Quantitative B and T cell abnormalities in four patients presenting with mucormycosis and prior asymptomatic COVID-19 infection

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SUMMARY

India saw an unprecedented and rapid rise of invasive coronavirus-associated mucormycosis (CAM) during the delta COVID-19 surge. There is little known about the pathophysiology and if there is a direct causation between the COVID-19 infection and invasive CAM. While the traditional risk factors such as uncontrolled diabetes and other immunocompromising conditions are recognised, there could be several COVID-19-induced phenomena that may predispose the patients to develop CAM and are yet unrecognised. It has been proposed that prior severe COVID-19 is associated with invasive CAM. This could be due to the increased use of immunomodulators or the direct effects of the COVID-19 infection. We report four patients with CAM during the delta surge who did not have prior known COVID-19 infection but on subsequent testing had positive antibodies suggesting past asymptomatic infection. We report the quantitative abnormalities in lymphocyte subsets in all four patients and report CD19+ B cell lymphopenia and reduced percentage of CD27+ CD45RA+ naïve helper T cells. CAM may occur in patients after asymptomatic COVID-19 infection, in the absence of systemic corticosteroid and immunomodulator use, and may reflect underlying immune abnormalities possibly attributable to or unmasked by prior COVID-19 infection.

BACKGROUND

Coronavirus-associated mucormycosis (CAM) has emerged as a catastrophic complication of COVID-19 infection in certain countries and there is an urgent need to study the clinical correlates and outcomes associated with this deadly disease.[1] Some commonly understood risk factors are history of uncontrolled diabetes mellitus (DM), inappropriate corticosteroid use and severity of COVID-19 infection as patients requiring intensive care unit admission were more likely to develop CAM.[2] Patients with less severe or asymptomatic COVID-19 have also been reported to develop CAM.[3] It remains unknown if there are specific immune defects that may predispose patients to the infection. Diagnosis of CAM is clinical and microbiological confirmation by surgical sampling. If untreated, this is a rapidly progressive disease which is often fatal. Treatment needs to be prompt with antifungal therapy and surgical debridement. It has been associated with COVID-19 infection especially in the Indian subcontinent and it is hypothesised that it may be related to excessive and inappropriate corticosteroid use during the pandemic.[2] In this case series, we present cases of CAM who had asymptomatic and remote COVID-19 infection, and characterise their immune cell subset differentiation.

CASE PRESENTATION

We report four cases of rhino-orbital mucormycosis, who on subsequent testing, had evidence of remote COVID-19 infection as they tested positive for SARS-CoV-2 IgG. We performed flow cytometric analysis of peripheral blood B and T cell subsets for immune compartment assessment. Sinonasal biopsies of affected tissues were performed and potassium hydroxide smear of the tissues was studied in the microbiology laboratory. All four patients had Mucorales infection of orbit and sinuses diagnosed by the presence of typical non-septate hyphae in histopathological analysis of the biopsies. None of the patients had acute COVID-19 infection at the time of presentation or any symptomatic COVID-19 in the past and none of the patients were vaccinated. None of the patients were hospitalised due to COVID-19 and since the COVID-19 infection was asymptomatic, the timing from its onset to mucormycosis is unknown. In view of risk factors, detailed history was obtained which revealed that none of the patients had received systemic corticosteroids prior to presentation and had no other significant comorbidities including HIV infection. Three out of four patients had DM. It was uncontrolled in one of the three patients in whom it was diagnosed on presentation and had not begun treatment (table 1). Quantitative assessment of immunoglobulins and lymphocyte subsets using flow cytometry was performed in all four patients. All laboratory testing was performed at a single laboratory at the National Institute of Immunohematology (Indian Council of Medical Research), KEM Hospital, Mumbai, India. Serum immunoglobulins were reported to be within reference range for IgG, IgA and IgM. All patients demonstrated quantitative abnormalities in lymphocyte subsets with CD19+ B cell lymphopenia seen in three of four patients. A reduced percentage of CD27+ CD45RA+ naïve helper T cells was seen in all four patients (table 2).

TREATMENT

All the patients underwent repeated surgical debridement. One of the patients had to undergo total orbital exenteration and extensive maxillary debridement. The other three patients were managed by endoscopic sinus debridement and

Table 1 Patient demographics

	Patient 1	Patient 2	Patient 3	Patient 4
Gender	F	F	M	M
Covid-19 Reverse Transcription-Polymerase Chain Reaction(RT-PCR) on admission for CAM	Negative	Negative	Negative	Negative
Vaccine status	Unvaccinated	Unvaccinated	Unvaccinated	Unvaccinated
History of diabetes mellitus (disease control)	None (not applicable)	Recent (untreated)	5 years (controlled)	15 years (controlled)
Corticosteroid use	After presentation of proptosis	None prior to diagnosis	None prior to diagnosis	None prior to diagnosis

CAM, coronavirus-associated mucormycosis.

Table 2 Immune assessment in patients who presented with CAM

	Patient 1	Patient 2	Patient 3	Patient 4	Reference range
Serum immunoglobulins IgA, IgM, IgG	Normal	Normal	Normal	Normal	
Absolute lymphocyte count (cells/mm^3)	1581	**818**	1096	2366	1400–3300
CD19+ B lymphocytes (cells/mm^3)	**47**	**33**	**44**	213	110–570
CD3+ lymphocytes (cells/mm^3)	1344	**614**	**953**	1656	1000–2200
CD3+ CD4+ T lymphocytes (cells/mm^3)	569	**123**	**383**	710	530–1300
CD3+ CD8+ T lymphocytes	648	**82**	515	710	330–920
CD3− CD16+/56+ Natural Killer (NK) cells	158	**65**	88	175	70–480
CD27+ CD45RA+ naïve helper T cells (%)	**37**	**24**	**9**	**3**	37–97
CD27+ CD45RA+ naïve cytotoxic T cells (%)	55	14	**10**	**6**	20–95

CAM, coronavirus-associated mucormycosis.

retrobulbar amphotericin B therapy. This was followed by treatment with systemic antifungals, three with liposomal amphotericin B and one with amphotericin deoxycholate. All four patients received oral posaconazole following amphotericin treatment for at least 2 months.

Patient's perspective

One day my eye suddenly started hurting a little with watering and my vision became blurred. My family took me to a nearby doctor in my village who could not figure out what was happening. I was sent to an eye doctor and given some medicines but my eye started popping out and I started to see lesser and lesser. Then I was sent to the city where a doctor did a test on my eye, with a small operation and a CT scan following which I was sent to a big tertiary care hospital because it was the terrible black fungus which was affecting many after COVID-19. My family and I were confused as I never had COVID-19. By then I had lost sight in that eye. The doctors admitted me and did some more scans. I was told that they would have to take my eye and some more of my cheek to save my life. I felt like my life was over. My mother and brother were crying. In a little over a month since I started having symptoms, I lost my eyeball and felt that life was over. We are poor. I have stopped attending school. But my doctors have made all medications and treatment available to me free of cost and have promised me that they would plan a complete reconstruction after 2 months also free of cost. I had to have my blood taken for tests every other day and one tube was put in my neck later on for the medications. At present they say that there is no black fungus in my body and I have been asked to start school again. Now my medications are over, I am awaiting a final scan so I can go back to my village and restart school. I am scared because of my disfigured face and have to keep it covered. My mother is worried that no one will marry me. How will the people in my village react to my appearance? But I am ok. So many others in the hospital who had black fungus were not so lucky.

OUTCOME AND FOLLOW-UP

Only one patient had recurrence of sinus disease and needed further surgical debridement within 15 days of discharge. At 2 months of follow-up, all were free of recurrence and off the oral antifungal medications. At 1 year of follow-up, one patient had died and the patient who underwent exenteration is undergoing extensive orbital reconstruction. The other two patients were symptom free. Flow cytometry was not repeated during the recovery phase as it would reflect the response to surgery.

DISCUSSION

We characterise the B and T cell subsets in patients with CAM with past asymptomatic COVID-19 infection. The contribution of adaptive immunity in mucormycosis has been studied in HIV population, where CD4 lymphopenia predisposes to fungal infections.[4] T cell responses are also induced in response to mucormycosis infection, suggesting adaptive immune responses may develop and play some role in antipathogen immunity.[5] The

Learning points

- Although severity of COVID-19 infection is associated with coronavirus-associated mucormycosis (CAM), the latter can occur even in patients who have had asymptomatic COVID-19 infection.
- Quantitative T cell abnormalities in our case series include reduced naïve helper T cells, which were seen in all four cases. Whether this identifies a patient population at risk of CAM or is the result of CAM requires further studies.
- Early diagnosis and prolonged treatment with repeated imaging and debridement as necessary are key to improve outcomes in CAM.

role of B cells in antifungal immunity is less understood. In an antigen-specific T/B cell in vitro co-culture system, activated B cells enhance *Candida albicans*-mediated T cell responses.[6] It remains unclear if the immune cell phenotypes seen in our case series were the result of past COVID-19 infection or if these were inherent to the patients predisposing them to increased risk of CAM. In COVID-19, even in the absence of mucormycosis infection, T cells are significantly reduced, especially the naïve CD4+ T cells. The degree of depletion corelates with the severity of COVID-19. Studies have mainly been carried out in moderate to severe disease and little is known about T cell subsets in asymptomatic disease. Activated SARS-CoV-2-specific T cells during recovery convert to phenotypes such as long-lived memory cells, although expression of markers is associated with activation and exhaustion persists.[7] The changes noted in our case series may represent the exhausted T cell subset. It may be possible that patients exhibiting delayed recovery of the T cell compartment may be the ones at increased risk of CAM. The latter hypothesis remains untested. Three out of the four patients studied here had DM, which is a known risk factor of CAM as we have reported earlier in the data from our online registry studying CAM-associated clinical epidemiological factors.[8] Of the three diabetics, two were controlled on medication and one recently diagnosed. They had no other significant comorbidities or known risk factors for mucormycosis. Reduced naïve helper T cells seen in all four of our patients however have not been seen in patients with known prior diabetes and coinfections.[9] We suspect this to be post-COVID-19 immune paralysis state in our series. It is also unclear if the patients eventually had immune recovery as we did not repeat the flow cytometry in patients post-treatment for CAM. In COVID-19-associated immune paralysis, however, there is evidence of recovery weeks to months after the primary infection.[7]

Despite the limitations, our findings are hypothesis generating, and more understanding of these immune deficits will be very helpful in understanding the role of adaptive immune system in CAM. To the best of our knowledge, this report is the first in the literature examining immunological subsets in CAM following asymptomatic COVID-19 infection.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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