Review Article

Lectin pathway and complement activation in COVID-19: A Systematic Review

ABSTRACT

Background: The COVID-19 pandemic has aroused interest in understanding the immunological mechanisms involved in the response to SARS-CoV-2. The lectin pathway stands out in this scenario, since understanding the activation of this pathway during COVID-19 infection can provide valuable information about correlated immunological mechanisms, especially in the uncontrolled inflammatory response of severe cases.
Aim: Thus, the objective was to synthesize the available information on the role of the lectin pathway in the activation of the complement system and its relevance in the immune response and pathogenesis of COVID-19 infection.
Methods: This is a systematic review carried out according to the PRISMA guidelines using the PICOS strategy. The quality of the studies and risk of bias were evaluated using the Checklist Hawker. After the analysis, 11 pertinent studies with the objectives of this study were included.
Results: Patients with COVID-19 had high levels of mannose-binding lectin recognition protein (MBL), especially those with thromboembolic complications. MBL reduction due to certain genotypes (BB or AB) was associated with a more severe form of the disease. The interaction between VL recognition proteins and SARS-CoV-2 virus proteins suggests the activation of the complement system through this pathway. The interaction of SARS-CoV-2 protein N with the VL activator potentiates complement activation. There is a correlation between the genetic polymorphisms in the MBL2 gene and the expression of MBL in the tissues during infection.
Conclusion: Although some studies have not found consistent correlations between complement markers and disease severity, it is consensus that complement system activation is present in patients with COVID-19, and high levels of activation products are associated with more severe forms of the disease, suggesting that inadequate regulation of the complement system may contribute to the uncontrolled inflammatory response and serious complications of COVID-19.

Keywords: COVID; lectin pathway; complement system; activation.

1. INTRODUCTION

The complement system (SC), an essential part of the innate immune response, plays a crucial role in eliminating pathogens and modulating the inflammatory response [1,2] It is a cascade of serum and membrane proteins that can be activated by different pathways: classical (VC), alternative (VA) and lectin (VL) [3, 4,5, 6].

The lectin pathway is activated when pattern recognition proteins (PRPs), such as soluble lectins Mannose-Binding Lectin (MBL), phytin and colectins, bind to pathogen-

associated molecular patterns (PAMPs) present on the viral surface, as well as on other surfaces of pathogens, such as bacteria and fungi [7, 8, 9]. This link between PRPs and PAMPs triggers a series of events that result in activation of the complement system. After binding to PAMPs, activated PRPs form complexes with the associated serine proteases, such as MASP-1 and MASP-2 (mannose binding protein associated with serine protease-1 and 2, respectively) [10, 11, 12, 13]. These Serinas proteases cleaved the complement proteins C4 and C2, forming C3 convertase (C4b2a). The C3 convertase complex, in turn, cleaves the C3 protein into C3a and C3b. C3b binds to the C3 convertase complex, forming C5 convertase, which cleaves the C5 protein into C5a and C5b[14, 15, 16, 17]. C5b binds to the pathogen membrane and then sequentially mounts proteins C6, C7, C8 and C9, forming the membrane attack complex (MAC). MAC creates pores in the pathogen membrane, resulting in loss of integrity and eventually cell lysis [18, 19, 20, 21]. In addition to direct lysis of the pathogen, activation of the lectin pathway of the complement system also triggers an inflammatory response. The release of complement activation products such as anaphylotoxin peptides and C3 degradation products promotes chemotaxis of immune system cells such as neutrophils and macrophages to the site of infection. These cells phagocyte and destroy the pathogen, contributing to the elimination of infection [23, 24,25].

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, remains a global public health challenge. Understanding the immunological mechanisms underlying viral infection has become essential in the search for effective therapeutic strategies. Among the immunological pathways involved in the antiviral response, CS activation through VL has received increasing attention due to its role in modulation of the inflammatory response and elimination of pathogens [26, 27,28]. Research indicates that deregulated immune system activation in patients with COVID-19 may contribute to the severity of infection. In this sense, studies have revealed that complement system VC is activated in all patients with COVID-19, while hyperactivation of VL and VA is associated with disease severity [29, 30, 31, 32]. In addition, it has been demonstrated that increased levels of MBL are associated with severe forms of the disease, suggesting a potential role of this pathway in the pathogenesis of COVID-19 [33,34, 35]. Depending on this, CS activation has been related to the exacerbated immune response, endothelial dysfunction and acute lung injury, characteristics often observed in severe cases of COVID-19 [36,37, 38].

Thus, the objective of this systematic review of the literature was to synthesize the information available in the literature on the role of the lectin pathway in the activation of the complement system against the immune response and pathogenesis of COVID-19 infection.

2. METHODOLOGY

This systematic review aimed to gather, analyze, and summarize the relevant evidence available on the mentioned topic, following clear selection and eligibility criteria. To clearly define the key elements of the research question and guide the selection of studies to be included in the review, the PICOS strategy was used, which consists of the components P (population), I (intervention/exposure), C (comparison), O (outcome) and S (type of study) systematic and organized analysis of the included studies [39]. More details on the components of the PICOS anagram can be found in Table 1. In addition, to ensure a consistent methodological approach, the review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol [40].

Abbreviation	Description	Question components
P	Population	Patients who have been diagnosed with COVID-19
I	Interview/exposure	Activation of the lectin pathway
C	Comparison	Healthy x COVID-19
O	Outcome	Influence on the clinical Picture of the patient
S	Study type	Experimental and observational

Table 1. Survey question components based on the PICOS strategy.

Based on the components of the research question described in Table 1, the search for scientific evidence in the databases PubMed, SCIELO, Scopus, Science Direct, Embase and BVS using the terminology MeSH (Medical Subject Headings) was started. For this, the following search strategy was used: (("Lectin Complement Pathway" OR "Mannose Binding Lectin Complement Pathway") AND ("COVID-19" OR "SARS-CoV-2").

All studies were analyzed for eligibility, considering the following criteria: (i) clear and concise approach to the relationship between complement activation through the lectin pathway and COVID-19 and (ii) carrying out studies in humans or animals. The following criteria were considered for the exclusion of studies to ensure the relevance and coherence of the studies selected for this analysis: reviews; studies testing therapies without any correlation with the lectin pathway and complement activation; book chapters; conference abstracts; case reports; letters; reviews and meta-analyses; studies related to other pathologies; and methods validation studies.

The selection process of the studies was conducted in two phases using the Rayyan platform [41]. In the first phase, two reviewers independently analyzed all titles and abstracts

obtained in the searches. References considered as "potentially eligible" advanced to the second phase, which consisted in evaluating the full text to confirm their eligibility. A third reviewer resolved any dissension.

Due to the review approach adopted in this paper, there was no need to submit or obtain approval from an ethics committee in research. However, all the studies analyzed underwent a quality assessment using the Hawker Checklist, which includes a comprehensive analysis of the ethical issues involved. The Checklist, developed by Hawker et al. (2002) [42], covers nine distinct domains: 1) abstract/title; 2) introduction and objectives; 3) methods and data; 4) sampling; 5) data analysis; 6) ethics and prejudice; 7) results; 8) transferability and generalization; and 9) implications and usefulness. Each of these domains was evaluated for each study, using a scale of four quality categories: very poor (1 point), poor (2 points), regular (3 points) and good (4 points). The scores obtained in each study were summed up, resulting in an overall quality score that was classified as follows: (A) high quality = 30-36 points; (B) average quality = 24-29 points; (C) low quality = 9-24 points. Data from the quality evaluation of the included studies were presented as mean standard deviation of the score obtained independently by two researchers.

3. RESULTS AND DISCUSSION

In total, 666 papers published through the search were identified. After applying the eligibility criteria, 11 studies were selected for inclusion in this review, as detailed in Figure 1.

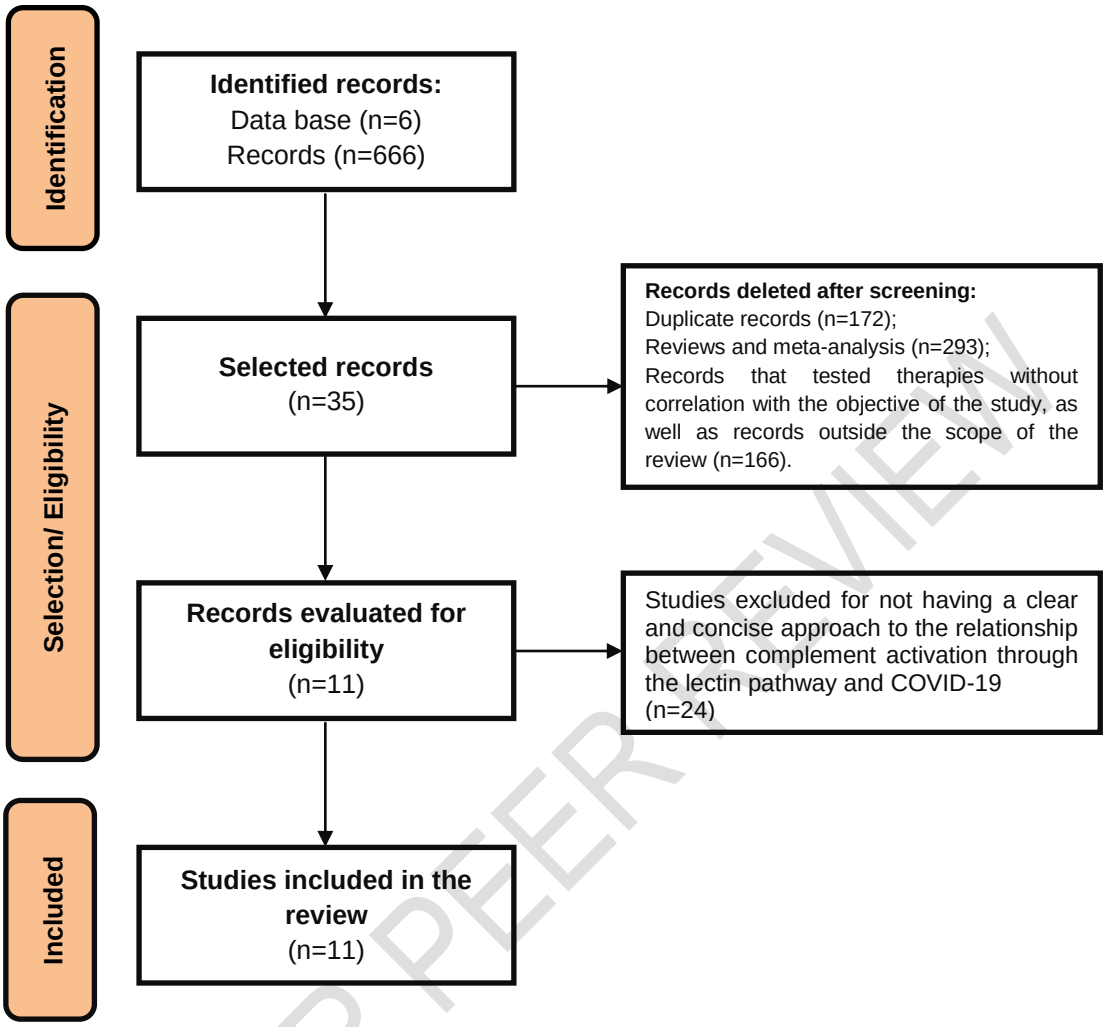

Figure 1. Flowchart for the stages of this systematic review, adapted from PAGE et al., 2022.

Studies were developed in different countries, covering several research methodologies. In the United Kingdom and China, experimental studies were conducted. In Switzerland, a prospective observational cohort study was conducted. In France, a retrospective cohort study was chosen. In the United States, the study adopted was longitudinal, following a group over time. In Sweden, a prospective single centre observational study was conducted. In Denmark, a study combining experimental and cross-sectional observational methods was conducted. In Norway, a prospective observational study was conducted. In Brazil, a comparative observational study was chosen. While in Turkey, a case-control study was conducted. Finally, in Germany, a cross-sectional observational study was conducted. Each research was structured according to the specific

objectives and questions. Thus, we sought to clarify the treatment of each of the works that were selected. Table 2 provides an overview of the objectives and main results obtained in each study, allowing a general understanding of the contributions and findings made in different countries and with different methodologies.

References	Objectives	Main results
Eriksson *et al.*, 2020 [43]	Investigate the role of MBL in coagulopathy associated with COVID-19.	Patients with COVID-19 have elevated levels of MBL; MBL is not related to survival, mechanical ventilation or kidney injury, but is linked to thrombosis. Patients with thromboembolic event have higher levels of MBL, as well as higher MBL activity and higher C3 deposition. MBL was correlated with D dimer and activated thromboplastin time.
Holter *et al.*, 2020 [44]	To describe the activation of CS and to investigate its association with the development of respiratory failure in hospitalized patients with COVID-19.	Most patients with COVID-19 demonstrated continuous and significant activation of the complement system, with values above the reference limit in hospitalization and in the following 10 days. Persistent activation of the complement system during hospitalization, with elevated levels of sC5b-9, C5a, C4d, C3bc and C3bBbP. Modest increase in MBL on days 3 to 5, consistent with an acute phase reaction. Patients with respiratory failure have higher levels of sC5b-9 and C4d on admission.
Medetalibeyoglu *et al.*, 2021 [45]	To investigate whether variant B of the MBL2 gene in codon 54 (rs1800450) is associated with variations in the clinical course of COVID-19 infection.	Genotype BB most common in cases of COVID-19, with no difference in genotypes AB and AA. Genotype BB or AB have increased risk of severe disease and hospitalization in ICU compared to genotype AA. As MBL protein levels decrease due to BB or AB genotypes, the course of COVID-19 may become more severe in some respects.
Malaquias *et al.*, 2021 [46]	To investigate the role of MBL and ficolin-3 (FCN3) in the activation of CS and to compare it between the pandemic infection of the Influenza A virus subtype H1N1 (H1N1pdm09), patients infected with SARS-CoV-2 and a control group.	Patients with COVID-19 have increased expression of MBL and FCN3 compared to H1N1 and control. Histopathological analysis of patients with COVID-19 revealed the presence of diffuse proliferative alveolar damage, fibrinous immune thrombosis and neutrophilic endothelitis, indicating a possible activation of complement and localized inflammation. Specific genotypes of the MBL2 gene were associated with higher expression of MBL in patients with COVID-19. There was an increase in FCN3 expression in the COVID-19 group and in the H1N1 group compared to the control group.
Ali *et al.*, 2021 [47]	To investigate the contribution of VL in the immune response against recombinant SARS-CoV-2 proteins.	VL recognition molecules such as MBL, FCN-2 and CL-11 bind to SARS-CoV-2 S and N proteins, leading to subsequent activation of LP-mediated C3b and C4b deposition. SARS-CoV-2 protein N binds directly to MASP-2 and activates the complement. Inhibition of VL with anti-MASP-2 antibody blocks complement activation. VL-dependent C3b deposition on the cell surface is inhibited by the anti-MASP-2 antibody.
Defendi *et al.*, 2021 [48]	To investigate the activation pathways of CS and its components involved in the immune response in patients with COVID-19.	Patients with COVID-19 had significant activation of the alternative pathways and lectin, with elevation of C3, C4, C5 and Factor B. MBL protein concentration was decreased in this group. All patients had MBL protein expression deficiency, indicating that the lectin pathway can be compromised in patients with COVID-19. Complement system VC is activated in all patients with COVID-19, while hyperactivation of VL and VA is associated with disease severity.
Charitos *et al.*, 2021 [49]	To investigate the role of CS in hospitalized patients with COVID-19 with varying degrees of disease severity.	There was no significant difference in VL activity, MBL and FCN-3 concentrations related to disease severity. Lower VA activity in patients who died or needed invasive ventilation. Slightly lower VC activity in more severe cases. Concentration of C1INH correlated with length of stay, inflammatory markers and disease severity at admission, but not during follow-up.
Niederreiter *et al.*, 2022 [50]	To evaluate complement activation in the kidney and lung of patients with severe COVID-19, as well as to investigate complement component deposition in different areas of these organs.	Pulmonary injury in patients with severe COVID-19 was more intense compared to the control group, including different stages of diffuse alveolar damage. Positive COVID-19 patients showed wide renal alterations, with greater tubular lesion. Kidney injury was correlated with the severity of lung injury. Robust deposition of complement components (C1q, C3c and C3d) in the lungs and kidneys. Strong deposition of MASP-2 in the lungs and kidneys. Positive correlation between complement deposition and severity of pulmonary and renal lesions.
Gao *et al.*, 2022 [51]	To investigate the role of binding pathogenic coronavirus N proteins (SARS-CoV, MERS-CoV and SARS-CoV-2) to MASP-2 in complement activation.	SARS-CoV, MERS-CoV and SARS-CoV-2 N proteins interact with MASP-2. Pathogenic coronavirus N proteins potentiate MBL-dependent activation of MASP-2, leading to hyperactivation of the LP complement cascade in vitro, in mice and in patients with COVID-19.

Devalaraja-Narashimha *et al.*, 2023 [17]	To evaluate the levels of complement activation products and complete proteins in hospitalized patients with COVID-19 and to investigate whether complement pathway markers are associated with clinical outcomes.	Activation of all complement pathways in hospitalized patients with COVID-19. These patients had high levels of complement activation products (C3a, C4a, C5a, sC5-9) compared to controls. Such biomarkers were higher in patients with severe forms of the disease. Increased C5b-9 deposition in patients with COVID-19. High levels of sC5b-9, C3a, AP factor Bb and low levels of MBL were associated with higher mortality.
Götz *et al.*, 2023 [18]	Develop a sensitive and reliable ELISA assay to investigate the possible association between MASP-2 and COVID-19.	Generation of monoclonal antibodies of mice against recombinant and native serum MASP-2. The sandwich MASP-2 ELISA was validated for accuracy, dilution linearity, parallelism, inter- and intra-assay variation and recovery capacity. There is no significant difference in MASP-2 concentrations between convalescent and control samples. MASP-2 levels were higher in hospitalized patients with COVID-19. The concentration of MASP-2 correlated positively with the concentrations of the complement terminal complex, ficolin-2, ficolin-3 and C-reactive protein.

Table 2. Description of the main results of the selected studies.

Based on the main findings of the 11 articles analyzed, there is an indication of a relationship between the activation of CS through VL and the pathogenesis of COVID-19. However, to reach this conclusion, it was necessary to evaluate the methodological quality of the studies performed (Table 3). Overall, the studies presented clear and consistent research objectives, concise information on ethical issues and possible biases, detailed justifications for the study, detailed descriptions of the research environment, Detailed explanations on the configuration and analysis of the data to ensure replicability, well-defined sampling criteria, and adequate literary reviews to contextualize the study. However, some studies had a limited sample size. Therefore, all studies were evaluated as being of high quality.

References	Average ±SD*	Quality
Ali et al., 2021	30,5 ± 0,7	High
Charitos et al., 2021	33,5 ± 0,3	High
Defendi et al., 2021	33 ± 0,5	High
Devalaraja-Narashimha et al., 2023	32,5 ± 0,5	High
Eriksson et al., 2020	30 ± 0,3	High
Gao et al., 2022	33 ± 0,4	High
Götz et al., 2023	31,5±0,5	High
Holter et al., 2020	31±0,5	High
Malaquias et al., 2021	32,5±0,4	High
Medetalibeyoglu et al., 2021	34,5±0,4	High
Niederreiter et al., 2022	30,5±0,8	High

***SD**: Standart deviation.

Table 3. Evaluation of the quality of the studies according to the Checklist Hawker.

In chronological order, the oldest study included in this review, conducted by Eriksson et al. (2020), investigated the role of MBL in thrombosis and coagulopathy in critical patients with COVID-19. A cohort of 65 patients with COVID-19 in critical condition, admitted to the ICU of a tertiary hospital center, was analyzed. Plasma levels of MBL were measured on the first day of hospitalization using an internal immunoenzymatic assay. The activity of the MBL pathway was evaluated using Manana as activator, measuring the binding capacity of the MBL and the C3 deposition of the complement. The results showed that patients with COVID-19 had high levels of MBL compared to healthy controls. MBL activity was also increased in patients with COVID-19, especially in those who developed thromboembolic complications. No associations were found between plasma levels of MBL and survival, need for mechanical ventilation or acute kidney injury. MBL showed a strong correlation with plasma D-dimer levels, indicating a clinical association with thrombosis. A significant correlation between MBL and activated partial thromboplastin time was also observed. There was no correlation between MBL and markers of cardiac function, respiratory function, or inflammation. There was a significant correlation between MBL and the total complement factor C3, but not with the activation product C3d or with C1q, suggesting a specific association between MBL and thrombosis. However, these observations do not establish a causal relationship between MBL and thrombosis. These results indicate a potential role of complement system MBL in coagulopathy associated with COVID-19. It is important to emphasize that the study by Eriksson et al. (2020) had a limited sample size and focused mainly on thromboembolic complications, without evaluating other clinical manifestations of COVID-19.

From another perspective, the prospective observational study conducted by Holter et al. (2020) investigated complement activation in patients with COVID-19, as well as its relationship with the development of respiratory failure. Two cohorts of hospitalized adult patients were included, while healthy blood donors were used as the reference group. Complement activation products such as C3bBbP, C3bc and the sC5b-9 complement terminal complex were quantified by enzyme immunoassays. VL was also evaluated by MBL detection. The results indicated that 59% of patients developed respiratory failure, with high levels of inflammation and coagulation parameters. During hospitalization, most samples showed persistent activation of the complement system, with elevated levels of sC5b-9, C4d, C3bc and C3bBbP. Although MBL levels were like those of the normal population, there was a modest increase on days 3 to 5, suggesting an acute phase reaction. Only one patient had hereditary MBL deficiency, with persistently low levels, and did not develop respiratory failure. Patients with respiratory failure on admission had higher levels of sC5b-9 and C4d compared to patients without respiratory failure. VL does not seem to be crucial for

complement activation in these patients. The findings indicate a prolonged and substantial activation of the complement, including the lectin pathway, in hospitalized patients with COVID-19, with the terminal complement sC5b-9 associated with respiratory failure and systemic inflammation. It is important to note that the study by Holter et al. (2020) had a relatively small sample size and used healthy blood donors as a reference group, which may limit comparisons with hospitalized patients. In addition, the study demonstrated a persistent and significant activation of the complement system, including the lectin pathway, which complements and contrasts the findings of Eriksson et al. (2020) that found an increased activity of the MBL pathway, which but not necessarily a prolonged activation.

The study by Medetalibeyoglu et al. (2021) sought to investigate the relationship between the rs1800450 variant of the MBL2 gene and the development and severity of COVID-19 infection. The study was conducted as a case-control study, analyzing the clinical courses of COVID-19. SARS-CoV-2 infection was confirmed by RT-PCR. We included 284 cases of COVID-19 and 100 healthy controls, matched by age and ethnicity. MBL2 gene genotyping (rs1800450) was performed by PCR. The results showed that the BB genotype was more common in cases of COVID-19 compared to controls. Patients with BB or AB genotype had a higher risk of severe disease and need for ICU compared to those with AA genotype. These results suggest that the reduction of MBL protein due to BB or AB genotypes is associated with a more severe course of COVID-19. However, the study has limitations, such as the small sample size in some analyzes and the lack of generalization of the results due to the specific population studied. The association between the rs1800450 polymorphism and the severity of infection can be explained by the functional deficiency of the MBL protein and the reduction in complement activation by lectin pathway. This results in a less efficient immune response in the elimination of SARS-CoV-2 and greater susceptibility to infection. Thus, the study highlights the association between the rs1800450 variant of the MBL2 gene and the severity of COVID-infection19, highlighting the importance of genetic factors in the immune response and indicating the need for further research in different populations to confirm these findings.

Given the role of the lectin pathway, focusing on the present study, the study conducted by Malaquias et al. (2021)precisely investigated the role of this pathway in activation during SARS-CoV-2 infection, comparing it with infection by Influenza A subtype H1N1 (H1N1pdm09) and a control group. The study used immunohistochemical analysis of lung tissue samples obtained post-mortem to evaluate the expression of lectin ligand mannose (MBL) and ficolin-3, as well as inflammatory cytokines. The results revealed significant differences in tissue expression of MBL and FCN3 between the groups studied, indicating a potential role of the lectin pathway in complement activation during SARS-CoV-2

infection. Histopathological analysis of pulmonary tissue samples also revealed distinct characteristics between groups, such as diffuse proliferative alveolar damage, fibrinous immune thrombosis and neutrophilic endothelitis in the COVID-19 group. In addition, genotypic analysis revealed an association between certain genotypes of the MBL2 gene and a higher tissue expression of MBL in the COVID-19 group, suggesting the influence of genetic polymorphisms on the complement system response by lectin during infection, as demonstrated by Medetalibeyoglu et al. (2021). Importantly, the use of post-mortem samples may not fully reflect the conditions during infection. These findings highlight the relevant role of the lectin pathway in complement activation during SARS-CoV-2 infection, according to the results obtained by Erikssson et al. (2020) who also reported an increased expression of MBL in patients with COVID-19.

Still regarding the pathway of interest, the study by Ali et al. (2021)investigated the participation of VL in the activation of SC, specifically in the immune response against recombinant proteins of SARS-CoV-2. The expression of recombinant S and N proteins of the virus was performed in mammalian cell lines, using plasmids provided by a specialized company. The study also employed recombinant truncated MASP-2 and a humanized antibody called HG4, which inhibits the cleavage of the VL-mediated C4 component. Samples of pre-pandemic serum were collected to obtain a pool of non-immune serum. HEK 293T cells were cultured and transfected with plasmid DNA containing the sequences of the proteins of interest. The cells were subsequently collected and analyzed by flow cytometry. In the opsonization assay, cells were opsonized with non-immune serum in the presence or absence of HG4, and C3b binding was detected using a human anti-C3c antibody conjugated with FITC. Fluorescence intensity was measured by flow cytometry. Additionally, ELISA plates were coated with purified SARS-CoV-2 recombinant S and N proteins, and the binding of lectin pathway recognition proteins (MBL, FCN2 and CL-11) was evaluated. The results were obtained by reading in an ELISA reader. The findings indicated that VL recognition proteins bind to S and N proteins of SARS-CoV-2, suggesting the activation of the complement system through this pathway, as demonstrated by Eriksson et al. (2020) and Medetalibeyoglu et al. (2021). A dose-dependent and saturatable deposition of C3b and C4b was observed in the immobilized SARS-CoV-2 proteins, which indicates activation of the complement by VL. Inhibition of VL with the antibody HG4 significantly reduced the functional activity of this pathway. A direct interaction between SARS-CoV-2 N protein and MASP-2 was also observed, resulting in the cleavage of the C4 component by VL. Protein S expression on the surface of HEK 293T cells resulted in higher C3b deposition, which was reduced by VL inhibition with HG4. In short, the study results suggest that VL plays a role in the immune response against SARS-CoV-2 proteins, resulting in activation and deposition of

C3b and C4b by this pathway. These findings provide a deeper understanding of the mechanisms of the immune response to SARS-CoV-2. Importantly, although interactions between virus proteins and VL proteins have been demonstrated in vitro, these results may not fully reflect the complexity and immune response in vivo. In addition, the study used recombinant proteins instead of the complete virus, which may limit the generalization of the results to the actual clinical situation.

The study conducted by Defendi et al. (2021), in the same line, but with another cut, sought to investigate the role of the three pathways of complement activation in tissue injury during COVID-19 infection. The retrospective study included 74 hospitalized patients with COVID-19 confirmed by RT-PCR. Complement activation pathways and components were analyzed, as well as their relationship with clinical outcomes. The results revealed different complement activation profiles in patients. The group with significant activation of VA and VL showed a high mortality rate, which was corroborated by the study by Eriksson et al. (2020), in which increased activity of MBL was associated with severe complications, such as thromboembolic. In addition, MBL protein deficiency was observed in all groups, which suggests a impairment of the lectin pathway. This deficiency may be a result of liquid consumption due to activation of the lectin pathway or possible tissue deposition, corroborating the hypothesis proposed later by Devalaraja-Narashimha et al. (2023). Thus, the study results suggest that the activation of VA and VL plays an important role in the pathogenesis and severity of COVID-19. It is important to note that the study has limitations, such as small sample and retrospective analysis in a single center.

On the other hand, the prospective observational cohort study conducted by Charitos et al. in 2021demonstrated that the concentration of MBL was not associated with severity of infection. Serum concentrations of pattern recognition receptors (PRP) of VL and its predominant inhibitor, C1INH, were investigated in patients with COVID-19, in which 154 hospitalized patients with confirmed infection by SARS-R were includedCoV-2 during the first wave of the pandemic. Complement activity by VC and VA was evaluated, as well as concentrations of MBL and FCN-3. The results showed that complement activity by VA was slightly lower in patients who died or who required invasive ventilation. While complement activity by VC was significantly lower in more severe cases. Concentrations of MBL, FCN-3 and C1INH at admission were not associated with adverse clinical outcomes, but higher concentrations of C1INH were correlated with inflammatory markers and subsequent use of tocilizumab. The study has some limitations due to the lack of data on other VL proteins. However, the results suggest a marked activation of complement by AV in critical patients with COVID-19, with a significant reduction of in vitro activity of the same pathway. Lectin

pathway activity does not seem to play a relevant role in progression to severe forms of the disease.

The study by Niederreiter et al. (2022) investigated complement activation in the kidney and lung of patients with severe COVID-19. Through the analysis of post-mortem tissues, the deposition of complement components, pathological changes, and the correlation between the severity of pulmonary and renal lesions were examined. The results showed that patients with severe COVID-19 had significantly more intense lung lesions compared to the control group. These changes included diffuse alveolar damage, thickening of alveolar septa, proliferated fibroblasts, and mononuclear inflammation. In renal tissue, tubular lesions, tubular necrosis, mild glomerular sclerosis, interstitial infiltrates, and fibrosis were observed. Complement deposition analysis revealed a strong presence of components C1q, C3c and C3d in the lungs and kidneys of patients with severe COVID-19. In addition, MASP-2, the VL activator, was also detected in large quantities. These findings suggest a consistent activation of the 3 complement pathways during SARS-CoV-2 infection, as proposed by Devalaraja-Narashimha et al., (2023). There was a positive correlation between complement deposition and severity of lung and kidney lesions, indicating the possible role of complement in the pathology associated with severe COVID-19. However, it is important to emphasize that the study had a small sample and was based on post-mortem tissue analysis, limiting the generalization of the results and the understanding of complement activation in milder cases of the disease.

Gao et al. (2022), through the investigation of the interaction between highly pathogenic coronavirus N proteins (SARS-CoV, MERS-CoV and SARS-CoV-2) and MASP-2, sought to examine their role in exacerbated complement activation through VL. The methodology included amplification of coronavirus N genes in serum samples of patients, chemical synthesis of these genes, amplification of the MASP2 gene from a cDNA library of human hepatocytes and several analyzes, such as immunoprecipitation, immunotransfer, protein purification, C4 cleavage assays and complement deposition. The results showed that the N proteins of SARS-CoV, MERS-CoV and SARS-CoV-2 interact with MASP-2. This interaction was observed in vitro with agarose granules conjugated with N protein and immunoprecipitation with human or mouse serum, as well as in lung tissues of COVID-19 victims. Additional analyses revealed specific regions in MASP-2 and N protein that are essential for this interaction. In addition, pathogenic coronavirus N proteins potentiated MASP-2-dependent complement activation, increasing C4 cleavage and MASP-2 deposition in infected cells. This activation of the complement was blocked by antibodies specific to protein N or MASP-2, as well as by MASP-2 inhibitors. Experiments in mice revealed that exposure to pseudotyped HIV with the Spike protein of SARS-CoV resulted in a significant

increase in the production of leukotriene B4 (LTB4), an inflammatory metabolite. This inflammatory effect mediated by N protein was suppressed by anti-MASP-2 and C1INH antibodies. The results suggest that the highly pathogenic coronavirus N protein interacts with MASP-2, potentiating complement activation by VL, which may contribute to the exacerbated inflammatory response during coronavirus infection and to the pathogenesis of these diseases, as demonstrated in patients with COVID-19 included in the study by Niederreiter et al. (2022). However, it is important to highlight that the analysis was mainly performed in vitro and in lung tissues of COVID-19 victims, which may limit the generalization of the results to the population. In addition, the study focused specifically on the interaction between coronavirus N proteins and MASP-2, leaving room for investigation of other complement activation pathways and other mechanisms involved in the pathogenesis of COVID-19. The results obtained by Gao et al., (2022) are corroborated by the study by Gotz et al. (2023), which highlights the essential role of MASP-2 in the complement cascade triggered by the virus.

In another scenario, Devalaraja-Narashimha et al. (2023)sought to evaluate, comprehensively, levels of complement activation products and proteins in hospitalized patients with different severity of COVID-19 disease. The authors also investigated whether CS markers were associated with adverse outcomes, such as mortality and the need for prolonged supplemental oxygen. The methodology included 89 hospitalized patients, grouped according to disease severity, in an adaptive, phase 2/3, randomized, double-blind, placebo-controlled study. Patients were treated with intravenous sarilumab or placebo. Serum samples were collected for cell deposition assays and complement pathway protein analysis. The results showed high levels of complement activation products in patients with COVID-19, especially those with more severe forms of the disease, as well as in the study by Niederreiter et al. (2022). Patients with higher levels of these biomarkers had higher disease severity, need for prolonged supplemental oxygen and higher mortality rate. In contrast, patients who died had significantly lower MBL levels at all time points compared to those who survived, and MBL levels were also inversely correlated with time to improve oxygen. In addition, serum from patients with COVID-19 demonstrated thrombotic potential in endothelial lung cells. The results of the study also suggest that reduced levels of MBL can be attributed to the liquid consumption resulting from the activation of the lectin pathway, as well as to the possible tissue deposition. These findings suggest that activation of complement pathways is associated with disease severity and adverse outcomes in hospitalized patients with COVID-19. However, more research is needed to confirm these findings and better understand the role of complement pathways in the pathogenesis and prognosis of COVID-19, considering other variables and biomarkers related to the disease.

Finally, the study by Gotz et al. (2023) investigated the role of MASP in COVID-19 and other diseases. The researchers generated mouse monoclonal antibodies against MASP-2 and established a reliable ELISA assay to quantify it. Plasma samples were obtained from healthy controls, patients convalescent with COVID-19 and patients with COVID-19 followed prospectively. The results showed that the antibodies were able to detect recombinant MASP-2 and native serum MASP-2. In addition, the ELISA assay was accurate, with a working range of 0.16 to more than 20 ng/mL of MASP-2. Hospitalized patients with COVID-19 had significantly higher levels of MASP-2 compared to control and convalescent groups, according to the results of Niederreiter et al. (2022) and Gao et al. (2022). These levels correlated with the C-reactive protein and with the PRMs: ficolin-2 and ficolin-3, as well as with the generation of TCC. The results suggest that MASP-2 plays a role in the complement cascade initiated by SARS-CoV-2 and may be a potential therapeutic target and prognostic marker.

4. CONCLUSION

The reviewed studies provide significant evidence on the role of the lectin pathway in the activation of the complement system against the immune response and pathogenesis of COVID-19 infection. Patients with COVID-19 had high levels of MBL, indicating activation of the lectin pathway during infection. The deficiency of MBL, due to certain genotypes, was associated with a more severe course of the disease, probably due to the reduction in the activation of the complement by the lectin pathway.

The interaction of lectin pathway recognition proteins with SARS-CoV-2 proteins suggests the activation of the complement system through this pathway. The presence of elevated levels of complement activation products was observed in patients with COVID-19, especially those with severe forms of the disease, associated with increased severity, prolonged need for supplemental oxygen and higher mortality rate.

Additional studies highlighted the hyperactivation of the complement cascade due to the interaction between SARS-CoV-2 N proteins and MASP-2. In addition, evidence suggests that lectin pathway recognition molecules bind to SARS-CoV-2 virus proteins, leading to subsequent activation of the complement. Complement component depositions were also observed in affected organs, suggesting a possible role of complement in tissue injury and disease severity. However, the results were not always consistent in all studies, highlighting the complexity of the immune response in COVID-19 and the need for additional research for a more comprehensive understanding of these mechanisms.

Despite the limitations of the reviewed studies, including the limited sample size and observational nature, it is consensus that the activation of the complement system is present in patients with COVID-19 and that levels of complement activation products are associated with more severe forms of the disease. Therefore, understanding the activation of the complement system via lectin can be crucial in the development of effective therapeutic strategies for patients with COVID-19. Therefore, the reviewed studies contribute to the knowledge about the importance of the lectin pathway in the immune response and pathogenesis of COVID-19 infection. However, further research is needed for a more complete understanding of these mechanisms and for the development of targeted therapeutic approaches.

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