European Review for Medical and Pharmacological Sciences 2022; 26: 710-714

Utility of serum Galactomannan in diagnosing COVID-19 patients with suspected IPA: an observational study in resource limited settings

S. DAS[1], C. GUPTA[2], C. JAIN[1], G. RAI[1], M.A.A. ALQUMBER[3], P.K. SINGH[1], R. SHARMA[4], A. TYAGI[5], R. SALHOTRA[5], S. NARANG[6], N.P. SINGH[1], S.A. DAR[7]

[1]Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India
[2]Department of Infectious Disease, All India Institute of Medical Sciences, New Delhi, India; Department of Infectious Disease, Northern Railway Central Hospital, New Delhi, India
[3]Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Albaha University, Albaha, Saudi Arabia
[4]Department of Community Medicine, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India
[5]Department of Anaesthesiology and Critical Care, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India
[6]Department of Medicine, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India
[7]Research and Scientific Studies Unit, College of Nursing, Jazan University, Jazan, Saudi Arabia

Abstract. – OBJECTIVE: To study the utility of Galactomannan (GM) antigen as a screening marker for diagnosing invasive pulmonary aspergillosis (IPA) in coronavirus disease 2019 (COVID-19) patients.

PATIENTS AND METHODS: The serum samples from patients with severe COVID-19 diseases admitted to the Critical Care Unit were collected on the 5th day of admission for GM screening. The samples were analysed by enzyme linked immune sorbent assay (ELISA) and GM index of more than 1 was considered as positive. All GM positive patients were serially followed until discharge or death.

RESULTS: The GM was raised in serum of 12 out of 38 patients, indicating an incidence of possible COVID-19 associated IPA (CAPA) in 31.57% of patients. The median age of these CAPA patients was 56.5 years, males were significantly more affected than females. The inflammatory marker serum ferritin was raised in all 12 patients (median value of 713.74 ng/ml), while IL-6 was raised in 9 patients (median value of 54.13 ng/ml). None of these patients received antifungals. Their median length of hospital stay was 20 days (IQR: 12, 34 days). All these patients succumbed to the illness.

CONCLUSIONS: The serum GM appears to be sensitive diagnostic tool to identify early IPA in COVID-19 patients and pre-emptive antifungal therapy could play a role in salvaging these patients.

Key Words:
COVID-19, Invasive pulmonary aspergillosis, Galactomannan, Pre-emptive.

Introduction

Invasive Pulmonary Aspergillosis (IPA) is typically known to occur in severely immunosuppressed hosts. Viruses particularly influenza virus, human cytomegalovirus, respiratory syncytial virus have recently been identified as emerging risk factors for IPA. Influenza associated invasive Aspergillosis (IAIA) was rarely diagnosed before 2009 H1N1 pandemic, subsequently there was sharp increase in cases post influenza pandemic, probably due to improved diagnostics[1]. In a large study[2] conducted in Europe over a period of 7 years, 19% of cases of IAIA were reported identifying influenza as an independent risk factor for IPA. The underlying impaired host adaptive response is considered an important factor for the development of IAIA, and if left untreated, mortality can be high in such patients[3].

Corresponding Authors: Shukla Das, MD; e-mail: shukladas_123@yahoo.com
Chhavi Gupta, MD; e-mail: chhavi13.86@gmail.com

Paralleling with IAIA, Coronavirus disease 2019 (COVID-19) associated pulmonary Aspergillosis (CAPA) has been identified as a distinct entity during the current COVID-19 pandemic. The influenza virus is believed to damage the respiratory epithelium permitting the invasion by fungal agent. Additionally, lymphopenia with impaired activity of macrophages and natural killer cells with associated cytokine imbalances creates immunosuppressive environment for secondary infections to develop[4]. Considering the underlying immune dysfunction a similar hypothesis can be predicted for development of IPA in COVID-19 patients. The degree of immune dysfunction in COVID-19 pneumonia is unknown but a study[5] showed considerable decrease in number and function of T cells. Severe COVID-19 illness, as in influenza, causes acute respiratory distress syndrome (ARDS). However, COVID-19 is characterised by extreme immune dysregulation with hyperinflammatory response or cytokine storm, often requiring administration of corticosteroids or immunosuppressants. These factors eventually make the patients vulnerable to invasive fungal infections[6]. Diagnosing CAPA clinically will remain a big challenge as symptoms are non-specific, performing bronchoscopy is difficult and imaging tools may not differentiate whether respiratory distress and pneumonia are due to COVID-19 or invasive fungal infection (IFI), unlike in IAPA.

There is wide variation in the reported incidences of CAPA ranging from 4% to 35%[7,8] in mechanically ventilated patients, though median time to onset of CAPA is not clearly defined. Also, whether the diagnosis should be based on fungal biomarkers for initiating pre-emptive antifungals is still not clear. Furthermore, the role of prophylactic antifungals in COVID-19 patients admitted to Intensive Care Units (ICUs) still remains undefined, as it may result in antifungal resistance over a period of time. In this study we attempted to identify IPA by using Galactomannan antigen (GM) detection as a predictive biomarker in COVID-19 patients.

Patients and Methods

We conducted a prospective observational pilot study during the first wave of COVID-19 in ICU of a tertiary care hospital, Delhi over period of two months (November-December 2020). Thirty-eight patients of severe COVID-19 diseases (saturation <94% on room air or respiratory rate >30 breaths/minute) admitted in ICU were enrolled for this study. Detailed history, clinical findings and relevant laboratory investigations of patients were collected at the time of enrolment in a predesigned case record form. The study was performed according to ethical clearance from UCMS and GTB Hospital, Delhi and written informed consent from the relatives of patients enrolled in the study was collected before the study. Serum samples were collected from all patients on 5th day of admission for GM screening. The serum samples were analysed by ELISA from Xema GalMAg ELISA, Russia. The GM index of more than 1 was considered as positive based on the ELISA kit recommendations. All GM positive patients were clinically followed until discharge or death.

The SPSS version 20.0 software (IBM, Armonk, NY, USA) was used to perform all statistical analyses. Quantitative data were presented as mean (for parametric data) or median and interquartile range. Non-parametric and categorical data were presented as numbers of cases and percentages. Mann-Whitney U test was used to assess the statistical significance of the difference between the two groups regarding quantitative data. The value of $p<0.05$ was taken as statistically significant.

Results

Out of 38 patients with severe COVID-19 diseases, serum GM was found to be raised in 12 patients. The incidence of possible CAPA patients as estimated by raised serum GM in the study population was 31.57% [95% CI = 26.88 to 37.09%]. The median age of COVID-19 patients with CAPA was 56.5 years (IQR: 25, 70 years). Males were significantly more affected with CAPA than females ($p<0.01$). Diabetes mellitus was the most common comorbidity seen in 66.6% of patients followed by hypertension (55%); one patient had chronic obstructive lung disease and another was suffering from bronchial asthma. All patients had severe COVID-19 illness, six patients were mechanically ventilated and six other were managed with either non-invasive ventilation or high flow nasal oxygen therapy. All patients received systemic corticosteroids, remdesivir and low molecular weight heparin, as per the management protocol. The inflammatory marker-ferritin was raised in all 12 patients with median value of 713.74 ng/ml (IQR: 363.35, 1220.4), while interleukin-6 (IL-6) was raised in 9 patients with median value of 54.13 ng/ml (IQR:8.12, 78.72). The bronchoalveolar lavage and computed tomography studies could not be performed due to the risk of aerosolization. None of the patients received antifungals. The median length of their hospital stay was 20 days (IQR:12, 34 days), though all patient succumbed to the illness (Table I).

Discussion

In the past one and a half year of COVID-19 pandemic, IPA (a well-known complication in immunocompromised patients) has been identified as a unique entity in COVID-19 patients, being referred to as CAPA. An early study from China[9] reported 7 of 221 COVID-19 patients in ICU developed IPA. White et al[10] reported 14.1% cases of CAPA amongst 135 ICU patients in United Kingdom with a mortality rate of 53%; while Italy reported 27.7% cases and 44% mortality amongst ICU patients[11]. In Asian subcontinent, 21.7% of cases of IPA were reported amongst 23 ICU patients from Pakistan[12]. The actual incidence of CAPA in India has not yet been reported. The time to detection of invasive Aspergillosis (IA) in COVID-19 patients post-admission to the ICU ranged from 0 to 35 days with median of 8 days[10]. The hypothesis of alveolar damage facilitates fungal invasion; Blot et al[13] emphasized that CAPA cannot be ignored.

The ECMM/ISHAM proposed consensus criteria for clinical guidance and research for CAPA which included host criteria of COVID-19 positive patients and their temporal relation (preceeding 2 weeks); criteria of abnormal clinical features or abnormal imaging (pulmonary infiltrates) not attributed to any other cause and microbiological criteria[14]. As per the AspICU criteria, proven CAPA is based on histopathology, direct microscopy and culture indicating invasive fungal disease (IFD); probable CAPA is based on culture or GM in bronchoscopic lavage, and possible CAPA is based on culture and GM on non-bronchoscopic lavage samples[14]. However, understanding the high risk of exposure, bronchoscopy is not always feasible in COVID-19 infections. Furthermore, there are considerable overlapping imaging features between COVID-19 pneumonia and associated invasive fungal infections; hence, imaging cannot be relied upon for identifying CAPA[14]. The restrictions in bronchoscopy and limitations of doing a fungal culture of respiratory secretions in COVID-19 patients become a deterrent factor of utilization of an otherwise valuable tool in identifying IPA.

For decades, fungal biomarkers like GM, beta- D glucan (BDG) have been used for diagnosing IFD especially in transplant and oncology settings. It is believed that these biomarkers are detectable even before the onset of clinical symptoms and hence are useful in guiding pre-emptive treatment. GM is the carbohydrate constituent of the cell wall of *Aspergillus spp.* released by the fungus during cell growth[15]. White et al[10] has shown BDG, a pan fungal biomarker, to be reliable marker while serum GM was associated with low sensitivity[10]. Based on these findings we attempted to identify CAPA patients early, by using GM as a pre-emptive biomarker by uniformly testing all severe COVID-19 illness patients having persistent breathlessness on 5th day of admission. Amongst 38 patients, 12 patients had elevated serum GM levels (above cut off), hence they were categorized as possible CAPA. There was a significant male preponderance, but due to limited sample size it would be difficult to strongly associate male predilection for CAPA. All patients had severe COVID-19 illness and the majority were diabetic with

Table I. Characteristics of patients with possible IPA in COVID-19 illness.

Patient Number	Age	Sex	Comorbidities	Serum IL-6 (ng/ml)	Serum Ferritin (ng/ml)	Galactomannan (GM) Index	Mode of Oxygen	Final Outcome	Length of stay (days)
1.	51	M	DM	1.2	728.9	6.027523	MV	Death	34
2.	58	M	DM, COPD	198.5	300.9	1.519266	MV	Death	20
3.	70	M	DM, HTN	50.9	550.7	1.699083	MV	Death	16
4.	68	M	HTN	20.5	551.4	2.445872	MV	Death	17
5.	55	M	HTN	4	1343	1.018349	MV	Death	15
6.	76	M	DM	35.5	561.1	1.122936	MV	Death	24
7.	48	M	HTN	21.4	905.4	1.818349	HFNC	Death	12
8.	66	F	DM	38.8	144	1.493578	NIV	Death	12
9.	25	F	BA	88	260.3	1.777982	HFNC	Death	27
10.	50	M	DM	4	1343.1	1.018349	HFNC	Death	25
11.	70	M	DM	50.9	550.7	1.699083	HFNC	Death	20
12.	55	M	HTN	135.9	1325.4	1.117431	NIV	Death	18

Abbreviations: M: Male; F: Female; DM: Diabetes mellitus; COPD: Chronic Obstructive Pulmonary Disorder; HTN: Hypertension; BA: Bronchial Asthma; ng/ml: nanogram per millilitre; MV: Mechanical Ventilation; NIV: Non-Invasive Ventilation; HFNC: High Flow Nasal Cannula.

markedly raised ferritin and IL-6 levels. Association of COVID-19 illness and diabetes is known to cause collision and further collusion of two diseases and extensive use of corticosteroids have added fuel to the fire[16]. Under such circumstances, high fungal spore burden in Indian ICUs makes patients vulnerable to acquiring fungal infections[17]. In our study, despite high serum GM, the decision for starting antifungals was left at the discretion of treating clinicians, unfortunately none of the patients eventually received antifungals. Several tertiary care hospitals of our country faced an unprecedented burden during peak of the COVID-19 pandemic and all treating clinicians were preoccupied with the aim to provide oxygen therapy and supportive care to the COVID-19 patients. The harsh reality of manpower crunch, lack of resources and limited availability of guidelines on CAPA probably led to neglect of this aspect of the critical care.

COVID-19 being a dynamic illness, patients can rapidly progress from mild pneumonia to ARDS, though in our study no direct correlation could be established between oxygenation status of patients with CAPA. As patients in our study were identified as possible CAPA, severe respiratory distress and poor hemodynamic condition led to 100% mortality. The absence of timely antifungal treatment prolonged hospital stay with a median length of 20 days. It is difficult to attribute mortality directly to CAPA as COVID-19 related ARDS may itself be responsible for fatal outcome. In our study, based on the GM cut off of >1, optical density with a sensitivity of 95.4% and specificity of 99.7%, we could identify nearly 31.6% of possible CAPA patients as early as 5th day of ICU admission. Hence, serum GM appears to be sensitive diagnostic tool to identify IPA in COVID-19 patients early and pre-emptive antifungal therapy could play an important role in reducing mortality in such patients. Our study suffers from certain limitations especially small sample size, failure to perform GM test serially due to resource constraints and absence of mycological evidence from respiratory samples. However, despite these limitations, the current study outscores the importance of screening critically ill COVID-19 patients with serum fungal biomarkers for early identification of IPA and adds to the growing literature on CAPA. Though bronchoscopy with bronchoalveolar lavage is a preferred tool with a better sensitivity, risk of aerosolization amongst health care workers reduces the access to this approach.

Conclusions

Screening serum GM appears to be a sensitive diagnostic tool for early identification of possible CAPA patients for pre-emptive antifungal therapy to reduce mortality in COVID-19 patients. In resource constrained settings, raised serum GM above cut-off accompanied with clinical criteria should set off an alarm for the clinicians to initiate antifungal therapy in COVID-19 patients. Nonetheless, studies with a larger study population are required to substantiate these findings.

Funding

No funding or grant was received for this study.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Vanderbeke L, Spriet I, Breynaert C, Rijnders BJA, Verweij PE, Wauters J. Invasive pulmonary aspergillosis complicating severe influenza: epidemiology, diagnosis and treatment. Curr Opin Infect Dis 2018; 31: 471-480.
2) Martin-Loeches I, M JS, Vincent JL, Alvarez-Lerma F, Bos LD, Sole-Violan J, Torres A, Rodriguez A. Increased incidence of co-infection in critically ill patients with influenza. Intensive Care Med 2017; 43: 48-58.
3) Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, Lagrou K, Verweij PE, Van de Veerdonk FL, Gommers D, Spronk P, Bergmans DCJJ, Hoedemaekers A, Andrinopoulou ER, van den Berg CHSB, Juffermans NP, Hodiamont CJ, Vonk AG, Depuydt P, Boelens J, Wauters J; Dutch-Belgian Mycosis study group. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. Lancet Respir Med 2018; 6: 782-792.
4) Bermejo-Martin JF, Martin-Loeches I, Rello J, Antón A, Almansa R, Xu L, Lopez-Campos G, Pumarola T, Ran L, Ramirez P, Banner D, Ng DC, Socias L, Loza A, Andaluz D, Maravi E, Gómez-Sánchez MJ, Gordón M, Gallegos MC, Fernandez V, Aldunate S, León C, Merino P, Blanco J, Martin-Sanchez F, Rico L, Varillas D, Iglesias V, Marcos MÁ, Gandía F, Bobillo F, Nogueira B, Rojo S, Resino S, Castro C, Ortiz de Lejarazu R, Kelvin D. Host adaptive immunity deficiency in severe pandemic influenza. Crit Care 2010; 14: R167.
5) Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian DS. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis 2020; 71: 762-768.
6) Choudhary S, Sharma K, Silakari O. The interplay between inflammatory pathways and COVID-19: A critical review on pathogenesis and therapeutic options. Microb Pathog 2021; 150: 104673.

7) Lamoth F, Glampedakis E, Boillat-Blanco N, Oddo M, Pagani JL. Incidence of invasive pulmonary aspergillosis among critically ill COVID-19 patients. Clin Microbiol Infect 2020; 26: 1706-1708.

8) Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F, Hallek M, Jung N, Klein F, Persigehl T, Rybniker J, Kochanek M, Böll B, Shimabukuro-Vornhagen A. COVID-19 associated pulmonary aspergillosis. Mycoses 2020; 63: 528-534.

9) Zhang G, Hu C, Luo L, Fang F, Chen Y, Li J, Peng Z, Pan H. Clinical features and short-term outcomes of 221 patients with COVID-19 in Wuhan, China. J Clin Virol 2020; 127: 104364.

10) White PL, Dhillon R, Cordey A, Hughes H, Faggian F, Soni S, Pandey M, Whitaker H, May A, Morgan M, Wise MP, Healy B, Blyth I, Price JS, Vale L, Posso R, Kronda J, Blackwood A, Rafferty H, Moffitt A, Tsitsopoulou A, Gaur S, Holmes T, Backx M. A National Strategy to Diagnose Coronavirus Disease 2019-Associated Invasive Fungal Disease in the Intensive Care Unit. Clin Infect Dis 2021; 73: e1634-e1644.

11) B Bartoletti M, Pascale R, Cricca M, Rinaldi M, Maccaro A, Bussini L, Fornaro G, Tonetti T, Pizzilli G, Francalanci E, Giuntoli L, Rubin A, Moroni A, Ambretti S, Trapani F, Vatamanu O, Ranieri VM, Castelli A, Baiocchi M, Lewis R, Giannella M, Viale P; PREDICO Study Group. Epidemiology of Invasive Pulmonary Aspergillosis Among Intubated Patients With COVID-19: A Prospective Study. Clin Infect Dis 2021; 73: e3606-e3614.

12) Nasir N, Farooqi J, Mahmood SF, Jabeen K. COVID-19-associated pulmonary aspergillosis (CAPA) in patients admitted with severe COVID-19 pneumonia: An observational study from Pakistan. Mycoses 2020; 63: 766-770.

13) Blot S, Rello J, Koulenti D. Diagnosing invasive pulmonary aspergillosis in ICU patients: putting the puzzle together. Curr Opin Crit Care 2019; 25: 430-437.

14) Koehler P, Bassetti M, Chakrabarti A, Chen SCA, Colombo AL, Hoenigl M, Klimko N, Lass-Flörl C, Oladele RO, Vinh DC, Zhu LP, Böll B, Brüggemann R, Gangneux JP, Perfect JR, Patterson TF, Persigehl T, Meis JF, Ostrosky-Zeichner L, White PL, Verweij PE, Cornely OA; European Confederation of Medical Mycology; International Society for Human Animal Mycology; Asia Fungal Working Group; INFOCUS LATAM/ISHAM Working Group; ISHAM Pan Africa Mycology Working Group; European Society for Clinical Microbiology; Infectious Diseases Fungal Infection Study Group; ESCMID Study Group for Infections in Critically Ill Patients; Interregional Association of Clinical Microbiology and Antimicrobial Chemotherapy; Medical Mycology Society of Nigeria; Medical Mycology Society of China Medicine Education Association; Infectious Diseases Working Party of the German Society for Haematology and Medical Oncology; Association of Medical Microbiology; Infectious Disease Canada. Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet Infect Dis 2021; 21: e149-e162.

15) Lamoth F. Galactomannan and 1,3-beta-d-Glucan Testing for the Diagnosis of Invasive Aspergillosis. J Fungi (Basel) 2016; 2.

16) Feldman EL, Savelieff MG, Hayek SS, Pennathur S, Kretzler M, Pop-Busui R. COVID-19 and Diabetes: A Collision and Collusion of Two Diseases. Diabetes 2020; 69: 2549-2565.

17) Rudramurthy SM, Singh G, Hallur V, Verma S, Chakrabarti A. High fungal spore burden with predominance of Aspergillus in hospital air of a tertiary care hospital in Chandigarh. Indian J Med Microbiol 2016; 34: 529-532.