Prospective and systematic screening for invasive aspergillosis in the ICU during the COVID-19 pandemic: a proof of principle for future pandemics

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Prospective and systematic screening for invasive aspergillosis in the ICU during the COVID-19 pandemic, a proof of principle for future pandemics

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Abstract

The diagnostic performance of a prospective, systematic screening strategy for COVID-19 associated pulmonary aspergillosis (CAPA) during the COVID-19 pandemic was investigated. Patients with COVID-19 admitted to the ICU were screened for CAPA twice weekly by collection of tracheal aspirate (TA) for *Aspergillus* culture and PCR. Subsequently, bronchoalveolar lavage (BAL) sampling was performed in patients with positive screening results and clinical suspicion of infection. Patient data were collected from April 2020–February 2022. Patients were classified according to 2020 ECMM/ISHAM consensus criteria. In total, 126/370 (34%) patients were positive in screening and CAPA frequency was 52/370 (14%) (including 13 patients negative in screening). CAPA was confirmed in 32/43 (74%) screening positive patients who underwent BAL sampling. ICU mortality was 62% in patients with positive screening and confirmed CAPA, and 31% in CAPA cases who were screening negative. The sensitivity, specificity, positive and negative predictive value (PPV & NPV) of screening for CAPA were 0.71, 0.73, 0.27, and 0.95, respectively. The PPV was higher if screening was culture positive compared to PCR positive only, 0.42 and 0.12 respectively. CAPA was confirmed in 74% of screening positive patients, and culture of TA had a better diagnostic performance than PCR. Positive screening along with clinical manifestations appeared to be a good indication for BAL sampling since diagnosis of CAPA was confirmed in most of these patients. Prospective, systematic screening allowed to quickly gain insight into the epidemiology of fungal superinfections during the pandemic and could be applicable for future pandemics.

Introduction

COVID-19 associated pulmonary aspergillosis (CAPA) has been described as a complication of COVID-19 in critically ill patients admitted to the intensive care unit (ICU). Despite uncertainties regarding CAPA classification and pathogenesis,[1] most reports show that COVID-19 patients with CAPA have high mortality rates compared to patients without CAPA.[2–4] Since the start of the COVD-19 pandemic a CAPA screening programme was implemented in the ICU of our hospital consisting of twice weekly culture and PCR on tracheal aspirate (TA) as a routine procedure. The screening programme for CAPA was evaluated after the first COVID-19 peak (April 2020–May 2020) and was found to be a feasible and simple method to monitor patients.[5] Since the first peak many changes have taken place in both viral virulence and therapeutic management of COVID-19 in ICU patients.

Immunosuppressive agents now play an important role in the treatment of patients with COVID-19 including corticosteroids and interleukin-6 Inhibitors (anti-IL-6). Treatment with immunosuppressants is a known risk factor for invasive aspergillosis.[6] Therefore, an increased frequency of CAPA could be expected in COVID-19 periods when ICU patients were treated with immunosuppressive agents. At the same time SARS-CoV-2 variants have appeared with different virulence profiles.[7–9]

Therefore, the purpose of this study was to investigate the yield of screening for CAPA and the frequency of CAPA in the ICU over a 2-year period in which medical management of COVID-19 changed and new SARS-CoV-2 variants appeared. Also, the diagnostic performance of various diagnostic tests for CAPA screening and diagnosis in relation to mortality were investigated. This evaluation may aid in designing fungal infection monitoring programmes during future pandemics.

Methods

Study population

All adult patients with COVID-19 admitted to the ICU in the Leiden University Medical Center (LUMC, Leiden, The Netherlands) were screened for CAPA twice weekly by collecting TA for *Aspergillus* culture and PCR (Figure 1). Confirmation bronchoalveolar lavage (BAL) sampling was performed in patients with positive screening results if clinically indicated (respiratory deterioration not caused by a pulmonary

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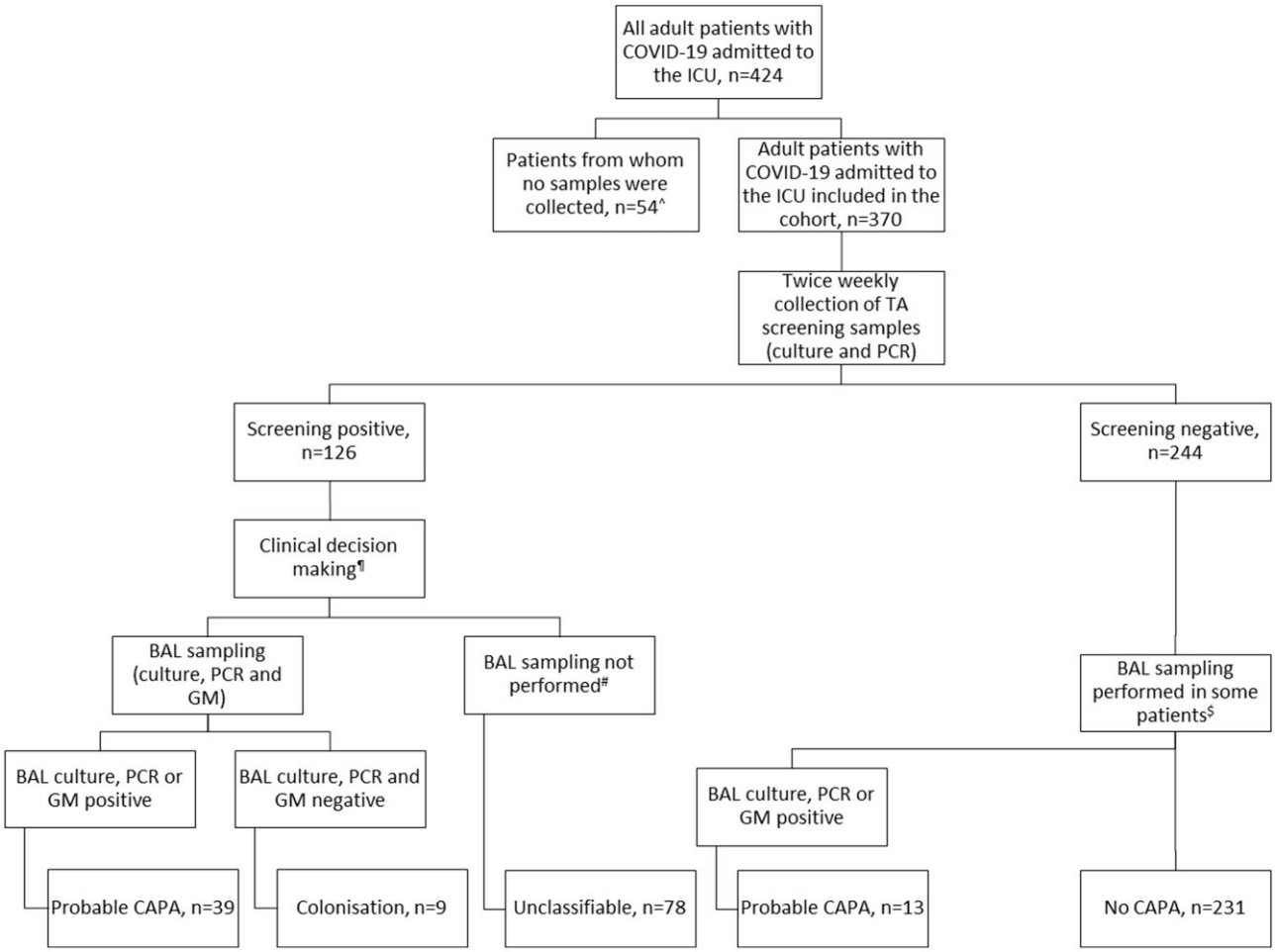


Figure 1. Screening method for detection of CAPA*. *According to 2020 ECMM/ISHAM consensus criteria.[11] ¶ Clinical decision-making was based on a combination of screening results and clinical manifestations (respiratory deterioration or clinical/radiological signs of infection). # BAL sampling was not performed if patients improved clinically, if there was another explanation for respiratory deterioration (pulmonary embolism for example), or if patients died. $ BAL sampling was performed in patients with negative screening if there was a clinical indication to perform a BAL (respiratory deterioration). ^ Of the 54 patients not included in the cohort median ICU stay was 3 days (IQR 2–5) and reason for discharge was transfer to another hospital $n = 13$, discharge to nursing department $n = 33$ or home $n = 1$, or death $n = 7$. CAPA: COVID-19 associated pulmonary aspergillosis, TA: tracheal aspirate, BAL: bronchoscopic alveolar lavage, GM: galactomannan.

embolism) and feasible (depending on oxygen saturation levels). *Aspergillus* culture, PCR and in some cases galactomannan (GM) were performed on BAL samples. The screening programme was implemented as temporary routine care.

From September 2020 patients with COVID-19 were treated with dexamethasone and from January 2021 with dexamethasone and tocilizumab.[10] In patients who deteriorated with an unknown cause, hyperinflammation was suspected and these patients were mostly treated with methylprednisolone.

Culture, susceptibility testing, PCR, resistance PCR, and GM

Methods of culturing, susceptibility testing, (resistance) PCR testing, and GM testing have been described in a previous manuscript.[5] In short, TA and BAL samples were inoculated on various agar plates and incubated for 2–10 days. Undiluted samples were inoculated onto culture media, using a one µl sterile inoculation needle. For TA samples, this was done directly, for BAL samples this was performed after sedimentation of undiluted sample. Triazole resistance screening was performed with VIPcheck™ and MIC testing was performed with the microbroth dilution method according to EUCAST. The PCR (AsperGenius®, PathoNostics®, Maastricht, The Netherlands) was performed for detection of *Aspergillus fumigatus* complex, *Aspergillus terreus* and *Aspergillus* species, and TR34/L98H and Y121F/T289A resistance mutations. Galactomannan testing was performed using the Platelia™ *Aspergillus* Ag (Bio-rad laboratories, Marnes-la-Coquette, France).

Classification of patients (Figure 1)

CAPA classified according to the 2020 ECMM/ISHAM consensus criteria[11] was considered the gold standard. Patients were classified according to the 2020 ECMM/ISHAM consensus criteria,[11] with one minor modification: all positive BAL PCR results were considered positive irrespective of cycle threshold value. Patients were considered colonised with *Aspergillus* if TA samples were *Aspergillus* positive, but confirmatory BAL samples remained negative. Patients were considered unclassifiable if screening was positive but no BAL was performed. A BAL was not performed in patients in

whom CAPA was not suspected at all or in patients who were too ill for bronchoscopy. When comparing patients with CAPA to patients without CAPA, the group of unclassifiable and colonised patients were included in the no CAPA group. According to our local screening strategy, BAL sampling was performed in patients with a clinical suspicion of CAPA, and therefore unclassifiable patients were included in the group of patients with no CAPA. A BAL performed within 3 days after positive screening was considered a confirmatory BAL. In our centre non-bronchoscopic lavages were not performed, thus patients could not be classified as possible CAPA.

Data collection

From the laboratory information system (LIMS) data were extracted about culture, susceptibility, PCR, and GM results from April 2020 until February 2022. Clinical data about age, sex, admission to the ICU of the Leiden University Medical Center, and mortality were extracted from electronic hospital databases.

SARS-CoV-2 variants

To describe the dominant variant at different time points, data from national[7] and local pathogen surveillance, and SNP typing were investigated. Structural pathogen surveillance has been performed since June 2021. Before then, SNP typing was performed for research purposes.[12]

Ethics

The study was approved by the hospitals' Institutional Review Board, the COVID-committee (CoCo): CoCo 2020-044. Patients were only included if they had consented via an approved opt-out procedure active in our institution. If patients were not able to consent because they were intubated, the opt-out procedure for clinical data for the National Intensive Care Evaluation (NICE) was applied.

Statistical analyses

Screening outcome, CAPA frequency and concordance between screening outcome and CAPA classification were the primary study outcome parameters. This was assessed by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of screening for CAPA. Secondary outcomes included time to event (ICU admission until collection of TA and until first positive TA or BAL sample), ICU mortality, and 60-day mortality.

Categorical variables were described as numbers and percentages per category, numerical continuous variables were described as medians and interquartile ranges. Categorical data were compared with the Fisher's exact test or Chi-square test depending on sample size and numerical data were compared with the Mann Whitney U-test (two groups) or Kruskal-Wallis test (> two groups). Proportion confidence intervals were calculated with the Wilson score interval. Kaplan-Meier survival curves for 60-day mortality were constructed and groups were compared using the log-rank test. ICU mortality was defined as in-hospital ICU mortality (31 patients, of whom 3 were classified as CAPA, were transferred to another ICU and censored at discharge). Statistical analyses were performed with IBM SPSS Statistics for Windows version 25.0.

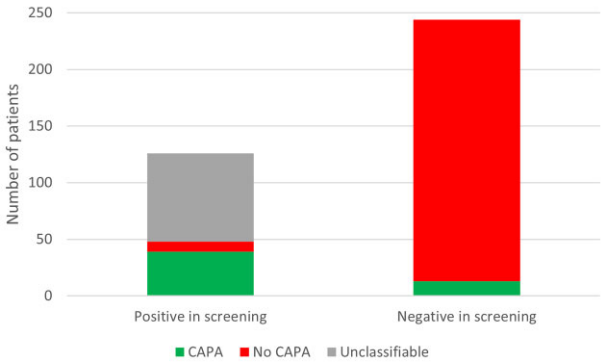


Figure 2. Screening outcome and CAPA* classification. * According to 2020 ECMM/ISHAM consensus criteria.[11] CAPA: COVID-19 associated pulmonary aspergillosis, screening: screening by collection of tracheal aspirate for culture and PCR. Patients were considered unclassifiable if screening was positive but no BAL was performed. Of note: CAPA was diagnosed in 32/43 (74.4%) of patients in whom BAL was performed within 3 days, in 32/126 (25.4%) of screening positive cohort, and in 32/370 (8.6%) of total cohort.

Results

Screening outcome and CAPA frequency

From April 2020 until February 2022 370 patients were routinely screened for CAPA from a total number of 424 patients with COVID-19 admitted to the ICU (Figure 1). The cohort consisted out of 270 (73%) males and median age was 62.5 years (IQR: 54–70). Of the patients who were screened 126/370 (34.1%) were positive in screening. Patients positive in screening were older than patients negative in screening (64.5 vs 60 years, $P = 0.037$). The median time from ICU admission at our medical centre until collection of first TA sample was one day and until collection of the first positive TA was 3 days. A BAL was performed within 3 days of positive screening in 43/126 (34.1%) of these patients and CAPA was confirmed in 32/43 (74.4%) (Figure 2).

Overall CAPA frequency was 52/370 (14.1%), and 32/52 (62%) cases were diagnosed through the screening programme. CAPA cases not detected by screening had a positive BAL sample obtained more than 3 days after a positive TA sample (seven patients) or were negative in screening (13 patients).

Influence of CAPA treatment regimen and viral subtype on screening outcome

Figure 3depicts screening outcome and CAPA classification[11] by COVID-19 period based on viral subtype dominance and standard treatment regimen (see also Supplemental Table S1). The frequency of screening positive patients was higher in COVID-19 periods when patients were treated with immunosuppressive agents (from June 2020 onwards), but not statistically significant ($P = 0.451$). Frequency of CAPA varied from 9.7–18.6% in the different COVID-19 periods without a statistically significant difference between the groups (proportion CAPA vs no CAPA/unclassifiable patients, $P = 0.385$).

Diagnostic value of screening

In the patients with a positive TA sample, PCR was positive more often than culture (91% and 51%, respectively) (Supplemental Table S2). The PPV and NPV of screening can

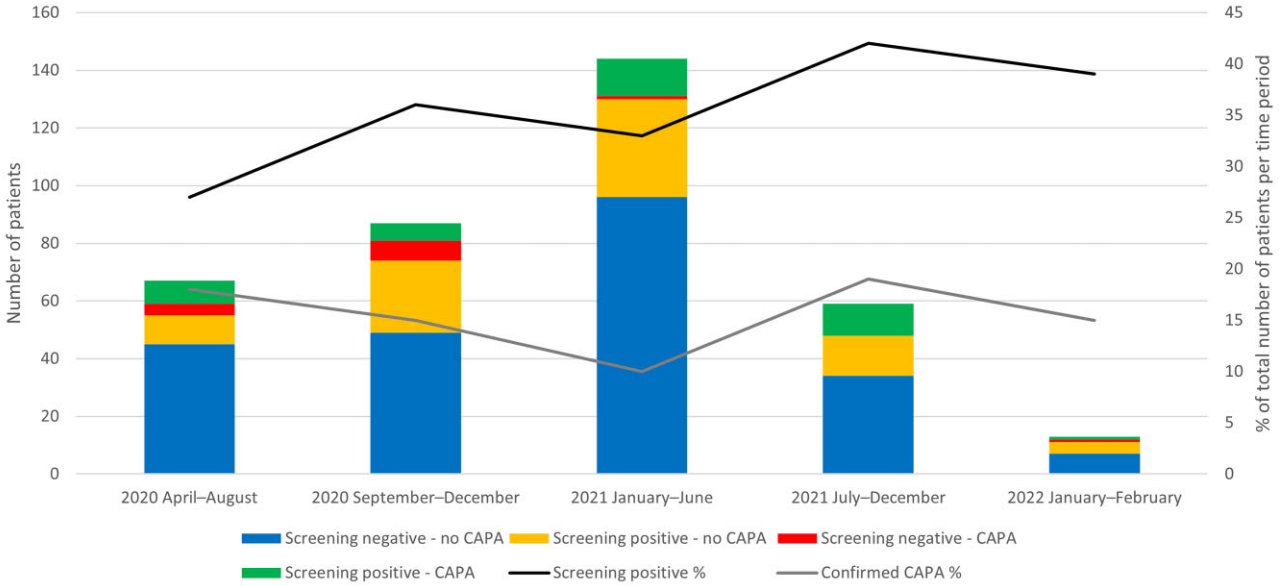


Figure 3. Screening outcome and CAPA* classification per COVID-19 period¶. * According to 2020 ECMM/ISHAM consensus criteria.[11] ¶ Based on viral subtype dominance and standard treatment regimen: 2020 April–August [baseline], 2020 September–December [corticosteroids], 2021 January–June [viral subtype Alpha, corticosteroids + anti-IL-6], 2021 July–December [viral subtype Delta, corticosteroids + anti-IL-6], and 2022 January–February [viral subtype Omicron BA1, corticosteroids + anti-IL-6]. Anti-IL-6: interleukin-6 Inhibitors, CAPA: COVID-19 associated pulmonary aspergillosis, screening: screening by collection of tracheal aspirate for culture and PCR. The group 'screening positive - no CAPA' consisted out of patients in whom CAPA was ruled out by negative BAL results $n = 9$ and patients who were unclassifiable $n = 78$.

Table 1. Sensitivity, specificity, positive, and negative predictive value of screening for CAPA* with TA.

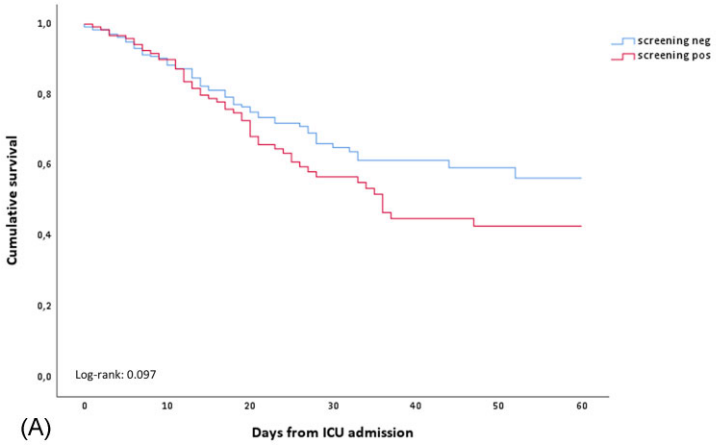
	CAPA, *n* (%)	No CAPA, *n* (%)	Total, *n* (%)	Sensitivity	Specificity	PPV	NPV
Screening total							
Screening positive	32 (71)	87 (27)	119 (33)				
Screening negative	13 (29)	231 (73)	244 (67)				
Total	45 (100)	318 (100)	363 (100)	32/45, 0.71	231/318, 0.73	32/119, 0.27	231/244, 0.95
Screening culture positive							
Screening positive	24 (65)	33 (13)	57 (19)				
Screening negative	13 (35)	231 (88)	244 (81)				
Total	37 (100)	264 (100)	301 (100)	24/37, 0.65	231/264, 0.88	24/57, 0.42	231/244, 0.95
Screening only PCR positive							
Screening positive	8¶ (38)	54 (18)	62 (20)				
Screening negative	13 (62)	231 (81)	244 (80)				
Total	21 (100)	285 (100)	306 (100)	8/21, 0.38	231/285, 0.81	8/62, 0.12	231/244, 0.95

*According to 2020 ECMM/ISHAM consensus criteria.[11] ¶Of the eight patients who were only PCR screening positive, three had a culture positive TA sample at another moment and in the remaining five TA were never culture positive. CAPA: COVID-19 associated pulmonary aspergillosis, NPV: negative predictive value, PPV: positive predictive value, TA: tracheal aspirate.
A BAL performed within 3 days after positive screening was considered a confirmatory BAL for CAPA diagnosis. Patients classified as CAPA with a positive BAL sample collected at another moment were excluded from the analysis (screening positive with a negative confirmatory BAL sample ($n = 2$) or no BAL collected within 3 days after positive screening ($n = 5$)).

be found in Table 1. The PPV and NPV of screening for different hypothetical attack rates of CAPA can be found in Supplemental Table S3. CAPA cases negative in screening were most often only GM positive in BAL (11 of 13 cases).

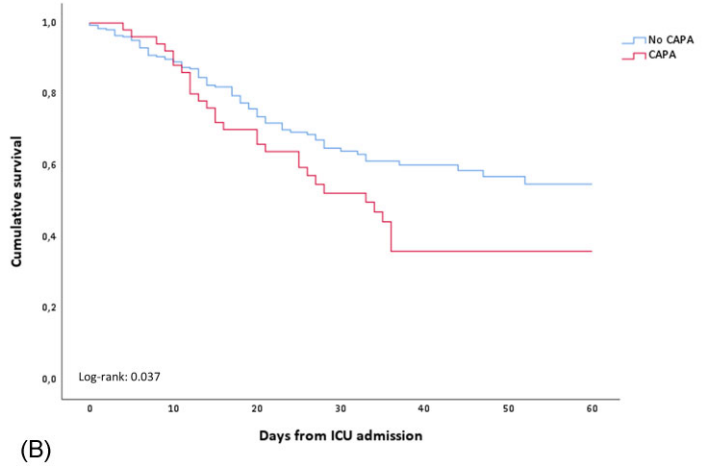
Screening and mortality

Overall ICU mortality was 119/423 (28.1%) and mortality in patients who were screening positive was 49/126 (38.9%). ICU mortality in patients with confirmed CAPA was 28/52 (53.8%) and was higher than patients with no CAPA/unclassifiable patients 84/318 (26.4%), $P < 0.001$. ICU mortality in the CAPA population missed by TA screening was 4/13 and was lower than mortality in patients with CAPA detected by screening (31% vs 62% $P = 0.054$, Supplemental Table S4). Kaplan-Meier survival curves of patients positive or negative in screening and patients with or without CAPA are depicted in Figure 4. ICU mortality in patients with no CAPA and unclassifiable patients was similar (61/240 (25.4%) vs 23/78 (29.5%), $P = 0.479$), whilst a difference was found in patients with CAPA and unclassifiable patients (28/52 (53.8%) vs 23/78 (29.5%), $P = 0.005$). Kaplan-Meier survival curves of patients with or without CAPA or unclassifiable patients are depicted in Supplemental Figure S1.



(A)

	Day 0-10	Day 10-20	Day 20-30	Day 30-40	Day 40-50	Day 50-60	Day 60
Screening negative (number/ death/censored)	244/22/38	184/24/55	105/12/33	60/4/19	37/1/14	22/1/7	14/0/14
Screening positive (number/ death/censored)	126/12/11	103/18/20	65/13/15	37/7/4	26/1/7	18/0/5	13/0/13



(B)

	Day 0-10	Day 10-20	Day 20-30	Day 30-40	Day 40-50	Day 50-60	Day 60
No CAPA (number/death/	318/30/47	241/31/74	136/17/43	76/5/21	50/2/18	30/1/9	20/0/20
CAPA (number/death/	52/4/2	46/11/1	34/8/5	21/6/2	13/0/3	10/0/3	7/0/7

Figure 4. a. Life table and Kaplan-Meier survival curves of patients who were positive and negative in screening (60-day mortality from ICU admission). Neg: negative, pos: positive. **b.** Life table and Kaplan-Meier survival curves of patients classified as CAPA∗ and patients without CAPA or unclassifiable patients (60-day mortality from ICU admission). ∗ According to 2020 ECMM/ISHAM consensus criteria.[11]

Discussion

From April 2020 until February 2022 34% of COVID-19 ICU patients in our hospital were culture or PCR positive in CAPA screening. In approximately 1/3 of patients a BAL was performed within 3 days after positive screening results and CAPA was confirmed in most of these patients (32/43 (74%)). Overall CAPA frequency was 14%. COVID-19 treatment regimen or viral subtype had no effect on screening outcome or CAPA frequency.

Screening for CAPA had a high NPV of 0.95. The overall PPV was 0.27 and was higher for culture-positive TA screening samples (with or without positive PCR) than for PCR positive only TA screening samples (0.42 and 0.12, respectively). Based on our findings, screening by culture alone seems acceptable. The PPV of a positive TA culture was 0.58 in another study,[13] but definitions for CAPA included possible cases based on TA GM as well, complicating a comparison. Also, predictive values are by definition dependent on disease prevalence in a population. The observed CAPA attack rate of 14% was similar to previous (Dutch) studies,[2,14–16] but higher than the prevalence reported in a systemic review and meta-analysis (10% (95%-CI: 8–13%) with wide variation among studies (0–34%)).[4] Hence, local validation of a screening strategy is mandatory. Screening by culture is a cheaper alternative than screening by PCR, but the time-to-result will be reduced by PCR.

Given the fact that *Aspergillus* and other invasive fungal infections can complicate various viral respiratory diseases, we calculated NPV and PPV of our screening strategy for different hypothetical frequencies of IA following viral infection (see Supplemental Table S3). However, our screening strategy included clinical decision making regarding BAL follow up diag-

nostics, which was influenced by increasing knowledge about the disease. We acknowledge that this factor can be variable between hospitals, even within a country and also over time during a pandemic.[2] Therefore, our strategy cannot simply be adopted to screen for IA in the context of viral pandemic preparedness. The high percentage of confirmed CAPA (74% of patients undergoing confirmatory testing) demonstrates that in most patients the indication for a BAL was correct. Screening might reduce the indications for BAL sampling. However, the effect on reduction of BAL sampling is unknown since there was no comparator arm.

Most CAPA patients missed by screening were only BAL GM positive (11/13). Diagnosis of CAPA in this group remains uncertain given that mortality was low compared to patients with BAL culture and/or PCR confirmed CAPA (33%, 61%, and 64%, respectively). Nevertheless, diagnosis of CAPA even remains uncertain in patients with culture and/or PCR confirmed CAPA as histopathological evidence of CAPA is limited. Lower mortality in patients with only BAL GM confirmed CAPA has been observed before[2] questioning this diagnostic marker for CAPA if it is the only positive test result. No association was found between BAL GM concentration and 30-day ICU mortality in a study that compared Aspergillus test profiles of CAPA patients and controls.[17] Another study showed that BAL GM was often found as an isolated marker in patients with CAPA and poorly repeatable in sequential samples.[18] Whether a GM only result is a consequence of test aspecificity or a marker of low fungal burden is unknown.

Whether screening results in lower mortality due to earlier diagnosis of CAPA cannot be concluded given the observational nature of our study. In a multicentre study, that compared hospitals with a pre-emptive screening strategy for CAPA to those with a reactive diagnostic strategy, no benefit of screening was observed.[2] However, as it was a retrospective study screening protocols varied per hospital and because it was observational, bias or confounding is likely. Furthermore, mortality is high in patients with COVID-19 with CAPA, but determining the attributability of IA in the cause of death remains difficult.[19]

Moreover, in the case of CAPA, there is debate about the clinical entity itself.[20] This is due to the variably reported attack rate of CAPA,[1,4,20] the limited histopathological evidence of IA[21,22] and the observation that treatment with an antifungal agent did not improve the outcome in CAPA cases.[2,23] A number of studies have suggested that CAPA should probably be seen as a disease with a continuous spectrum from colonisation to tissue invasion and angioinvasion in which multiple factors contribute to *Aspergillus* becoming invasive.[17,24] However, distinguishing CAPA patients with IPA from those without IPA is challenging.[1] Besides knowledge on local epidemiology and clinical manifestations, combination testing might improve diagnosis of CAPA especially if tests show concordant results.[1,18] For the purpose of research standardisation, the 2020 ECMM/ISHAM consensus criteria[11] were urgently needed, but an improvement based on recent findings may be warranted given the limited specificity of BAL GM. Interestingly, after large scale COVID-19 vaccination, patients with CAPA are more likely to have an EORTC/MSGERC host factor for IA.[25]

The strength of our study is that our screening and confirmation strategy was unchanged over time, after some reluctance to perform BAL sampling in the first period. Hence, we present a reliable overview of frequency of positive screening and confirmed CAPA over time. Our study has a number of limitations. Firstly, our cohort includes patients who were unclassifiable because no BAL sampling was performed. We assumed that this wasn't done because there was no clinical CAPA suspicion and these patients were included in the group not classified as having CAPA but this may not be true in all cases. Secondly, the COVID-19 periods were based on viral subtype dominance and SARS-CoV-2 treatment regimens in that period. Clinical information was limited so on patient level exact treatment for COVID-19 and SARS-CoV-2 variant were unknown.

In conclusion, screening for CAPA by regular and systematic collection of TA is a feasible and simple bedside method to monitor COVID-19 patients admitted to the ICU and can be performed by culture alone. A pre-emptive screening strategy for CAPA or other fungal superinfections of severe viral respiratory tract infections should include screening results in combination with clinical decision making, and should ideally be standardised. Further research is necessary to improve accuracy of CAPA diagnosis and to evaluate whether screening improves survival.

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None.

Author contributions

Rebecca van Grootveld (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing), Judith van Paassen (Investigation, Methodology, Writing – review & editing), Eric C.J. Claas (Investigation, Methodology, Writing – review & editing), Laura Heerdink (Data curation, Writing – review & editing), Ed J. Kuijper (Investigation, Methodology, Writing – review & editing), Mark G.J. de Boer (Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing), and Martha T. van der Beek (Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing)

Supplementary material

Supplementary material is available at *Medical Mycology* online.

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Conflict of interest

None of the authors have a conflict of interest to declare.

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