Review article

Hematologic Abnormalities Associated with Post-Acute COVID-19 Sequelae or "long-COVID"- a Systematic Review

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Objective: SARS-CoV-2 emerged late 2019 and quickly spread globally. Acute COVID-19 effects were quickly elucidated; however, some patients were found to suffer from persistent symptoms in the absence of an acute infection. This places unnecessary pressure on healthcare systems and affects patient quality of life. Literature indicated lymphopenia, hyperferritinemia and coagulopathies were common among those with persistent symptoms. This systematic review aims to summarize the association between these hematologic abnormalities and long-COVID.

Methods: A systematic search of five electronic databases, PubMed, Google Scholar, Science Direct, Griffith University library and Cochrane, was conducted using specified search terms described in the methods section. Studies were refined using the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) tool. Data was retrieved from studies that passed the risk of bias (ROB) and met the inclusion and exclusion criteria, as follows; number of participants (≥10), hematologic testing, timing of testing, and studies with full text available in English.

Results: The search strategy identified 14 studies that passed the ROB, met the inclusion and exclusion criteria, and were selected for the systematic review. Though some patients experiencing long-COVID had lymphopenia, hyperferritinemia and coagulopathies, there was inconsistencies found. Some patients with long-COVID had limited evidence of hematologic abnormalities.

Discussion: Lymphopenia was a frequent anomaly identified in post-acute COVID, however, not exclusive to long-COVID patients. New research has shown the absence of specific T and B lymphocyte subsets may be exclusive to long-COVID patients, along with the sustained activation of other immune cells. Evidence has also emerged showing sustained inflammation beyond the acute infection in long-COVID patients. Coagulopathies have been shown to persist due to an elevated D-dimer in post-acute COVID-19 analyses.

Conclusion: There is evidence of hematologic features that are exclusive to long-COVID, however, research is still limited. The cause and effect of these abnormalities are yet to be determined. With future directions, further supporting evidence may emerge elucidating the potential hematological causes and mediators of long-COVID.

Key words: Long-COVID, Persistent symptoms, Lymphopenia, Iron dysregulation, Coagulopathy.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic has resulted in global consequences, including deaths, lockdowns, economic breakdowns, and more. One consequence of COVID-19 referred to as "long-COVID," occurs when symptoms of the viral infection persist even after the acute infection, or virus, has been cleared. A clear definition of the timeline associated with long-COVID has not yet been established and varies between articles (discussed later). Long-COVID symptoms include fatigue, shortness of breath, general malaise and more.[1-3] These lingering symptoms affect the patient's quality of life and, thus, adds unnecessary additional pressure on the healthcare system due to the extra care required for patients following the acute phase of the disease. The purpose of this study is to assess the changes seen in the blood components, such as red cells, white cells, and platelets, of COVID-19 patients who have recovered from an active infection and are suffering from long-COVID symptoms. Elucidating how long-COVID occurs, how it affects the blood, how to predict it, and how it could possibly be treated could relieve the burden on both healthcare and patients. Samples are often used repeatedly up to several times to recreate measurements and/or to determine additional results of multiple analytes.

Study Aims and Objectives

This systematic review aims to analyze and compare current literature regarding long-COVID with a focus on hematologic parameters to determine the commonly seen changes and the possible associated pathophysiology to assist in future care and rehabilitation. This will be achieved via the following objectives:

1. A planned systematic review of the literature to assess and compare the most frequent abnormal hematologic findings in long-COVID.
2. Comparisons between literature methods and findings to determine the reliability of abnormal hematologic markers for predicting long-COVID and severity.
3. Discussions of the theorized pathophysiology to elucidate possible directed therapy or rehabilitation for affected patients to assist in recovery and return to baseline quality of life.

Multiple studies investigating hematologic parameters in patients with long-COVID were critically evaluated and compared. Establishing disease or diagnostic patterns could provide critical information to determine prognosis and guide patient therapy or rehabilitation.

SARS-CoV-2 and Hematologic System

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded, positive sense, enveloped RNA virus belonging to a large family of coronaviruses.[4] With an unestablished, but highly debated origin, SARS-CoV-2 emerged in late 2019 and in March 2020, the World Health Organization (WHO) declared SARS-CoV-2 a global pandemic. Numerous studies of SARS-CoV-2 have provided key information regarding COVID-19 transmission, pathogenesis, and possible treatment options.[5,6] An unexpected aspect of the pandemic, however, was the persistence of symptoms in the absence of an acute COVID-19 infection. This phenomenon has been coined "long-COVID," "chronic COVID," or post-acute COVID syndrome (PACS). Although many patients experience mild respiratory symptoms during the acute phase, select studies have found that many patients are affected by long-COVID. This places significant pressures on healthcare systems and the patient's quality of life.[3,7,8] The hematologic system is central to the basic functions of the human body and based on recent literature, SARS-CoV-2 affects the hematologic system in various ways. This analysis provides insight into the hematologic pathophysiology of long-COVID, providing some key prognostic indicators that may help predict the severity of disease, which can be used for directed therapy and rehabilitation for future patients.

Background/Literature Review

Long-COVID, the persistence of symptoms in the absence of an acute COVID-19 infection,

affects the quality of life of many patients. Establishing patterns in hematologic abnormalities could be used to determine suitable treatment and possible rehabilitation strategies for patients.

Definition of Long-COVID

An official definition for long-COVID has not yet been established. Some studies have followed the National Institute for Health and Care Excellence (NICE) definition of long-COVID, which, in collaboration with other institutes, have defined "ongoing symptomatic COVID-19" as persistent signs and symptoms lasting 4 to 12 weeks, while signs and symptoms lasting more than 12 weeks which cannot be explained by differential diagnoses is defined as "post-acute COVID-19 syndrome (PASC)."[1,3,9,10] Other studies have defined long-COVID as symptoms persisting in the absence of the virus or post-acute COVID-19 if symptoms persist for 3-12 weeks and chronic COVID-19 if symptoms persist for more than 12 weeks.[11-13] For the purposes of this review, long-COVID was defined as persistent COVID-19 symptoms with a negative COVID-19 polymerase chain reaction amplification (PCR) test or at least 1 month following the onset of symptoms (where PCR results were not available). This definition was used to establish a timeline to identify the significant diagnostic changes associated with the persistence of COVID-19 symptoms.

Risk Factors for Long-COVID

Comorbidities are known to increase the severity of COVID-19 and may also be the cause of some patients' suffering from long-COVID. Some of these comorbidities are listed include age (more than 60 years of age), obesity, diabetes mellitus (DM), hypertension, ischemic heart disease, chronic obstructive pulmonary disease (COPD), asthma, and chronic kidney disease (CKD).[1,14,15] The presence of these comorbidities may have pre-existing effects on hematologic parameters or contribute to the abnormalities seen in long-COVID.

Hematologic Abnormalities in Long-COVID

After extensive evaluation of the literature, the most common hematologic abnormalities found in long-COVD included lymphopenia, hyperferritinemia, and coagulopathies. Figure 1 depicts a basic schematic of the hematologic abnormalities identified in this systematic review.

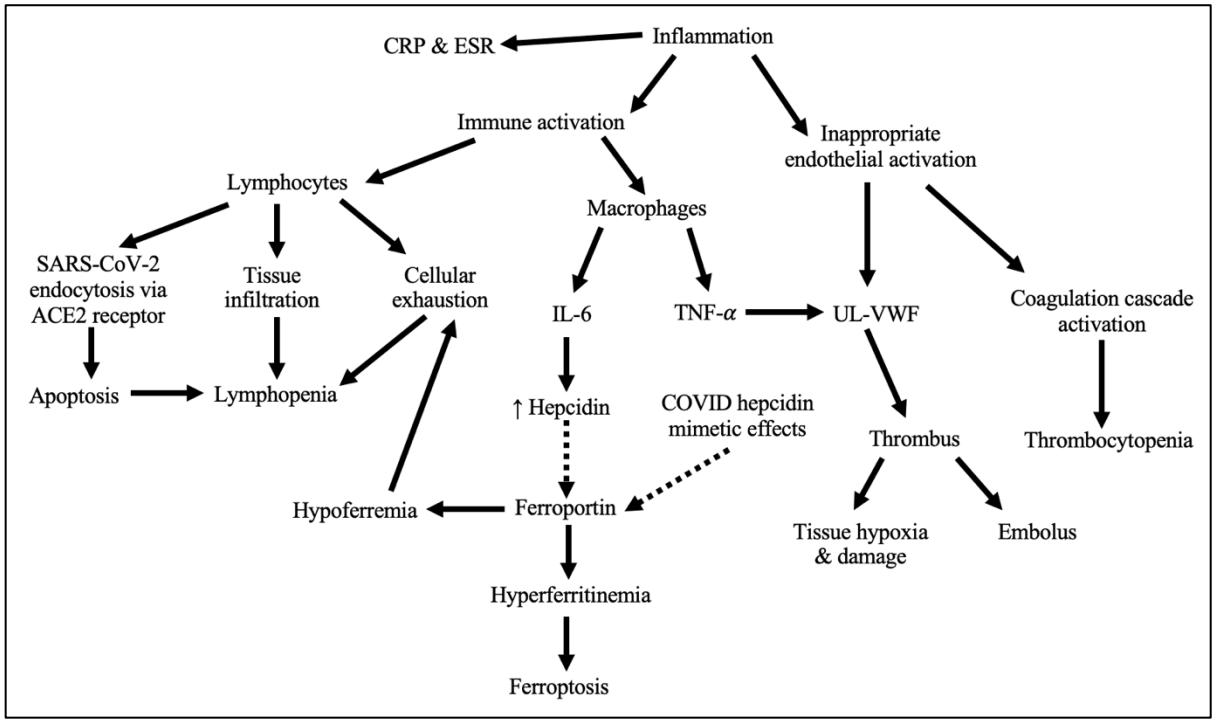

Figure 1: Summary depicting the effects of COVID-19 on various aspects of the hematologic system. C-reactive protein (CRP); erythrocyte sedimentation rate (ESR) tumor necrosis factor-alpha (TNF-α); ultra-large von Willebrand factor (UL-VWF); interleukin-6. (IL-6). Made by authors.

Cytokine Storm

Various studies have shown SARS-CoV-2 induces a cytokine storm, which is an excessive cytokine release due to uncontrolled immune regulation.[4,16] Some of the cytokines noted in COVID-19 includes proinflammatory and procoagulant molecules, such as interleukin (IL)-2, IL-6, interferon-gamma (IFN-γ), interferon-beta (IFN-ß), and tumor necrosis factor alpha (TNF-α) to list a few.[16-18] These cytokines have a multitude of effects on the hematological system. Another notable inflammatory marker that is elevated in SARS-CoV-2 infections is C-reactive protein (CRP). CRP is an acute phase protein and is increased during inflammation due to the release of IL-6.[19] Elevated CRP typically decreases when an active infection is cleared by the immune system, however in long-COVID patients CRP remains elevated indicating a potential significance to the persistent of a patient's symptoms.

Lymphopenia

Lymphopenia, a significant decrease in lymphocyte counts, was noted as a common anomaly found in long-COVID patients.[1,15,20] It was also found in acute infections and was shown to be a good predictor of COVID-19 severity, where patients with lymphopenia were found to have more severe symptoms.[14,20] Lymphopenia is abnormal in viral infections as lymphocytes are known to be one of the primary immune cells elevated in viral infections and involved in the clearance of the virus.[21] It should also be noted that neutrophilia with abnormalities in granulocytes and monocytes were seen in addition to lymphopenia in post-acute infections, which was postulated to contribute to morbidity via facilitation of infections caused by other microorganisms due to the patient's immunocompromised state.[20,22] These leukocyte abnormalities may exacerbate the negative effects of post-COVID contributing to long-COVID symptoms.

Numerous theories have been postulated as to the cause of lymphopenia seen in COVID-19 infections. A narrative review by Korompoki *et al*. indicated that lymphopenia may result from cell lysis due to SARS-CoV-2 infecting the cells via endocytosis mediated by binding of the viral spike protein to angiotensin converting enzyme 2 (ACE2) receptors.[17] Another theory suggested that the cytokine storm seen in COVID-19, as noted earlier, resulting in hyperinflammation was associated with cytopenia and may induce lymphocyte apoptosis.[16,17] Ramakrishnan *et. al*. postulated the possibility of "COVID-associated immune exhaustion," which occurs in chronic viral infections due to prolonged antigen stimulation.[1] Lymphocytic infiltration may also contribute to lymphopenia in COVID-19 patients. Lymphocytic infiltration has been reported in multiple organs, including the lungs, hepatic portal tract, kidneys, and myocardium.[1]

In addition to lymphopenia, iron is central to erythropoiesis and lymphocyte activity. Lymphocytes require iron to produce an effective immune response to infections when the cell initially interacts with the viral protein (antigen) or is primed.[23] Therefore, iron dysregulation may also contribute to lymphopenia and long-COVID symptoms.

Iron Dysregulation

Iron dysregulation has been associated with severe acute COVID-19 infections, producing hyperferritinemia (elevated ferritin concentrations), and has been shown to persist for up to 2 months post-acute infection.[22,24] During inflammation, the cytokine IL-6 is released, which stimulates hepcidin synthesis. Therefore, the hepcidin stimulation functions as a host defense mechanism by limiting iron availability to invading organisms.[11,23,24]

Hepcidin, a peptide hormone produced by the liver, is central to iron homeostasis; it functions to inhibit iron absorption by inactivation of ferroportin, the transmembrane protein responsible for iron exportation out of cells.[21,25] Increases in hepcidin inhibits the release of iron from cells, thus, increasing ferritin concentrations, causing hyperferritinemia, in both serum and macrophages.[11,23] This hyperferritinemia alters iron metabolism, affecting red blood cell (RBC) indices and leading to apoptosis, termed ferroptosis, which is a

type of necrosis induced by excessive iron. Ferroptosis may also cause neighboring tissue damage, which further exacerbates inflammation creating a vicious cycle and continued inflammatory response.[11,24,26] Interestingly, a study by Ehsani found sequence similarities between the SARS-CoV-2 spike protein and hepcidin, suggesting SARS-CoV-2 could possibly have a hepcidin-mimetic effect, further exacerbating the effects of hepcidin.[27] This brings into question whether hepcidin upregulation is due to host defense or a pathological process due to COVID-19.

Coagulopathy

It is well established that inflammation plays a crucial role in infections and often activates clotting and impairs fibrinolysis promoting thrombosis. In long-COVID, it has not been clearly defined whether inflammation is the cause or effect of coagulopathy. Coagulopathy is the dysregulation of the coagulation system resulting in inappropriate coagulation or bleeding. A simplified diagram of the coagulation cascade with select anticoagulant factors and part of the fibrinolytic system is presented in Figure 2. Coagulopathies are the most frequently identified hematologic abnormality in COVID-19 infections, including low platelet counts (thrombocytopenia), low fibrinogen (fibrinogenemia), and an elevated D-dimer.[4,16] This has been coined "COVID-induced coagulopathy" (CIC) or "COVID-19-associated coagulopathy" (CAC).[4,17]

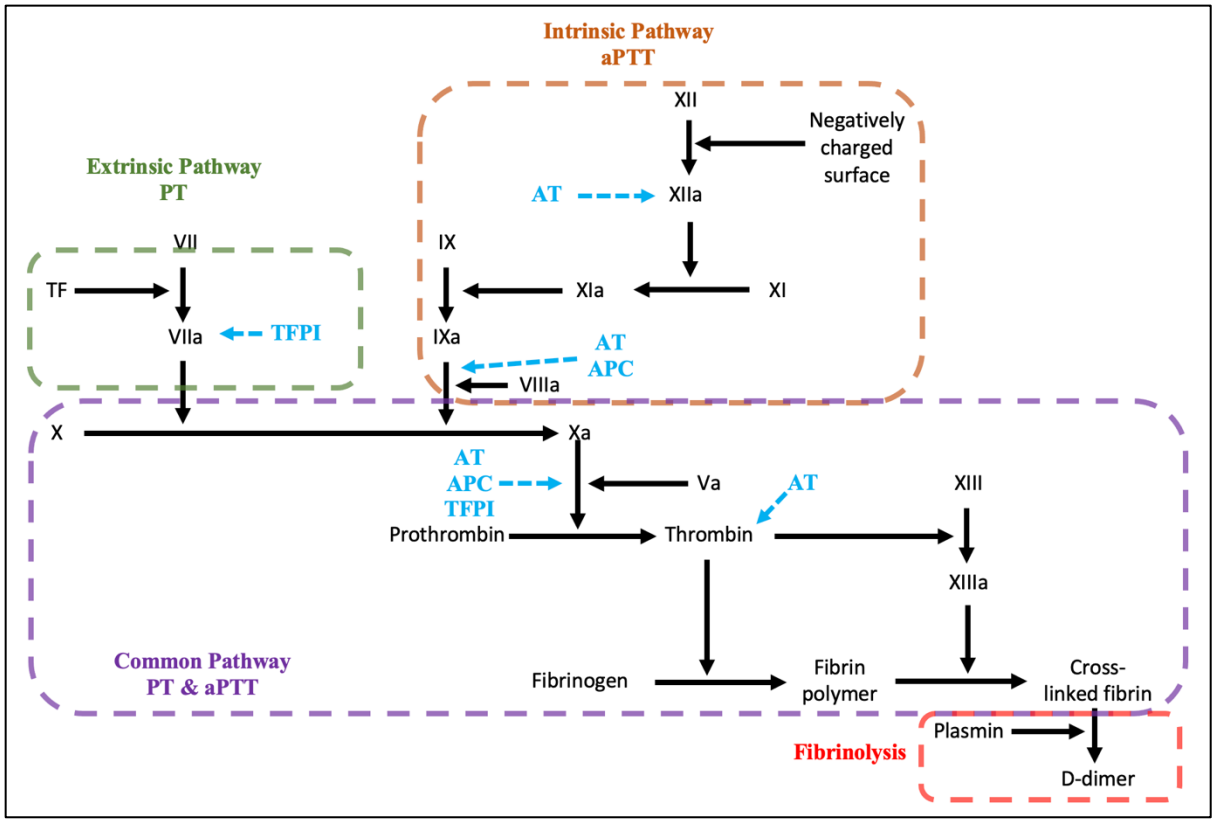

Figure 2: Simplified diagram depicting the coagulation cascade with the intrinsic pathway (measured using activated partial thromboplastin time [aPTT] in orange, the extrinsic pathway (measured using prothrombin time [PT]) in green, and the common pathway (measured using both PT and aPTT) in purple. Select anticoagulant factors and where they act on the cascade are presented in blue. A small segment of the fibrinolytic system is also depicted (red) for convenience.
Abbreviations: Aantithrombin (AT); activated protein C (APC); tissue factor pathway inhibitor (TFPI).
Modified from Keohane EM, Otto CN, Walenga JM. Rodak's Hematology: Clinical Principles and Applications. Sixth edition. ed. St. Louis, Missouri: Elsevier; 2020.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT), which are laboratory tests used to analyze the coagulation system, may be prolonged in acute COVID-19.[4] These features were found to be due to a prothrombotic state, in conjunction with hypo fibrinolysis, caused by COVID-19.[28] A study by Pretorius *et al*. demonstrated that inflammation induced hypercoagulation, hyperactive platelets (figure 3), and ineffective

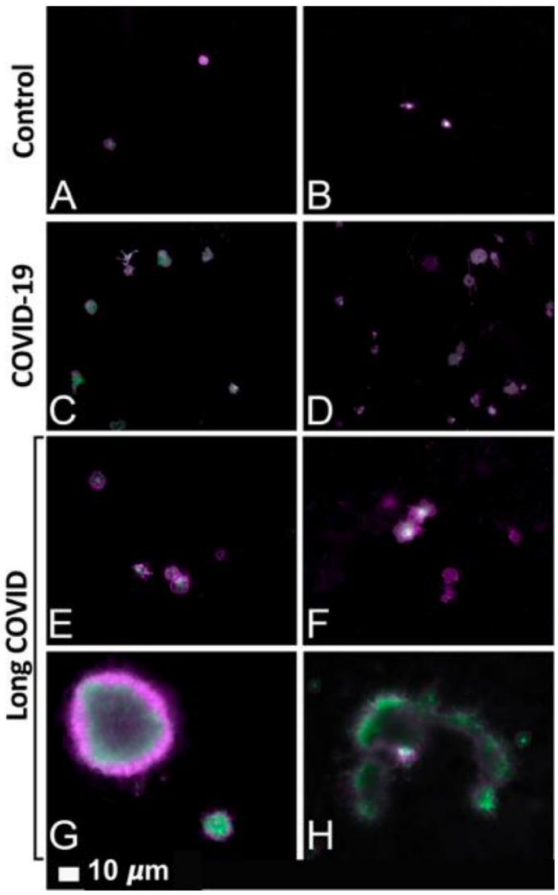

Figure 3: Fluorescence microscopy depicting the hyperactivity of platelets in acute COVID (C & D) and long-COVID (E to H) in comparison to minimally activated control platelets (A & B). From Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, et al. (2021). Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. Cardiovasc Diabetol, 20(1), 172. PMC8381139 (Open access journal).

fibrinolysis occurs in both acute and long-COVID patients.[28] Hyperactive platelets, which results in inappropriate coagulation, may be the cause of thrombocytopenia in some long-COVID patients; however, it may also be due to platelet aggregation with leukocytes and/or engulfment by leukocytes.[28,29] Furthermore, with persistent symptoms such as shortness of breath noted up to 6 months post-acute infection, the symptoms may be due to fibrinolytic-resistant clotting, which blocks blood flow causing ineffective oxygen exchange.[28] Again, the cytokine storm induced by SARS-CoV-2 may be central, due to disease pathology and the resulting hyperinflammatory state, leading to inappropriate endothelial cell activation and coagulation cascade activation.[4,28] Furthermore, release of TNF-α has been shown to induce the release of ultra large von Willebrand factor (UL-VWF) multimers from vascular endothelial cells.[29] UL-VWF is normally fragmented into small multimers, producing functional VWF, which is required for platelet adhesion. The UL-VWF augments thrombus formation due to the larger size. The cytokine storm also causes dysregulation of the anticoagulant systems, where antithrombin III, tissue factor pathway inhibitor (TFPI) and protein C have been affected (Figures 1 and 2).[4]

Elevated markers of fibrinolysis were also identified, suggesting fibrinolysis was taking place, however, ineffective compared to coagulation.[28] Serum amyloid A (SAA) type 4 was found to be significantly increased in fibrinolytic-resistant clots of long-COVID samples. SAA is an acute phase protein, which increases during inflammation and has been shown to bind fibrin, promoting coagulation and thrombus formation.[30]

Thrombosis can lead to serious complications, including vascular occlusion resulting in tissue hypoxia due to the lack of blood flow and oxygen, which was seen in select patients who suffered from ischemic strokes, limb ischemia and myocardial infarctions.[4,17] This indicates abnormal coagulation parameters are significant in post-acute COVID-19 infections and should be investigated during the recovery phase to prevent serious consequences.

Methods

A systematic search for literature, which assessed the hematologic parameters and

discussed possible connections to post-acute COVID-19 symptoms, was completed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta Analyses) guidelines.[31]

Search Strategy

The search strategy used key terms associated with the scope of this study. The key terms were combined in searches to further refine literature. Key terms included "post-acute COVID" OR associated synonyms, which was then combined with "hematology" OR "bio-markers" OR "coagulation" OR "lymphocyte" OR "inflammation" OR other terms and associated synonyms as defined in table 1.

Table 1: Primary key terms with synonyms used in data collection

Key term	Synonyms
Post-acute COVID	Post-acute COVID syndrome, long-COVID, COVID long-hauler(s), post-acute COVID sequelae, chronic COVID. ± hyphens. Interchange COVID with "Covid" OR "SARS-CoV-2" OR "coronavirus" ± "19" OR "nCoV2"
Hematology	Hematologic, clinical hematology. Interchange European (haematology) and American (hematology) spelling.
Biomarkers	Laboratory biomarker(s), parameter(s), laboratory parameter(s), clinical laboratory parameter(s), markers. Interchange laboratory with "lab".
Coagulation	D-dimer, fibrin degradation products (FDP), plasmin, plasminogen.
Lymphocyte	Leukocyte, leucocyte, lymphopenia, leukopenia.
Ferritin	Hyperferritinemia, hyperferritin, iron, iron dysregulation.
Hemolysis	Hemolytic anemia Interchange European (haemolytic) and American (hemolytic) spelling.
Anemia	Anemic. Interchange European (anaemia) and American (anemia) spelling.

Five electronic databases were used to search the relevant terms and synonyms. The databases used included:

- PubMed
- Google Scholar*
- Science Direct**
- Griffith University Library***
- Cochrane

*Time range was adjusted to articles from 2020 to present to reduce non-specific results.

**Advanced search was used for Science Direct. Key terms were searched in the "Title, abstract or author-specified keywords" to reduce non-specific results, due to high numbers of out-of-scope results.

***Search was refined to "Journal Articles."

Inclusion Criteria

The PRISMA guidelines were used to formulate and refine the study methods (Figure 4).[32] Initially, duplicates were removed using EndNote 20. Studies were then screened, and selected studies were sought for retrieval and assessed for eligibility. Eligibility was determined as follows:

1. Number of patients (at least 10 participants),
2. Tests completed (hematologic parameters, e.g., D-dimer, hemoglobin, leukocyte counts, etc.), and
3. Timing of testing (negative COVID-19 PCR or minimum 1 month after onset of symptoms).

Literature was limited to English and original research articles. Though other types of articles were excluded, their reference lists were analyzed for relevant articles.

Exclusion Criteria

Reports without hematologic parameters were excluded. Single case studies and articles without full text were excluded. Studies reporting on acute-phase parameters were excluded (parameters during an active infection). No restrictions were placed on sex, age, or ethnicity.

Results

Literature Search Results

Figure 4 depicts the results of the PRISMA guided database searches, which identified a total of 3221 publications using the search terms presented in table 1. After removal of

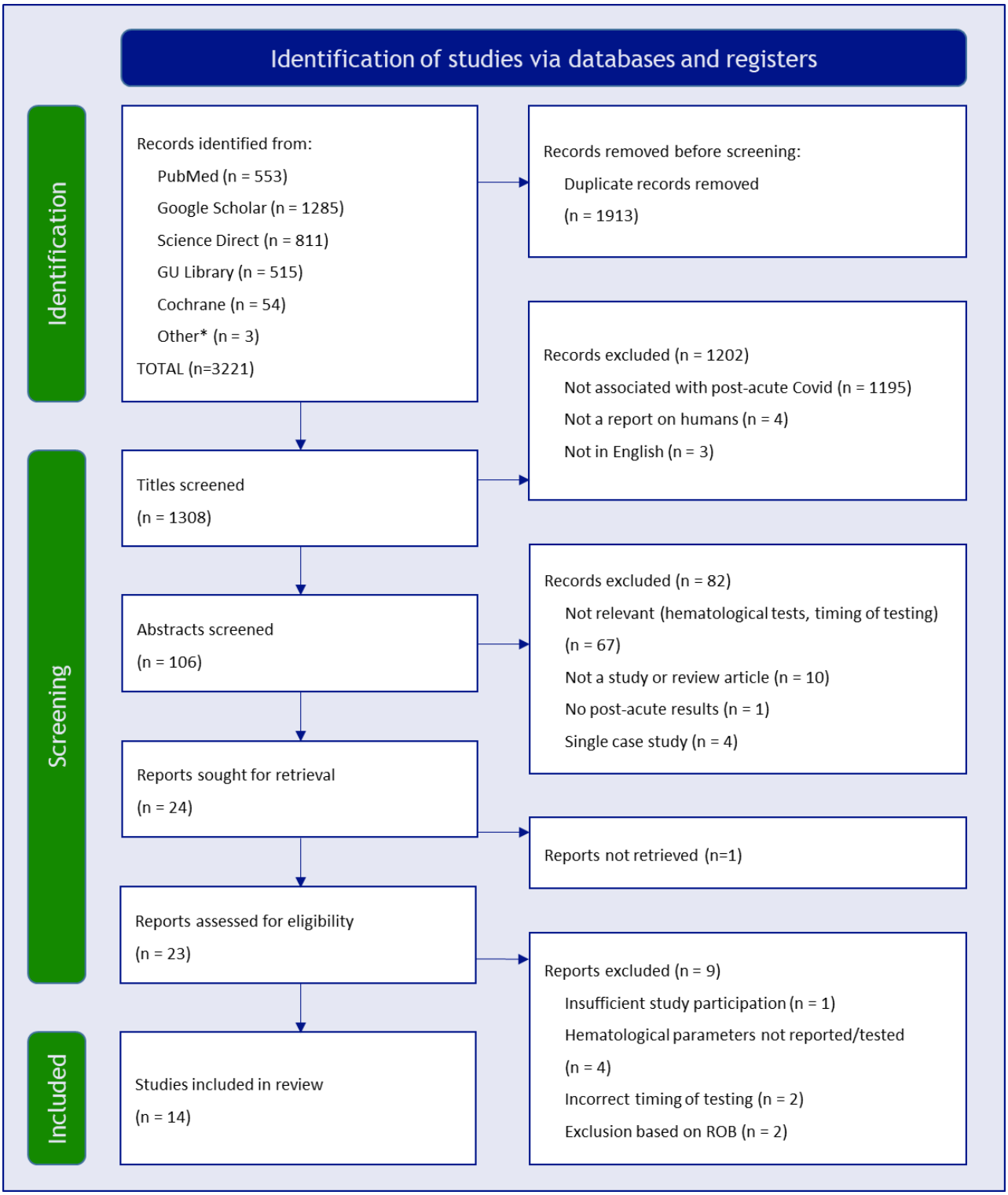

Figure 4: PRISMA flow chart using key terms as outlined in Table 1 (12/11/2021).31 Adapted from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Int J Surg, 88, 105906.
Key:* Studies sourced and retrieved from other articles.
Abbreviations: Griffith University (GU); risk of bias (ROB).

duplicates, there was 1308 potentially eligible articles. Initial screening of titles and abstracts resulted in 24 possible articles, which were sought for retrieval and assessed for eligibility. After eligibility and risk of bias (ROB) assessment, a total of 14 articles were included in this review.

Quality Assessment and Risk of Bias

Studies meeting the inclusion criteria were assessed using the Specialist Unit for Review Evidence (SURE) Critical Appraisal Tool (CAT) to limit bias.[33] The SURE CAT method was selected as it presents a straightforward appraisal of the selected studies. Bias was assessed by awarding a score of "1" to any checklist criteria met and a "0" for unmet criteria. A percentage for each study evaluated was allocated based on met criteria and only studies with a score equal to or above 70% were included (Tables 2a and 2b).

Sixteen studies were evaluated against the 10 checklist criteria, each was scored as 1 (criteria met) or 0 (criteria not met). Only studies meeting ≥70% of criteria were included. Fourteen studies passed appraisal and were included in the systematic review. Two articles had a score of <70%. These studies were excluded as they did not have a score of ≥70% and were therefore deemed unreliable. Although all the studies included in the review reported statistical significance, only 6 out of the 14 studies included reference ranges (RR) in the reported results (Table 3). Without the respective RR, it was difficult to determine the clinical significance of the reported results. Due to the variable scopes of the included studies, Table 3 provides a summary of the articles and indicates if the respective articles included RR in the reported results for comparison in this analysis.

Table 2a: Table of Critical Appraisal with Checklist Criteria Adapted from SURE CAT (Specialist Unit for Review Evidence (SURE), 2018).[33]

Critical Appraisal Checklist Criteria	Articles							
	2	9	10	11	12	13	15	24
Is the study design clearly stated?	1	1	1	1	1	1	1	1
Does the study address a clearly focused question?	1	0	1	1	1	1	0	1
Are participant characteristics provided?	1	1	1	1	1	1	1	1
Number of participants (≥10)	1	1	1	1	1	1	1	1
Test timing (negative COVID PCR or min. 1 month after symptom onset)	1	0	1	1	1	1	1	1
Are the statistical methods well described?	1	1	1	1	1	1	0	1
Were coagulation or D-dimer tests performed?	1	1	1	1	1	1	1	0
Were ferritin or inflammation markers (e.g., CRP) analysed?	1	0	1	1	1	1	1	1
Were leukocyte or lymphocyte analysis performed?	0	1	1	1	1	1	1	1
Platelet-related disorders or confounding addressed?	1	0	0	0	1	1	0	0
Results out of 10	9	6	9	9	10	10	7	8
Percentage Score (%)	90	60	90	90	100	100	70	80
Included or Excluded	In.	Ex.	In.	In.	In.	In.	In.	In.

Abbreviations: Polymerase chain reaction (PCR); C-reactive protein (CRP); included (In); excluded (Ex).

Table 2b: Table of Critical Appraisal with Checklist Criteria Adapted from SURE CAT (Specialist Unit for Review Evidence (SURE), 2018).[33]

Critical Appraisal Checklist Criteria	Articles							
	28	37	38	39	40	41	42	67
Is the study design clearly stated?	1	1	1	1	1	1	1	1
Does the study address a clearly focused question?	1	1	1	0	1	1	1	1
Are participant characteristics provided?	0	1	1	1	1	1	1	0
Number of participants (≥10)	1	1	1	1	1	1	1	1
Test timing (negative COVID PCR or min. 1 month after symptom onset)	1	1	1	1	1	1	1	1
Are the statistical methods well described?	1	1	1	0	1	1	1	0
Were coagulation or D-dimer tests performed?	1	1	1	1	1	1	1	0
Were ferritin or inflammation markers (e.g., CRP) analysed?	1	1	1	1	1	1	1	0
Were leukocyte or lymphocyte analysis performed?	0	1	1	1	1	1	1	1
Platelet-related disorders or confounding addressed?	1	0	1	1	1	1	1	0
Results out of 10	8	9	10	8	10	10	10	5
Percentage Score (%)	80	90	100	80	100	100	100	50
Included or Excluded	In.	In.	In.	In.	In.	In.	In.	Ex.

Abbreviations: Polymerase chain reaction (PCR); C-reactive protein (CRP); included (In); excluded (Ex).

Table 3: Scope of included studies in analysis and indication if reference ranges (RR) were included in respective studies.

Article	Scope of study	RR
2	Grouped COVID recovered patients based on DD levels (normal vs high).	Y
10	Compared post-severe COVID-19 in patients at discharge, 1 and 3 months.	Y
11	Post-COVID-19 assessment of patients with PS after hospital discharge.	Y
12	Compared symptomatic and asymptomatic long-COVID-19 patients.	N
13	Compared patients whose musculoskeletal symptoms were aggravated post-COVID-19 infections vs. no change post-COVID-19.	N
15	Assessed post-COVID-19 patients with PS post-hospital discharge.	N
24	Post-hospital COVID patients; compared mild, moderate and severe patients.	N
28	Investigated coagulopathies in long-COVID using proteomics.	N
37	Investigated persistent endothelial activation in long-COVID-19. Compared convalescent patients with controls.	Y
38	COVID-19 recovered patients with persistent cardiac symptoms; compared positive CMR and negative CMR imaging patients.	N
39	Assessed post-hospital discharge COVID-19 patients.	N*
40	Compared post-COVID patients with normal vs. abnormal CT scans.	Y
41	Post-COVID-19 recovery assessment in patients with PS vs non-PS.	Y
42	Post-COVID-19 recovery assessment in patients with PS vs non-PS	N*

* No RR provided, however, indicated if results were within, above or below RR.
Abbreviations: No (N); yes (Y); cardiac magnetic resonance (CMR); persistent symptoms (PS); D-dimer (DD); computed tomography (CT).

Data

The data extraction information was adapted from the Centre for Reviews and Dissemination guidelines (2009).[34] Tables 4a, 4b, and 4c present the extracted data of the included studies. The information extracted includes:

- General information: date & identification features of study
- Study characteristics: study design
- Participants: number, age, gender, confounding factors
- Analysis: parameters tested/reported

Variable units were used throughout all studies, for the purposes of accuracy, the units reported by the studies were kept as is.

Table 4a: Extracted Data included in Review

Articles	N	Age (years, mean)	Gender	Study	N in laboratory tests
2	150	47.3 ±15.4	F = 85, M = 65	Retrospective	150
10	199	60.5 ±13.9	F = 73, M = 126	Prospective	Variable◆
11	75	72 ±7	F = 33, M = 42	Retrospective	75
12	315	47.9 ±14.8	F = 158, M = 157	Retrospective	351
13	280	47.45 ±13.92	F = 183, M = 97	Retrospective	182
15	384	59.9 ±16.1	F = 38, M = 62	Retrospective	Variable◆
24	109	58 ±14	F = 44, M = 65	Retrospective	109
28	11	55.7 ±5.8	F = 8, M = 3	Observational	11
37	50	50 ±17	F = 20, M = 30	Retrospective	50
38	109	58 ±14	F = 44, M = 65	Retrospective	109
39	767	63 ±13.6✧	F = 252, M = 515	Retrospective	Variable◆
40	94	48.11 ±11.9	F = 40, M = 54	Retrospective	94
41	1021	Variable◆	F = 256, M = 764	Retrospective	Undefined◆
42	116	41✧	F = 17, M = 99	Retrospective	Undefined

◆ See respective article for further details
✧ Median
Abbreviations: Female (F); male (M).

Table 4b: Extracted Data included in Review (Continued)

Articles	Test timing	Confounding factors
2	6 weeks post symptoms or HD	HTN (18%), DM (9.3%) ◆
10	1- and 3-months post HD	44.4% HTN✣, 25.6% CVD✣, & more◆.
11	60 days post HD	N/A
12	1 month post-acute	20.6% smokers, 42.2% had comorbidities◆
13	Neg. COVID-19 PCR.	Anticoagulant therapies indicated
15	Median 54 days	41.9% HTN, 9.7% IHD & more◆
24	Mean 60 (±12) days post symptom onset	N/A
28	2 months post symptom onset	N/A
37	Median 68 days post-symptom	62% had comorbidities◆
38	Mean 60 (±12) days post symptom onset	8% HTN. CVD patients excluded.
39	Median 81 days post HD	HTN (21.7%), CAD (9.5%) ◆
40	1 year post HD	HTN (17.02%), DM (9.57%) ◆
41	Neg. COVID-19 PCR.	5.7% HTN
42	Median 66 days post symptom onset	Patients with comorbidities excluded

◆ See respective article for further details

✣ Not all participants included

Abbreviations: Hospital discharge (HD); hypertension (HTN); diabetes mellitus (DM); cardiovascular disease (CVD); polymerase chain reaction (PCR); ischemic heart disease (IHD); coronary artery disease (CAD).

Table 4c: Extracted Data included in Review (Continued)

Articles	D-dimer	Ferritin	CRP	Lymphocyte
2	327 ng/mL	N/A	1.23 mg/mL	N/A
10	1 mo. 446 µg/L 3 mo. 322 µg/L	1 mo. 179 µg/L 3 mo. 95 µg/L	1 mo. 5.6 mg/L 3 mo. 2.7 mg/L	1 mo. 29.6% 3 mo. 31.4%
11	900.71 ng/mL	496.24 ng/mL	9.12 mg/L	N/A★
12	0.44*	65.3*	62.55*	26.7*
13	S: 0.38* AS: 0.26*	S: 117* AS: 75*	S: 5.4* AS: 4.45*	S: 1.82* AS: 2.16*
15	384 ng/mL	169 µg/L	1 mg/L	1.94 x 109/L
24	N/A	Mild = 139 µg/L Mod = 260 µg/L Severe = 317 µg/L	Mild = 0.2 mg/dL Mod = 0.2 mg/dL Severe = 0.4 mg/dL	Mild = $5.7x10^9$/L Mod = $6.1x10^9$/L Severe = $6.4x10^9$/L
28	★	N/A	✦	N/A
37	377 ng/mL	N/A	1.1 mg/mL	★
38	0.28 µg/mL	N/A	1.4 mg/L	1.6x109/L
39	700 ng/mL ±1021	N/A	0.36 mg/dL ±0.85	⍰
40	NCT = 290 µg/L ACT = 290 µg/L	N/A	NCT = 5 mg/L ACT = 15 mg/L	NCT = $1.69x10^9$/L ACT = $1.18x10^9$/L
41	Measured, but not defined◆	Measured, but not defined◆	Measured, but not defined◆	Measured, but not defined◆
42	⋆	PS: 191.48 µg/L NPS: 177.03 µg/L	PS: 0.41 mg/dL NPS: 0.41 mg/dL	PS: 29.62% NPS: 31.77%

★ Other associated parameters (e.g., fibrinolytic markers, inflammation markers, or leukocyte counts).

* No units provided.

✦ Reports fold change between healthy controls and long-COVID.

⍰ Reports number of patients within specified ranges.

◆ See respective article for further details.

Abbreviations: C-reactive protein (CRP); month (mo); symptomatic (S); asymptomatic (AS); moderate (Mod); normal computed tomography (NCT); abnormal computed tomography (ACT); persistent symptoms (PS); non-persistent symptoms (NPS).

Discussion

Two years after the emergence of the novel SARS-CoV-2 virus, some patients continue to experience symptoms in the absence of an active infection. After a systematic literature search and ROB assessment, 14 studies that investigated hematological parameters in patients with persistent symptoms (PS) were included in the analysis. Though the included articles addressed statistical significance, clinical significance was often omitted or overlooked. The statistical significance of the included articles may indicate the reliability of the results; however, clinical significance indicates the impact the results have in clinical practice.[35] The statistical significance of results addresses the second aim of this review, while the clinical significance addresses the third aim; therefore, both

statistical and clinical significance are taken into consideration.

Lymphopenia

Lymphopenia is frequently reported in acute COVID-19 infections and has been hypothesized to contribute to symptoms seen in the post-acute phase.[16,36] Twelve of the 14 included studies reported lymphocyte or leukocyte counts (Table 4c). Out of these 12 studies, 5 reported no statistically significant difference in lymphocyte/leukocyte counts between their respective comparison groups.[11,12,24,37,38] RR was included in the studies by Fogarty, *et al.* and Pasini, *et al.*, and showed all participants to be within the reported RR.[11,37] The remaining 7 studies found either statistically or clinically significant differences. Bakilan, *et al.* and Venturelli, *et al.* found statistically significant differences in lymphocyte/ leukocyte count between their comparison groups, however, did not include RR with their results; therefore, it could not be established if the results were clinically significant.[13,39] Darcis, *et al.* and Zhao, *et al.* found statistically significant differences between the comparison groups and included RR in the results.[10,40] When comparing the results to the included RR, the lymphocyte/leukocyte counts were still within the accepted RR, indicating although there is a statistical difference between the groups, it was not clinically significant. The study by Mandal, *et al.* reported 7.3% of the 247 participants showed persistent lymphopenia and Mannan, *et al.* found lymphopenia in 3% of patients experiencing PS, however, ~50% of asymptomatic patients also had lymphopenia.[15,41] In the study by Varghese, *et al.*, 12% of participants were found to have lymphopenia, where 31% of these participants had PS and 9% had none.[42] Furthermore, the results reported 91.07% of the cohort was within RR, however, it was undefined what percentage had PS and what percentage did not.[42] These results indicate some patients will experience lymphopenia with post-acute COVID-19; however, it is not a common abnormality nor a reliable indicator of PS. Further follow-up results were not available; it is undetermined if the lymphocyte population returned to within RR for patients with lymphopenia.

Although Varghese, *et al.* indicated not all patients with lymphopenia had PS post-COVID, a significant difference was noted in immunoglobulin A (IgA) concentration between patients with and without PS.[42] The study indicated IgA concentrations at certain time points in disease progression may be central to PS. High concentrations of IgA during the acute phase can indicate or predict severe disease, while high concentrations post-acute indicates less PS. IgA antibodies are produced by B lymphocytes, or plasma cells, in the lamina propria, and transported to the mucosal surface via receptors to aid in the defense against invading pathogens.[19] Although lymphopenia was not found to be a common factor in patients with PS, the association of PS and reduced IgA may indicate either a pathogenic mechanism of SARS-CoV-2 affecting B lymphocytes or an ineffective immune response. Only one study evaluated the immunoglobulins post-COVID-19 infections; future analyses can elucidate if this is in fact a common factor among other cohorts.

A study by Gao, *et al.* analyzed the frequencies of T lymphocyte subsets in both acute and convalescent patients.[43] The results showed decreased lymphocytes, total T cells, CD4+ T cells and CD8+ T cells during the acute phase of infection and a further reduction noted post-acute infection. B lymphocytes were not assessed. Similarly, a review by Ramakrishnan, *et al.* indicated the ability of SARS-CoV-2 to impair T lymphocyte functionality, leading to immune exhaustion, thus facilitating long-COVID symptoms.[1] This is supported in the study by Peluso, *et al.*, which found patients with PS had decreased CD8+ T lymphocyte responses over time.[44] In contrast to these findings, an additional study found patients with PS had increased and sustained T lymphocyte activity in the late convalescent phase and patients without PS had a gradual decrease in T lymphocyte activity over time.[45]

This suggests immune overactivity may be a cause of PS. No difference was found in B lymphocyte activity between patients with or without PS in the study by Files, *et al*.[45] Interestingly, the study by Phetsouphann, *et al*. analyzed 24 cell clusters 3 months post-acute COVID-19 infection, which showed 5 lymphocyte subsets were absent in long-COVID patients.[18] A further 3 remained absent when analyzed at the 8-month interval, which included CD8+ and CD4+ T lymphocytes and B lymphocyte subsets. Furthermore, CD8+ T lymphocyte activation and exhaustion markers were also found to be higher in long-COVID patients. It was also identified that sustained monocyte and plasmacytoid dendritic cell activity occurred in the long-COVID cohort compared to the matched controls.[18] These results indicate there is a decrease in certain lymphocytes in long-COVID patients with a chronic and sustained activation of a CD8+ T cell subset, monocytes and plasmacytoid dendritic cells, which may contribute to long-COVID symptoms. Other theories described in literature surrounding the cause of lymphopenia include viral bone marrow suppression or immunosuppression, resulting in not only lymphopenia, but also at times neutropenia and thrombocytopenia.[17,46] There are multiple studies reporting neutropenia, however, it was not addressed in the articles included for this review.[47-49]

Blood Morphological Changes

Studies with morphological analysis of peripheral blood (PB) smears on long-COVID patients are limited. There are, however, studies analyzing morphology during the acute phase. Of particular interest are the dysplastic myelocyte features, such as neutrophils with pseudo-Pelger-Huët anomalies, which are atypical for viral infections and have only been evident in human immunodeficiency virus (HIV) infections.[50-52] Analysis of the blood morphological features in PB of long-COVID patients could be useful to determine the persistence of abnormal cells and potential contributors to PS.

Although studies on the PB of post-acute COVID-19 patients are significantly limited, flow cytometry cell analysis has been completed. The study by Kubankova, *et. al* investigated 14 post-acute COVID-19 patients who were, on average, 7 months post-infection, using real-time deformability cytometry (RT-DC).[53] RT-DC is a fast and high-throughput method of analysis the phenotypical features of cells. The study found marked changes in cell phenotypes during the acute phase, including smaller erythrocytes with decreased deformability, monocytes with increased cell size, and lymphocytes with decreased stiffness. Some of these abnormalities were also noted in the post-acute COVID-19 group indicating the effects of COVID-19 persists in the hematologic system for some time. There was a significant difference in the deformation of erythrocytes between the post-acute COVID-19 cohort and the acute and healthy cohorts; the erythrocytes had not returned to "healthy state" in the post-acute COVID-19 group.[53] A study by Thomas, *et al*. found oxidative stress induced by COVID-19 infections resulted in damage of essential erythrocyte proteins.[54] Mature erythrocytes cannot repair or resynthesise these proteins; the persistence or survival of these damaged cells, possibly due to lack of splenic clearance or inefficient damage to induce hemolysis, may contribute to ineffective oxygen transport, resulting in the symptoms seen in long-COVID sufferers.[54] Furthermore, the study by Kubankova, *et al*. found lymphocyte size and deformation was not significantly different from the heathy control group, however, the analysis of neutrophil parameters indicated significant changes between the post-COVID-19 and healthy groups, including cell cross-sectional area, volume, and deformation.[53]

Interestingly, the study by Kannan and Soni, which analyzed the PB smears of acute-phase COVID-19 patients, found one patient, approximately 100 days post COVID-infection, had presented with neutrophilic nuclear abnormalities, coined by the authors as

acquired neutrophilic nuclear projections (ANNP).[55]

There are no clear diagnostic criteria regarding lymphopenia, PB abnormalities and long-COVID yet, however, there is evidence the absence of certain lymphocyte subsets or sustained activation of immune cells may have a connection to long-COVID symptoms. In addition, lymphocyte abnormalities, dysregulated inflammation was also reported in long-COVID patients, which results in high ferritin and CRP.

Iron Dysregulation and C-Reactive Protein (CRP)

Ferritin and CRP are seen in the acute-phase response during inflammation.[19] Of the 14 studies included, 8 reported results for ferritin analysis (Table 4c). Only one study found no significant difference in ferritin between their comparison groups.[13] The remaining 7 studies reported a significant difference in ferritin results between the respective comparison groups. Four studies reported a statistically significant increase in ferritin; however, RR was not stated and, therefore, it could not be determined if there was any clinical significance.[12,15,24,42] The remaining three studies showed a clinically significant increase in ferritin. Darcis, *et al*. reported ferritin concentrations above the RR at hospital discharge, which normalized at the 1 and 3-month assessment. ~37% of symptomatic and ~40% of asymptomatic participants in the study by Manna, *et al*. had ferritin concentrations above RR, indicating possible persistent inflammation in the absence of an active COVID-19 infection.[10,41] Similarly, ferritin concentrations were above RR in both male and female participants in the study by Pasini, *et al*.[11] As stated earlier, the SARS-CoV-2 spike protein was found to have sequence similarities to hepcidin, which may play a role in the hyperferritinemia seen in post-acute COVID-19.

Furthermore, Sonnweber, *et al*. identified iron deficiency anemia in 30% of their participants two months post-acute COVID-19. Of these participants, 90% had severe acute COVID-19 infections.[24] Eighty percent of participants in another study had clinically significant low hemoglobin concentrations.[11] This suggests it may be beneficial to include iron studies as part of a panel for laboratory investigations into long-COVID.

All studies reported CRP results. Six studies reported no significant increase in CRP, or the CRP results were within the RR.[2,13,24,37,38,42] Three of the studies reported a statistically significant increase in CRP in patients with PS, however, it was unclear if the increase was clinically significant (no RR for comparison).[12,15,28] The remaining 5 studies reported CRP above the RR. Darcis, *et al*. found CRP decreased at 1 month and within RR at the 3 month follow up, indicating a gradual return to normal concentration.[10]

The results indicate hyperferritinemia and elevated CRP may be a consequence of COVID-19 infections, however, these markers are non-specific and, hence, may not be specific to long-COVID and PS. Nevertheless, the presence of hyperferritinemia and elevated CRP in recovered patients, with or without PS, indicates there is iron dysregulation and/or persistent post-acute inflammation. Evidence of persistent and sustained inflammation was evident in a study by Phetsouphanh, *et al*., which found persistently elevated IFN-β and IFN-λ1 in the long-COVID cohort compared to matched controls.[18] Inflammation is known to affect the coagulation system, resulting in coagulopathies. This has been noted in both acute and post-acute COVID-19 infections.

Coagulopathies

Coagulopathies are a well-known consequence of COVID-19. There is evidence SARS-CoV-2 invades vascular endothelial cells, resulting in endothelial dysfunction, which triggers a procoagulant environment and, along with the hyperinflammatory response, results in endothelitis.[17,28] This systematic analysis revealed that three of the 14 studies found D-dimer results to be within RR or found no

statistical significance (Table 4c).[37,38,40] Eight studies found either statistically or clinically significant increases in D-dimer in affected participants. Two studies had further follow-up results and reported a decrease in the D-dimer over time, indicating the resolution of COVID-19 induced coagulopathy. The study by Mannan, *et al*. Mannan, *et al*. [41], which compared symptomatic and asymptomatic participants, found elevated D-dimer in both cohorts at similar frequencies; approximately 40%. Similarly, found high D-dimer in ~38% of their participants.[39] In addition, Pretorius, *et al*. reported significant failure in the fibrinolytic processes in convalescent patients, which was evidenced by the presence of clots that were resistant to fibrinolysis.[28] These results indicate there is a combination of hypercoagulation and hypo fibrinolysis occurring in some post-COVID patients.

Although an increased D-dimer is not exclusive to patients with PS, it is still a significant marker. This is highlighted in a retrospective case study concerning an 82-year-old Japanese male whose autopsy findings indicated the patient died due to portal and mesenteric vein thrombosis.[56] This thrombosis caused portal hypertension, which consequently resulted in extensive gastrointestinal necrosis. The patients' D-dimer was reported to be consistently elevated, which emphasizes the importance of investigating persistent coagulopathies in post-acute COVID-19 cases, particularly persistently elevated D-dimer in post-acute COVID-19, which may be valuable in determining patient care and treatment to prevent fatal thrombotic events.

Other markers of the coagulation system may also provide insights into the hypercoagulable state of some patients. Interestingly, although the D-dimer has been found to be within the RR of some patients, a significant increase in factor VIII has been found in convalescent patients.[37] No RR was reported however; thus, clinical significance is undetermined.[37] Similarly, another study also found significantly increased factor VIII in convalescence patients.[17] A comparison of fibrinogen between participants with and without PS indicated higher fibrinogen (hyperfibrinogenemia) in individuals with PS (311.65±78.52 mg/dL) compared to those without (294.34 ±48.33 mg/dL).[42] Though these results are not statistically significant, it may indicate there is more deranged coagulation occurring in individuals experiencing PS. Interestingly, the finding of hyperfibrinogenemia was noted to be contrary to other literature, which reported fibrinogenemia, indicating there may be variations in coagulopathy patterns among long-COVID patients.[4,16] Furthermore, an article by Fan, *et al*. reported significant thrombotic events in 4 young patients (median 38.5 years of age). Laboratory analysis of these patients showed increased factor VIII, VWF, D-dimer and hyperfibrinogenemia.[57] Although these results are not exclusive to patients with PS, analysis of patients' coagulation profile, including D-dimer, fibrinogen, factor VIII, and VWF, may be beneficial in determining post-acute COVID-19 care and to prevent significant thrombotic events. Although lymphopenia, iron dysregulation/inflammation and coagulopathies are the predominant reported abnormalities in long-COVID, some studies have also found other abnormalities secondary to COVID-19 infections.

Abnormalities Secondary to COVID-19 Infections

Clinically significant abnormalities secondary to COVID-19 infections in post-acute patients has briefly been noted in the literature. Abnormalities include alterations in glucose metabolism, development of hemophagocytic lymphohistiocytosis (HLH) and autoimmune diseases.

Abnormal Glucose Metabolism

An increase in hemoglobin A1c (HbA1c) has been noted in post-acute COVID-19 patients who had no prior diabetes mellitus (DM) diagnoses.[36,58] HbA1c is a glycated from of hemoglobin, which becomes elevated when plasma glucose levels are increased for long periods of time, as seen in DM.[21] Multiple

studies have found patients with long-COVID have indications of altered glucose metabolism as evidenced by increases in HbA1c.[58,59] HbA1c has been shown to increase blood viscosity, endothelial inflammation and vascular dysfunction, thus, elevated HbA1c may be the cause of or contribute to the coagulopathies and sustained inflammation seen in post-acute COVID-19.[60]

Hemophagocytic Lymphohistiocytosis (HLH)

Although rare, another consequence of COVID-19 infection is secondary hemophagocytic lymphohistiocytosis (HLH), a life-threatening and rapidly progressive inflammatory syndrome leading to multiorgan failure.[61,62] Characteristics commonly seen in HLH includes excessive cytokines, cytopenia, and hyperferritinemia.[62] These features have been seen in long-COVID, as discussed previously. Due to the high mortality rate seen with HLH, this is certainly a significant consequence associated with COVID-19 that should be given due consideration when assessing patients.[63]

Autoimmune Diseases

There have been multiple reports of immune-related diseases developing after resolution of COVID-19 infections. One study reported seven cases of warm and cold autoimmune hemolytic anemia (AIHA), which developed after confirmed COVID-19 infection and without differential diagnosis.[64] Furthermore, a case report presented a patient with immune thrombocytopenia (ITP) secondary to COVID-19.[65] Viral-induced ITP is caused when antibodies produced by B lymphocytes in response to the viral infection are cross-reactive with thrombocytes, resulting in the antibodies binding to and causing the destruction of thrombocytes, leading to thrombocytopenia.[66]

Limitations of the Review

There are limited studies investigating the long-term effects of SARS-CoV-2 infection. Many of the studies did not include the results of all participants or had variable numbers of participants at different time points, thus, the study may not present an accurate representation of the study groups. Furthermore, the participants were not grouped based on age, ethnicity, sex, or comorbidities, therefore, it could not be determined if one group or characteristic was more prone to PS compared to others. None of the studies defined COVID-19 variants, therefore, it is unestablished whether one strain is more likely to cause long-COVID compared to others.

Future Directions

New emerging studies have found absent lymphocyte subsets or activity in long-COVID patients with sustained activation of other immune cells; studies correlating these results may allow for predictions of long-COVID and potential directed therapeutics. Further research into the cause and prevalence of elevated HbA1c and HLH in post-acute COVID patients may assist in determining the significance and requirements for further observation in potentially affected patients. Many of the studies included in this review were not solely focused on hematologic parameters, therefore, future analyses which focus on the hematologic system would be beneficial, which include the PB smears of long-COVID patients. Furthermore, there are many COVID-19 variants; none of the studies addressed which strains were detected or which were predominant in the respective cohorts. Future studies may reveal one COVID-19 strain implicated in long-COVID more often than another. Finally, although comorbidities were addressed in the included studies, patients were not grouped according to comorbidities; elucidating which comorbidities are more often associated with long-COVID and whether there is a causal relationship may help with prognosis, recovery, and rehabilitation.

Conclusion

Although lymphopenia was not found to be exclusive to long-COVID patients, new studies are emerging with evidence of certain features exclusive to long-COVID. These studies have

shown there is an absence of T and B cell subsets, along with sustained activation of monocytes and plasmacytoid dendritic cells, in long-COVID, however these are not found in non-long-COVID cohorts. Collated evidence also suggests there is sustained inflammation occurring in long-COVID, which may drive the persistent signs and symptoms, including coagulopathies for which these is strong evidence, due to the elevated D-dimer seen in the majority of COVID-19 recovered patients. There is still limited research addressing long-COVID and the effects seen in the hematological system, however, the evidence presented to date indicates the promise of elucidating the potential hematological causes and mediators of long-COVID.

References

1. Ramakrishnan RK, Kashour T, Hamid Q, Halwani R, Tleyjeh IM. (2021). Unraveling the Mystery Surrounding Post-Acute Sequelae of COVID-19. Front Immunol, 12,686029. PMC8278217

2. Townsend L, Fogarty H, Dyer A, Martin-Loeches I, Bannan C, Nadarajan P, *et al*. (2021). Prolonged elevation of D-dimer levels in convalescent COVID-19 patients is independent of the acute phase response. J Thromb Haemost, 19(4), 1064-1070. PMC8013297

3. Suvvari TK, Kutikuppala LVS, Tsagkaris C, Corriero AC, Kandi V. (2021). Post-COVID-19 complications: Multisystemic approach. J Med Virol, 93(12), 6451-6455. PMC8427008

4. Singh S, Zuwasti U, Haas C. (2020). Coronavirus-Associated Coagulopathy: Lessons From SARS-CoV1 and MERS-CoV for the Current SARS-CoV2 Pandemic. Cureus, 12(11), e11310. PMC7714748

5. Acuti Martellucci C, Flacco ME, Cappadona R, Bravi F, Mantovani L, Manzoli L. (2020). SARS-CoV-2 pandemic: An overview. Adv Biol Regul, 77, 100736. PMC7832554

6. Welte T, Ambrose LJ, Sibbring GC, Sheikh S, Mullerova H, Sabir I. (2021). Current evidence for COVID-19 therapies: a systematic literature review. Eur Respir Rev, 30(159).

7. Moreno-Perez O, Merino E, Leon-Ramirez JM, Andres M, Ramos JM, Arenas-Jimenez J, *et al*. (2021). Post-acute COVID-19 syndrome. Incidence and risk factors: A Mediterranean cohort study. J Infect, 82(3), 378-383. PMC7802523

8. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, *et al*. (2021). Post-acute COVID-19 syndrome. Nat Med, 27(4), 601-615.

9. Kozak R, Armstrong SM, Salvant E, Ritzker C, Feld J, Biondi MJ, *et al*. (2021). Recognition of long-covid-19 patients in a canadian tertiary hospital setting: A retrospective analysis of their clinical and laboratory characteristics. Pathogens (Basel), 10(10), 1246.

10. Darcis G, Bouquegneau A, Maes N, Thys M, Henket M, Labye F, *et al*. (2021). Long-term clinical follow-up of patients suffering from moderate-to-severe COVID-19 infection: a monocentric prospective observational cohort study. International Journal of Infectious Diseases, 109, 209-216.

11. Pasini E, Corsetti G, Romano C, Scarabelli TM, Chen-Scarabelli C, Saravolatz L, *et al*. (2021). Serum Metabolic Profile in Patients With Long-Covid (PASC) Syndrome: Clinical Implications. Front Med (Lausanne), 8, 714426. PMC8339407

12. Akinci Ozyurek B, Sahin Ozdemirel T, Akkurt ES, Yenibertiz D, Saymaz ZT, Büyükyaylacı Özden S, *et al*. (2021). What are the factors that affect post COVID 1st month's continuing symptoms? International journal of clinical practice (Esher), 75(11), e14778-n/a.

13. Bakilan F, Gokmen IG, Ortanca B, Ucan A, Eker Guvenc S, Sahin Mutlu F, *et al*. (2021). Musculoskeletal symptoms and related factors in postacute COVID-19 patients. Int J Clin Pract, 75(11), e14734. PMC8420386

14. Luo XH, Zhu Y, Mao J, Du RC. (2021). T cell immunobiology and cytokine storm of

COVID-19. Scand J Immunol, 93(3), e12989. PMC7645942

15. Mandal S, Barnett J, Brill SE, Brown JS, Denneny EK, Hare SS, *et al*. (2021). 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax, 76(4), 396-398. PMC7661378

16. Henderson LA, Canna SW, Schulert GS, Volpi S, Lee PY, Kernan KF, *et al*. (2020). On the Alert for Cytokine Storm: Immunopathology in COVID-19. Arthritis Rheumatol, 72(7), 1059-1063. PMC7262347

17. Korompoki E, Gavriatopoulou M, Fotiou D, Ntanasis-Stathopoulos I, Dimopoulos MA, Terpos E. (2021). Late-onset hematological complications post COVID-19: An emerging medical problem for the hematologist. Am J Hematol.

18. Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CML, Patel SK, *et al*. (2022). Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nat Immunol.

19. Parham P, Janeway C. *The immune system*. Fourth edition. ed. New York, NY: Garland Science, Taylor & Francis Group; 2015.

20. Frater JL, Zini G, d'Onofrio G, Rogers HJ. (2020). COVID-19 and the clinical hematology laboratory. Int J Lab Hematol, 42 Suppl 1, 11-18. PMC7264622

21. Keohane EM, Otto CN, Walenga JM. *Rodak's Hematology: Clinical Principles and Applications*. Sixth edition. ed. St. Louis, Missouri: Elsevier; 2020.

22. Rahman MA, Shanjana Y, Tushar MI, Mahmud T, Rahman GMS, Milan ZH, *et al*. (2021). Hematological abnormalities and comorbidities are associated with COVID-19 severity among hospitalized patients: Experience from Bangladesh. PLoS One, 16(7), e0255379. PMC8315496

23. Girelli D, Marchi G, Busti F, Vianello A. (2021). Iron metabolism in infections: Focus on COVID-19. Semin Hematol, 58(3), 182-187. PMC8305218

24. Sonnweber T, Boehm A, Sahanic S, Pizzini A, Aichner M, Sonnweber B, *et al*. (2020). Persisting alterations of iron homeostasis in COVID-19 are associated with non-resolving lung pathologies and poor patients' performance: a prospective observational cohort study. Respir Res, 21(1), 276. PMC7575703

25. Pagani A, Nai A, Silvestri L, Camaschella C. (2019). Hepcidin and Anemia: A Tight Relationship. Front Physiol, 10, 1294. PMC6794341

26. Cavezzi A, Troiani E, Corrao S. (2020). COVID-19: hemoglobin, iron, and hypoxia beyond inflammation. A narrative review. Clin Pract, 10(2), 1271. PMC7267810

27. Ehsani S. (2020). COVID-19 and iron dysregulation: distant sequence similarity between hepcidin and the novel coronavirus spike glycoprotein. Biol Direct, 15(1), 19. PMC7563913

28. Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, *et al*. (2021). Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. Cardiovasc Diabetol, 20(1), 172. PMC8381139

29. Becker RC, Sexton T, Smyth S, International C-TBCI. (2021). COVID-19 and biomarkers of thrombosis: focus on von Willebrand factor and extracellular vesicles. J Thromb Thrombolysis. PMC8336902

30. Fernandez JA, Deguchi H, Elias DJ, Griffin JH. (2020). Serum amyloid A4 is a procoagulant apolipoprotein that it is elevated in venous thrombosis patients. Res Pract Thromb Haemost, 4(2), 217-223. PMC7040552

31. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, *et al*. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. Ann Intern Med, 151(4), W65-94.

32. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*.

(2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Int J Surg, 88, 105906.

33. Specialist Unit for Review Evidence (SURE) 2018. Questions to assist with the critical appraisal of systematic reviews available at: http://www.cardiff.ac.uk/specialist-unit-for-review-evidence/resources/critical-appraisal-checklists

34. Akers, J., & University of York. (2009). Systematic reviews: CRD's guidance for undertaking reviews in health care. York: CRD, University of York.

35. Ranganathan P, Pramesh CS, Buyse M. (2015). Common pitfalls in statistical analysis: Clinical versus statistical significance. Perspect Clin Res, 6(3), 169-170. PMC4504060

36. Al-Aly Z, Xie Y, Bowe B. (2021). High-dimensional characterization of post-acute sequelae of COVID-19. Nature, 594(7862), 259-264.

37. Fogarty H, Townsend L, Morrin H, Ahmad A, Comerford C, Karampini E, *et al*. (2021). Persistent endotheliopathy in the pathogenesis of long COVID syndrome. Journal of thrombosis and haemostasis, 19(10), 2546-2553.

38. Huang L, Zhao P, Tang D, Zhu T, Han R, Zhan C, *et al*. (2020). Cardiac Involvement in Patients Recovered From COVID-2019 Identified Using Magnetic Resonance Imaging. JACC Cardiovasc Imaging, 13(11), 2330-2339. PMC7214335

39. Venturelli S, Benatti SV, Casati M, Binda F, Zuglian G, Imeri G, *et al*. (2021). Surviving COVID-19 in Bergamo province: a post-acute outpatient re-evaluation. Epidemiol Infect, 149, e32. PMC7873454

40. Zhao Y, Yang C, An X, Xiong Y, Shang Y, He J, *et al*. (2021). Follow-up study on COVID-19 survivors one year after discharge from hospital. International Journal of Infectious Diseases, 112, 173-182.

41. Mannan A, Mehedi HMH, Chy N, Qayum MO, Akter F, Rob MA, *et al*. (2021). A multi-centre, cross-sectional study on coronavirus disease 2019 in Bangladesh: clinical epidemiology and short-term outcomes in recovered individuals. New Microbes New Infect, 40, 100838. PMC7834423

42. Varghese J, Sandmann S, Ochs K, Schrempf IM, Frommel C, Dugas M, *et al*. (2021). Persistent symptoms and lab abnormalities in patients who recovered from COVID-19. Sci Rep, 11(1), 12775. PMC8211641

43. Gao M, Liu Y, Guo M, Wang Q, Wang Y, Fan J, *et al*. (2021). Regulatory CD4(+) and CD8(+) T cells are negatively correlated with CD4(+) /CD8(+) T cell ratios in patients acutely infected with SARS-CoV-2. J Leukoc Biol, 109(1), 91-97.

44. Peluso MJ, Deitchman AN, Torres L, Iyer NS, Munter SE, Nixon CC, *et al*. (2021). Long-term SARS-CoV-2-specific immune and inflammatory responses in individuals recovering from COVID-19 with and without post-acute symptoms. Cell Rep, 36(6), 109518. PMC8342976

45. Files JK, Sarkar S, Fram TR, Boppana S, Sterrett S, Qin K, *et al*. (2021). Duration of post-COVID-19 symptoms is associated with sustained SARS-CoV-2-specific immune responses. JCI Insight, 6(15). PMC8410022

46. Proal AD, VanElzakker MB. (2021). Long COVID or Post-acute Sequelae of COVID-19 (PASC): An Overview of Biological Factors That May Contribute to Persistent Symptoms. Front Microbiol, 12, 698169. PMC8260991

47. Bouslama B, Pierret C, Khelfaoui F, Bellanne-Chantelot C, Donadieu J, Heritier S. (2021). Post-COVID-19 severe neutropenia. Pediatr Blood Cancer, 68(5), e28866. PMC7883096

48. Mank VMF, Mank J, Ogle J, Roberts J. (2021). Delayed, transient and self-resolving neutropenia following COVID-19 pneumonia. BMJ Case Rep, 14(5). PMC8117979

49. Hernandez JM, Quarles R, Lakshmi S, Casanas B, Eatrides J, McCoy E, *et al*. (2021). Pancytopenia and Profound Neutropenia as a Sequela of Severe SARS-CoV-2 Infection (COVID-19) With Concern for Bone Marrow Involvement. Open Forum Infect Dis, 8(2), ofab017. PMC7880265

50. Nazarullah A, Liang C, Villarreal A, Higgins RA, Mais DD. (2020). Peripheral Blood Examination Findings in SARS-CoV-2 Infection. Am J Clin Pathol, 154(3), 319-329. PMC7454310

51. Zini G, Bellesi S, Ramundo F, d'Onofrio G. (2020). Morphological anomalies of circulating blood cells in COVID-19. Am J Hematol, 95(7), 870-872. PMC7262044

52. Ong J, Ramanan R, Hocking J, Morgan S. (2020). An unexpected cause of Pseudo-Pelger-HuEt anomaly. Pathology, 52, S35-S36.

53. Kubankova M, Hohberger B, Hoffmanns J, Furst J, Herrmann M, Guck J, *et al*. (2021). Physical phenotype of blood cells is altered in COVID-19. Biophys J, 120(14), 2838-2847. PMC8169220

54. Thomas T, Stefanoni D, Dzieciatkowska M, Issaian A, Nemkov T, Hill RC, *et al*. (2020). Evidence of Structural Protein Damage and Membrane Lipid Remodeling in Red Blood Cells from COVID-19 Patients. Journal of Proteome Research, 19(11), 4455-4469.

55. Kannan G, Soni M. (2021). Leukocyte morphological changes in COVID-19, a peripheral smear study and analysis at a tertiary health care centre in India. Apollo Medicine, 18(3), 158-161.

56. Hosoda T, Orikasa H. (2022). A fatal case of extensive gastrointestinal necrosis due to portal and mesenteric vein thrombosis in the post-acute phase of COVID-19. J Infect Chemother, 28(1), 108-111. PMC8529290

57. Fan BE, Umapathi T, Chua K, Chia YW, Wong SW, Tan GWL, *et al*. (2021). Delayed catastrophic thrombotic events in young and asymptomatic post COVID-19 patients. J Thromb Thrombolysis, 51(4), 971-977. PMC7648538

58. Andrade Barreto AP, Duarte LC, Cerqueira-Silva T, Barreto Filho MA, Camelier A, Tavares NM, *et al*. (2021). Post-Acute COVID Syndrome, the Aftermath of Mild to Severe COVID-19 in Brazilian Patients. medRxiv, 2021.2006.2007.21258520.

59. Morris D, Patel K, Rahimi O, Sanyurah O, Iardino A, Khan N. (2021). ANCA vasculitis: A manifestation of Post-Covid-19 Syndrome. Respir Med Case Rep, 34, 101549. PMC8580553

60. Saleh J. (2015). Glycated hemoglobin and its spinoffs: Cardiovascular disease markers or risk factors? World J Cardiol, 7(8), 449-453. PMC4549778

61. Al-Samkari H, Berliner N. (2018). Hemophagocytic Lymphohistiocytosis. Annu Rev Pathol, 13, 27-49.

62. Soy M, Atagunduz P, Atagunduz I, Sucak GT. (2021). Hemophagocytic lymphohistiocytosis: a review inspired by the COVID-19 pandemic. Rheumatol Int, 41(1), 7-18. PMC7315691

63. Flower L, Laundy N, Khosravi M, Buckley J, Gale A, Kumar ID, *et al*. (2021). Haemophagocytic lymphohistiocytosis secondary to COVID-19: a case series. Lancet Rheumatol, 3(11), e744-e747. PMC8367191 JJM, RT, and VQ had full access to all the data in the study and had final responsibility for the decision to submit for publication. There was no funding source for this study.

64. Lazarian G, Quinquenel A, Bellal M, Siavellis J, Jacquy C, Re D, *et al*. (2020). Autoimmune haemolytic anaemia associated with COVID-19 infection. British journal of haematology, 190(1), 29-31.

65. Davoodian A, Umeh C, Novatcheva E, Sassi GP, Ahaneku H, Kundu A. (2021). Severe Immune Thrombocytopenia Post-COVID-19: A Case Report. Cureus, 13(11), e19544. PMC8668258

66. Raadsen M, Du Toit J, Langerak T, van Bussel B, van Gorp E, Goeijenbier M. (2021). Thrombocytopenia in Virus Infections. J Clin Med, 10(4). PMC7924611

67. Garg P, Arora U, Kumar A, Malhotra A, Kumar S, Garg S, *et al*. (2021). Risk factors for prolonged fatigue after recovery from COVID-19. J Med Virol, 93(4), 1926-1928.