THE LABORATORY PERSPECTIVE OF LEUKOCYTE DIFFERENTIAL, LYMPHOCYTE COUNT AND CELL POPULATION DATA IN EVALUATION OF SARS-COV2: A COMPREHENSIVE SYSTEMATIC REVIEW

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ABSTRACT

*Background***:** The pathology of COVID-19 involves a dysregulation of the adaptive immune system, with hematological abnormalities being frequently reported in affected patients since the onset of the pandemic. Specifically, leukocyte differentials in SARS-CoV-2 infected individuals often exhibit neutrophilia, lymphopenia, and morphology alterations. This study aims to serve a comprehensive systematic review to examine the role of leukocyte differential, lymphocyte count, and CPD parameters to evaluate SARS-COV2 in literatures of the last 10 years.

*Methods***:** The review adhered to PRISMA 2020 standards and analyzed full-text English literature from 2014 to 2024. It excluded editorials, review papers from the same journal, and submissions lacking a DOI. Literature sources included PubMed, SagePub, SpringerLink, and Google Scholar.

*Result***:** A total of 937 articles were retrieved from online databases (PubMed, SagePub, SpringerLink and Google Scholar). After three rounds of screening, five articles directly relevant to the systematic review were selected for full-text reading and analysis.

*Conclusion***:** The comprehensive assessment of leukocyte count, lymphocyte count, and cell population data provides invaluable insights into the immune dysregulation and disease severity in COVID-19 infection. Understanding the intricate interplay between these hematological parameters can guide clinicians in early detection, risk stratification, and tailored therapeutic interventions for patients battling COVID-19.

KEYWORDS: *COVID-19, leukocyte, lymphocyte, neutrophils*

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sparked the global outbreak of coronavirus disease 2019 (COVID-19), first identified in December 2019. The World Health Organization (WHO) declared it a pandemic on March 11, 2020, reflecting its rapid global spread. Over the ensuing six months, the pandemic has persisted unabated, imposing significant strain on healthcare systems worldwide, resulting in a substantial death toll, and causing widespread economic disruption.[1]

COVID-19 presents a spectrum of manifestations, ranging from asymptomatic infection to severe pneumonia, acute respiratory distress syndrome (ARDS), and sepsis leading to multiorgan failure and death. Initial clinical symptoms commonly include fever (77%–98%), dry cough (46%–82%), myalgia or fatigue (11%–52%), and dyspnea (3%–31%). The majority of patients progress to pneumonia, with 20%–30% of cases advancing to respiratory failure necessitating ventilatory support. In pneumonia cases, dyspnea typically manifests about eight days after symptom onset. Radiographic abnormalities, such as ground-glass opacities and focal consolidation, are often observed in pneumonia patients through CT or chest X-ray imaging.[2]

Respiratory failure and myocardial damage, attributed to myocarditis, are major causes of death. Additionally, acute kidney injury, secondary infections, and coagulopathy occur in roughly 50% of nonsurvivors. Mortality rates are higher among older individuals and those with underlying conditions like hypertension, diabetes mellitus, coronary heart disease, chronic lung disease, and cancer.[3]

The pathology of COVID-19 involves a dysregulation of the adaptive immune system, with hematological abnormalities being frequently reported in affected patients since the onset of the pandemic. Specifically, leukocyte differentials in SARS-CoV-2 infected individuals often exhibit neutrophilia, lymphopenia, and morphology alterations, which could serve as valuable screening markers. Moreover, cell population data (CPD) derived from modern analyzers provide morphometric parameters characterizing leukocytes, aiding in the identification and classification of various cell types based on volume, granularity, and nucleic acid content.[4]

Given the emergence of COVID-19, several studies have utilized CPD data for timely diagnosis, monitoring, prognosis, and assessing disease severity. The haematology laboratory plays an important role in the prognostication of patients with COVID-19. This prospective observational study focused on evaluating the utility of leukocyte differential and CPD parameters in distinguishing SARS-CoV-2 infected patients from those with other infections.[4] This study aims to serve a comprehensive systematic review to examine the role of leukocyte differential, lymphocyte count, and CPD parameters to evaluate SARS-COV2 in literatures of the last 10 years.

METHODS

PROTOCOL

The author carefully followed the rules laid out in the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020. This was done to make sure the study met all its standards. The selection of this methodological approach was specifically aimed at ensuring the precision and reliability of the conclusions drawn from the investigation.

CRITERIA FOR ELIGIBILITY

This systematic review examined the role of leukocyte differential, lymphocyte count, and CPD parameters to evaluate SARS-COV2 in literatures of the last 10 years. This study meticulously analyzed data on literatures to provide insights and enhance patient treatment strategies. The primary objective of this paper is to highlight the collective significance of the identified key points.

Inclusion criteria for this study entail: 1) Papers must be in English, and 2) Papers must have been published between 2014 and 2024. Exclusion criteria comprise: 1) Editorials; 2) Submissions without a DOI; 3) Previously published review articles; and 4) Duplicate entries in journals.

SEARCH STRATEGY

The keywords used for this research are "COVID-19", "leukocyte", "lymphocyte", "neutrophils". The Boolean MeSH keywords inputted on databases for this research are: *(("covid 19"[All Fields] OR "covid 19"[MeSH Terms] OR "covid 19 vaccines"[All Fields] OR "covid 19 vaccines"[MeSH Terms] OR "covid 19 serotherapy"[All Fields] OR "covid 19 nucleic acid testing"[All Fields] OR "covid 19 nucleic acid testing"[MeSH Terms] OR "covid 19 serological testing"[All Fields] OR "covid 19 serological testing"[MeSH Terms] OR "covid 19 testing"[All Fields] OR "covid 19 testing"[MeSH Terms] OR "sars cov 2"[All Fields] OR "sars cov 2"[MeSH Terms] OR "severe acute respiratory syndrome coronavirus 2"[All Fields] OR "ncov"[All Fields] OR "2019 ncov"[All Fields] OR (("coronavirus"[MeSH Terms] OR "coronavirus"[All Fields] OR "cov"[All Fields]) AND 2019/11/01:3000/12/31[Date - Publication])) AND ("leucocytic"[All Fields] OR "leukocytes"[MeSH Terms] OR "leukocytes"[All Fields] OR "leucocyte"[All Fields] OR "leucocytes"[All Fields] OR "leukocyte"[All Fields] OR "leukocytic"[All Fields]) AND ("lymphocytes"[MeSH Terms] OR "lymphocytes"[All Fields] OR "lymphocyte"[All Fields] OR "lymphocytic"[All Fields] OR "lymphocyts"[All Fields]) AND ("neutrophil s"[All Fields] OR "neutrophiles"[All Fields] OR "neutrophilic"[All Fields] OR "neutrophillic"[All Fields] OR "neutrophils"[MeSH Terms] OR "neutrophils"[All Fields] OR "neutrophil"[All Fields] OR "neutrophile"[All Fields])) AND (y_10[Filter]).*

DATA RETRIEVAL

The authors assessed the studies by reviewing their abstracts and titles to determine their eligibility, selecting relevant ones based on their adherence to the inclusion criteria, which aligned with the article's objectives. A consistent trend observed across multiple studies led to a conclusive result. The chosen submissions had to meet the eligibility criteria of being in English and a full-text.

This systematic review exclusively incorporated literature that met all predefined inclusion criteria and directly pertained to the investigated topic. Studies failing to meet these criteria were systematically excluded, and their findings were not considered. Subsequent analysis examined various details uncovered during the research process, including titles, authors, publication dates, locations, study methodologies, and parameters.

QUALITY ASSESSMENT AND DATA SYNTHESIS

Each author independently evaluated the research presented in the title and abstract of the publication to determine which ones merited further exploration. The subsequent stage involved assessing all articles that met the predefined criteria for inclusion in the review. Decisions on including articles in the review were based on the findings uncovered during this evaluation process. This criterion aimed to streamline the paper selection process for further assessment, facilitating a comprehensive discussion of previous investigations and the factors that made them suitable for inclusion in the review.
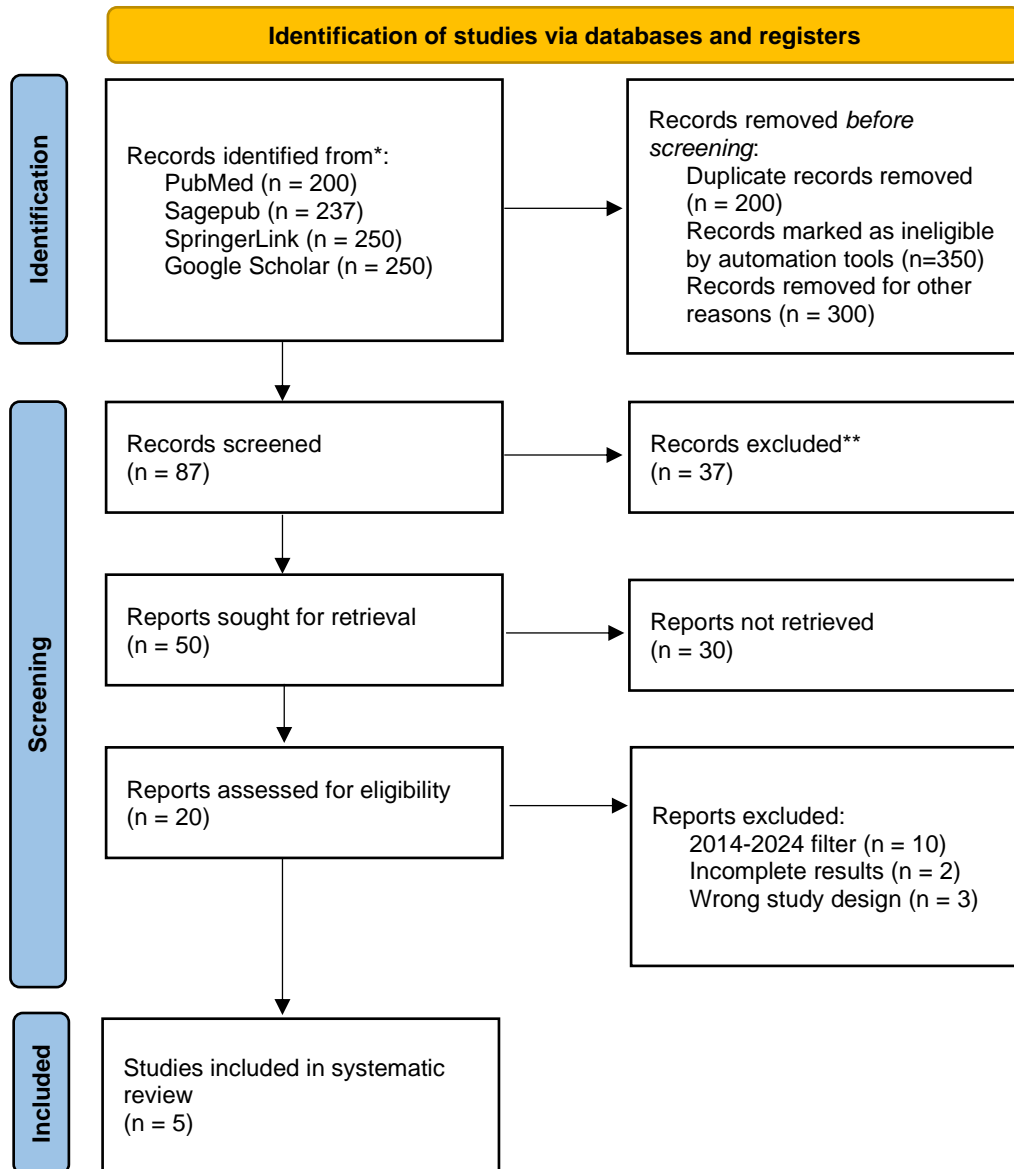
Figure 1. Article search flowchart

RESULT

The initial number of articles retrieved from online databases (PubMed, SagePub, SpringerLink, and Google Scholar) is 937 articles. After conducting three levels of screening, five articles that directly relate to the current systematic review have been chosen for further assessment through full-text reading and analysis. Table 1 presents the selected literature included in this analysis.

Table 1. The literature included in this study

Author	Origin	Method	Sample	Result
Patel., et al.[5] (2021)	India	Observational study	50 covid-19 patients	Total white blood cell (WBC) count ranged from 3.4 to 23.21 x10^9/L, with a median of 7.3 x10^9/L. Leukopenia was noted in 2 cases (4%), while 10 cases (20%) had leucocytosis. Neutrophilic predominance was observed in cases with high WBC counts. The absolute neutrophil count ranged from 0.87-19.7 x10^9/L. Absolute lymphocyte count ranged from 0.37-3.28 x10^9/L, with eighteen patients (36%) exhibiting lymphopenia.

Balzanelli, et al.[6] (2021)	Italy	Case control study	138 patients	The study analyzed leukocyte count, lymphocyte count, and cell population across four distinct clinical groups. In the positive–positive (PP) group, consisting of 45 confirmed COVID-19 patients, a decline in lymphocytes (%) was observed alongside significant associations with inflammatory markers, including increased neutrophils, T-killer, T-active, T-suppressor, and T-CD8+CD38+ cells. Similarly, the negative–positive (NP) group, comprising 37 individuals with COVID-like symptoms but negative RT-PCR results, showed altered lymphocyte counts and significant associations with inflammatory markers
Zhang, et al.[7] (2021)	China	Cohort study	34 covid-19 patients	The study aimed to predict COVID-19 severity and patient outcomes by analyzing peripheral blood samples from 34 patients in early 2020. While the number of monocytes did not significantly differ between COVID-19 patients and healthy individuals, notable morphological and functional differences were observed, particularly in patients requiring prolonged hospitalization and ICU admission. COVID-19 patients exhibited larger-than-normal monocytes, specifically a distinct population with high forward scatter (FSC-high). These FSC-high monocytes, expressing CD14+CD16+, displayed mixed M1/M2 macrophage polarization, with heightened expression of CD80+ and CD206+ and secreted elevated levels of IL-6, IL-10, and TNF-α compared to normal controls.
Fumagalli, et al.[8] (2023)	Italy	Retrospective cohort stidy	11,052 patients	The study included 11,052 patients admitted to the emergency room, with a median age of 67 years and 48% female. Of these, 59% were discharged, and 41% were hospitalized. After a median follow-up of 306 days, 86% were alive, and 14% deceased. Elevated hazard ratios were associated with age >73 years, in-hospital admission, and ER admission during SARS-CoV2 surges. Gender and various blood cell parameters, including leukocyte count and lymphocyte count, were significant. Two models were developed: the benchmark-BCDC model, including basophils and platelet count (AUROC 0.74), and the tailored-BCDC model, including monocyte counts and platelet hematocrit (AUROC 0.79). Overall, baseline discretized BCDC analysis offers valuable insights into the survival outcomes of emergency room patients.

Spijkerman, et al.[9] (2021)	Netherland	Prospective cohort study	103 patients	The study aimed to investigate whether COVID-19 patients exhibit characteristics of hyperinflammation in the neutrophil compartment. A prospective study was conducted on suspected COVID-19 patients presenting at an academic hospital's emergency room. Blood samples collected within 2 days of hospital admission were analyzed using automated flow cytometry and compared with samples from later time points. Contrary to expectations, COVID-19 patients did not show neutrophilia or eosinopenia. Additionally, neutrophil activation markers did not differ significantly between COVID-19-positive patients and those diagnosed with other bacterial or viral infections, nor did they differ among COVID-19 severity groups. However, all patients, regardless of COVID-19 status, exhibited a decrease in neutrophil maturation markers, indicating an inflammation-induced left shift in the neutrophil compartment. This shift was associated with disease severity in COVID-19 patients.

Patel., et al.[5] (2021) showed that COVID-19 patients exhibited notable reductions in lymphocyte count, indicating lymphopenia, along with mild decreases in white blood cell count (leukopenia) and platelet count (thrombocytopenia). However, other hematological parameters did not display significant alterations.

Balzanelli, et al.[6] (2021) showed that alterations in peripheral lymphocyte subsets correlated with COVID-19 clinical characteristics and progression. T-lymphocyte and B-lymphocyte subset levels could serve as independent predictors of COVID-19 severity and treatment efficacy.

In a study conducted by Zhang et al.[7] (2021), peripheral blood samples were analyzed using flow cytometry. While the number of monocytes did not significantly differ between COVID-19 patients and healthy individuals, significant morphological and functional differences were observed, especially in patients requiring prolonged hospitalization and ICU admission. COVID-19 patients displayed larger-than-normal monocytes, particularly a distinct population characterized by high forward scatter (FSC-high).

Fumagalli, et al.[8] (2023) explored factors influencing COVID-19 survival outcomes. They analyzed a range of blood cell characteristics, including leukocyte count and lymphocyte count, along with demographic factors like age, gender, and hospital admissions. The findings indicated that besides age and hospital admission status, leukocyte count and lymphocyte count emerged as significant predictors of survival.

Spijkerman, et al.[9] (2021) showed that COVID-19 patients did not show neutrophilia or eosinopenia. Additionally, neutrophil activation markers did not differ significantly between COVID-19-positive patients and those diagnosed with other bacterial or viral infections, nor did they differ among COVID-19 severity groups. However, all patients, regardless of COVID-19 status, exhibited a decrease in neutrophil maturation markers, indicating an inflammation-induced left shift in the neutrophil compartment.

DISCUSSION

Technological innovations have allowed haematology analysers to generate quantitative CPD on the morphological and functional characteristics of circulating blood cells. Leukocyte count, lymphocyte count, and cell population data (CPD) serve as fundamental pillars in unraveling the dynamics of the immune response and disease progression in COVID-19 infection. These hematological parameters provide crucial insights into the underlying mechanisms of the disease, offering valuable prognostic indicators and aiding in the formulation of effective therapeutic strategies.[10]

Leukocyte count stands as a pivotal marker for gauging the overall immune response, reflecting the systemic inflammatory state triggered by viral invasion. Similarly, lymphocyte count plays a pivotal role in assessing the adaptive immune response, particularly the functionality of T lymphocytes crucial in combating viral pathogens.[4] In COVID-19, a reduction in lymphocyte count, commonly observed as lymphopenia, underscores the severity of the disease and is strongly associated with adverse clinical outcomes.

Previous study revealed reductions in lymphocyte count (indicating lymphopenia), leukopenia, and thrombocytopenia in COVID-19 patients, highlighting the significance of these hematological parameters in assessing disease severity and prognosis.[5] Moreover, Spijkerman et al.[9] (2020) provided insights into the neutrophil compartment, revealing a nuanced inflammatory response characterized by a decrease in neutrophil maturation markers. This observation suggests a unique immune dysregulation in COVID-19, potentially influencing disease severity and clinical outcomes.[9]

Furthermore, Balzanelli et al.[6] (2021) demonstrated correlations between alterations in peripheral lymphocyte subsets and COVID-19 clinical characteristics, suggesting their utility as independent predictors of disease severity and treatment efficacy. Meanwhile, Zhang et al.[7] (2021) investigation into monocyte characteristics uncovered significant morphological and functional differences, particularly in patients with severe disease manifestations. Notably, COVID-19 patients exhibited larger-than-normal monocytes, indicative of a hyperactive immune state, further emphasizing the complexity of the immune response in COVID-19 infection.

CONCLUSION

In summary, the comprehensive assessment of leukocyte count, lymphocyte count, and cell population data provides invaluable insights into the immune dysregulation and disease severity in COVID-19 infection. Understanding the intricate interplay between these hematological parameters can guide clinicians in early detection, risk stratification, and tailored therapeutic interventions for patients battling COVID-19.

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