SYSTEMATIC REVIEW

Predicting SARS-Cov-2 - Procoagulant/Coagulation Related Protein Interaction: A Systematic Review

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ABSTRACT

Introduction: Patients with COVID-19 tend to have procoagulant states and a higher risk of blood clotting. This systematic review aimed to provide an overview of studies that used the computational docking method to predict the interaction between procoagulant/coagulation human proteins and SARS-CoV-2. **Method:** A systematic review was conducted using six databases (Scopus, Cochrane, PubMed, Clinical key, Emerald, and Google scholar). The review included original articles, that applied computational docking methods for viewing the potential interaction between host protein and SARS-CoV-2 in COVID-associated coagulopathy. **Results:** Five of 715 articles were included. In this systematic review, the assessment of high-quality aspects included multi-target protein assessment, the utilization of crystal structure for the generation of target interaction, the use of CAPRI validated protein-protein docking tools combined with other software, molecular dynamic simulation, and multi-host protein databases. The systematic review revealed the prediction interaction between host procoagulant/coagulation related protein and SARS-CoV-2 protein as follows: VKORC1 - ORF7a, Thrombin - Protein S, ORF3a-heme. Protein S has three potential Heme-binding motifs (HBMs) and protein 7a contains two HBMs. Thrombin and Factor Xa were structurally similar to Mpro. Human gene variants influence the affinity interaction between SARS-CoV-2 and human proteins. **Discussion:** This systematic review provided a list of SARS-CoV-2 protein targets (ORF7a, Protein S, ORF3a, Mpro) that had the potential to interact with human proteins through different mechanisms of action of procoagulant state and blood clotting. This will expand the diagnostic, clinical monitoring, and therapeutic options for SARS-CoV-2 infection.

Keywords: Computation; Molecular; COVID-19; Blood Coagulation

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INTRODUCTION

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2). This viral infection has caused a global pandemic. It has spread to more than 200 countries worldwide. Patients with COVID-19 infections may tend to have procoagulant states and a higher risk of blood clots. Coagulation disorders are challenging to treat. This is an important feature of disease severity (1,2). Autopsies identified clots in vessels of COVID-19 patients' lungs, liver, heart, and kidney. Excessive activation of platelets and coagulation proteins may cause thrombocytopenia and high levels of blood clots. SARS-CoV-2 infection evokes a systemic inflammatory response and causes procoagulant-anticoagulant homeostatic abnormalities (1–4). Heme may also increase procoagulant activity and induce coagulation activation. Hemolytic anemia has been reported in patients with severe COVID-19. In this pathological state, free heme is released from hemoglobin. It promotes coagulation activation (5–7).

Based on a recent systematic review-meta-analysis study, COVID-19 coagulopathy and its complications have been considered to correlate with severe disease and a higher risk of death. Thromboembolism was significantly associated with the COVID-19 mortality rate. The mechanism of SARS-CoV-2 protein interactions with human procoagulant/coagulopathy proteins is still being understood (8). Improving our understanding of SARS-CoV-2 infections will expand the diagnostic, patient care, and therapeutic options. A computational approach is a time-saving method with the best chance of identifying potential interactions between SARS-CoV-2 and the host protein. This systematic review aimed to provide an overview of studies that have used computational approaches to predict the interaction between human procoagulant/ coagulation-related proteins and SARS-CoV-2.

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MATERIALS AND METHODS

The procedure, search strategy, and study selection
The protocol of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was applied in this systematic review. Problem (P), Intervention (I), Control (C), and Outcome (O) in this study were COVID-19 (P), SARS-CoV-2 protein/gene(I), human procoagulant/coagulopathy related protein/gene(C), and computational approaches for protein-protein interaction(O). We searched in six databases (Scopus, Cochrane, PubMed, Clinical key, Emerald, and Google scholar) with keywords ("DOCKING") AND ("GENE" OR "PROTEIN") AND ("COAGULATION") AND ("COVID"). Our systematic review included original studies (published since 2020) that used computational approaches for SARS-CoV-2–Procoagulant/coagulation protein interactions. Studies with a wet laboratory approach, non-English language articles, duplicate articles, and the inaccessibility of full-text were excluded. Two authors independently conducted article screening, reviewed articles, and assessed the quality of articles. In the case of discrepancy results, a third author was involved for a consultation to reach a consensus.

Quality assessment

Based on potential issues of bias in the computational protein-ligand study, Mohamed proposed five aspects (Mohamed tools) for assessing quality in a systematic review (9). We used Mohamed tools to assess the study quality in our systematic review. To identify protein-protein interactions, we modified two items of the Mohammed tools, which were docking tools and resources of databases. In Mohamed-tools "the use of AutoDock-Vina combined with other molecular docking-tools" was considered as one of the criteria of a high-quality study(9). Compared to AutoDock-Vina, Autodock tool had a similar performance. However, big ligand analysis remains a challenge for these two docking tools (10). Therefore, we changed the option with Critical Assessment of Predicted Interaction (CAPRI) validated protein-protein docking tools (11). Furthermore, we also changed "Mohamed's item "resources for approved drugs" (9) into "resources for host protein". Multiple databases give a better view of the structure and function of proteins (12). Therefore, in this systematic review, the assessment of high-quality aspects included multi-target protein analysis, the utilization of crystal structure for assessing the target interaction, the use of CAPRI validated protein-protein docking tools combined with other software, multi-host protein databases, and molecular dynamic simulation.

RESULTS

Study Selection

Five of 715 articles were included in this systematic review (Figure 1). Methods/protocol, software, protein target, and human procoagulant/coagulation protein varied among the included studies (Table I and II).

Figure 1: Flow diagram of the systematic review process following PRISMA

Study Quality

The result of the quality assessment were in Figure 2 and Table I. The total number of high-quality aspects was 4 (1 article), 3 (1 article), 2 (2 articles), and1 (1 article). None of the studies performed molecular dynamic simulation.

Data Synthesis

Potential protein target candidates of SARS-CoV-2 were Protein S, ORF3a, ORF7a, and Mpro (13–17). This systematic review revealed the best prediction interaction between host procoagulant/coagulations-related protein and SARS-CoV-2 protein as follows: VKORC1-ORF7a, Thrombin-SER494 in protein S, and ORF3a-heme (Table II).

DISCUSSION

The potential SARS-CoV-2 proteins identified in this study were protein S, ORF3a, ORF 7a, and Mpro (Table 2). Protein S attaches to the host angiotensin-converting enzyme 2 (ACE2). Protein S is essential for receptor recognition. ORF3a is a protein that contributes to activating NLRP3, ASC ubiquitination, caspase 1, and IL-1β maturation. ORF7a is a type I transmembrane protein (18,19). ORF7a binds to human CD14+ monocytes, leading to decreased HLA-DR/DP/ DQ levels and increase proinflammatory cytokine levels (20). Mpro is essential for SARS-CoV-2 gene transcription, viral ribonucleic acid translation, and viral replication(21).

Table I: Quality of studies included in the systematic review

Design of target proteins		Target template Modelling			Docking Tools			Molecular dynamic simulation		Resources of host protein		Total high-quality aspects	References
1	2	3	4	5	6	7	8	9	10	11	12		
	V	V			V				V	NR		2	(13)
	V	V	V			V	V		V		BIOGRID, NCBI	4	(14)
	V	V	V				V		V	RCSB PDP		2	(15)
	V	V	V				V		V		NCBI, RSCB PDB	3	(16)
V		V			V				V	RCSB PDP		1	(17)

1=Mono-target; 2=Multi-target; 3=Crystal structure; 4=Homology modelling; 5=Co-crystal ligand; 6=Autodoct-Vina/Autodock; 7=CAPRI validated protein-protein docking-tools combined with other software; 8=Others; 9=Yes; 10=No; 11=mono-resource; 12=multi-resources; NR=Not reported; RSCB PDB=RSCB Protein Bank Database; BIOGRID=the biological general repository for interaction datasets; NCBI=national center for biotechnology information; underlined text=criteria of high quality aspect (which was number 2,3,7,9,12).

Table II: Human Procoagulant/ Coagulation Proteins Interaction with SARS Cov-2 Proteins

Human protein references	ORF 10	ORF 7a	ORF 3a	ORF 1ab	N	S	M	E	M pro	Main findings
Thrombin (13)						V				Thrombin links with Protein S and also binds with ACE2.
VKORC1 (14)		V								VKORC1-ORF7a possesses a strong binding affinity. VCORC1 gene variants might influence this interaction.
SERPING 1 (14)		X								
PABPC 4 (14)		X								
Heme (15)		V				V	X	X		Protein S is a moderate binder and ORF7a is a poor.
Heme (16)	V		V							Heme-iron binding site was Arg134 of ORF3a, Ile304 of N, and Cys44 of E. The important interactions were ORF3a-cytochrome c (G41S), and oxidized hemoglobin/ deoxyhemoglobin - ORF1ab-ORF3a-ORF10.
Human-Oxy-Hemoglobin (16)	V		V	V	V					
Human-Deoxy- Hemoglobin (16)	V		V	V	V					
Cytochrome c (G41S) (16)			V							
Human erythrocyte catalase (16)			X							
Human Erythrocyte NADH-cytochrome b5 Reductases(16)			X							
Factor Xa (17)									V	Similarities in structural comparison were identified among Mpro and human protein (Factor Xa and thrombin), which were presented by low root mean-square deviation values (2,49 Ă and 2.57Ă).
Thrombin (17)									V	

V=potential candidate; X=dismissed as candidate because of lack of structural data or no similar motif.

ORF 7a-human VKORC1 possessed a strong binding affinity (Table 2). VKORC1 plays an essential role in supporting coagulation activity through the vitamin K cycle (22). Gene variants of VKORC1 may contribute to the binding mechanism. The gene variants may affect splicing, minimum free energy (MFE) value of messenger ribonucleic acid (mRNA), and micro ribonucleic acid (miRNA). Splicing alterations were found at -695C T, +1391 C>T, and +2110T>C. The change in splicing may affect mRNA and alter potential binding affinity to the virus. Furthermore, a potential increase in mRNA MFE value was identified in VKORC -1975G>C and +1100 C>T. A greater MFE value may give greater feasibility for mRNA degradation. Changes were also determined

using miRNA analysis. In this case, miRNA increases in –2834C>A and -1675G>A, and miRNA decreases in -3537C>G. An elevation in miRNA may lower protein expression, and vice versa. The interaction between VKORC1 and ORF7a may inhibit the activation of clotting proteins (14). This may have affect the substances metabolized by VKORC1. The anticoagulant warfarin, inhibits the Vitamin K-epoxide reductase complex. Warfarin is also metabolized in CYP2C9 (a part of the CYPP450 family). VKORC1 and CYP2C9 are related to warfarin sensitivity and dose requirement (23,24). The significant features of symptomatic COVID-19 were cytokine release, proinflammatory development, and lower CYP450 isoenzyme levels. These features may decrease warfarin metabolism (25–27). Decreasing warfarin dose requirements may be required for patients with COVID-19 (25). Interestingly, patients with COVID-19 tend to have a lower vitamin K status as compared to healthy subjects (28). It is essential to understand the effect of SARS-CoV-2 infection on warfarin dose adjustment.

Another interaction between SARS-Cov-2 and the host protein is Protein S-thrombin. The docking result showed that thrombin is linked with Ser494 in Protein S and also Glu37 in human receptor ACE2 (13). SARS-Cov2 protein S (Ser494, Phe 486, Leu 455) supports hotspot 31 of the ACE2 receptor. Ser494 also strengthenened the stability of hotspot 353 (29,30). In vitro tests, using cell-based bioassays, showed that human protease (thrombin and factor Xa) can promote viral entry by direct proteolytic cleavage of SARS-CoV-2 Protein S (31). Factor X is vitamin K dependent. It is expressed mainly in the liver and in other cells which express ACE-2. Factor X, a localized serine protease, cleaves protein S, which binds to the host cell receptor. Spike protein 2 is released. This supports the fusion of the host cell membrane and Sars-CoV-2. The Sars-CoV-2 genetic material was inserted into the human cells. It replicates in the host cells. New Sars-CoV-2 particles are released and cause infection of other host cells, inflammation, and coagulation (32). Therefore, anticoagulation therapy may suppress viral entry (31).

Thrombin bursts have also been reported in cases of severe COVID-19 (33). Sars-Cov-2 may also affect coagulation through Mpro (17). Thrombin is highly similar to the active site of Mpro, leading to the potential for off-target binding of small molecules (34). Human proteases (Factor Xa and thrombin) had fold similarity with SARS-CoV-2 Mpro, which were presented by low root-mean-square deviation values (2,49 Ă and 2.57Ă). These superposed structures may cause perturbations in the coagulation (17). Disorder in pro-clotting factors caused a significantly lower value of antithrombin and a higher D-dimer level and fibrinogen. The higher value of D-dimer showed worse progression of COVID-19 severity due to disseminated intravascular coagulation or thrombosis. These features may be caused by activation of the coagulation system (4,17,35). Blocking coagulation proteins are potential targets for anticoagulant therapy in COVID-19 (34).

SARS-Cov-2 proteins (Protein S and ORF3a) are also linked to Heme. Direct interactions of SARS-Cov-2 proteins with heme are presented by Heme Binding Motifs (HBMs). Protein S has three HBMs, a positive net charge (+2), and several potential hydrophobic residues that moderately support heme binding. ORF7a possesses only two hydrophobic residues that support heme binding. ORF7a is a poor heme binder with two HBMs. Therefore, Protein S becomes a more interesting candidate than OFR7a for interaction with heme (15). Heme is also essential for Protein S to avoid antibody immunity. Free heme is toxic. An endogenous tetrapyrrole product, biliverdin, is produced for heme detoxification. High-affinity binding of N-terminal domain (NTD) of Protein S to biliverdin stabilizes the NTD structure and produce the SARS-CoV-2 envelope glycoprotein. This leads to reduces the reactivity of Protein S and blocks the neutralizing antibody response to the virus (36).

Heme is composed of protoporphyrin IX and iron. Heme binds to hemoglobin and cytochrome c (Cyt c) to play crucial roles in human biological processes (37). Dysregulation of heme-iron metabolism and hemoglobin dysfunction has been identified in COVID-19 (38). The heme-iron binding sites identified in the conserved-domain analysis, were Arg134 of ORF3a, Ile304 of protein N, and Cys44 of protein E. The conserved domains of ORF3a were also found in Cyt c. ORF3a does not interact with wild-type Cyt c, but it fully embeds in Cyt c G41S variant and blocks heme. The interaction of ORF3a-Cyt c (G41S) may contribute to the respiratory problems in COVID-19. Based on this result, subjects with Cyt c (G41S) may have a greater risk of disease severity. The docking results also showed that ORF1ab, ORF3a, and ORF10 were bound to oxidized hemoglobin and deoxyhemoglobin. ORF3a and ORF10 of SARS Cov-2 are embeded in deoxyhemoglobin and directly bind to the heme of the beta chain. This result may explain the invasion of the virus on hemoglobin (16). CD26, CD147, and ACE2, which are located on erythrocytes and blood cell precursors, also play important roles in the interaction between hemoglobin molecules and SARS Cov-2 (38). SARS-Cov-2 induces hemoglobin and heme-iron dysfunction. Consequently, it may cause anemia, hyperferritinemia, an increase in free toxic heme in circulation, downregulation of heme oxygenase-1, hypoxemia, hypoxia, an increase in oxidative stress, inflammatory stress, and multi-organ damage (38–40). The incidence of anemia is not frequent in patients infected with SARS-CoV-2. Low levels of hemoglobin have been identified in severe disease (6,7,41). Free heme increases oxidative stress by scavenging nitric oxide, catalyzing the Fenton reaction, and activating inflammasome (40). Excess-free heme in

circulation may induce thrombin generation (5).

The COVID-19 pandemic is a challenging phenomenon in the healthcare system (42). During this pandemic, hospitals experienced a large number of COVID-19 patients. Interprofessional collaboration was adopted to promote patient care. COVID 19 can cause many clinical manifestations and complications in multiple organ systems. Therefore, a multi-professional team is involved in COVID-19 management (43). The SARS-CoV-2 infection causes coagulation disorders. Patients with COVID coagulopathy have a higher incidence in severe cases and have a poor prognostis (44). The health care team is a vulnerable group that is not only exposed to the hazards of the pathogen but also psychological stress. Updating knowledge of SARS CoV-2 is important for health services (45,46). Our systematic review results can be beneficial for future clinical investigations.

All computational docking methods have inherent limitations to describe protein interactions models. Therefore, investigation in wet laboratories and clinical studies need to be conducted for further exploration.

CONCLUSION

This systematic review provided a list of SARS CoV-2 protein targets (ORF7a, Protein S, ORF3a) that had the potential to interact with human protein (VKORC-1, thrombin, and heme) through different mechanisms of action of procoagulant state and blood clotting. Therefore, it will expand the diagnostic, clinical monitoring, and therapeutic options in SARS-CoV-2 infection.

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