nr 027-2020. CAPA classification (possible/probable) was performed by using the 2020 ECMM/ISHAM consensus criteria,[23] using a combination of microbiology, imaging and clinical factors. Statistical analyses were performed with SPSS statistics (IBM version 25). For the unpaired two-tailed t tests, a *p*-value of less than .05 was considered statistically significant.

2.1 | Diagnostics

SARS-CoV-2 PCR was performed by in-house PCR or by Cepheids GeneXpert Xpress SARS-CoV-2 PCR as described by Wolters et al.[24] Triazole susceptibility screening was done using VIPcheck™ (Mediaproducts BV). MICs of *Aspergillus fumigatus* isolates were determined with broth microdilution using CLSI standards.[25] Fungal PCR targeting the *Cyp51A* gene was done using AsperGenius™ (PathoNostics). 1-3 β-d-glucan (BDG) testing was done using the Fungitell assay (Associates of Cape Cod Inc). Galactomannan (GM) testing was done using Platelia Aspergillus (Bio-Rad) and/or Aspergillus lateral flow device (AspLFD, OLM Diagnostics).

3 | RESULTS

Case characteristics and diagnostics performed are presented in Table 1. Of the 33 admitted patients to our ICU in the first wave, 15.2% (*n* = 5) developed possible or probable CAPA. In the second wave out of 33 patients, 24.2% (*n* = 8) developed possible or probable CAPA, totalling 19.7% (13/66) in both waves combined. We found a significantly lower percentage of admitted COVID-19 patients needing mechanical ventilation on the ICU in the second wave (33/265 vs 33/508; *p*-value < .01) with a non-significant increase in CAPA patients in the second wave (5/33 vs 8/33; *p*-value = .36). Mortality between groups was similar (2/5 vs 4/8; *p*-value = .75). We found two patients (15.4%) with an environmental TR_{34}/L98H azole-resistant *Aspergillus fumigatus* isolate.

4 | DISCUSSION

In this series, we found a significant reduction in COVID-19 patients needing mechanical ventilation on the ICU in the second wave. This reduction is probably partly attributable[26] to the 10-day 6 mg intravenous dexamethasone SARS-CoV-2 therapy[27] introduced after the first wave, indicated for patients with severe COVID-19 and associated symptoms longer than 7 days. From these patients, however, a larger percentage were diagnosed with CAPA in the second wave (24.2% vs 15.2%), although not significant. Prolonged use of corticosteroids is known to be a risk factor for invasive fungal disease.[17] However, numbers are too small to determine whether there is a true difference in CAPA incidence in mechanically ventilated patients between the two waves, and whether it could be attributed to dexamethasone SARS-CoV-2 therapy. None of the CAPA patients in this case series had prior immuno-compromising conditions, and diabetes mellitus was not overrepresented in either group.

TABLE 1 CAPA patient cohort characteristics and diagnostics

First wave COVID-19, March through May 2020					
Characteristics	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Patient #5**
CAPA classification.[23]	Possible	Possible	Possible	Possible	Probable
Gender	Male	Male	Male	Female	Male
Age (years)	70	74	68	74	65
Medical history	-Diabetes mellitus type 2 -Proctitis ulcerosa -Lacunar infarction left hemisphere	-Hypertension -Colitis ulcerosa -3rd degree AV block (pacemaker)	-Diabetes mellitus type 2 -Mitral valve insufficiency -Hyperthyroidism	Polyarthrosis	None
Immuno-compromising condition	None	None	None	None	None
Symptom onset (days before ICU admission)	Day-13: fever, dyspnoeic	Day-5: fever, dry cough, malaise	Day-15: fever, dry cough, muscle ache, dyspnoeic	Day-16: fever, dry cough, dyspnoeic. Day-13: diarrhoea	Day-7: malaise, dyspnoeic
ICU admission duration	23 days	18 days (death)	32 days	18 days (death)	30 days
Acute renal failure	Yes: CVVH	Yes: CVVH	Yes: CVVH	Yes: CVVH	No
Imaging	X-ray findings related to COVID-19	CT chest findings related to COVID-19	CT chest findings related to COVID-19	CT chest findings related to COVID-19	CT chest findings related to COVID-19. Additional developing cavitary lesion right lower lobe suspect for fungal disease
ARDS					
Prone positioning	Yes	No	Yes	Yes	Yes
ECMO	No	No	No	No	No
Microbiology					
Serum GM (>0.5)	Negative (<0.1)	Negative (0.2)	Negative (<0.1)	Negative (<0.1)	Negative (<0.1)
Fungal culture	TA Aspergillus fumigatus	TA Aspergillus fumigatus	TA Aspergillus fumigatus	TA Aspergillus fumigatus	BAL: Aspergillus fumigatus
Susceptibility	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)	VIPcheck™ positive: Voriconazole I, Itraconazole R, Posaconazole I	Azole susceptible (VIPcheck™ negative)
Fungal PCR	N/A	N/A	N/A	PCR TA: Aspergillus fumigatus TR_{34}/L98H	N/A

(Continues)

TABLE 2 (Continued)

First wave COVID-19, March through May 2020					
Characteristics	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Patient #5**
TA/BAL GM (≥1)	TA: positive (4.7)	TA: positive (4.7)	TA: negative (0.5)	TA: positive (>3.0)	BAL: negative (0.3)
TA/BAL AspLFD	TA: positive (<15 min)	TA: negative (45 min)	TA: positive (<15 min)	TA: positive (<15 min)	BAL: negative
BDG	N/A	N/A	N/A	1590 pg/ml	N/A
Virology	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive
SARS-CoV-2 therapy	Hydroxychloroquine	None	Hydroxychloroquine	Hydroxychloroquine	Hydroxychloroquine
Antifungal therapy	Voriconazole	Voriconazole	Voriconazole	Empiric caspofungin/ voriconazole; L-amphotericin B	Voriconazole

Second wave COVID-19, mid-September through mid-December 2020								
Characteristics	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Patient #5**	**Patient #6**	**Patient #7**	**Patient #8**
CAPA classification [23]	Possible	Probable	Probable	Probable	Probable	Probable	Probable	Probable
Gender	Male	Male	Male	Male	Male	Female	Male	Female
Age (years)	67	56	77	65	54	59	68	78
Medical history	None	None	-Angina pectoris -Hypertension -Diabetes mellitus type 2	-Myocardial infarction	-Carotid endarterectomy -Fontaine 3 peripheral arterial disease	-COPD gold II	-Multiple myocardial infarctions -Heart failure -Impaired renal function	None
Immuno-compromising condition	None	None	None	None	None	None	None	None
Symptom onset (days before ICU admission)	Day-5: malaise, sore throat, headache	Day-21: fever, dry cough, dyspnoeic	Day-4: fever, dry cough, dyspnoeic	Day-12: fever, dry cough, dyspnoeic	Day-6: fever, dry cough, dyspnoeic	Day-7: malaise, dry cough, dyspnoeic	Day-17: fever, malaise, dyspnoeic	Day-7: fever, malaise, throat ache, dyspnoeic
ICU admission duration	29 days (still admitted)	56 days	41 days	41 days (death)	13 days (death)	40 days (death)	30 days (death)	42 days
Acute renal failure	No	No	No	No	Yes: CVVH	No	Yes: CVVH	No

(Continues)

TABLE 2 (Continued)

Second wave COVID-19, mid-September through mid-December 2020								
Characteristics	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Patient #5**	**Patient #6**	**Patient #7**	**Patient #8**
Imaging	X-ray findings related to COVID-19	CT chest findings related to COVID-19. Additional developing nodular lesions suspect for fungal disease	CT chest findings related to COVID-19	CT chest findings related to COVID-19	CT chest findings related to COVID-19	CT chest findings related to COVID19	CT chest findings related to COVID-19	CT chest findings related to COVID-19
ARDS								
Prone positioning	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
ECMO	No	No	No	No	No	No	No	No
Microbiology								
Serum GM (>0.5)	Negative (<0.1)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Fungal culture	TA: Aspergillus fumigatus	BAL: Aspergillus fumigatus	BAL: Aspergillus fumigatus	BAL: Aspergillus fumigatus	BAL: Aspergillus fumigatus	BAL: Aspergillus fumigatus	BAL: Aspergillus fumigatus	BAL: Aspergillus fumigatus
Susceptibility	Azole susceptible (VIPcheck™ negative)	VIPcheck™ positive: Voriconazole R, Itraconazole R, Posaconazole I	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)	Azole susceptible (VIPcheck™ negative)
Fungal PCR	PCR TA: Aspergillus fumigatus	PCR BAL: Aspergillus fumigatus TR_{34}/L98H	N/A	PCR BAL: Aspergillus fumigatus	N/A	PCR BAL: Negative	PCR BAL: Aspergillus fumigatus	PCR BAL: Aspergillus fumigatus
TA/BAL GM (≥1)	TA: positive (3.5)	BAL: negative 0.3	BAL: negative 0.5	BAL: positive > 5.9	N/A	BAL: negative 0.1	BAL: negative 0.2	BAL: positive 4.3
TA/BAL AspLFD	TA: negative	BAL: Positive (<15 min)	BAL: positive (<15 min)	BAL: positive (<15 min)	BAL: positive (<15 min)	BAL: negative	BAL: positive (<15 min)	BAL: positive (<15 min)
BDG	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Virology	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive	SARS-CoV-2 positive
SARS-CoV-2 therapy	Dexamethasone Remdesivir	Dexamethasone	Dexamethasone Remdesivir	Dexamethasone	Dexamethasone	Dexamethasone	Dexamethasone	Dexamethasone Remdesivir

(Continues)

TABLE 2 (Continued)

Second wave COVID-19, mid-September through mid-December 2020								
Characteristics	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Patient #5**	**Patient #6**	**Patient #7**	**Patient #8**
Antifungal therapy	Voriconazole	Empiric caspofungin/ voriconazole; L-amphotericin B	Empiric caspofungin/ voriconazole; L-amphotericin B	L-amphotericin B; voriconazole	Voriconazole	Voriconazole	Empiric caspofungin/ voriconazole; Voriconazole	Empiric caspofungin/ voriconazole; L-amphotericin B

Note: BDG testing: Fungitell assay, Associates of Cape Cod Inc, East Falmouth, MA, USA; Fungal PCR: AsperGenius™, PathoNostics, Maastricht, the Netherlands; GM testing: Platelia Aspergillus, Bio-Rad, Marnes-La-Coquette, France; Triazole susceptibility testing: VIPcheck™, Mediaproducts BV, Groningen, The Netherlands; Aspergillus lateral flow device: AspLFD, OLM diagnostics, Dagenham, United Kingdom.

Abbreviations: BAL, Bronchoalveolar lavage; BDG, 1-3 β-d-glucan; CVVH, continuous venovenous hemofiltration; ECMO, extracorporeal membrane oxygenation; GM, galactomannan; TA, Tracheal aspirate.

In the first COVID-19 wave, tracheal aspirates were used instead of bronchoalveolar lavage (BAL) as the use of bronchoscopy had been restricted in the COVID-19 setting.[28,29] This likely explains the difference in possible versus probable CAPA classification in patients from the first and second waves. The consensus case definition of IAPA/CAPA from Verweij and colleagues were adapted for clinical decision making before the 2020 ECMM/ISHAM consensus criteria[23] were published, and yielded a similar number of CAPA patients in our cohort.[16] In line with the majority of other published case series, the EORTC/MSGERC consensus definition[17] and *Asp*ICU algorithm[18] for diagnosing invasive aspergillosis were unsuitable.[19,20,30] The 15.2% incidence of CAPA in our ICU in the first wave is comparable to the 19.4%[3] and 21.4%[4] published recently from other Dutch centres. These centres employ similar treatment regimes, and avoid empiric fungal prophylaxis. Of note, van Biesen et al.[4] used a novel, non-directed, BAL method and did not apply existing diagnostic algorithms, making direct comparison with this study difficult.

By combining published case series, 16.5% (n = 151/917) of all SARS-CoV-2 patients requiring mechanical ventilation in the ICU developed CAPA (Table 2). The 47% (n = 62/132) mortality found in these case series is comparable to a recent study who report 52.5% mortality including both case series and case reports.[31] Not all patients were discharged from the hospital in several studies, possibly underestimating mortality. In IAPA, a similar overall mortality has been described (51%),[21] with a subset of IAPA tracheobronchitis patients having a reported mortality of over 90%.[32] To our knowledge, invasive tracheobronchitis has not been reported in CAPA patients, underscoring how IAPA and CAPA are mechanistically distinct clinical entities.

In diagnosing CAPA, little was known on the performance of serum GM and the 'panfungal' marker BDG. Serum GM testing in neutropenic non-CAPA patients with proven invasive aspergillosis has been shown to have a sensitivity of around 70%, and 25% in the non-neutropenic host.[33] CAPA patients are generally non-neutropenic and sensitivity of serum GM reported in these patients are similarly low (15.6%–21%).[30,31] Whilst BDG testing is nonspecific, its sensitivity in the ICU population for invasive fungal disease has been shown to be high (88%).[34] Combining the case series reported, we found that only 20.9% had a positive serum GM (Table 2). In contrast, serum BDG was reported positive in 75.8% of CAPA patients tested (Table 2) albeit infrequently reported. Altogether, only 60.2% and 21.8% of mechanically ventilated suspected CAPA patients were tested for serum GM and serum BDG, respectively. In the first wave of our prospective case series, none of the patients had a positive serum GM. Because of the apparent low sensitivity of serum GM in this cohort and the lacking specificity of serum BDG, our hospital decided to limit the use of serum GM and BDG in the second wave.

Epidemiological data dominate the choice of primary antifungal therapy, whilst development of resistance to antifungals in *Aspergillus* species is a growing concern.[35] Based on surveillance data, azole-resistance in the Netherlands has been estimated to be around 11%,[36,37] in line with our findings in this series. Therefore, if *A fumigatus* susceptibility is unknown, empiric treatment is started with an echinocandin plus voriconazole or liposomal amphotericin B if toxicity

TABLE 2 Overview of CAPA patients in published case series

Country	Diagnostic criterium CAPA	Patients CAPA/ total cohort (%)	Serum GM positive/ tested (%)	Serum BDG positive/ tested (%)	Mortality (%)
Belgium[5]	Verweij et al.	7/34 (20.6)	0/3 (0)	N/A	4/7 (57.1)
China[11]	EORTC/MSGERC	4/50 (8)	N/A	N/A	N/A
Denmark[10]	AspICU algorithm	2/27 (7.4)	0/2 (0)	N/A	2/2 (100)
France[8]	AspICU algorithm	19/106 (17.9)	1/12 (8.3)	N/A	7/19 (36.8)
France[9]	N/A[a]	9/27 (33.3)	1/9 (11.1)	4/8 (50)	4/9 (44.4)
Germany[6]	AspICU algorithm	5/19 (26.3)	2/5 (40)	N/A	3/5 (60)
Italy[7]	Verweij et al.	30/108 (27.8)	1/16 (6.3)	N/A	13/30 (43.3)
Mexico[12]	AspICU algorithm	14/144 (9.7)	6/14 (42.9)	N/A	8/14 (57.1)
Switzerland[13]	Verweij et al.	3/80 (3.8)	1/3 (33)	1/2[b] (50)	1/3 (33.3)
The Netherlands[3]	Verweij et al.	6/31 (19.4)	0/3 (0)	N/A	4/6 (66.7)
The Netherlands[4]	N/A[a]	9/42 (21.4)	N/A	N/A	2/9 (22.2)
The Netherlands - this case series	ECMM/ISHAM consensus criteria	13/66 (19.7)	0/6 (0)	1/1 (100)	6/13 (46.2)
United Kingdom[1]	Verweij et al.[a]	15/ 122(12.3)	2/3 (66.7)	7/7 (100)	8/15 (53.3)
United Kingdom[2]	AspICU algorithm	15/61 (24.6)	5/15 (33.3)	12/15 (80)	N/A
Total		151/917 (16.5)	19/91 (20.9)	25/33 (75.8)	62/132 (47.0)

Note: For better comparison, only mechanically ventilated patients were included from listed case series. Studies not adequately describing if patients were mechanically ventilated were excluded from this table.

Abbreviations: BDG, 1-3 β-d-glucan; CAPA, COVID-19–associated pulmonary aspergillosis; GM, Galactomannan.

[a]Novel criteria are discussed in these publications.

[b]Unpublished: personal communication with the authors, with gratitude.

or co-infection with Mucorales is suspected. From our cohort, two patients had an environmental TR_{34}/L98H azole-resistant isolate, one of these elsewhere published as a case report.[20] This mutation has also been identified in CAPA patients in Ireland,[38] France[39] and the UK[2] underscoring the challenges faced in patient management, the importance of early diagnostics and (inter)national surveillance programs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Eelco Meijer: Conceptualization (equal); Formal analysis (lead); Writing-original draft (equal). **Anton Dofferhoff:** Conceptualization (equal); Data curation (equal); Writing-review & editing (equal). **Oscar Hoiting:** Conceptualization (equal); Data curation (equal); Investigation (lead); Writing-review & editing (equal). **Jacques F. Meis:** Conceptualization (equal); Resources (lead); Supervision (lead); Writing-original draft (equal).

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