Calprotectin as a possible biomarker in various lung diseases

ABSTRACT:

Calprotectin (CLP) comprises two calcium-binding proteins from the S-100 protein family, S100A8 and S100A9. CLP involves various cellular processes in lung health and disease, including anti-microbial functions, pro- and anti-tumor properties, angiogenesis, DNA damage response, and extracellular matrix remodeling. The systematic review explores the potential of calprotectin as a diagnostic and prognostic biomarker for various respiratory diseases. The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and comprehensively searched electronic databases. Of 290 initially identified studies, 13 were included Olumide Ijishakin in the review, covering conditions such as cystic fibrosis (CF), lung cancer, COVID-19, asthma, and interstitial lung disease. For CF, fecal calprotectin showed promise as a non-invasive biomarker for diagnosing and monitoring pulmonary exacerbations. In lung cancer, calprotectin and other markers demonstrated potential for identifying high-risk individuals. In COVID-19, calprotectin levels were associated with disease severity and mortality risk. For asthma, calprotectin's role in neutrophil activation and neutrophilic asthma development was investigated. The review emphasizes the importance of robust biomarkers in improving disease management, reducing mortality rates, and enhancing patient care for respiratory conditions. Further research and validation studies are needed to establish the clinical utility of calprotectin as a biomarker for respiratory diseases.

KEYWORDS: Calprotectin, Lung Diseases, Cystic Fibrosis, COVID-19, Asthma, Lung Cancer, Interstitial Lung Disease

INTRODUCTION:

Respiratory diseases are ailments that affect the lungs and other organs of the respiratory system. These diseases can be caused by a variety of factors, including bacterial infections, viruses, smoking, second-hand smoke, and environmental contaminants(US Department of Health & Services, 2014). Each respiratory disease has unique characteristics and challenges that require developing and utilizing specific biomarkers. For example, biomarkers can help detect lung cancer at an early stage, personalize treatment strategies for cystic fibrosis, identify COVID-19 infection for effective control measures, diagnose and manage interstitial lung disease, or optimize asthma management (Dobler, 2019). These biomarkers provide valuable insights that enable timely intervention, targeted therapies, and enhanced patient care. The availability of robust and accessible biomarkers is vital in improving disease management, reducing mortality rates, and enhancing the quality of life for individuals affected by these respiratory conditions (Garcia-Rio et al., 2022).

“Calprotectin (CLP) comprises two calcium-binding proteins from the S-100 protein family, S100A8 and S100A9” (Kruzliak, Novák, Novák, & Fodor, 2014). “CLP has anti-inflammatory and anti-bacterial properties and is mainly expressed by neutrophils but can also be found in other cells. It is released extracellularly by activated or damaged cells, mediating various physiological and pathological responses. High levels of CLP have been found in many infectious and inflammatory diseases, including sepsis, inflammatory bowel disease, myocardial infarction, and rheumatological diseases. CLP has been implicated in the inflammatory process for over 20 years” (Ayling & Kok, 2018). However, its role in the pathogenesis of respiratory diseases and its usefulness as a biomarker for lung diseases have only recently gained attention.

“CLP is involved in various cellular processes in lung health and disease, including anti-microbial functions, pro- and anti-tumor properties, angiogenesis, DNA damage response, and extracellular matrix remodeling” (Kotsiou, Papagiannis, Papadopoulou, & Gourgoulianis, 2021). “CLP can be introduced into daily clinical practice for respiratory diseases. High levels of CLP have been found in many infectious and inflammatory diseases, which are closely associated with disease severity” (Mahler, Meroni, Infantino, Buhler,

& Fritzler, 2021). CLP also plays a critical role in various cellular processes, such as cell cycle progression, proliferation, differentiation, chemotaxis, migration, and survival.

METHODS:

The study in this research followed the 2020 guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement, ensuring a thorough and transparent approach. The process of selecting studies followed the PICO framework, which stands for Population, Intervention, Comparator, and Outcomes, aiding in establishing criteria for including relevant articles. An extensive search was conducted in electronic databases, including PubMed and Google Scholar, to identify relevant studies covering articles published from January 2012 to May 15, 2023. The inclusion criteria included randomized controlled trials, observational studies, and cohort studies involving patients with cardiovascular diseases. The main focus was on assessing calprotectin's potential as a prognostic and diagnostic biomarker for lung diseases. Various keywords related to Calprotectin, S1008/A9, or MRP8/14, lung disease, and pleural effusions were used in the search, employing Boolean logic (using "and/or") to refine the results. Only studies published in English were considered, while case reports, case series, animal studies, and reviews were excluded. Data synthesis and reporting were carried out using a combination of tabular and textual formats. A standardized form for data extraction was used to collect essential information from the included studies, such as author names, publication year, study design, sample size, population characteristics, outcome measures assessed, study results, limitations, and conclusions. This structured approach facilitated systematic data extraction and comparison across the studies. Additionally, throughout the systematic review process, Mendeley's referencing management tool was utilized to handle citations and full-text articles effectively. Figure 1 presents the PRISMA flowchart illustrating the included studies.

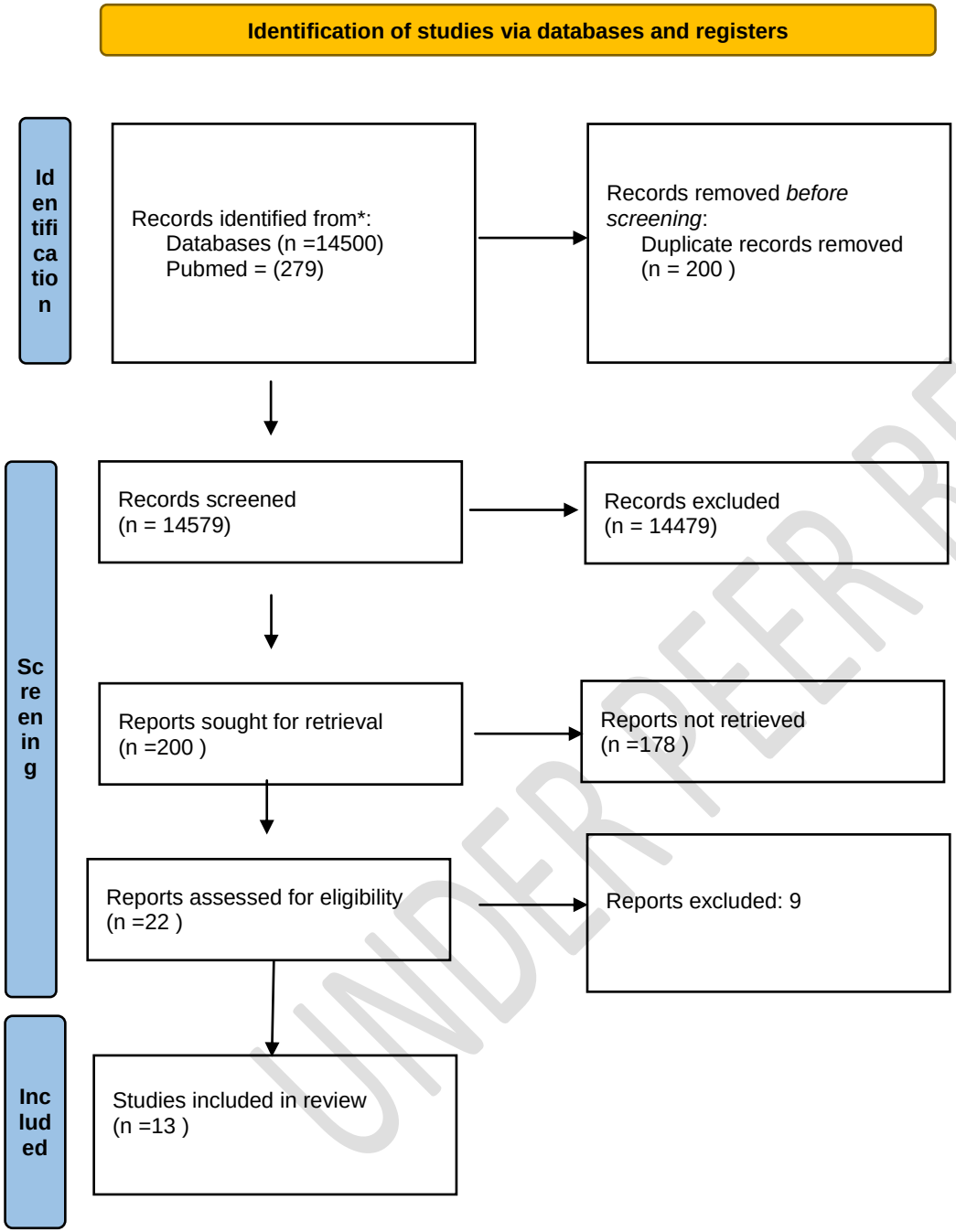

Figure 1. PRISMA Flowchart Depicting the Study Selection Process.

RESULTS:

During the database search, a thorough collection of 290 studies was initially identified. After removing 200 duplicate entries, 200 studies underwent screening based on their titles and abstracts. Among these, 178 records were excluded as they did not meet the predetermined inclusion criteria. The remaining 22 articles were then fully assessed for eligibility. Out of these, nine articles were subsequently excluded as they needed to meet the inclusion criteria. Ultimately, 13 studies were considered appropriate for inclusion in the systematic review, as depicted in Figure 1. The studies included different lung diseases, including Cystic Fibrosis (CF), lung cancer, COVID-19, Asthma, and Interstitial Lung Disease. The patients are predominantly male (n=906). While most of the participants belong to the age group of more than 50, all studies on pulmonary exacerbations recruited children below 18 years of age. Detailed description of included studies is shown in Table 1.

Table 1: Descriptive analysis of included studies.

Author, year	Methodology	Lung disorder	Biomarkers Tested	Participants	Demographics	Male	Mean Age	outcomes
(Imanzadeh et al., 2022)	**longitudinal study**	Cystic fibrosis (CF) pulmonary exacerbations (PEx)	Calprotectin	30 CF patients (1-18 years) without current infectious gastroenteritis	Male 16, Female 14, Age 11.5	16	11.5	The initial fecal calprotectin level in CF patients receiving antibiotics was 651.13 ± 671.04, significantly decreasing two weeks after antibiotic therapy and following recurrence (171.81 ± 224.40, 607.93 ± 549.89, respectively; $P < 0.01$).

(Jung et al., 2021)	longitudinal study	Cystic fibrosis (CF) pulmonary exacerbations (PEx)	C reactive Protein (CRP), Calprotectin	19 subjects (56 stable, 46 PEx visits)	Male =12, Age=40.8, BMI=23.4	12	40	CRP and calprotectin could discriminate stable vs. PEx visits with good performance and appear promising as diagnostic biomarkers but further validation studies are required prior to implementing these diagnostic thresholds.
(Parisi et al., 2017)	case control	Cystic fibrosis (CF)	Calprotectin	54 CF patients and 50 healthy controls	Male 29, female 25 (CF), Male 29 Female 21 (Control) Mean Age 18	29	18	FC levels were elevated above the cut-off value and significantly higher than in healthy controls. Among CF patients, FC was significantly higher in patients older than 18 years, with pancreatic insufficiency, underweight status, *Pseudomonas Aeruginosa* airways colonization, CF-related diabetes mellitus, reduced lung function, or high number of pulmonary exacerbations.
(Blanco-Prieto et al., 2015)	Prospective study	lung cancer	HB-EGF, EGF, EGFR, sCD26, VEGF, and Calprotectin	72 lung cancer patients of different histological types and 56 control subjects (healthy individuals and patients with benign pulmonary pathologies)	male =58, female 12 patient, 33 male 23 females controls	58		A remarkable discriminatory capacity was observed for EGF, sCD26, and especially for Calprotectin, these three molecules constituting a marker panel boasting a sensitivity of 83% and specificity of 87%, resulting in an associated misclassification rate of 15%.

(Luo et al., 2015)	single centre	malignant PE (MPE) and 56 benign PE (BPE)	calprotectin and CXCL12	malignant PE (MPE) and 56 benign PE (BPE)	Male =85	85		Calprotectin and CXCL12 levels of patients with MPE were significantly lower than that of BPE and tuberculous PE ($P<0.05$). The area under the curve (AUC) of calprotectin and CXCL12 was 0.683 and 0.641 in MPE and BPE, and a combination of calprotectin ≤500.19 ng/mL and CXCL12 ≤6.11 ng/mL rendered a sensitivity and specificity of 48.72% and 78.57%, respectively.
(Botana-Rial et al., 2020)	multicentre trial	malignant pleural effusion (MPE) and benign pleural effusion (BPE)	calprotectin	425 patients BPE in 223 cases (53.7%) or MPE in 137 patients (33%).	Male 262, Female 153	262		96% sensitivity and 60% specificity, with a negative and positive predictive value, and negative and positive likelihood ratios of 96%, 57%, 0.06, and 2.4
(Shi et al., 2021)	comparative	COVID-19	Calprotectin (S100A8/A9)	COVID-19 (n = 172), with controls	female 75, male 98, mean age 61	75	61	Patients with COVID-19 (n = 172) had markedly elevated levels of calprotectin in their blood. In longitudinal samples, calprotectin rose as oxygenation worsened.
(Cardiero et al., 2022)	retrospective	COVID 19	Calprotectin	195(156 hospitalized in the infectious disease unit and 39 in the intensive care unit (ICU))	Male 94, Age 55	94	55	ROC curves analysis for calprotectin levels and neutrophil count revealed a good discriminatory power toward survival (area under the curve of 0.759 and 0.843, respectively) and identified the best cut-off (1.66 mg/L and 16.39 × 103/μL, respectively). Kaplan–Meier analysis

								confirmed the prognostic role of high calprotectin levels and neutrophil count in death prediction
(Kassianidis et al., 2022)	case control	COVID-19	calprotectin, pro- and anti-inflammatory cytokines, interferons	181 patients and 40 non-infected comparators	156 females	35		Levels of the proinflammatory interleukin (IL)-8, IL-18, matrix metalloproteinase-9, platelet-derived growth factor (PDGF)-B and calprotectin (S100A8/A9) were significantly higher in patients with ARDS and MV. Levels of the anti-inflammatory IL-1ra and IL-33r were also increased; IL-38 was increased only in asymptomatic patients but significantly decreased in the more severe cases
(Kumar et al., 2022)	observational study	COVID 19	serum cystatin C and serum calprotectin	95 COVID-19 patients	Male 70, female 25	70		median cystatin C levels were significantly higher on the first day in the severe group ($P <$ 0.001) and in patients with cardiovascular disease ($P < 0.05$), chronic lung disease ($P = 0.009$) and among patients who died ($P <$ 0.05). It remained raised on day 3 in severe ($P < 0.05$) and deceased ($P < 0.05$) group. Serum calprotectin levels were significantly higher in patients with chronic lung disease ($P =$

								0.008) and in those who died ($P < 0.05$).
(Quoc et al., 2021)	single centre, mechanistic	Asthma	Calprotectin (S100A9)	187 adult asthmatic patients 57 healthy controls (HCs)				S100A9 serum levels were higher in patients with neutrophilic asthma than in those with non-neutrophilic asthma. There was a positive correlation between serum S100A9 levels and sputum neutrophil counts. Patients with higher S100A9 levels had lower PC20 methacholine values and a higher prevalence of severe asthma.
(Caimmi et al., 2019)	observational study	**Interstitial Lung Disease in Systemic Sclerosis**	fecal calprotectin	129 outpatients with SSc.	Female 109, Mean age 63	109	63	Patients with ILD (35, 27.1%) had higher values of FC ($p < 0.001$).

(Lin et al., 2022)	observational study, single cetre	interstitial lung diseases	S100A9) and Klebs von den Lungen-6 (KL-6)	This study included 98 patients, 37 patients with idiopathic pulmonary fibrosis (IPF), 12 with hypersensitivity pneumonitis, 13 with connective tissue disease-associated ILD, and 36 with sarcoidosis (SAR): stage I (18), stage II (9), stage III (5), and stage IV (4).	61 Male	61		The expression of KL-6 in BALF was significantly higher in IPF patients than other 3 groups (all P-value < .05). However, there was no significant difference in the levels of S100A9 in BALF and serum between the 4 groups (P-value > .05).

ROC-Receiver Operating Characteristic: A graphical plot illustrating the diagnostic ability of a binary classifier system.
ARDS-Acute Respiratory Distress Syndrome: A severe lung condition causing shortness of breath and low oxygen levels in the blood.
EGF-Epidermal Growth Factor: A protein that stimulates cell growth and differentiation by binding to its receptor, EGFR. EGF plays a crucial role in the wound-healing process and the initiation of hair follicle generation.
EGFR-Epidermal Growth Factor Receptor: A cell surface protein that binds to epidermal growth factor. Abnormalities in EGFR can lead to its constant activation, which can result in uncontrolled cell division – a characteristic of many cancers.
VEGF-Vascular Endothelial Growth Factor: A protein that is known to stimulate the formation of blood vessels (angiogenesis). VEGF plays a key role in both normal and pathological angiogenesis, including the growth of tumors and their metastases.
MV-Mechanical Ventilation: A method to assist or replace spontaneous breathing using a machine called a ventilator.

Cystic Fibrosis:

Cystic fibrosis (CF) is a genetic disorder that affects the respiratory and digestive systems, producing thick and sticky mucus in the lungs and other organs. Given the complex nature of CF, the development of effective diagnostic biomarkers is crucial to enable early detection, personalized treatment strategies, and improved quality of life for individuals living with this condition (De Boeck, Vermeulen, & Dupont, 2017). Imanzadeh et al., 2022 conducted a study on 30 patients with cystic fibrosis (CF), which causes progressive lung function reduction and pulmonary exacerbations. The study aimed to investigate using fecal calprotectin, a non-invasive biomarker of intestinal inflammation, as a diagnostic tool for CF patients. The patients' fecal calprotectin levels were evaluated before and after systemic antibiotic treatment following pulmonary exacerbations. The results showed that antibiotic treatment reduced fecal calprotectin levels and improved respiratory and gastrointestinal symptoms. The study suggests that fecal calprotectin can be used as a diagnostic tool and an indicator of treatment response in CF pulmonary exacerbations(Imanzadeh et al., 2022). Jung et al., 2021 discuss the underdiagnosis of pulmonary exacerbations (PEx) in individuals with cystic fibrosis (CF). The study included 19 subjects with CF, and the diagnostic performance of absolute and fold-change CRP and calprotectin cut-offs to differentiate stable and PEx visits were assessed. The optimal thresholds to identify PEx were determined based on Youden's index. A

step-wise algorithm was able to improve diagnostic performance. The study suggests that CRP and calprotectin could be promising as diagnostic biomarkers for PEx, but further validation studies are required before implementing these diagnostic thresholds (Jung et al., 2021). Recent studies have shown a correlation between bowel and lung disease in CF patients. Parisi et al., 2017 analyzed FC levels in CF patients and correlated them with different disease phenotypes. "A cohort of 54 CF patients and 50 healthy controls were enrolled in the study. FC levels were measured in stool samples using an ELISA kit. The results showed that FC levels were significantly higher in CF patients than healthy controls. FC levels were also significantly higher in CF patients who were older than 18 years, had pancreatic insufficiency, were underweight, had Pseudomonas aeruginosa airways colonization, had CF-related diabetes mellitus, had reduced lung function, or had a high number of pulmonary exacerbations" (Parisi et al., 2017).

Lung cancer:

Lung cancer is a malignant tumor that originates in the tissues of the lungs, primarily caused by smoking and exposure to certain pollutants. It is deadly cancer with the highest mortality rate, primarily due to late-stage diagnoses and limited treatment options. The current lack of reliable and widely available biomarkers for early detection further underscores the urgent need for innovative diagnostic tools to improve survival rates and enhance patient care (Adams et al., 2023). Blanco-Prieto et al., 2015 aimed to identify high-risk individuals among patients attending Pulmonary Services who may have symptoms related to the respiratory system. A non-invasive serum test was conducted to measure six cancer-associated molecules in the blood of lung cancer patients and control subjects (healthy individuals and patients with benign respiratory pathologies). The study found that three of these molecules (EGF, sCD26, and calprotectin) had a high discriminatory capacity and could be used as a marker panel to identify patients at high risk for lung cancer. An algorithm and nomogram were developed to generate classification scores based on the risk of a patient developing lung cancer. The authors suggest that the efficacy of this three-marker panel should be tested in a larger population (Blanco-Prieto et al., 2015). In another study Luo et al., 2015 aim to determine the clinical efficacy and diagnostic accuracy of a combination of two biomarkers, calprotectin, and CXCL12, for predicting malignancy in patients with exudative PE. The levels of calprotectin and CXCL12 were measured in 95 individuals with exudative PE, including 39 malignant PE (MPE) and 56 benign PE (BPE). Receiver-

operating characteristic (ROC) curves were used to evaluate the accuracy of these biomarkers for discriminating MPE from BPE or tuberculous PE. Univariate and multivariate logistic regression analyses were performed to test the association between calprotectin and CXCL12 levels and MPE. The results showed that calprotectin and CXCL12 in the pleural fluid are informative diagnostic biomarkers for predicting patients with MPE (Luo et al., 2015). The difficulty in distinguishing between malignant pleural effusion (MPE) and benign pleural effusion (BPE) and the need for new and effective biomarkers for diagnosis. Botana-Rial et al., 2020 attempted to validate calprotectin as a diagnostic biomarker for pleural effusion (PE) in clinical settings. Calprotectin levels were measured in pleural fluid samples collected from 425 patients, with 223 cases having BPE and 137 having MPE. The results showed that calprotectin levels were significantly higher in BPE than in MPE, with an area under the curve of 0.848. A cut-off value of ≤ 6,233.2 ng/mL had a sensitivity of 96% and specificity of 60%. Multivariate analysis showed that low calprotectin levels were a better discriminator of PE than any other variable. The study confirms that calprotectin is a useful diagnostic biomarker for patients with PE of uncertain cause and could complement cytological methods in clinical practice (Botana-Rial et al., 2020).

COVID 19:

COVID-19 is a highly contagious respiratory disease caused by the coronavirus SARS-CoV-2.. Reliable and accessible biomarkers for COVID-19 detection can aid in early diagnosis, monitoring disease progression, identifying high-risk individuals, and informing effective treatment strategies, ultimately mitigating the impact of the pandemic (Hedberg et al., 2023). The SARS-CoV-2 virus causes COVID-19 by attaching to a specific receptor called ACE2 in human cells. This leads to an immune response that includes the release of various molecules, including cytokines and chemokines, which recruit inflammatory cells to fight the virus. However, this response can also lead to a worsening of the clinical situation if not adequately controlled. Some molecules released during this response include IL-6, IL-1β, and S100A8/A9 (Chapuis et al., 2022).

Cardiero et al., 2022 found that COVID-19 patients who had higher levels of calprotectin and neutrophil count at the time of hospitalization had a higher risk of dying from the disease. The Kaplan-Meier evaluation affirmed the efficacy of calprotectin levels

and neutrophil count in forecasting mortality. Specifically, individuals with calprotectin concentrations exceeding 1.66 mg/L or those with a neutrophil count surpassing 16.39 × 103/μL had a less favorable outcome compared to those with diminished levels. When both factors are considered concurrently, the precision in pinpointing patients with a heightened death risk is enhanced. Notably, a combined influence of both the neutrophil count and calprotectin levels above the specified limit was evident in determining patients who did not survive.

The researchers suggest that these biomarkers could be helpful in predicting mortality in COVID-19 patients (Cardiero et al., 2022). Shi et al., 2021 investigated the potential role of neutrophils in severe cases of COVID-19. The text mentions that elevated levels of blood neutrophils/ Calprotectin S100A8/9 may be associated with worsening oxygenation in COVID-19. However, it is unclear whether neutrophils are causing the inflammation and respiratory failure or if they are simply bystanders. To better understand the potential role of neutrophils in COVID-19, the researchers measured levels of a neutrophil activation marker called S100A8/A9 (calprotectin) in hospitalized patients. They found that patients with COVID-19 had markedly elevated calprotectin levels in their blood and that calprotectin was tracked with other markers of inflammation and disease severity. The researchers also found that calprotectin levels were significantly higher in patients who progressed to severe COVID-19, requiring mechanical ventilation, suggesting that neutrophils may play a role in perpetuating inflammation and respiratory compromise in COVID-19 (Shi et al., 2021). Kassianidis et al., 2022 investigate the levels of various blood molecules associated with inflammation and acute respiratory distress syndrome (ARDS) and how their presence correlates with disease severity. The study analyzed serum levels of various mediators (such as cytokines and interferons) in patients with COVID-19 at different stages of severity (asymptomatic, moderate, severe, and ARDS/MV) to identify associations with critical illness and pathways associated with mortality. The results showed that specific proinflammatory mediators were significantly higher in patients with ARDS and MV, while specific anti-inflammatory mediators also increased. Multivariate ordinal regression analysis identified pathways associated with worse outcomes, including IL-6, IL-33, and calprotectin. Calprotectin levels increased serially among patients who progressed to ARDS and MV (Kassianidis et al., 2022).

Kumar et al., 2022 investigated the potential use of two biomarkers, serum cystatin C and serum calprotectin, as prognostic markers

for COVID-19. Serum cystatin C and serum calprotectin are biomarkers that can indicate the severity and outcome of COVID-19. D-dimer, lactate dehydrogenase (LDH), and C-reactive protein (CRP) are other biomarkers that have been proposed as prognostic markers for COVID-19. The study was an observational cohort study that included 95 COVID-19 patients admitted to a dedicated COVID care facility. The patients' serum cystatin C and serum calprotectin levels were measured at different time points and compared between severe (NEWS 2 score ≥5) and non-severe (NEWS 2 score <5) groups, survivors and deceased, and based on comorbidities. The results showed that median cystatin C levels were significantly higher in the severe group, patients with cardiovascular disease, chronic lung disease, and those who died. Serum calprotectin levels were significantly higher in patients with chronic lung disease and those who died (Kumar et al., 2022).

Asthma:

Asthma is a chronic respiratory condition characterized by inflammation and narrowing of the airways, resulting in recurring episodes of wheezing, coughing, and shortness of breath. Diagnostic biomarkers play a vital role in the effective management of asthma. By identifying specific markers associated with airway inflammation and responsiveness, these biomarkers can aid in early diagnosis, monitoring disease activity, guiding treatment decisions, and improving overall asthma control, thus enhancing the quality of life for individuals living with asthma (Levy et al., 2023). Quoc et al., 2021 discuss the role of S100A9 in the activation of neutrophils and the development of neutrophilic asthma (NA). The study found that levels of S100A9 were higher in the blood of NA patients compared to non-NA patients and that there was a positive correlation between S100A9 levels and the number of neutrophils in sputum samples. The study also found that S100A9 activated airway epithelial cells, stimulated the formation of neutrophil extracellular traps (NETs), and induced macrophages to polarize towards an inflammatory M1 phenotype. The study suggests that S100A9 may be a potential biomarker and therapeutic target for NA (Quoc et al., 2021).

Interstitial lung disease (ILD)

Interstitial lung disease (ILD) refers to a group of lung disorders characterized by inflammation and scarring of the lung tissues. Given

the diverse nature of ILD and the challenges in accurate diagnosis, identifying reliable diagnostic biomarkers is crucial. Developing effective biomarkers can facilitate early detection, enable targeted therapies, and enhance patient management, ultimately improving outcomes for individuals affected by interstitial lung disease.

Caimmi et al., 2019 investigated the relationship between fecal calprotectin (FC) and interstitial lung disease (ILD) in systemic sclerosis (SSc). SSc is an autoimmune disease that affects connective tissues, and ILD is a common complication of SSc that affects the lungs. The study enrolled 129 SSc patients, collected data on their disease characteristics, including lung involvement, and measured their FC levels. The results showed that patients with ILD had higher FC levels than those without ILD. The study also found that several factors, including disease subset, skin score, disease duration, severity scores, steroid treatment, and FC levels, were associated with an increased risk of ILD. Diverticulosis, a condition characterized by small pouches in the colon, was found to be protective against ILD. The study suggests that ILD is independently associated with increased FC levels in SSc (Caimmi et al., 2019). Lin et al., 2022 investigated whether S100A9 and Klebs von den Lungen-6 (KL-6) can be used as biomarkers for the differential diagnosis and disease progression of four common interstitial lung diseases (ILDs): idiopathic pulmonary fibrosis (IPF), hypersensitivity pneumonitis, connective tissue disease-associated ILD, and sarcoidosis (SAR). The study collected data from patients who underwent fiber-optic bronchoscopy and bronchoalveolar lavage (BAL) at a hospital in China between 2012 and 2020. The data included clinical parameters such as pulmonary function tests, levels of S100A9 and KL-6 in BAL fluid and serum, and the percentage of different cell types in BAL fluid. The study found that KL-6 expression in BAL fluid was significantly higher in IPF patients than in the other three groups, while there was no significant difference in S100A9 levels. However, S100A9 levels in BAL fluid were positively correlated with KL-6 expression and the percentage of neutrophils in IPF patients and negatively correlated with lung function parameters and oxygen levels in SAR patients. The study concludes that measuring both KL-6 and S100A9 levels in BAL fluid can be useful for differential diagnosis of the four ILDs (Lin et al., 2022).

DISCUSSION:

Diagnostic biomarkers are critical in various respiratory diseases, including lung cancer, cystic fibrosis, COVID-19, interstitial lung disease, and asthma. These diseases, each with unique characteristics and challenges, necessitate developing and utilizing reliable biomarkers for accurate diagnosis and improved patient outcomes (Jain, 2017). Whether it is detecting lung cancer at an early stage, personalizing treatment strategies for cystic fibrosis, identifying COVID-19 infection for effective control measures, diagnosing and managing interstitial lung disease, or optimizing asthma management, diagnostic biomarkers provide invaluable insights that enable timely intervention, targeted therapies, and enhanced patient care (Baines, Pavord, & Gibson, 2014). The availability of robust and accessible biomarkers is vital in improving disease management, reducing mortality rates, and enhancing the quality of life for individuals affected by these respiratory conditions.

Twelve out of thirteen studies reported significantly higher levels of calprotectin ($p<0.05$) in patients as compared to controls, with only the exception of Lin et al ., 2022 who reported no significant association of calprotectin expression with Interstitial lung disease (Lin et al., 2022). The reason can be due to the low sample size n=98 in four subgroups of patients and conducted at a single center. The results suggest that calprotectin can be used as a molecular biomarker not only for detecting various lung pathologies but also act as a discriminatory factor to evaluate the severity of the disease.

An important consideration is to look at the type of sample needed to evaluate the role of CLP as possible biomarker. Studies have reported higher expression of CLP in fecal, sputum and serum samples (Imanzadeh et al., 2022)(Gray et al., 2010). It is essential to identify a single suitable body fluid to analyze the potential suitability of CLP as a diagnostic biomarker.

Although the reviewed studies suggest higher levels of calprotectin in analyzed patients as compared to controls. But most studies included in the review have small sample sizes and are often conducted at a single center, which limits the generalizability and validity of the results. There is a need for larger independent cohorts to validate the findings and include a more diverse population.

Calprotectin's relevance in the context of COVID-19 is particularly noteworthy. As COVID-19 primarily affects the respiratory system, causing inflammation and damage to the lung tissues, the inflammatory marker calprotectin has been under investigation for

its potential as a diagnostic and prognostic tool. Elevated levels of calprotectin, found in serum samples of COVID-19 patients, suggest a heightened inflammatory response, which is a hallmark of the disease, especially in severe cases. For clinicians, the advantage of utilizing calprotectin as a biomarker for COVID-19 lies in its potential for early detection of severe disease manifestations. Patients with elevated levels of calprotectin could be identified as those at increased risk for complications, allowing for timely therapeutic interventions and closer monitoring. Additionally, by tracking the levels of calprotectin during the course of the disease and treatment, clinicians can gauge the patient's response to therapies and adjust treatment strategies accordingly.

CONCLUSION:

In conclusion, this systematic review identified 13 studies investigating diagnostic biomarkers for various respiratory diseases, including cystic fibrosis, lung cancer, COVID-19, asthma, and interstitial lung disease. The studies highlighted the potential utility of fecal calprotectin in cystic fibrosis for diagnosing pulmonary exacerbations and monitoring treatment response. In lung cancer, serum markers such as the epidermal growth factor (EGF; a protein that plays a crucial role in cell growth, proliferation, and differentiation), sCD26, and calprotectin showed promise in identifying high-risk individuals. In contrast, pleural fluid calprotectin demonstrated diagnostic value in distinguishing between benign and malignant pleural effusion. For COVID-19, calprotectin and neutrophil count were associated with disease severity and mortality, and cytokines like IL-6 and IL-33, along with calprotectin, were linked to worse outcomes. In asthma, S100A9 emerged as a potential biomarker for neutrophilic asthma. These findings emphasize the importance of calprotectin as a diagnostic biomarker in improving early detection, personalized treatment, and overall management of respiratory diseases. Further research and validation studies are necessary to establish its clinical utility.

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