American Journal of Otolaryngology and Head and Neck Surgery

Research Article
Published: 26 Nov, 2025

Platelets Soluble-CD40L a Bridge Between Coagulopathy and Host Defence Dysfunction in Patients with Invasive Fungal Rhinosinusitis

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Abstract

Objectives: To assess the role of platelet soluble CD40L in the Invasive Mucormycosis Fungal Rhinosinusitis (IMFRS) pathogenesis with its related immunological and coagulation pathways dysfunctions.

Methods: This prospective case-control study included 124 subjects divided into four groups: first group was control group of 27 patients, 29 patients confirmed with COVID-19 patients and recently diagnosed type 2 Diabetes Mellitus (T2DM), 33 patients confirmed with COVID-19 and recently diagnosed T2DM with IMFRS, and 35 patients confirmed with COVID-19 but they were non-diabetic group. RT-PCR for COVID-19 was done for all patients. The diagnosis of mucormycosis was established via histopathological examination of the affected nose and paranasal sinus tissue. Immunophenotyping assessment of NK cells (CD16, CD56) and T-cell subtypes CD3, CD8, and CD4. Interferon-gamma and soluble CD40 Ligand were estimated.

Results: sCD40L was the highest in IMFRS patients and correlated positively with HBA1c, MPV, PDW, INR and D-dimer. Natural killer cells percentage was lower, whereas CD56 and CD16 expression were higher in all 3 diseased groups. CD16 expression was significantly higher in IMFRS than COVID-19 diabetic patients. IMFRS patients displayed the lowest CD3, CD8 and CD4 lymphocytes expression. Interferon gamma was higher in all diseased groups with non-significant differences comparing any two groups of diseased patients.

Conclusion: sCD40L significantly increased in IMFRS group in comparison with all groups. That could be obvious that fungal infection had precipitating factor to dampen the innate and adaptive immune system, by decreasing natural killer cells, CD3, CD8, and CD4 percentages.

Keywords: Invasive fungal rhinosinusitis; Mucormycosis; sCD40L; Platelets

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Received Date: *11 Nov 2025*
Accepted Date: *22 Nov 2025*
Published Date: *26 Nov 2025*

Citation:
Ragab A, Abd ELatty A, Ahmedy IA, Youni YAHS, Elkholy HY, Abdel Tawab AM. Platelets Soluble-CD40L a Bridge Between Coagulopathy and Host Defence Dysfunction in Patients with Invasive Fungal Rhinosinusitis. Am J Otolaryngol Head Neck Surg. 2025; 8(1): 1267.

Introduction

Invasive Mucormycosis Fungal Rhinosinusitis (IMFRS) is a life-threatening angio-invasive infection caused by mucormycetes fungi that has been increasing worldwide. Despite the used therapy, which includes toxic antifungal therapy and aggressive surgical debridement, the global mortality rates can reach more than 50% [1]. In COVID-19 and non-COVID-19 mucormycosis, the pathogenesis starts in patients who lack phagocytes or have impaired number and phagocytic activity [2].

Multiple factors can interact to facilitate starting and propagation of the disease. In such patients, diabetes mellitus and corticosteroid use can diminish the phagocytic activity of White Blood Cells (WBCs), e.g., macrophage migration, and fungal tissue invasion [3]. Angioinvasion, which causes vascular thrombosis and subsequent tissue necrosis, may be triggered by any of these conditions. The death of diseased tissues due to ischemia may inhibit the transport of leukocytes and antifungal drugs to infection sites [4].

On the other hand, in COVID-19 patients, this viral infection often causes endothelial injury

and inflammation, which may predispose to subsequent blood vessel thrombosis allowing more tissue ischemia. Fungal growth and invasion can occur due to associated leukopenia, lymphopenia, and reduction in CD4+ and CD8+ levels [5].

So, whether thrombosis due to COVID-19 is the starting point of the disease that led to tissue ischemia and subsequent tissue invasion by Mucorales or the host defense dysfunction that promote fungal growth, adhesion, and invasion of the blood vessels. Therefore, novel strategies for preventing and treating Mucormycosis may result from a better knowledge of the processes involved [6].

CD40-ligand (sCD40L) has more specific cleaved form called soluble CD40L. This form was usually detected in plasma which has the same function of CD40L. They had obvious role in the inflammatory pathway for many diseases. Detected CD40L was studied to show its role in B-cell cycle till apoptosis, and antibody production by binding to its receptor CD40 on B-cells. Significantly, sCD40L and CD40L showed enhancement in the local inflammatory response in the Sino nasal mucosa by impairing peripheral blood B-cell function [7].

Also, study of the host defense dysfunctions, related immunophenotyping, and blood coagulopathy abnormalities in such patients and the differences between COVID-19 with and without diabetes and their comparison with IMFRS patients can identify the changed parameters and subsequently the pathogenesis of the diseases. CD40L can be expressed on platelets within few seconds of activation in vitro and in the process of thrombus formation *in vivo*. Also, platelet CD40L can encourage the cells lining the blood vessels to express adhesion molecules and to secrete chemokines, thus creating signals for recruitment and WBC extravasation in the affected area [1,8] So, studying its role is crucial to be acknowledged in IMFRS as it was not studied yet.

So, the aim of the present prospective case control study was to assess the role of platelet sCD40L in IMFRS pathogenesis and its related immunological and coagulation pathways dysfunctions.

Material and Methods

This prospective case-control study was carried out in the period from February 2021 to October 2023 after approval of the ethical committee and informed consent was taken from all participant before they share in this work. This study included 124 patients of the four designed groups according to the following inclusion criteria: Group I was control group of 27 patients as normal control subjects. Volunteers were healthy adults who did not smoke and had normal medical history, negative PCR for COVID-19 and laboratory investigations that were done to all included patients. Group II was for 29 patients confirmed with COVID-19 patients and recently diagnosed type 2 Diabetes Mellitus (T2DM). Group III was for 33 patients confirmed with COVID-19 and recently diagnosed T2DM with IMFRS. Group IV was for 35 patients confirmed with COVID-19 but they were non-diabetic group. All patients with history of autoimmune, or cardiovascular diseases, plus previous chronic sinusitis, nasal polyposis or nasal surgery were excluded from the study. All included participants with history of antiplatelet or anticoagulant medication were also excluded.

Computer randomization of sampling technique was done to select all patients even control group to be at same age level (above 30 years). All these patients were collected from the presented persons to had results of COVID-19 test with or without nasal and chest manifestations. Positively diagnosed COVID-19 patients were hospitalized and grouped also randomly into the remaining three groups: one group for management of COVID-19 and T2DM, second group for management of COVID-19, T2DM, and IMFRS, third group for management of COVID-19 only. All these patients had admitted in the ward at isolated rooms. All these patients had S1 staging for chest CT findings as the Quantitative lung computed tomography imaging features for severity assessment of COVID-19 [9].

RT-PCR is the ideal test for confirmation of COVID-19 that was done to all patients. The sample for RT-PCR was done by a swab from nasopharyngeal or respiratory secretions, also Computed Topography (CT) chest was used in diagnosis, grading severity of disease, guiding treatment and detecting complication.

Each patient underwent a detailed history, including demographic data and detection of many risk factors. A thorough general examination plus full chest and otorhinolaryngological examination were done. Detection of any area with black discoloration in nasal passages was done under endoscopic examination. The diagnosis of mucormycosis in IMFRS group was established via histopathological examination of the affected paranasal sinus tissue. The specimens were taken from the viable and necrotic tissues from the affected area of nose and paranasal sinuses under local examination. Computed topography and Magnetic resonance imaging (T1, T2, and with Fat suppression) were done to all patients. Magnetic resonance imaging is the ideal for detection of any orbital or intracranial involvement even with subclinical manifestations.

Patients diagnosed with IMFRS had scored using Computed Tomography Severity Scoring Index (CTSI). All these patients had moderate form of score index in computed tomography for nose and paranasal sinuses. Based on the severity of disease extension, Table 1 shows the Computed tomography severity scoring index. The involvement areas were divided into four major areas including nose ± paranasal sinuses; adjacent soft tissue infiltration; orbit and intracranial involvement. Points were granted for each involved area this were calculated together to give a score (1-25). For each patient, the final CTSI was calculated, and the disease was further classified as mild (CTSI 1-8), moderate (9-16) or severe (17-25) [10].

All the laboratory assessment was done at clinical pathology department of our university hospital during admission. All patients were subjected to blood sample collections for full routine laboratory investigations included Complete Blood Count with differential including red blood cells, Total Leucocytic Count (TLC), platelets, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Red Cell Distribution Width (RDW). C-Reactive Protein (CRP), serum ferritin, liver functions tests, albumin, kidney function tests, electrolytes assessment (potassium, and calcium) and glycosylated Hb (HbA1c). Fasting and 2 hours postprandial blood glucose level were done to all patients before start of any medication for diabetic patients.

All patients diagnosed with IMFRS started strict control of diabetes and medical treatment of fungal infection using frequent nasal wash with Voriconazole. Surgical debridement was done for all patients presented with IMFRS under general examination. Voriconazole was administered by 200 mg twice daily intravenous formulation initially and then was switched to an oral formulation for a period up to 3 months until disappearance of any signs of fungal

invasion clinically and radiologically. Radiological assessment was repeated every month. Follow-up was done using mainly serum creatinine during medical management of IMFRS.

Immunophenotyping assessment of NK cells (CD16, CD56) and T-cell subtypes CD3, CD8, and CD4 were counted during admission on CytoFLEX flow cytometer (Beckman Coulter Life Sciences). Interferon-gamma and soluble CD40 Ligand were estimated using ELISA. Blood coagulopathy assessment include platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), D-dimer, and INR.

Statistical analyses

Data analysis was done by IBM SPSS software package version 20.0. Mean, standard deviation plus maximum and minimum levels were measured for quantitative values. Anouva test, Chi square test, and Post Hoc test were performed for quantitative values. Kruskal Wallis test was used to compare different groups. Spearman coefficient was used to correlate between quantitative variables. P-value was indicating non-significant, significant, or highly significant as it equal consequencely more than 0.05, less than 0.05, or less than 0.001.

Results

Regarding demographic data; there are non-significant differences according to age and gender comparing four groups. At the same time, serum creatinine was the highest in COVID-19 diabetic group, followed by COVID-19 non-diabetic group. These levels of serum creatinine were higher after Voriconazole administration in group of IMFRS.

Concerning laboratory assessment; IMFRS patients had the highest HbA1c value, followed by COVID-19 diabetic patients. As regards hematological parameters, hemoglobin and MCV were significantly lower in IMFRS than COVID-19 diabetic and non-diabetic groups. At the same time, RDW, MPV and PDW had significantly higher levels in IMFRS patients than COVID-19 diabetic and control groups. Interferon-gamma, sCD40L, D-dimer and INR had the highest levels in all COVID-19 patients. Also, COVID-19 diabetic groups displayed a significantly higher level of D-dimer than COVID-19 non-diabetic patients. At the same time, there was no significant difference between IMFRS group and COVID-19 diabetic group according to Interferon-gamma, sCD40L, D-dimer and INR.

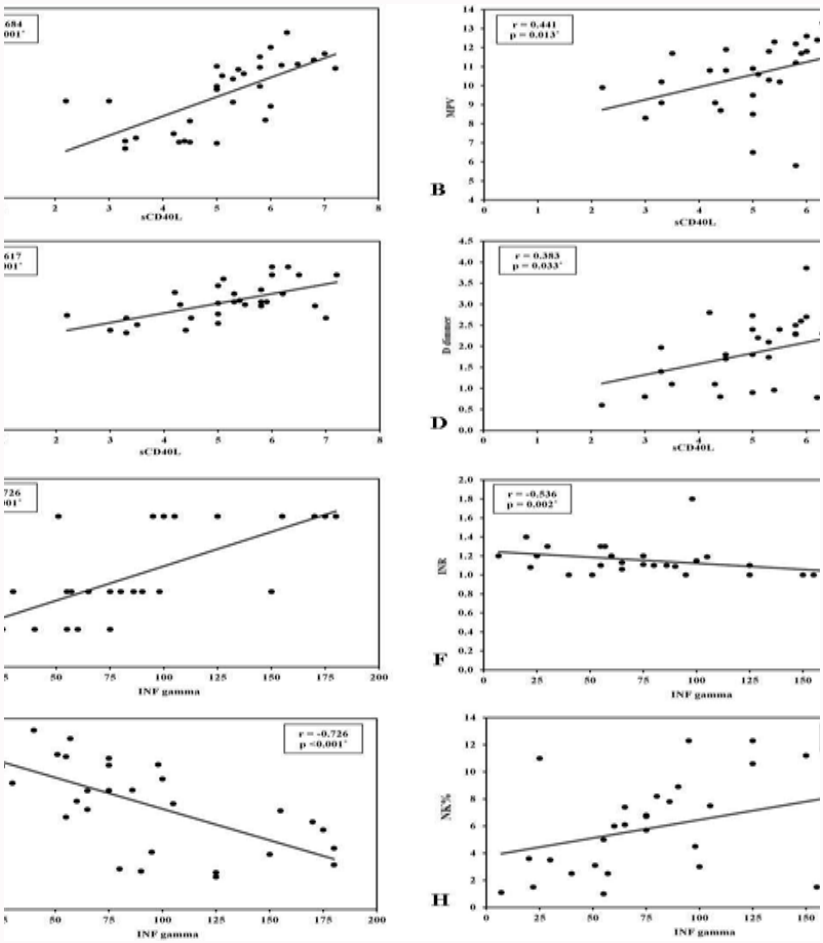

Figure 1: Includes the following:
A: Positive correlation between sCD40 L and HBA1c in both COVID-19 diabetic and IMFRS groups.
B: Positive correlation between sCD40 L and MPV in all groups.
C: Positive correlation between sCD40 L and PDW in all groups.
D: Positive correlation between sCD40 L and D-dimer in both COVID-19 diabetic and IMFRS groups.
E: Positive correlation between INF gamma and CRP in both COVID-19 diabetic and IMFRS groups.
F: Negative correlation between INF gamma and INR in both COVID-19 diabetic and COVID- 19 non- diabetic groups.
G: Negative correlation between INF gamma and CD56% in both COVID-19 diabetic and IMFRS groups.
H: Positive correlation between INF gamma and NK% in all groups.

Table 1: Computed tomography severity scoring index.

Items	Value
Disease limited to nose/PNS	
Mucosal disease in paranasal sinuses	1
Mucosal disease in nasal cavity and nasopharynx	1
Adjacent tissue invasion	
Hard palate erosion	1
Soft tissue infiltration anterior/posterior peri-antral fat	1
Soft tissue infiltration extending to PPF/SOF/IOF/Orbital apex/ITF Significant bilateral disease	2
Significant bilateral disease	2
Orbit	
Soft tissue/fat/muscle/NLD involvement	1
Bone erosion	2
Intraocular/optic nerve involvement	3
Intracranial disease	
Skull base invasion (erosion)	2
Cavernous sinus involvement	3
Internal carotid artery narrowing/occlusion	3
Intracranial complications (meningitis/cerebritis/abscess/infarct)	3

Mild: 1-8; Moderate: 9-16; Severe: 17-25.
PNS: Paranasal Sinuses; PPF: Pterygopalatine Fossa; SOF: Superior Orbital Fissure; IOF: Inferior Orbital Fissure; ITF: Infratemporal Fossa; NLD: Nasolacrimal Duct.

Moreover, sCD40L depicted the highest significant values in IMFRS group compared with all other groups Table 2 and 3.

Regarding Immunophenotyping assessment; TLC was significantly higher in IMFRS group than COVID-19 non-diabetic group. Lymphocytes were lower in the diseased than control group. However, IMFRS group only had the highest count of lymphocytes and monocytes. Finally, all patient groups had the highest neutrophils/lymphocytes ratio, while; platelets/lymphocyte ratio showed no significance between patient groups and control group Table 2.

Diseased group had noticeably fewer natural killer cells than control group by flowcytometry. Moreover, all patient groups showed a higher CD56 and CD16 expression level than control group. CD16 expression was significantly higher in IMFRS than COVID-19 diabetic group. CD3, CD8, and CD4 expression on lymphocytes was significantly higher in diseased than control groups. Interestingly, IMFRS group displayed the lowest CD3, CD8, and CD4 lymphocyte expression Table 3.

sCD40L correlated positively with HBA1c, MPV, PDW, and D-dimer in COVID-19 diabetic group and negatively with serum albumin, calcium, and serum Hb%. It also correlated positively with HBA1c, MPV, PDW, D-dimer, and liver function tests and negatively with serum albumin, bilirubin, calcium, and serum Hb% in IMFRS group. On the other hand, it correlated positively with age, MPV, and PDW and negatively with serum albumin, sodium, RDW, and INR in COVID-19 non-diabetic group. Besides, HBA1c displayed an independent risk factor for platelet sCD40L release.

On the other hand, Interferon-gamma correlated positively with CRP and NK% and negatively with serum albumin and CD56 expression on natural killer cells in both COVID-19 diabetic and IMFRS groups. While in COVID-19 non-diabetic group, it correlated positively with NK% only Figure 1.

Discussion

The Pandemic era of COVID-19 had increased flow of mucormycosis invading the paranasal sinuses and surrounding structures. After inhaling the sporangiospores into nasal mucosa, the infection proliferates from the sinuses to the brain via the orbital area [10]. Platelets had a role in antimicrobial host defense against fungal infections [11], e.g., the secretion of related anti-inflammatory mediators and cytokines. Also, attachment of Mucorales spore and hyphae activates aggregation of the platelets which initiate thrombus development [12].

In this study, we study the implication of sCD40L in mucormycosis. We found that sCD40L was the highest in IMFRS group and correlated positively with HBA1c, MPV, PDW and D-dimer. In addition, IMFRS group obtained the highest MPV and PDW as platelet activation markers Table 4. It has been reported that sCD40L also has a pro-inflammatory and induction of coagulation by inducing tissue factor expression on monocytes [13] and endothelial cells [14]. IMFRS group displayed the highest monocyte count values and significantly high INR and D-dimer compared to control group. These results reflect that mucormycosis affects primary and secondary hemostasis; the platelets, and the coagulation cascade.

CD4+ and CD8+ T-cells were exclusively generated during invasive mucormycosis, just like the innate immune response. IL-4, IFN-γ, IL-10, and IL-17 are only few of the cytokines that were produced by both types of T-cells [15,16].

To evaluate the immunological effect of sCD40L, Natural killer cell expression of both CD56 and CD16 was used to evaluate innate immunity. Also, the adaptive immune system was studied by estimating CD3, CD4, and CD8 expression on lymphocytes. IFN-γ was selected to evaluate the efficacy of both immune system pathways.

We depicted that, natural killer cell CD56 and CD16 expression

Table 2: Comparison between the different studied groups according to complete blood count.

Complete blood count	Control (n = 27)	(T2DM) COVID-19 patients (n = 29)	IMFRS patients (n = 33)	COVID-19 non-diabetic patients (n = 35)	Test of sig.	p
Hb (gm/dl)						
Mean ± SD.	13.3 ± 1	11.1 ± 1.7	9.6 ± 1.5	10.2 ± 2	F=	<0.001*
Median (Min. - Max.)	13.1 (12.1 - 16.4)	11.1 (7.6 -14.5)	9.5 (7.4 - 13.7)	10.3 (6.6 - 13.5)	31.971*	
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.002*, p_2=0.142, p_3=0.460				
MCV (µl)						
Mean ± SD.	89.6 ± 4.5	82.5 ± 6.5	78.8 ± 5.9	82.5 ± 7.3	H=	<0.001*
Median (Min. - Max.)	89 (81 - 97)	82 (71 - 98)	79 (66.5 - 93)	82 (67 - 98)	39.331*	
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.048*, p_2=0.944, p_3=0.041*				
MCH (pg)						
Mean ± SD.	29 ± 1.7	44.2 ± 92.2	27.2 ± 2.4	27.7 ± 1.9	F=	0.41
Median (Min. - Max.)	29 (26 - 32)	28 (21 - 541)	27 (22 - 31)	27.9 (24.6 - 31.2)	0.968	
RDW (fl)						
Mean ± SD.	13.2 ± 0.5	14.7 ± 1.8	15.5 ± 2.4	14.5 ± 2.2	H=	<0.001*
Median (Min. - Max.)	13.1 (12.2 - 13.8)	14.2 (11.8 - 18.5)	14.8 (12.6 - 22.4)	13.5 (11.7 – 18.5)	24.097*	
p_0		0.001*	<0.001*	0.021*		
Significance between groups		p_1=0.180, p_2=0.279, p_3=0.015*				
Platelets (1000cell / µl)						
Mean ± SD.	279.8 ± 83.1	169.6 ± 103.5	266.8 ± 130.9	186.5 ± 116.7	H=	0.401
Median (Min. -Max.)	289 (127 - 415)	130 (44 - 405)	268 (80 - 550)	139 (2 - 436)	2.939	
MPV (fl)						
Mean ± SD.	8.8 ± 1	9.8 ± 1.2	10.7 ± 1.8	8.5 ± 1.1	F=	<0.001*
Median (Min. -Max.)	8.9 (6.7 - 10.7)	9.8 (6.7 - 11.7)	10.9 (5.8 - 13.3)	8.3 (6.7 - 10.7)	17.486*	
p_0		0.022*	<0.001*	0.731		
Significance between groups		p_1=0.051, p_2=0.001*, p_3<0.001*				
PDW (%)						
Mean ± SD.	10.7 ± 1.3	17.4 ± 1.1	19.5 ± 1.4	13.9 ± 1.1	H=	<0.001*
Median (Min. -Max.)	10.5 (8.7 - 13.6)	17.6 (15.4 - 19.3)	19.4 (17.2 - 22.1)	13.8 (12.2 - 16.3)	108.292*	
p_0		<0.001*	<0.001*	0.001*		
Significance between groups		p_1=0.010*, p_2<0.001*, p_3<0.001*				
TLC (µl)						
Mean ± SD.	7.4 ± 1.5	10.2 ± 5.6	11.7 ± 6.3	8.1 ± 3.5	H=	0.005*
Median (Min. - Max.)	7.7 (4.3 - 9.8)	9.1 (2.5 - 25)	9.7 (3.8 - 33.7)	7.7 (0.7 - 16)	12.709*	
p_0		0.031*	0.001*	0.419		
Significance between groups		p_1=0.256, p_2=0.178, p_3=0.031*				
Neutrophils (µl)						
Mean ± SD.	4.5 ± 1.3	7.9 ± 4.5	9.3 ± 6.1	6.5 ± 3.1	H=	<0.001*
Median (Min. - Max.)	4.7 (2.5 - 7.1)	6.9 (1.4 - 17.6)	7.2 (2.5 -30.8)	6.2 (0.1 - 13.8)	21.250*	
p_0		<0.001*	<0.001*	0.007**		
Significance between groups		p_1=0.422, p_2=0.415, p_3=0.106				
Lymphocytes (µl)						
Mean ± SD.	2.2 ± 0.5	1.2 ± 0.9	1.6 ± 0.7	1.1 ± 0.6	H=	<0.001*
Median (Min. - Max.)	2.1 (1.4 - 2.9)	1 (0.2 - 3.4)	1.6 (0.6 - 3.6)	1.1 (0.2 - 2.7)	39.753*	

p_0		<0.001*	0.002*	<0.001*		
Significance between groups		p_1=0.022*, p_2=0.983, p_3=0.021*				
Neutrophils/lymphocytes ratio						
Mean ± SD.	2.1 ± 0.8	10.1 ± 9.3	7 ± 6	6.9 ± 5.5	H=	<0.001*
Median (Min. - Max.)	2.1 (0.9 - 3.9)	7.1 (1.8 - 45.5)	4.9 (1.6 - 30.8)	5.5 (0.3 - 31)	48.992*	
p_0		<0.001*	<0.001*	0.001*		
Significance between groups		p_1=0.182, p_2=0.350, p_3=0.690				
Platelets / lymphocytes ratio						
Mean ± SD.	132.6 ± 49.3	252.7 ± 297.8	178.5 ± 73.6	242.3 ± 278.8	H=	0.401
Median (Min. - Max.)	119.7 (43.8 -296.4)	118.8 (12.9 - 1535)	171.3 (72.4 - 317.9)	134.4 (2.8 -1380)	2.939	
Monocytes (µl)						
Mean ± SD.	0.6 ± 0.2	0.5 ± 0.2	0.9 ± 0.5	0.4 ± 0.2	H=	<0.001*
Median (Min. -Max.)	0.6 (0.2 - 0.8)	0.5 (0.2 -1.2)	0.9 (0.1 - 1.7)	0.4 (0.1 - 0.8)	27.825*	
p_0		0.599	0.017*	0.004*		
Significance between groups		p_1=0.003*, p_2=0.020*, p_3<0.001*				

F: F for One way ANOVA test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Tukey), H: H for Kruskal Wallis test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test), p: p value for comparing between the studied groups, p_0: p value for comparing between Control and each other groups, p_1: p value for comparing between COVID-19 diabetic and IMFRS, p_2: p value for comparing between COVID-19 diabetic and COVID-19 non diabetic, p_3: p value for comparing between IMFRS and COVID-19 non diabetic*: Statistically significant at p ≤ 0.05.

Table 3: Comparison between the different studied groups according to blood coagulopathy results and interferon gamma.

Variables	Control (n = 27)	(T2DM) COVID-19 patients (n = 29)	IMFRS patients (n = 33)	COVID-19 non-diabetic patients (n = 35)	Test of sig.	p
INR						
Mean ± SD.	1 ± 0	1.2 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	H=	<0.001*
Median (Min. - Max.)	1 (1 - 1)	1.2 (1 - 1.6)	1.1 (1 - 1.8)	1.1 (1 - 1.9)	34.973*	
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.298, p_2=0.131, p_3=0.638				
D-dimer (ng/ml)						
Mean ± SD.	0.2 ± 0.1	2 ± 0.9	1.9 ± 0.8	1.5 ± 0.9	H=	<0.001*
Median (Min. - Max.)	0.2 (0 - 0.5)	2.1 (0.6 - 3.8)	2 (0.6 - 3.9)	1.4 (0.3 - 3.6)	71.416*	
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.616, p_2=0.042*, p_3=0.126				
sCD40L (ng/ml)						
Mean ± SD.	1.3 ± 0.2	4.2 ± 1.2	5.1 ± 1.2	3.6 ± 1.1	F=	<0.001*
Median (Min. - Max.)	1.3 (1 - 1.7)	4.5 (1.9 - 6)	5.3 (2.2 - 7.2)	3.5 (1.8 - 5.5)	78.034*	
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.003*, p_2=0.130, p_3<0.001*				
INF gamma (pg/ml)						
Mean ± SD.	13 ± 7.1	99.7 ± 42.5	86.8 ± 50	86.8 ± 38.6	H=	<0.001*
Median (Min. - Max.)	12 (2 - 27)	90 (25 - 175)	75 (7 - 180)	76 (45 - 162)	66.869*	
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.300, p_2=0.312, p_3=0.979				

SD: Standard deviation, F: F for One way ANOVA test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Tukey), H: H for Kruskal Wallis test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test), p: p value for comparing between the studied groups, p_0: p value for comparing between Control and each other groups, p_1: p value for comparing between COVID-19 diabetic and COVID-19 associated IMFRS, p_2: p value for comparing between COVID-19 diabetic and COVID-19 non diabetic, p_3: p value for comparing between IMFRS and COVID-19 non diabetic, *: Statistically significant at p ≤ 0.05.

Table 4: Comparison between the different studied groups according to Immunophenotyping assessment.

Immunophenotyping assessment	Control (n = 27)	(T2DM) COVID-19 patients (n = 29)	IMFRS patients (n = 33)	COVID-19 non diabetic patients (n = 35)	H	p
CD56 (%)						
Mean ± SD.	5.7 ± 2.5	32.3 ± 13.9	34.2 ± 14.3	34.2 ± 12.1	69.365*	<0.001*
Median (Min. - Max.)	6 (2 - 10)	33 (10.2 - 54.6)	37.3(10.2 - 56.7)	33 (12.9 - 57)		
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.697, p_2=0.772, p_3=0.921				
CD16 (%)						
Mean ± SD.	10 ± 2.8	14.7 ± 5.3	20.6 ± 9.9	16.9 ± 6	31.638*	<0.001*
Median (Min. -Max.)	10 (4 - 15)	15.6 (6.5 - 29)	19.4 (2.5 -37.5)	17 (8 - 31.6)		
p_0		0.002*	<0.001*	<0.001*		
Significance between groups		p_1=0.027*, p_2=0.206, p_3=0.344				
CD3 (%)						
Mean ± SD.	69.6 ± 6.5	92.4 ± 3.2	84.7 ± 7.7	92.4 ± 2.6	77.620*	<0.001*
Median (Min. - Max.)	69 (52 - 82)	92 (85 - 98)	84.4 (70 - 98.5)	93 (86 - 97)		
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.001*, p_2=0.983, p_3=0.001*				
CD8 (%)						
Mean ± SD.	35.8 ± 7.2	48.9 ± 4.9	42.7 ± 6.4	46.6 ± 6.4	46.407*	<0.001*
Median (Min. - Max.)	37 (21 - 50)	49 (36 - 57)	43 (29 - 58)	46 (35 - 58)		
p_0		<0.001*	0.003*	<0.001*		
Significance between groups		p_1=0.001*, p_2=0.169, p_3=0.039*				
CD4 (%)						
Mean ± SD.	36.1 ± 7	42.5 ± 5.4	40.2 ± 7.6	45 ± 6.6	23.346*	<0.001*
Median (Min. - Max.)	36 (15 - 48)	42 (32 - 53)	40 (27 - 56)	45 (32 - 58)		
p_0		0.001*	0.037*	<0.001*		
Significance between groups.		p_1=0.212, p_2=0.194, p_3=0.011*				
NK (%)						
Mean ± SD.	12.4 ± 3.6	6.7 ± 3.2	6.1 ± 3.5	7.3 ± 3.7	38.385*	<0.001*
Median (Min. - Max.)	12.5 (6 - 18.3)	6.7 (1.1 - 11.3)	6.1 (1 - 12.3)	7.9 (1 - 13.6)		
p_0		<0.001*	<0.001*	<0.001*		
Significance between groups		p_1=0.506, p_2=0.579, p_3=0.223				

SD: Standard Deviation, H: H for Kruskal Wallis test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test) p: p value for comparing between the studied groups, p_0: p value for comparing between Control and each other groups, p_1: P value for comparing between COVID-19 diabetic and COVID-19 associated IMFRS: p_2: p value for comparing between COVID-19 diabetic and COVID-19 non diabetic: p_3: p value for comparing between IMFRS and COVID-19 non diabetic*: Statistically significant at p ≤ 0.05.

was significantly higher in all patient groups than control group. CD16 expression was significantly higher in IMFRS than in COVID-19 diabetic group. Moreover, IMFRS patients displayed the lowest expression of CD3, CD8, and CD4. CD3 is a T cell co-receptor that activates the cytotoxic T cell (CD8+ naive T cells) and T helper cells (CD4+ naive T cells). Its diminution explains CD4 and CD8 lymphocytes decrease and this can explain the vulnerability of mucormycosis in IMFRS patients. All patient groups had a higher Interferon-gamma level than control group. IMFRS displayed relatively the lowest value. It correlated positively with CRP and NK% and negatively with albumin, monocytes, and CD56 expression in IMFRS patients.

It had been postulated that hyphae inhibit expression of IFN-γ [17]. This account for IFN-γ being relatively the lowest in IMFRS group than other patient groups. Likewise, in a study that analyzed the response of CD4+ T cells, CD8+ T cells, and NK cells to fungal infection in immunocompromised individuals, both quantitatively and qualitatively. The authors' reports showed an insufficient NK cell recovery being less than 200/μl and lower reactive oxygen species production [18].

Researchers have shown that when exposed to fungi, the pathogen identification receptor CD56 dramatically decreases [19]. It had been hypothesized that high levels of cytolytic molecules were produced by CD16+ NK cells, and cytokine-secreting cells were CD56+ NK cells [20] These findings help elucidate the highly significant value of CD16 relative to CD56 and INF secretion, consequently in IMFRS patients in the current study. It reflects that the cytotoxic activity may somewhat be compromised compared to cytolytic properties of NK

in IMFRS patients. Moreover, sCD40L had been reported to inhibit the proliferation of autologous T cells co-cultured with Myeloid-derived suppressor cells and their ability to secrete IFNγ. This could hypothesize the lowest level T cell subsets and relatively lowest IFNγ in IMFRS patients in our study [21].

Furthermore, we found out that IMFRS patients with diabetes represented 80.6 % of all IMFRS cases and obtained the highest HbA1c value, followed by COVID-19 diabetic patients. It displayed an independent risk factor in platelet soluble CD40L release in IMFRS patients. The findings indicate that poorly controlled diabetes mellitus in COVID-19 diabetic patients is a risk factor for mucormycosis development, following many previous studies [22]. Impaired host/immune reaction in poorly controlled DM also carries risk of invasive fungal diseases [23] It also causes endothelial cell damage by Rhizopus and increases surface overexpression of glucose-regulated protein 78 and CotH invasins [24].

Conclusion

sCD40L significantly increased in IMFRS group in comparison with all groups. That could be obvious that fungal infection had precipitating factor to dampen the innate and adaptive immune system, by decreasing natural killer cells, CD3, CD8, and CD4 percentages.

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