

Routine Laboratory Testing Role For Covid-19 Identification: A Systematic Review

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) is the cause of undergoing pandemic. Molecular testing in the form of reverse transcription-polymerase chain reaction (RT-PCR) is considered as the diagnostic standard for detecting COVID-19. However, there are still certain issues with its application. We sought this study is to systematically review and summarize studies pertaining blood laboratory biomarkers for diagnosing COVID-19. **Methods:** The systematic review was conducted by following the PRISMA 2020 recommendations. We searched published articles in four databases (EbscoHost, Pubmed, Science Direct, and Scopus).that assessed the implementation of routine laboratory tests examination for diagnosing COVID-19 patients from March 2020 through September 2021. **Results:** Three studies were selected to be reviewed, with the number of participants ranging from 100 to 485 (total: 792). Peripheral blood count parameters were assessed in all studies. The monocyte-lymphocyte ratio had the highest accuracy. Several abnormalities of laboratory indicators such as white blood cell, neutrophil, lymphocyte, eosinophil, NLR, MLR, LDH, AST, ALT, and CRP were significantly different between COVID-19 positive patients to negative controls,. **Conclusion:** No single test could identify or distinguish COVID-19 from other pneumonia causes. However, laboratory biomarkers can be used as a complement to the COVID-19 diagnostic approach.

Keywords: COVID-19, Leukocyte, Routine laboratory test, Polymerase Chain Reaction

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first reported at the end of 2019, and responsible for one of the biggest outbreak ever faced by human.¹⁻⁴ COVID-19 has various symptoms, and some can even be asymptomatic. Initial symptoms presented are fever, cough, myalgia or fatigue, dyspnea, headache, hemoptysis, sputum production, and diarrhea. Commonly occurred symptoms of COVID-19 is pneumonia, varied from mild to severe.^{5,6}

The main test that serves that is accepted as the worldwide standard for detecting active COVID-19 is by the implementation of a molecular technique, named reverse transcription polymerase chain reaction (RT-PCR), which is oriented to detect genetic materials of the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the origin of COVID-19 development.⁷ Although seemingly simple, laboratory identification

of COVID-19 proved to be challenging to do, particularly in low resource country such as Indonesia due to expensive equipment needed, although testing resources should ideally be evenly distributed in each region and it still proved to be difficult thing to do and might contributed to second peak that Indonesia has experienced. Furthermore, RT-PCR have long turnaround time and thus unsuitable as mass screening test, also

the fact that false negative rates are as high as 20%.⁸⁻¹¹ This brings the need for cheap and readily accessible tests.¹² Previous studies have shown some alterations to the laboratory parameters of COVID-19 patients. These include, but not limited to, C-reactive protein (CRP), lactate dehydrogenase (LDH), routine blood testing (e.g. leukocyte, lymphopenia), and transaminases (liver enzyme).^{13,14} Therefore, this study aims to conduct a systematic review and summarize studies

about blood laboratory biomarkers that are readily accessible and also determine their potential for the diagnostic utility of COVID-19. Therefore, the objective of our study is to commence a systematic review which is oriented to summarize studies pertaining routine blood-related laboratory biomarkers that is widely available and also determine their potential for diagnostic utility of COVID-19.

METHODS

Search strategy

We implemented the updated recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 to conduct this study.¹⁵ The study procedure was authorized by all representatives of the review panel prior to the completion of evidence search. We investigate four databases, including Science Direct, Pubmed, Scopus, and EBSCOhost for published articles from March 2020 through September 2021 that assessed the implementation of routine laboratory tests examination for diagnosing COVID-19 patients. The search terms were utilized in the literature retrieval: Routine blood test OR Kidney Function AND Screening AND Diagnostic AND COVID-19. Each of the databases fetched different amount of articles, in order of Science Direct (956 hits), Pubmed (160 hits), Scopus (21 hits), and EbscoHost (4 hits), for a total of 801 articles.

Literature selection

We investigated the observational studies, including cross-sectional, case-control, and cohort (retrospective and prospective) researches which examined the role of routine laboratory parameters for rapid recognition of COVID-19 patients. All of the full-text articles were written in English to maintain reports accuracy. Reviews, case reports, brief reports,

protocols, clinical trials, abstracts, editorials, opinions, correspondence, presentations, and posters were excluded. In addition, articles evaluating the prognostic applicability of laboratory testing in COVID-19 patients or non-routine laboratory measurements were also considered ineligible. Duplicates were removed, and two researchers (TPU and RS) adjudicated both titles and abstracts independently employing Rayyan QCRI.¹⁶ Any differences were settled through discussion to achieve consensus. We selected 14 publications for full text suitability assessment from the 1,141 articles collected. In the aggregate, three studies fulfilled the criteria for data synthesis inclusion (**Figure 1**).

Data extraction

Data were obtained from a series of studies. Two reviewers (TPU and RS) autonomously extracted appropriate information through each study. Authorship status, year of publication, country of the research project, investigation populations, study setting, sample assessment procedures, and key findings were captured. In each paper, we also included a list of all variables that were evaluated. The questionnaire Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) was used for the appraisal. QUADAS-2 aids in assessment across a wide range of domains, including participant selection, index examination, reference range, and flow and timeline.¹⁷ Each component was assessed for

the possibility of bias, and the first three domains were also considered for the scope of application issues. Two reviewers evaluated each article independently, and disagreements were resolved through dialogues. We did not eliminate research papers based on quality assessment owing to the small number of qualified manuscripts; instead, quality evaluation data was used to demonstrate the strength of the available evidence. RESULTS

Characteristics of included studies

The main characteristics of the included studies are listed in (Table 1). The sample size ranged from 100 to 485 participants. The total number of participants summed from all included studies was 792, with 345 of them was diagnosed with COVID-19, all comprised of adults. Among those studies, eosinophil appears in all observations. Examination of leukocyte, other differential cell counts, and some leukocyte ratio was done in two studies, as well as the platelets. However, only one studies which examined the role of hemoglobin. Two studies observed the laboratory parameters directly and stating the collection methods, and one only taken the data from the medical records. Two studies were case-control,^{18,19} meanwhile one was cross-sectional²⁰ in design.

Study quality

The quality of most studies was average to high (Table 2). All studies enrolled patients which is tested for COVID-19 diagnosis, which is confirmed by the RT-PCR testing which is then classified as negative or positive. Most of them is case-control, although following randomization of subjects, so some risk of bias in patient selection was identified. All of the participants were analyzed in the end of the study. All of the studies have analyzed the peripheral or complete blood count, mainly in leukocyte-related parameters. Only one studies which assessed the inflammatory markers.¹⁸ The sensitivity dan specificity analysis only mentioned in one study.²⁰ In addition, the reference range of the laboratory markers only given in two studies.^{19,20}

Diagnostic Value of Several Laboratory Parameters for Detecting COVID-19

All studies have observed peripheral blood count parameters (either routine or complete), one of the studies only assessed a specific cell type, eosinophils¹⁹. Almost all leukocyte-related parameters and ratios showed significant differences ($p < 0.05$) between those with and without COVID-19. Thus, based on the sensitivity and specificity analysis, the monocyte-lymphocyte ratio (MLR) posed the best accuracy in differentiating COVID-19 patients from healthy subjects (specificity: 90.00%, sensitivity: 75.79), giving the high diagnostic value. A conflicting result was reported for the use of platelet as a putative COVID-19 diagnostic marker; a study in China²⁰ indicated substantial differences, whilst the result in Italy¹⁸ was insignificant. For the peripheral blood count, MLR, Neutrophil-lymphocyte ratio (NLR), and lymphocyte count may be employed as quick, cost-effective, and promising potential biomarkers to support the diagnosis of COVID-19.

Several other parameters, such as inflammatory markers (C-reactive protein/CRP and Lactate dehydrogenase/LDH) and hepatic function markers (Aspartate aminotransferase/AST, Alanine aminotransferase/ALT, Gamma-glutamyl transferase/GGT, and Alkaline phosphatase/ALP), have been observed to assist in the diagnosis of COVID-19 in low-resource settings. Inflammatory indicators have been found to differ significantly between persons who have COVID-19 and those who do not. Meanwhile, hepatic enzyme, AST, and ALT also showed significant differences in differentiating COVID-19 states (infected or not infected). Studies have shown that these parameters may provide about 70% of accuracy for the correct classification of RT-PCR results (positive and negative), thus potentially be used for identifying false-positive/negative RT-PCR and applied in the developing countries or in those countries without readily available resources as the alternative screening to prioritize RT-PCR use.¹⁸

DISCUSSION

In this systematic review of laboratory routine markers, we found WBC count such as neutrophil, lymphocytes and monocytes were significantly different in COVID-19 patients compared to controls. In particular, lower count of neutrophil in COVID-19 patient has highest specificity meanwhile monocyte/lymphocyte (MLR) ratio has highest sensitivity. Similar finding that COVID-19 positive patients had significantly lower WBC values compared to COVID-19 negative patients were reported previously by Olgat, et. al. in a study of 90 hospitalized patients.²¹ Lymphocyte count was also interesting with the observation lymphopenia as one the most common laboratory finding in patient with COVID-19. Lymphopenia and monocytopenia have also been reported as one of prognostic markers for severe cases of COVID-19 that requires admission to the intensive care unit.²¹ AST, ALT and LDH is particularly interesting as it seemingly better as diagnostic markers compared to blood count that is affected by various medical conditions. Previous study has also observed AST, ALT and LDH potential as diagnostic markers of COVID-19 with high LDH as the most accurate marker for predicting the diseases. High level of LDH was also associated with more severe progression of the disease.^{14,22} The strong association between LDH and the COVID-19 diseases might be the facts that this enzyme is a marker of lung damage and that COVID-19 is respiratory pathogen that primarily infects the lower respiratory tracts.¹⁸ MLR ratio although is a marker with highest sensitivity, previous studies observed it has acceptable efficiency to separate COVID-19 patients from healthy subjects, but failed to rule out influenza pneumonia patients. Meanwhile NLR although could be used as diagnostic markers, more study instead study the use of NLR as prognostic tools. Reason underlying this is although neutrophil levels are more commonly elevated during bacterial infection, during viral infection neutrophils could also be elevated via neutrophil-mediated innate immune responses and take part in clearance of the virus. Higher NLR is correlated with increase severity of

COVID-19 and might be due to viral load and immune response provoked.^{14,20,23}

Inflammatory markers such as C-reactive protein (CRP) that is found high in COVID-19 cases might be useful to differentiate the diagnosis with other form of viral pneumonias although unlikely able to differentiate the condition from bacterial pneumonias. High level of C-reactive protein in COVID-19 is due to cytokine storm that is known to occur in pathogenesis of the disease and is linked to higher severity and rates of mortality. In line with this, previous study indicates CRP as markers that could be useful for assessing disease mortality.²⁴

Even though at the time of writing RT-PCR currently serves as gold standard in diagnosing COVID-19, the test limitation comprised of long time needed for the result and expensive equipment became major limitations for large scale screening tool and rapid diagnosis of patients. In particular at developing country such as Indonesia, although testing infrastructure has increased dramatically some places still unable to have access to this test. Biomarker that is readily accessible, specially at public health center, has an advantage of faster result times and could serve as guide to isolate the patient and even some has potential as prognostic marker therefore likely severe patient could be referred faster.²³

Additional problem regarding the use of RT-PCR is the possibility of false-negative results (those infected with SARS-CoV-2 who have negative RT-PCR results). According to research, the proportion of false-negative results can range from 20% to 100%, with an average of 54% (happened in about 1 of 2 patients infected).^{8,10} The viral load (which is usually lower in asymptomatic people), the onset of disease symptoms (which is lowest at the third day after symptoms onset, 20%, and greatest four days before the onset), those with laboratory tests indicating noticeable inflammation (increase in white blood cells, platelets, and CRP), and technical problems (sampling errors) could all be aspects causing this phenomenon.^{8,9,25,26} As a result, while interpreting RT-PCR results, the

judgment about SARS-CoV-2 infection must be considered in conjunction with the progress of the disease and the clinical and epidemiological context.

Our study limitations are the lack of studies pertaining value of readily accessible diagnostic biomarkers and even more on the quality of the studies. Some of the study data are collected on early pandemic and the result might be affected by poor quality of RT-PCR which limit conclusion of the study.

It should also be noticed that no single blood laboratory marker have enough sensitivity and specificity to diagnose or exclude COVID-19. Previous review have concluded laboratory test were insufficient to accurately diagnose COVID-19. There are suggestion to combine laboratory markers to better discriminate COVID-19 from other form of respiratory infection therefore further studies and analyses are required however this is beyond the scope of the study. Even though blood markers might prove insufficient as diagnostic tools, however it could serve as an adjunct of diagnostic approach in differential of other respiratory infection such as influenza, particularly in low resource setting. Thus, diagnostic studies assessing value of readily accessible markers are still needed.^{21,27}

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CONCLUSION

Abnormal laboratory markers such as white blood cell, neutrophil, lymphocyte, eosinophil, NLR, MLR, LDH, AST, ALT and CRP were found significantly different in COVID-19 positive patients compared to control. However no single laboratory markers alone could diagnose or differentiate COVID-19 from other etiology of pneumonia. Despite this, laboratory biomarker can act as adjunct for diagnostic approach of COVID-19 and might be useful when combined together as clinical scoring algorithm.

Competing Interest

None declared

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None

Authors' Contributions

TPU and RS contributed significantly to the work's concept or design, data acquisition, and analysis. The paper's drafting and critical review were done by all of the writers. All authors reviewed and approved the final document.

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Table 1. Summary and key findings of included studies

Author (year)	Country	Study populations	Primary aim	Data collection tools	Key findings
Ferrari, et.al. (2020) ¹⁸	Italy	207 patients (105 with positive RT-PCR, 102 with negative RT-PCR)	Identify statistically significant differences for the use of blood test results for the identification of positive and negative COVID-19 patients.	CRP, AST, ALT, GGT, ALP and LDH: Roche Cobas 8000; WBC, platelets, leukocyte formula: Sysmex XE 2100	<ul style="list-style-type: none"> WBC (p<0.001), neutrophils (p = 0.001), lymphocytes (p=0.010), monocytes (p=0.001), eosinophils (p=0.003), basophils (p<0.001), CRP (p=0.034), AST (p=0.007), ALT (p=0.006), and LDH (p< 0.001) all were observed in significantly different levels between patients with negative and positive RT-PCR results ALP, Platelets, and GGT (p>0.050) did not show significant differences between negative and positive RT-PCR patients Lower count on leukocyte parameters (and its differential count) were observed in COVID-19 positive patients, meanwhile CRP, AST, ALT and LDH showed higher value in patients with negative RT-PCR results
Peng, et.al. (2020) ²⁰	China	485 patients (190 COVID-19 patients, 190 healthy subjects, and 105 inpatients)	Identify public health student participation in COVID-19 response activities	Indirect (medical records)	<ul style="list-style-type: none"> WBCs (p<0.001), neutrophil percentages (p<0.001), lymphocytes (p<0.001), lymphocyte percentages (p<0.001), monocyte percentage (p<0.001), platelets (p<0.001), hemoglobin (p<0.001), NLR (p<0.001), MLR (p<0.001), and PLR (p<0.001) were significantly different in COVID-19 patients as compared with healthy subjects Although the neutrophil and monocyte percentage showed significant results, it is not applied to the absolute count of these cells The highest specificity to differentiate COVID-19 patients and health subject was observed in the neutrophil (95.79%), meanwhile greatest sensitivity was examined for the MLR. Overall, MLR showed an acceptable efficiency to separate COVID-19 patients from healthy subjects.
Tanni, et. al. (2020) ¹⁹	United States	100 patients (50 with COVID-19, 50 with influenza)	Investigate the diagnostic and prognostic value of eosinopenia in COVID-19-positive patients.	Coulter counter	<ul style="list-style-type: none"> Lower eosinophil count on presentation for COVID-19 patients vs. Influenza (35/μL vs. 100/μL) Absent eosinophil count was observed in 60% of COVID-19 patients, while in Influenza cases, only 16% with absent eosinophil detection.

Table 2. QUADAS-2 Risk of Bias Assessment

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Ferrari	Unclear	High	Unclear	Low	Low	Low	Low
Peng	Low	Low	Low	Low	Low	Unclear	Low
Tanni	Unclear	Low	High	Low	Low	Low	Low

Figure 1. PRISMA 2020 flow diagram of search and data extraction

