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Association between Myeloperoxidase Enzyme Levels in Parenchymal Lung Disease and Airway Disease in Paediatrics

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ABSTRACT

Background: The most prevalent pro-inflammatory biomarker found in neutrophilic granulocytes is myeloperoxidase enzyme. In order to fight various microbial activities, proinflammatory factors and oxidative stress at the infection site release it from these cells. Several reactive oxygen and nitrogen species are produced as a result of MPO's antimicrobial actions.

Aim and objectives: This study aimed to assess the diagnostic value of myeloperoxidase elevation in parenchymal lung disease and airway disease in children.

Subjects and methods: This cross-sectional investigation was carried out at the Pediatrics Department of the Suez Canal University Hospitals Faculty of Medicine. 99 patients were split into 3 groups: Group A had 33 patients with pneumonia, group B had 33 patients with bronchiolitis and group C had 33 patients with bronchial asthma in addition to 33 healthy cases as control group.

Results: Serum and salivary MPO levels were reliable in the diagnosis of pneumonia, but only serum MPO level can be used to diagnose patients with asthma. However, serum and salivary MPO levels failed to diagnose patients with bronchiolitis. Also, salivary MPO failed to diagnose patients with asthma. Moreover, serum and salivary MPO levels failed to predict outcome among pneumonia, bronchiolitis and asthma patients.

Conclusion: Both serum and salivary myeloperoxidase levels were significantly differed between pneumonia, bronchiolitis, bronchial asthma and control groups, suggesting the potential role of MPO in the diagnosis of parenchymal lung disease and airway disease in pediatrics.

Keywords: Myeloperoxidase enzyme, Parenchymal lung disease, Airway disease, Paediatrics.

INTRODUCTION

The heme-containing peroxidase enzyme myeloperoxidase (MPO) is widely distributed in neutrophils and, to a lesser degree, monocytes. The powerful oxidant hypochlorous acid, which is produced by enzymatically active MPO in combination with hydrogen peroxide and chloride, is a major factor in the oxygen-dependent microbicidal action of phagocytes. Furthermore, tissue damage in a number of disorders, especially those that are marked by acute or chronic inflammation, has been connected to increased MPO-derived oxidant generation [(1)]. Migrating neutrophils may release active MPO during inflammatory responses. MPO levels have been found to be elevated in a variety of lung diseases, including pneumonia, asthma, and bronchiolitis [(2)].

Chronic inflammation, mostly caused by the overabundance and activation of neutrophils and other inflammatory cells, is what defines asthma. Large volumes of MPO are released by activated neutrophils, and this causes the Myeloperoxidase-Hydrogen Peroxide-Chloride system to produce more reactive oxygen species. Because of their direct oxidative damage to epithelial cells and their cell shedding properties, reactive oxygen species (ROS) may cause lung injury. By inducing bronchial hyperreactivity and directly inducing mast cell histamine release and mucus secretion from airway epithelial cells, which in turn causes the preparations of airways to contract, it has been demonstrated that reactive oxygen species (ROS) are linked to the pathophysiology of asthma. This effect is amplified when the epithelium is damaged or removed [(3)].

One common respiratory infection that causes bronchiolitis in infants under two years old is the respiratory syncytial virus (RSV). When bronchial epithelial cells are infected by respiratory viruses, this leads to enhanced neutrophil recruitment and activation as well as epithelial activation. By releasing their own chemokines, superoxide anion, and granular enzymes, primarily MPO, neutrophils can exacerbate inflammation by raising the level of H_2O_2, destroying extracellular matrix structures, and inducing the release of additional inflammatory cytokines (TNF-α, IL-8, and tumor necrosis factor-α) [(4)]. This study aimed to evaluate the diagnostic value of Myeloperoxidase elevation in parenchymal lung disease and airway disease in children.

PATIENTS AND METHODS

This cross sectional research was conducted in the Emergency Department and Inpatient Ward of the Paediatrics Department of Suez Canal University Hospital (Inpatient-PICU). Patients' samples were collected throughout an eight months period, starting from October 2022 to May of 2023.

Clinical cases who presented to the Emergency Department with respiratory distress then diagnosed

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with pneumonia and bronchiolitis or bronchial asthma according to the clinical guidelines of Suez Canal Hospitals Pulmonology Unit by examination and confirmation of diagnosis by chest X-ray for all patients (number = 99). Then another group of 33 normal healthy children who presented to Suez Canal Pediatrics Clinic for follow up to be compared with the above three groups. Patients were split into 3 groups: Group (A) included patients with pneumonia, group (B) included patients with bronchiolitis and group (C) that included patients with bronchial asthma.

Inclusion criteria: Children between 6 months to 12 years (males and females) who presented to Suez Canal University Hospital (ER-inpatient) with signs of respiratory distress (RD) after stabilization of the case.

Exclusion Criteria: Children with congenital heart disease or other congenital anomalies, children with foreign body aspiration, patients with a history of frequent aspiration as gastro-esophageal reflux disease (GERD) and cerebral palsy, other causes of respiratory distress as renal acidosis, children with history of immunodeficiency disorders and patients who refused enrolment in the study.

Sample Size: The sample size was computed using the formula below [(5)]:
Where,

$$n = \left[\frac{Z_{\alpha/2} + Z_{\beta}}{\frac{1}{2}\log\frac{1+r}{1-r}}\right]^2 + 3$$

n= sample size, Z α/2 = 1.96 (The crucial figure that separates the Z Distribution's center 95% from its tail 5%), Zβ =0.84 (The crucial figure that divides the Z distribution's bottom 20% from its upper 80%) and r = correlation between MPO in relation to the patients' lung function=-0.608 [(6)]. The final sample size was 120 patients, with 10% as drop-out rate, total sample equals 132 patients.

All patients were subjected to: Full history taking, full examination (General examination, chest examination and signs of RD) and laboratory and radiological investigation (Sample collection, storage and MPO estimation by ELISA for the serum and saliva samples).

Grading of RD [(7)]: Grade I (mild distress); rapid respiratory rate; nasal faring (Alae nasi), **grade II** (Moderate distress): Grade I plus intercostal and substernal retraction, **grade III** (Severe distress): Expiatory grunting and **grade IV** (Advanced distress): Central cyanosis or disturbed consciousness.

Ethical considerations: Faculty of Medicine's Pediatrics Department, Suez Canal University and Hospital Administration Research Ethics Committee both approved the research. Prior to involving any participant informed written permission was acquired from the Child Guardian. The study was conducted in accordance with Declaration of Helsinki.

Statistical analysis

Version 26 of the Statistical Program for Social Science (SPSS Inc., Chicago, IL, USA) was utilized to analyze the data. The mean and standard deviation were utilized to characterize the quantitative variables. Number and percent were utilized to describe qualitative characteristics. The Student t test was utilized to compare parametric quantitative variables between the two groups. When frequencies were less than five, the Fisher's exact test or the Chi-square (X^2) test was utilized to compare the qualitative variables. Utilizing Spearman rank correlation coefficients, the relationship between two normally distributed data was evaluated. A significance level of $P \leq 0.05$ was considered significant.

RESULTS

Serum MPO level at 41.8 ng/ml level had significant sensitivity of 65.5% and specificity of 67.4% in diagnosing patients with pneumonia, while salivary MPO at 16.6 ng/ml level had significant sensitivity of 75.5% and specificity of 70.5% in diagnosing patients with pneumonia (Table 1).

Table (1): Validity of serum and salivary MPO in diagnosing patients with pneumonia

MPO_ Level(ng/ml)	AUC	Cut-off	Asymptotic Sig.[b]	Sensitivity	Specificity	PPV	NPV
Serum level	.692	41.8	.001	65.5%	67.4%	63.1%	61%
Saliva level	.437	16.6	.280	75.5%	70.5%	60.4%	65.8%

Serum MPO level at 34.3 ng/ml level had insignificant sensitivity of 39.4% and specificity of 53.2% failed to diagnose patients with bronchiolitis. Also, salivary MPO at 13.5 ng/ml level had insignificant sensitivity of 54.5% and specificity of 23.4% failed to diagnose patients with bronchiolitis (Table 2).

Table (2): Validity of serum and salivary MPO in diagnosing patients with bronchiolitis group

MPO_ Level(ng/ml)	AUC	Cut-off	Asymptotic Sig.[b]	Sensitivity	Specificity	PPV	NPV
Serum level	.491	34.3	.883	39.4%	53.2%	45.7%	46.7%
Saliva level	.316	13.5	.002	54.5%	23.4%	41.6%	34.5%

Serum MPO level at 42.6 ng/ml level had significant sensitivity of 71.9% and specificity of 72.9% in diagnosing patients with bronchial asthma, while salivary MPO failed to diagnose patients with bronchial asthma (Table 3).

Table (3): Validity of serum and salivary MPO in diagnosing patients with bronchial asthma

MPO_ Level(ng/ml)	AUC	Cut-off	Asymptotic Sig.[b]	Sensitivity	Specificity	PPV	NPV
Serum level	.791	42.6	.000	71.9%	72.9%	72.6%	72.9%
Saliva level	.333	12.2	.005	62.5%	21.9%	41.5%	36.9%

Serum MPO level failed to predict outcome in the study patients, while salivary MPO at 14 ng/ml level had insignificant sensitivity of 57.1% and specificity of 42.9% in prediction of the outcome among the study patients (Table 4).

Table (4): Validity of salivary and serum MPO level in predicting outcome (death or PICU admission)

MPO_ Level(ng/ml)	AUC	Cut-off	Asymptotic Sig.[b]	Sensitivity	Specificity	PPV	NPV
Serum level	0.457	49.7	0.612	28.6%	34.5%	72.6%	72.9%
Saliva level	0.505	14	0.951	57.1%	42.9%	41.5%	36.9%

Serum level of MPO showed statistical significant difference between study group with the lowest level among control group, while salivary MPO was significantly lower among bronchiolitis group ($p<0.001$). Post hoc test result revealed that variation in salivary MPO level was due to control group, while in other groups it didn`t show statistical significant differences with the lowest mean among bronchiolitis group. While, serum MPO level showed statistical significant difference between pneumonia group and bronchiolitis group, and bronchial asthma group. All groups showed statistical significant difference with control group with the highest mean level among control (Table 5).

Table (5): Comparison of myeloperoxidase level among the studied group

MPO level$_{(ng/ml)}$	Pneumonia group (n=33)	Bronchiolitis group (n=33)	Bronchial asthma group (n=33)	Control group (n=33)	P-value
Serum level$_{(ng/ml)}$ Mean ± SD	44.76±11.7[d]	31.17±1.3[ad]	49.53±9.3[bd]	0.89±0.16	**<0.001*[1]**
Salivary level$_{(ng/ml)}$ Mean ± SD	16.21±3.1[d]	14.1±4.8[d]	15.49±3.9[d]	45.53±8.1[d]	**<0.001*[1]**

1. ANOVA test. 2. Post hoc test. *Statistically significant as $p<0.05$. a. substantial variation with group A c. substantial variation with group B b substantial variation with control group d. substantial variation with group C

Table (6) showe4d that the median age of studied cases was significantly lower among children with bronchiolitis than other groups with statistical significant difference ($p<0.001$). Males showed predominance in pneumonia and control groups, while females were more in bronchial asthma and bronchiolitis group with insignificant differences among the study groups.

Table (6): Socio-demographic data of the studied groups

	Pneumonia group (n=33)	Bronchiolitis group (n=33)	Bronchial asthma group (n=33)	Control group (n=33)	P-value
Age (years)					
Median (IQR)	2 (3)	1 (0.85)	5 (4.5)	7 (3.5)	<0.001*[1]
Gender					
Female	14(42.4%)	20(60.6%)	21(63.6%)	14(42.4%)	0.191[2]
Male	19(57.6%)	13(39.4%)	12(35.4%)	19(57.6%)	
Residence					
Rural	7(21.2%)	6(18.2%)	2(6%)	19(57.57%)	0.231[2]
Urban	26(78.8%)	27(81.8%)	31(94%)	14(42.43%)	

1. Kruskal Wallis test. 2. Chi square test. *Statistically significant as $p<0.05$

The fever was significantly higher among children with pneumonia with statistical significant difference (p=0.019). Secretions were significantly higher among bronchiolitis groups, while dry cough was significantly higher among bronchial asthma group with statistical significant difference (p<0.001). Wheezing was significantly higher among bronchial asthma, while crepitation was significantly higher among bronchiolitis and pneumonia groups with statistical significant difference (p<0.001). While, there was statistically insignificant difference between the study groups in degree of respiratory distress (RD). Children with bronchial asthma had the highest percentage of children with grade (I). While, children with bronchiolitis had the highest percentage of RD grade (II). Children with pneumonia had the highest percentage of children with RD grade (III). Only one children with pneumonia had grade (IV) (Table 7).

Table (7): Clinical picture of the studied groups

	Pneumonia group (n=33)	Bronchiolitis group (n=33)	Bronchial asthma group (n=33)	P-value
Fever	30 (90.9%)	13(39.4%)	13(39.4%)	0.019*[1]
Cough				
Dry cough	9 (27.3%)	5 (15.2%)	25 (75.75%)	
Secretions	24 (72.7%)	28 (84.8%)	8 (24.24%)	<0.001*[2]
Degree of respiratory distress				
I	11 (33.4%)	13 (39.4%)	18 (54.5%)	
II	14 (42.4%)	16 (48.5%)	11 (33.4%)	0.358[2]
III	7 (21.2%)	4 (12.1%)	4 (12.12%)	
IV	1 (3%)	0 (0%)	0(0%)	
Clinical-finding (auscultation)				
Wheezing	3 (9.1%)	7 (21.2%)	24 (72.7%)	<0.001
Crepitation	26 (78.8%)	20 (60.6%)	6 (18.2%)	<0.001
Diminished airway entry	4 (12.1%)	6 (18.2%)	3 (9.1%)	0.538

1. Chi square test. 3. Fisher exact test. 2.*Statistically significant as p<0.05

According to radiological findings bronchial asthma group had the highest percentage of patients with normal chest radiograph. Pneumonia group showed pneumonic patches, while bronchiolitis group showed increased normal and bronchovascular markings with bronchopneumonia with statistical significant variation (p<0.001) (Table (8).

Table (8): Radiographic findings of the studied groups

	Pneumonia group (n=33)	**Bronchiolitis group (n=33)**	**Bronchial asthma group (n=33)**	**P-value**
Normal	0 (0%)	20 (60.6%)	23 (69.7%)	
Bronchopneumonia	6 (18.2%)	0		
Lobar bronchopneumonia	3 (9.1%)	0		
Increased bronchovascular markings	0	5 (15.2%)	3 (9%)	
Chest Hyperinflation + Bronchopneumonia	0	0	1 (3%)	
Increased bronchovascular markings with bronchopneumonia	0	8 (24.2%)	4 (12.12%)	**<0.001*[1]**
Pneumonic patches	24 (72.3%)	0	0	
Bronchovascular marking + lobar pneumonia	0	0	2 (6%)	

1 -Fisher exact test used. *Statistically significant as p<0.05

Hospital stay showed insignificant difference between groups. Most of patients in pneumonia group and bronchiolitis group had prolonged hospital stay. 24.2% of pneumonia group used mask. 15.1% in both pneumonia and bronchial asthma groups used CPAP. 9% of bronchial asthma group used ventilator. Most of patients in all groups used nasal O_2 support with statistical significant difference (p<0.001). All groups used nebulizer. Regarding outcome, two patients died and 4 patients were admitted to PICU in pneumonia group, two patients died and one patient was admitted to PICU in bronchial asthma group. In bronchiolitis group 6 patients were admitted to PICU with no death with statistical significant variation between study groups (p<0.001) (Table 9).

Table (9): Prognostic data of the studied groups

	Pneumonia group (n=33)	Bronchiolitis group (n=33)	Bronchial asthma group (n=33)	P-value
Hospital stay (days), Median (IQR)	7(5)	7(5)	6.5(5)	0.819[1]
Average time <5 days	10(30.3%)	13(39.4%)	17(51.5%)	
Prolonged time > 5days	23(69.7%)	20(60.6%)	16(48.5%)	
O_2 support				
No	**0**	**0**	**0**	**<0.001*[1]**
Nasal	**18(54.5%)**	**26(78.8%)**	**24 (72.7%)**	
Mask	**8(24.2%)**	**5(15.1%)**	**4 (12%)**	
CPAP	**5(15.1%)**	**2(6%)**	**5(15.1%)**	
Ventilator	**2(6%)**	**0**	**3 (9%)**	
Nebulizer use	**33(100%)**	**33(100%)**	**33(100%)**	**1.00[1]**
Outcome				
Death	**2(6%)**	**0(0%)**	**2(6.1%)**	**<0.001*[1]**
PICU	**4(12.1%)**	**6(18.2%)**	**1(3%)**	
Discharge	**27(81.9%)**	**27(81.8%)**	**30(90.9%)**	

1. Kruskall Wallis test. 2. Fisher exact test. *Statistically significant as p<0.05.

CPAP; continuous positive airway pressure, PICU; pediatric intensive care unit.

Severity of cases distribution among the study groups according to O_2 support, fever, cyanosis, chest findings, leukocytosis, hsCRP level, outcome, lymphocyte and hospital stay. According to fever, cyanosis, leucocytosis and hsCRP, most of patients were high in pneumonia group. According to O_2 support, most of patients who needed severe support were in bronchial asthma. According to hospital stay, most of patients who showed prolonged stay were in pneumonia group. According to outcome, most of patients discharged were in bronchial asthma group. Two patients died and 4 patients were admitted to PICU in Pneumonia group. Two patients died and one patient was admitted to PICU in bronchial asthma group. In bronchiolitis group, 6 patients were admitted to PICU with no death. In pneumonia group, most of patients had diagnostic chest finding, while most patients of bronchial asthma and bronchiolitis groups showed normal chest findings (Table 10).

Table (10): Classification of data according to severity of the studied variables among the studied groups

	Pneumonia group (n=33)	**Bronchiolitis group (n=33)**	**Bronchial asthma group (n=33)**	**P-value**
O_2 support				
Mild	26(78.7%)	31(93.9%)	28 (84.8)	<0.001*[1]
Moderate	5(15.1%)	2(6%)	5(15.1%)	
Severe	2(6%)	0	3 (9%)	
Fever (>39 ^{0}C)	19(57.6%)	17(51.5%)	12(36.4%)	0.034*[1]
Cyanosis	6(18.2%)	4(12.1%)	3(9.1%)	0.069[1]
Chest x ray findings	33(100%)	13(39.3%)	10(30.3%)	<0.001*[1]
Leucocytosis (n, %)	18(54.5%)	8(24.2%)	3(9%)	0.009*[2]
hsCRP (mg/L) >5	27(81.8%)	16(48.5%)	19(59.4%)	0.015*[2]
Lymphocyte (%), Median (IQR)	21(13)	56(13)	52.5(12)	<0.001*[1]
Hospital stay				
Average time (<5 days)	10(30.3%)	13(39.4%)	17(51.5%)	0.055[1]
Prolonged time (>5 days)	23(69.7%)	20(60.6%)	16(48.5%)	
Outcome				0.001*[1]
Death	2(6%)	0(0%)	2(6.1%)	
PICU	4(12.1%)	6(18.2%)	1(3%)	
Discharge	27(81.9%)	27(81.8%)	30(90.9%)	

1. Fisher exact test used. *Statistically significant as p<0.05

DISCUSSION

Myeloperoxidase (MPO) is a member of subfamily of peroxidases. The most prevalent pro-inflammatory indicator found in neutrophilic granulocytes is myeloperoxidase enzyme. Active MPO may be released during inflammatory reactions by migrating neutrophils. MPO levels have been found to be elevated in a variety of lung diseases, including pneumonia, asthma, and bronchiolitis [(1)].

Asthma is characterized by chronic inflammation, which is characterized by an influx and activation of inflammatory cells mainly neutrophils. Activated neutrophils release large amount of MPO, which act to release increased amounts of reactive oxygen species by the Myeloperoxidase-Hydrogen Peroxide-Chloride system [(2)]. Mycoplasma pneumoniae (M. pneumoniae) is one of the most common causes of pneumonia. Serum MPO levels appeared to be significantly elevated in patients with pneumonia [(3)].

One common respiratory infection that causes bronchiolitis in infants under two years old is the respiratory syncytial virus (RSV). By releasing granular enzymes, superoxide anion, and their own chemokines. Neutrophils may exacerbate inflammation [(4)].

This study's findings indicated that the median age of studied cases was significantly lower among children with bronchiolitis than other groups which is in agreement with literature with statistically significant difference ($p<0.001$).

Since the most common respiratory tract illness in babies is bronchiolitis. It affects over 80% of newborns under 6 months of age, it is also the most common cause of hospitalization for children under 2 years of age [(8)].

Males showed predominance in pneumonia and control groups, while females were more in bronchial asthma and bronchiolitis groups with insignificant differences among the study groups. This comes in agreement with an Egyptian study by **Fadl *et al.*** [(9)] in which they revealed that male gender was independently associated with the incidence of pneumonia among under-five children. The reason for gender variance may be attributed to girls' stronger immune systems than boys'. Additionally, there is evidence that males' early life peripheral airways are smaller, which may make them more susceptible to lower respiratory infections [(10)].

This study's findings indicated that fever was significantly higher among children with pneumonia, which is in agreement with literature with statistically significant difference ($p=0.019$). According to fever, most of patients who had fever were in pneumonia group then bronchiolitis group. Literature showed that a high fever (> 39 °C) was consistent with the children having pneumonia rather than bronchiolitis [(11)].

Regarding radiographic findings, the current study showed that bronchial asthma group had the highest percentage of patients with normal chest radiograph. Pneumonia group showed pneumonic patches, while bronchiolitis group showed increased normal and broncho-vascular markings with bronchopneumonia with statistically significant difference ($p<0.001$). **Chen *et al.*** [(12)] supported the reliability of chest X-ray in differentiating diseases of pneumonia, asthma, and bronchiolitis in children [(12)]. Moreover, **Lemine *et al.*** [(13)] showed that in children, lung ultrasonography is a more sensitive and reliable way to identify probable pneumonia cases than a chest X-ray. LUS is a very specific and sensitive diagnostic technique for children who have pneumonia that they got from the community (CAP) [(13)].

The results of the present research indicated that although hospital stay did not substantially vary across the analyzed groups, the kind of oxygen treatment and the outcome did. The results suggested that hospital stay, need for oxygen support and outcome were related to disease severity rather than the disease type. Moreover, according to O_2 support, most of patients who needed severe support were in bronchial asthma group.

In the current study serum MPO showed significantly lower levels among control group compared to patients' groups, however, salivary MPO showed significantly higher levels among control group compared to patients' groups ($p<0.001$).

Post hoc test result showed that difference in salivary MPO level was higher in control group, while in other groups it didn`t show statistically significant differences with the lowest mean among bronchiolitis group. While serum MPO level showed statistically substantial variation between pneumonia group and bronchiolitis group and bronchiolitis group and bronchial asthma group. All groups showed statistically substantial variation with control group with the highest mean level among bronchial asthma group. This is in contrast with **Kim *et al.*** [(14)] who revealed that children with asthma had a substantially greater sputum MPO concentration than the control group ($p=0.032$). They also came to the conclusion that a major factor influencing the severity of juvenile asthma is airway neutrophilic inflammation. According to an adult investigation by **Abdullah *et al.*** [(15)], individuals with asthma had noticeably higher blood MPO levels than healthy controls, which is consistent with the present findings. Compared to the control group, asthmatics had greater serum MPO levels, which may indicate a latent function for neutrophils in asthma. The greater MPO levels did not, however, substantially correspond with the asthma control levels. Another, adult study by **Hassan *et al.*** [(16)] showed that MPO levels were significantly greater in the bronchial asthma patients than controls. The same results were reported by **Aldhalmi *et al.*** [(17)]

Regarding the validity of MPO in discriminating patients with pneumonia, ROC curve analysis showed that serum level of MPO > 41.8 can be used as a marker with sensitivity of 65.5% and specificity of 67.4% in diagnosing patients with pneumonia. While, salivary

MPO at 16.6 ng/ml level had significant sensitivity of 75.5% and specificity of 70.5% in diagnosing patients with pneumonia. Moreover, serum MPO level at 42.6 ng/ml level had significant sensitivity of 71.9% and specificity of 72.9% in diagnosing patients with bronchial asthma. However, serum and salivary MPO levels failed to diagnose patients with bronchiolitis. Also, salivary MPO failed to diagnose patients with bronchial asthma.

CONCLUSION

This study showed that both serum and salivary myeloperoxidase levels differed significantly between pneumonia, bronchiolitis, bronchial asthma and control groups, suggesting the potential role of MPO in the diagnosis of parenchymal lung disease and airway disease in pediatrics.

DECLARATIONS

- **Consent for publication:** All authors agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** No conflicts of interest.

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