Medical Mycology, 2022, 60, myac026
https://doi.org/10.1093/mmy/myac026
Advance Access Publication Date: 8 April 2022
Original Article

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Usefulness of Sōna *Aspergillus* Galactomannan LFA with digital readout as diagnostic and as screening tool of COVID-19 associated pulmonary aspergillosis in critically ill patients. Data from a multicenter prospective study performed in Argentina

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Received 16 November 2021; Revised 17 February 2022; Accepted 6 April 2022; Editorial Decision 8 March 2022

Abstract

COVID-19-associated pulmonary aspergillosis (CAPA) incidence varies depending on the country. Serum galactomannan quantification is a promising diagnostic tool since samples are easy to obtain with low biosafety issues. A multicenter prospective study was performed to evaluate the CAPA incidence in Argentina and to assess the performance of the lateral flow assay with digital readout (Sōna *Aspergillus* LFA) as a CAPA diagnostic and screening tool. The correlation between the values obtained with Sōna *Aspergillus* LFA and Platelia® EIA was evaluated. In total, 578 serum samples were obtained from 185 critically ill COVID patients. CAPA screening was done weekly starting from the first week of ICU stay. Probable CAPA incidence in critically ill patients was 10.27% (19/185 patients when LFA was used as mycological criteria) and 9% (9/100 patients when EIA was used as mycological criteria). We found a very good correlation between the two evaluated galactomannan quantification methods (overall agreement of 92.16% with a Kappa statistic value of 0.721). CAPA diagnosis (>0.5 readouts in LFA) were done during the first week of ICU stay in 94.7% of the probable CAPA patients. The overall mortality was 36.21%. CAPA patients' mortality and length of ICU stay were not statistically different from for COVID (non-CAPA) patients (42.11 vs 33.13% and 29 vs 24 days, respectively). These indicators were lower than in other reports. LFA-IMMY with digital readout is a reliable tool for early diagnosis of CAPA using serum samples in critically ill COVID patients. It has a good agreement with Platelia® EIA.

Lay Summary

The incidence of COVID-associated pulmonary aspergillosis (CAPA) in critically-ill Argentinian patients was established (10.27%). Serum galactomannan quantification was useful as a screening tool for this mycosis. A good agreement between Platelia® EIA and Sōna *Aspergillus* LFA is reported.

Key words: CAPA, aspergillosis, COVID-19, Galactomannan, lateral flow.

Introduction

As for October 20th 2021, more than 240 million cases of coronavirus disease 2019 (COVID-19) were reported worldwide.[1]

COVID-19-associated pulmonary aspergillosis (CAPA) was reported as a new clinical complication in critically ill patients[2] with a reported incidence ranging from 3.5 to more than 26% with geographical variations.[3–9] In these patients, diagnosis is challenging. Radiological findings are nonspecific and bronchoalveolar lavages (BAL) for microbiology studies are usually not available. In addition, as in any non-neutropenic patients, galactomannan (GM) quantification and interpretation is controversial. These facts raised questions about the used criteria for CAPA diagnostics.[10–12]

One recent improvement in invasive aspergillosis diagnosis is the commercialization of a specific GM-lateral flow assay (LFA). These devices demonstrated excellent performance, becoming a viable option when the well-established EIA GM quantification is not available.[13,14] Moreover, these LFA devices reduced the turnaround time and cost.[15]

Although some reports were recently published[16–18] the incidence of CAPA in Argentina is barely known.[19] Data about this subject is important considering that Argentina is a big country, with important differences in access to the health system related to economic and demographic issues. A mixed health system (public, semi-public and private) has access to different techniques.

The aims of this work were to evaluate the CAPA incidence in Argentina and to assess the performance of the GM lateral flow assay (Sōna *Aspergillus* LFA-IMMY®) as a CAPA diagnostic and screening tool. A prospective multicenter study, including Hospitals and referral centers from diverse Argentinian regions with different economic and demographic characteristics, was performed for this purpose.

Methods

Participating center, inclusion criteria, and patient data

In this work, we studied samples obtained in three public hospitals from Buenos Aires city (Clínicas-UBA Hospital, Durand Hospital and Ramos Mejía Hospital) and in three referral regional centers, one from the northwest Argentina (Tucumán), one from the northeast region (Chaco-Corrientes) and one from central Argentina (Santa Fe-Entre Ríos). These three referral regional centers received samples from eight different regional Hospitals (public, semi-public and private hospitals). Moreover, Muñiz Hospital participated in processing samples obtained at the Durand Hospital (no patients were enrolled in Muñiz Hospital). All the participating centers were managed and financed by state governments with the exception of three centers, semi-public (n = 1) and private (n = 2).

The study included critically ill adult patients (>18 years-old) with a COVID-19 positive RT-PCR SARS-CoV-2 test admitted to ICU with respiratory support. These patients were admitted between March 15th 2020 and October 15th 2020 during the so-called first wave of SARS-CoV-2 infections in Argentina.

Patient's clinical and demographic data were collected including: age, genre, underlying disease (if any), ICU admission and discharge (or death) dates, microbiology laboratory results (mycology and bacteriology studies), corticosteroid and antibiotic treatments received, type of respiratory support (tracheotomy, intubation or other assisted ventilation) and radiological findings.

Samples and diagnostics methods

Serum samples were obtained as follows: during the first two weeks of ICU stay, one sample per week (days 2 or 3 and 9 or 10 of ICU stay) were taken. In the following weeks, two serum samples were obtained. All serum samples (and BAL if received) were subjected to GM quantifications using IMMY´s SŌNA LFA (from now on: GM-LFA) (IMMY Diagnostics, OK, USA). Moreover, BAL samples (when available) were subjected to the routine diagnostic procedures including microscopic examination and culture. BAL pellets were cultured in two Sabouraud–chloramphenicol agar slants (incubated for 30 days at 28 and 37°C and examined every day). The rest of the resuspended pellet was used for microscopic examination (Giemsa stain and direct examination with and without calcofluor-white and with and without 20% KOH).[20] Furthermore, four of the participating centers performed GM quantification by EIA (Platelia®, Biorad, from now on: GM-EIA) in parallel (see below for further details). GM-LFA and GM-EIA were performed strictly following the manufacturer instructions. Cut-off values for GM-LFA and GM-EIA positivity were an index value of 0.5 and 1.0 for serum

and BAL, respectively.[21,22] SŌNA cube reader (IMMY diagnostics, OK, USA) was used to accurately obtain the readout results of the GM-LFA.

Used definitions of CAPA and study characteristics

This was a prospective multicenter study. The 2020 ECMM/ISHAM consensus criteria for research and clinical guidance definitions of CAPA were followed.[23] Briefly, the entry criterion of the consensus is positive SARS-CoV-2 RT-PCR anytime during 2 weeks between hospital admission and ICU admission or within 72–96 h after ICU admission and acute respiratory distress syndrome. We also used the proposed grades (possible, probable and proven CAPA). A proven CAPA diagnosis requires normally sterile pulmonary samples (e.g., pulmonary biopsies). Due to the known difficulties to obtain these samples in severely ill COVID-19 patients, none of the described cases of this study could be categorized as proven CAPA. Probable CAPA diagnosis requires the demonstration of pulmonary nodules or infiltrates and/or cavitating infiltrates (by a chest CT scan) with no other attributable cause than SARS-CoV-2 infection together with one or more mycological evidence. Radiological series were analyzed locally (each hospital analyzed their own radiological reports as each hospital usually does). The followed consensus criteria included at least one of the following positive mycological tests: observation of filamentous fungal elements in BAL by microscopy, *Aspergillus* spp. isolated from BAL culture, GM ratio >0.5 in serum and/or ≥1.0 in BAL, PCR in BAL, 2 PCR in plasma, serum, or whole blood.[23] Therapeutic data were retrospectively collected and analyzed.

Statistical analyses and ethical approval

Data analysis was performed using the Statistical Package for Social Sciences Software (SPSS version 25.0; IBM SPSS statistics Inc., Chicago, IL, USA). For descriptive data as age, the statistical dispersion was measured as median. Continuous variables (e.g., GM quantifications) are expressed as means ± standard deviations and as median and ranges and analyzed by unpaired Student's *t*-test. Differences in proportions were determined by Fisher´s exact test or χ^2 test. GM-LFA and GM-EIA qualitative agreement was demonstrated by a Kappa statistic[24] and by determining a Spearman correlation. A *P*-value < 0.05 was considered significant. The participating centers ethics committees approved this study.

Results

CAPA epidemiology in Argentina

Between March 15th and October 15th 2020, 185 patients (65.95% males, n = 122) fulfilling the inclusion criteria were enrolled in this protocol (Ramos Mejia Hospital enrolled 33 patients, Clinicas-UBA: 38, Durand/Muñiz: 44, Tucuman referral center: 26, Chaco-Corrientes referral center: 21 and Santa Fe-Entre Rios referral center: 23).

Table 1. Underlying conditions of the patients included in this study.

Underlying diseases	N (%)
Hypertension	67 (36.21)
Diabetes mellitus	44 (23.78)
Obesity	39 (21.08)
Smoker/ex-smoker	21 (11.35)
Respiratory chronic disease*	14 (7.57)
Renal insufficiency	14 (7.56)
Oncohematological diseases and cancer	12 (6.48)
Autoimmune disease†	11 (5.95)
Hypothyroidism	7 (3.78)
Neurological disorders	6 (3.24)
Chagas-Mazza disease	4 (2.16)
Other‡	24 (12.97)

*8 asthma.
†6 rheumatoid arthritis.
‡Includes: HIV/AIDS, hepatitis B infection, healthcare worker, pregnancy, etc.

The patient's median age was 63 years (ranging from 17 to 86 years) (**Figure 1A**). Among these patients, 166 (89.72%) showed at least one of the known underlying diseases linked with severe COVID-19. Briefly, hypertension, diabetes mellitus (93.2% diabetes type II), obesity, smoker/ex-smoker, respiratory chronic respiratory diseases (mostly asthma) and renal insufficiency (mostly chronic renal insufficiency) were the pre COVID-19 conditions that the enrolled patients presented most frequently (Table 1).

The overall mortality percentage of this patient cohort was 36.21% (67 deaths over 185 enrolled patients). Mortality was statistically not related with the patient's age ($P = 0.1013$) (age of the discharged alive: 59.27 ± 13.15 years old vs. 62.79 ± 12.17 years old for those who died). The average length of stay in ICU was 24 days (ranging from 3 to 100 days). However, when the patients were stratified considering those who had a favorable evolution (UTI discharge) and those who died, the ICU stay showed significant differences ($P = 0.0016$). Patients who were discharged stayed longer in ICU (28.47 ± 19.38 days) than those patients who died (19.18 ± 15.45 days from ICU admission to death). Turning to the received treatments, the vast majority of the patients (86.22%) received corticosteroids (mainly dexamethasone 6–8 mg/day) for an average of 10.57 ± 3.58 days (ranging from 3 to 26 days) while an even higher percentage (n = 172, 92.97%) received at least one antibacterial drug (115/172 patients received multiple antibacterial drugs) during their ICU stay. Antifungal treatment was less common and only 20 patients received at least one antifungal (10.85%) (9/20 received an antifungal/s and antibacterial/s drug/s). The most commonly used antibacterial drugs were vancomycin (26.16% alone or in combination), colistin (20.34%), ceftazidime with or without avibactam (19.18%), meropenem/imipenem (18.02%), piperacillin

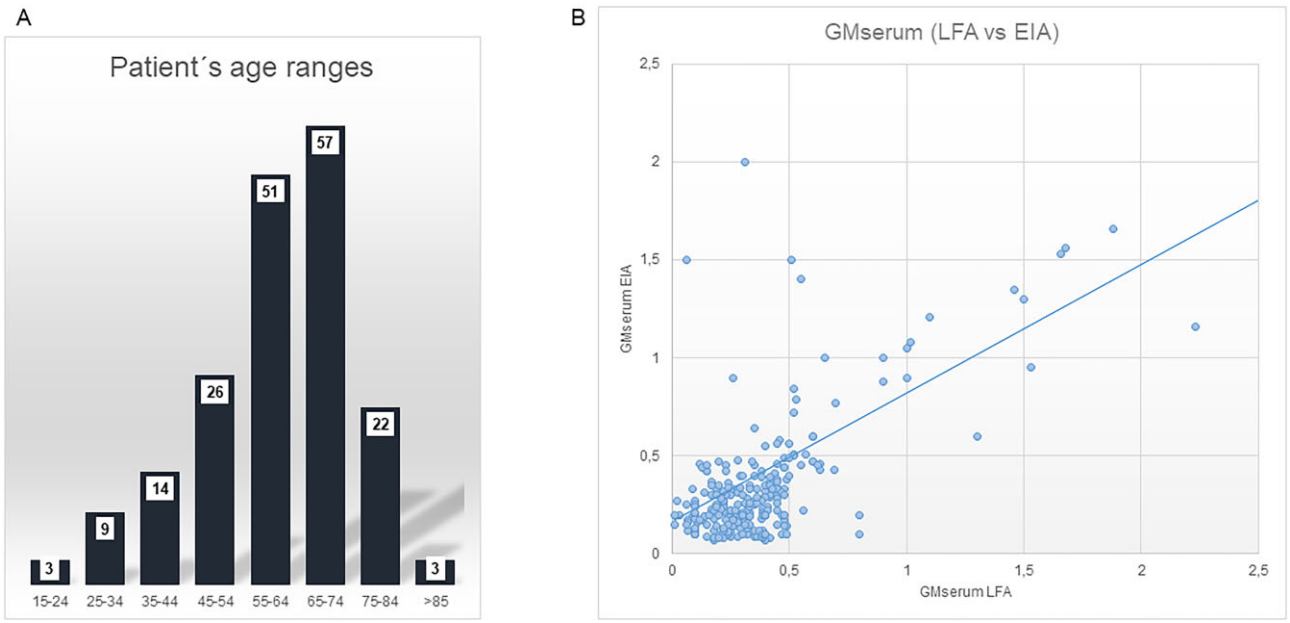

Figure 1. (A) Patient's age ranges. The numbers in the bars represent the number of patients in each particular age range. (B) Correlation between galactomannan indexes determined by EIA and by LFA. Values over 2.5 (n = 6) were excluded from the graphic to improve visibility.

tazobactam (15.70%), ampicillin sulbactam (6.97%), trimethoprim sulfamethoxazole (5.23%) and clarithromycin (4.65%).

Technical comparison of GM-LFA with GM-EIA

Galactomannan quantification by GM-LFA was performed in 578 serum samples obtained from the 185 patients included in the study (averaging 3.82 sera per patient ranging from 2 to 10 sera/patient). Moreover, 35 BAL specimens from 18 patients were analyzed (1.94 per patient).

Quantification of GM was also carried out in parallel by GM-EIA in 258/578 serum (obtained from 100 individual patients) and in 19/35 BAL samples (taken from 9 patients). Despite the used method, most of the serum samples showed low (<0.5) GM ratios, 497/578 (85.99%) and 211/258 (81.78%) for GM-LFA and GM-EIA, respectively.

We received 10 hemolyzed sera samples that were considered invalid by IMMY´s CUBE reader (n = 3) or showed result discrepancies between methods (n = 7) (>0.5 and <0.5 for GM-LFA and GM-EIA, respectively). After excluding these hemolyzed samples, a good agreement between GM-LFA and GM-EIA was statistically confirmed (248 samples were analyzed). The overall observed agreement was 92.16% (95% confidence interval (CI) from 0.606 to 0.836) with a Kappa statistic value of 0.721 (95% CI from 0.390 to 0.747) that represent a substantial agreement following Landis et al. criteria (Kappa strata 0.61–0.80)[24] and a moderate agreement by Spearman´s coefficient ($P < 0.0001$) (**Figure 1B**). Briefly, the results obtained from 235 serum samples were interpreted equally by using both methods (202 negative and 33 positives by both). The rest (n = 13) showed discrepant results. The most common discrepancies were detected when samples were positive by GM-LFA and negative by GM-EIA. Seven of these discrepancies were seen in samples showing borderline GM values (between 0.5 and 0.70 for GM-LFA and between 0.30 and 0.50 for GM-EIA). As an example, we can state the second serum sample of the 3rd week of ICU stay of patients 1 and 4, depicted in Table 2. Agreement between methods was not evaluated for BAL samples (low number of samples).

Probable CAPA diagnosis by GM-LFA, agents and treatment

Since the followed prospective protocol included multiple serum samples (once per week during the first 14 days of ICU stay and twice per week thereafter) and BAL microbiology analysis when available, we could evaluate the performance of GM-LFA testing in diagnosing CAPA. All 185 enrolled patients had at least two GM-LFA values (3.82 sera/patient on average). Moreover, 35 BAL samples from 18 patients were received for microscopy- and culture-based mycological tests. On the other hand, radiological imaging data was recorded from 164 out of the 185 included patients (138 were obtained after the analysis of CT scans and 26 were chest X-rays). Out of the 185 patients, 18 (9.72%) showed at least one serum sample with a GM-LFA >0.5 (Table 2). Moreover, one patient showed an *Aspergillus* spp. positive BAL culture with low serum GM-LFA (0.2 cube reader value). These 19 patients had documented pulmonary infiltrates with no other attributable cause than SARS-CoV-2 infection documented by chest CT scan. On the other hand, nine patients showed at least one serum sample with GM-EIA > 0.5 (9%, 9 out of the 100 patients tested by EIA). Thus, following the used definitions of CAPA these patients were diagnosed with probable CAPA

Table 2. Detail of the probable CAPA patients.

			Serum GM index			Other mycological tests							
Patient #	Age/sex	Underlying condition	Week in ICU	LFA	ELISA	Microscopy	Culture	PCR	BAL GM	Other microbiology results	Antifungal treatment received	Radiology	ICU stay (Days)
1	60/M	Hypertension/ diabetes Type II	1st	0.9	1.0	Septate hyphae	*Aspergillus* section *fumigati*	ND	1.5	Neg.	VRC	Bilateral infiltrates Grounded glass	21
			2nd	1.53	0.95	ND	ND	ND	ND				
			3rd	0.90	0.88	ND	ND	ND	ND				
				0.55	0.45	Neg	Neg	ND	0.3				
2	73/M	Pulmonary thromboem- bolism in 2004	1st	2.1	2.51	Septate Hyphae	*Aspergillus fumigatus sensu stricto**	+ (BAL and Blood)	2.4	*S. aureus/P. aeruginosa*	AMB 3 days followed by 6 weeks of VRC	Bilateral infiltrates Grounded glass	66
			2nd	1.5	1.30	ND	ND	+ (Blood)	ND				
			3rd	1	0.90	ND	ND	+ (Blood)	ND				
				0.63	0.43	Neg	Neg	· (BAL and Blood)	0.3				
			4th	0.4	0.55	ND	ND	· (BAL and Blood)	ND				
3	69/F	Emphysema	1st	0.4	0.35	Neg	Neg	· (BAL and Blood)	0.30	*P. aeruginosa*		Multiple foci in ground glass bilateral distribution and areas of consolida- tion of the lung parenchyma	26 (died)
			2nd	1	1.05	Neg	*Aspergillus fumigatus sensu stricto**	+ (BAL and Blood)	1.9		VRC		
			3rd	1.1	1.21	Neg	Neg	+ (BAL and Blood)	2.56				
				1.8	2.51	Neg	Neg	+ (BAL and Blood)	4.50				
4	68/M	Hypertension Type II diabetes Smoker	1st	4.24	3.82	Septate Hyphae	*Aspergillus terreus sensu stricto**	+ (BAL and Blood)	3.1	Neg.	VRC 3 days followed by ISAV for 6 weeks	Bilateral infiltrates Grounded glass	31
			2nd	1.46	1.35	ND	ND	+ (Blood)	ND				
			3rd	1.02	1.08	ND	ND	· (blood)	ND				
				0.46	0.58	Neg	Neg	· (BAL and Blood	0.32				
			4th	0.32	0.28	ND	ND	·(blood)	ND				
5	70/F	Hypertension/ diabetes Type II	1st	0.9	ND	Septate Hyphae	*Aspergillus* section flavi	ND	ND	*Klebsiella pneumoniae*	AMB-L	Bilateral infiltrate	11 (died)

Table 2. Continued.

			Serum GM index			Other mycological tests							
Patient #	Age/sex	Underlying condition	Week in ICU	LFA	ELISA	Microscopy	Culture	PCR	BAL GM	Other microbiology results	Antifungal treatment received	Radiology	ICU stay (Days)
6	77/M	Ulcerative colitis	1st	1.3	0.6	Septate Hyphae	*Aspergillus* section fumigati	ND	ND	Neg.	AMB-L	Lateral and basal consolidation. Grounded glass, predominantly peripheral and bilateral	22
			2nd	0.4	0.1	ND	ND	ND	ND				
7	53/M	ANCA vasculitis (pulmonar and renal)		0.5	0.4	Neg	*Aspergillus* section *fumigati*	ND	ND	Neg.	VRC	Grounded glass, bilateral infiltrates	35
8	69/M	Mantle lymphoma		0.2	ND	Neg	*Aspergillus* section *fumigati*	ND	ND	ND	AMB	Grounded glass, bilateral infiltrates	18
9	57/F	Obesity/ hypertension	1st week	1.03	ND	ND	ND	ND	ND	ND	None	Bilateral infiltrate	12 (died)
			2nd week	0.24	ND								
10	67/F	Obesity	1st week	0.60	0.47	ND	ND	ND	ND	ND	FLC VRC 6 weeks	Ground-glass opacities, combination with lung consolidations	83
			2nd week	1.20	9.24								
			3rd week	0,62	0,45								
				0.24	0,34								
			4th weed	0.30	0.1								
				0.12	0.46								

Table 2. Continued.

			Serum GM index			Other mycological tests							
Patient #	Age/sex	Underlying condition	Week in ICU	LFA	ELISA	Microscopy	Culture	PCR	BAL GM	Other microbiology results	Antifungal treatment received	Radiology	ICU stay (Days)
11	72/F	Hypertension dyslipi-demia	1st week	0.63	0.43	ND	ND	ND	ND	Neg	None	Bilateral ground glass infiltrates	28 (died)
			2nd week	2.23	1.16					KPC			
			3rd week	4.49	1.92								
				4.73	1.4								
12	63/F	Smoker	1st week	0,90	2,78	ND	ND	ND	ND	ND	None	Bilateral infiltrate	36 (died)
			2nd week	0,50	0,49								
			3rd week	0,30	0,21								
13	61/M	Type II diabetes Smoker COPD	1st week	1.1	ND	ND	ND	ND	ND	ND	None	Bilateral infiltrate	15
14	75/F	COPD Smoker Obesity Hypertension	1st week	0.6	ND	ND	ND	ND	ND	ND	None	Bilateral infiltrate	8 (died)
15	60/M	Type II diabetes Hypertension Smoker acute kidney failure	1st week	0.8	ND	ND	ND	ND	ND	*C. albicans* (blood)	FLC	Bilateral infiltrate	44
16	76/M	Smoker	1st week	4.5	ND	ND	ND	ND	ND	*C. tropicalis* (blood)	FLC	Grounded glass opacity	35

Table 2. Continued.

			Serum GM index			Other mycological tests							
Patient #	Age/sex	Underlying condition	Week in ICU	LFA	ELISA	Microscopy	Culture	PCR	BAL GM	Other microbiology results	Antifungal treatment received	Radiology	ICU stay (Days)
17	66/M	HIV	1st week	0.6	0.47	ND	ND	ND	ND	ND	None	Bilateral infiltrate	7 (died)
18	49/M	Hypertension Obesity	1st week	1.56	ND	ND	ND	ND	ND	KPC colonization	ITC	Bilateral ground glass infiltrates	23
			2nd week	1.66							VRC		
			3rd week	1.38									
				0.45									
19	49/M	Hypertension Type I Diabetes Obesity	1st week	1.68	1.56	ND	ND	ND	ND	Klebsiella pneumoniae	VRC	Bilateral interstitial infiltrates	19 (Died)
			2nd week	1.88	1.66								
			3rd week	0.65	1								
				1.66	1.53								

GM: galactomannan, LFA: Lateral flow assay (IMMY®), ELISA: Platelia (Biorad), BAL: bronco alveolar lavage, F: female, M: Male, ND: Not done, Neg. negative. COPD: Chronic obstructive pulmonary disease, VRC: voriconazole, AMB: amphotericin B, AMB-L: liposomal amphotericin B, ISAV: isavuconazole, VRC: voriconazole, FLC: fluconazole, ITC: Itraconazole, KPC: *Klebsiella pneumonie* harboring a carbapenemase.
*Molecular identification (calmodulin, beta-tubulin gene sequencing).

(if we include GM-LFA as a mycological criteria the incidence of probable CAPA: 10.27% and if we only include GM-EIA as criteria of probable CAPA its incidence was 9.0%).[23] Probable CAPA diagnosis using GM-LFA as criteria was performed during the first week of ICU stay in 18 out of the 19 patients (94.7%). Of the 18 patients who have high serum GM-LFA, twelve showed other positive mycological tests (hyphae at microscope and/or positive BAL culture and/or GM-EIA).

Out of these 19 probable CAPA patients, eight died (42.11%) (Table 2). This mortality percentage showed no statistical differences with the mortality of the non-CAPA patients (33.13%, $P = 0.3237$). The average length of ICU stay of these CAPA-patients was 29.32 ± 19.24 days (ranging from 7 to 83 days) and there were no statistical differences with the other COVID (non-CAPA) enrolled patients ($P = 0.620$).

Aspergillus spp. was recovered in culture from eight BAL samples. All but one positive *Aspergillus* cultures were obtained from patients with at least one positive GM-LFA (Table 2, patient 8 showed GM-LFA < 0.5 and positive *Aspergillus* culture). On the other hand, one patient showed a positive *Aspergillus* spp. culture with GM-LFA > 0.5 but negative GM-EIA (Table 2, patient 7). Five isolates were identified to section and three to species level as: *Aspergillus* section *Fumigati* (n = 4), *Aspergillus* section *Flavi* (n = 1), *Aspergillus fumigatus sensu stricto* (n = 2) and *Aspergillus terreus sensu stricto* (n = 1). Septated hyphae were seen in only 5 patients (all with positive GM-LFA) (Table 2).

Turning to antifungal treatments, 11/19 (if GM-LFA was used as criterion) and 5/10 (if only GM-EIA was used as criterion) of the probable CAPA patients received a drug active against *Aspergillus* spp. All the CAPA patients diagnosed by GM-LFA plus other methods (microscopy, culture, EIA, etc.) were treated with an *Aspergillus*-active antifungal (Table 2). On the other hand, five of the eleven patients diagnosed as probable CAPA by means of GM-LFA alone did not receive antifungal treatment and six died. Most of the patients that were treated received voriconazole (63.3%, n = 7) followed by amphotericin B (n = 4, 3 liposomal presentation), isavuconazole (n = 1) and itraconazole (n = 1). Some patients received multiple antifungal treatments. In one case, fluconazole pre-emptive treatment was changed to voriconazole after the high GM-LFA report. In some cases, treatment was initiated with itraconazole or amphotericin B until voriconazole was received (some centers had no voriconazole in their pharmacies on a regular basis). In one of the CAPA patients, voriconazole was replaced by isavuconazole when voriconazole serum level was considered not adequate (low concentration in serum) (Table 2).

Discussion

We present the results of a prospective multicenter study from different Argentinian regions with differences in climate, economic and demographic characteristics. The selection of centers was done in order to know CAPA incidence in Argentina including these heterogeneities. In addition, the evaluation of the IMMY®´s SŌNA *Aspergillus* LFA with cube reader lecture GM quantification method was done.

We studied 185 critically ill COVID-19 patients (ICU-with respiratory assistance). Age ranges and underlying diseases of our patients were similar to other worldwide reports.[19,25,26] It has to be highlighted that four (2.16%) of our patients had a diagnosis of Chagas disease (all with other comorbidities).

The overall mortality rate in our cohort was 36.21%. It was higher than some US centers (26.5%)[27] but similar to most reported mortality rates in COVID-19 ICU-mechanical ventilated patients.[27–30] In contrast to other series, age was not related with mortality in our group of patients[28] and the average length of ICU stay (24.43 days) was three-times longer than some reports from China (8 days[31] to 9 days[32]) and similar to others (outside China).[33,34]

Following the described ISHAM/ECMM consensus criteria, the incidence of CAPA in critically ill patients (ICU-with respiratory assistance) during the first COVID-19 wave across Argentina was 10.27% (if GM-LFA was used as mycological criterion) and 9.0% (if GM-EIA was used as the unique biomarker). This incidence is similar to the overall incidence of CAPA in COVID-19 patients on mechanical ventilation published by Fungiscope[19] and comparable with data from some European centers (11.43 ± 1.68% in UK, Germany and Belgium one center each). On the other hand, CAPA incidence in Argentina was lower than in some European countries (>20%).[19] These variations on incidence would be due to differential exposure to *Aspergillus*, different CAPA definition usage, regional differences in diagnostic capabilities, different clinical approaches and differences in awareness of CAPA (as suggested before for influenza-associated pulmonary aspergillosis).[12]

Using the classical mycology techniques (no biomarkers), we were able to diagnose eight probable CAPA patients. These patients showed positive BAL cultures and hyphae were observed in five samples. *Aspergillus* section Fumigati was the most commonly isolated agent (6 out of 8 isolates). Thus, the performance of these techniques in terms of the CAPA diagnosis was similar to other reports (lower but close to 50% of the cases).[19,35–40] The rest of the CAPA patients were diagnosed by GM detection in serum using LFA and EIA. Our data shows a good correlation of results between IMMY´s LFA with cube reader and BIO-RAD‘s EIA results, replicating previous reports.[15] However, discrepant results were obtained in 5.24% of the serum samples (13 out of 248 evaluated serum samples) where 7 of these samples showed borderline GM values. In a recently published study, Autier et al. demonstrated that GM-LFA values between 0.5 and 0.8 in serum samples lacked of specificity for CAPA diagnostics and would produce false-positive results.[41] These authors recommended that isolated borderline GM-LFA results should be confirmed by other mycological tests.[41] Similarly, in some of our patients with borderline GM-LFA serum values (e.g., patients

1 and 4 second serum of week 3, see Table 2), GM-LFA and GM-EIA in BAL samples were below the cut-off value and cultures and microscopy were negative confirming that serum GM were false positives. This example also confirms what was reported previously: respiratory samples would be better for probable CAPA diagnostics.[13,14,41]

In our series, CAPA diagnosis was mostly done during the first week of ICU stay. This quick diagnosis was possible by the implementation of the GM-LFA quantification as a screening tool in all the patients. Similarly, early diagnosis was described by Alanio et al.,[35] Helleberg et al.,[42] Bartoletti et al.[43] and Lahmer et al.[36] (between the 3rd and the 4th day of ICU stay). On the other hand, other reports from France, The Netherlands and Spain, showed that CAPA diagnosis (or onset) was slower (11–16 days after respiratory assistance initiation).[4,5,37] These differences on diagnosis speed seems to be related with the use of aspergillosis diagnostic tests on ICU admission (in some cases in the first 48 h[43]) or using these analyses when patients showed a deterioration on respiratory status.

It was reported that pulmonary aspergillosis increases the mortality of COVID-19 patients (e.g., 66.7 vs 32%[5] and 71.4 vs 36.8%[4] for CAPA and non-CAPA, respectively). Similarly, the mortality rate in our cohort was higher for CAPA patients (42.11 vs 33.13% for CAPA and non-CAPA, respectively). However, this difference was not statistically significant and it was lower than in the majority of previous reports.

As in most reports, there was no postmortem histological confirmation of pulmonary aspergillosis in our CAPA patients.[44] In others, it was demonstrated that a combination of tools is needed to support a CAPA diagnosis (conventional mycology, PCR and biomarkers).[40,45] It is clear that further studies and perhaps a more rigorous criterion for CAPA diagnosis are needed. Until then, we would support that serial and routine (weekly) GM quantification in critically ill COVID-19 patients would be useful for CAPA diagnosis and mortality reduction.[45,46]

Transparency declaration: Sōna Lateral Flow assays and cube readers were provided by IMMY® (Norman, OK, USA). IMMY® and their staff had no participation on study design, selection and collection of data, data analysis and interpretation, in the writing of the manuscript, in the decision to publish and in the selection of the journal.

Acknowledgments

This study was supported in part by Science, Technology and Productive Innovation Ministry (MinCyT; Argentina) grant PICT 2016-1985 to G.G.-E.

Author contributions

All authors contributed to investigation doing the tests, collecting samples and data. Conceptualization and formal analysis were done by G. G-E, G.G and N.B. F. G. G-E wrote the original draft of the manuscript. All authors edited and reviewed the manuscript and approved the final version.

Declaration of interest

The authors have declared no conflict of interest.

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