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# Differential laboratory findings of common respiratory viruses in hospitalized children: a retrospective study

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## ABSTRACT

**Objective:** The objective of this study was to comparatively investigate the effects of five common viral agents causing respiratory infections—influenza virus (IFV), human respiratory syncytial virus (hRSV), human rhinovirus (hRV), human metapneumovirus (hMPV), and human bocavirus (hBoV)—on laboratory parameters and clinical outcomes in children without underlying chronic diseases.

**Material and Methods:** A total of 983 children aged one month to eighteen years who presented to Ankara Bilkent City Hospital between January 2020 and December 2024 were retrospectively evaluated. Only children hospitalized with a single detected viral agent were included in the study. Clinical data and laboratory parameters were analyzed based on the identified viral pathogens.

**Results:** Among the 983 patients included, 62% were male. The most commonly detected viral agent was IFV, followed by hBoV, hRSV, hRV, and hMPV. In IFV infections, elevated levels of AST and ALT were observed. Significant elevations in partial pressure of $PCO_2$ and $HCO_3$ were detected in hRSV infections. In hBoV infections, inflammatory markers such as CRP, WBC, and NLR reached the highest levels. Furthermore, decreases in pH and increases in $PCO_2$ were significantly associated with intubation and intensive care admissions.

**Conclusion:** The distinct biomarker profiles exhibited by different viral agents may aid in guiding the clinical decision-making process. In particular, early assessment of biomarkers such as LDH, pH, $PCO_2$, and CRP at the time of hospital admission can be valuable for predicting disease severity and determining the need for intensive care in the clinical management of pediatric viral infections.

**Keywords:** Human bocavirus, Human metapneumovirus, Human respiratory syncytial virus, Human rhinovirus, Influenza virus, Lower respiratory tract infections

## INTRODUCTION

Lower respiratory tract infections (LRTIs) are among the most common causes of hospital admissions and prolonged stays during childhood. Viral pathogens, particularly in children under the age of five, are the primary etiological agents of LRTIs. The clinical manifestations of these infections can vary significantly, ranging from mild symptoms to severe illness requiring intensive care (1). In recent years, the identification of viral agents has been greatly enhanced by the widespread adoption of diagnostic techniques such as multiplex real-time PCR (mPCR), enabling a more comprehensive evaluation of the clinical and laboratory profiles associated with viral infections (2).

Common blood tests play a critical role among the parameters frequently utilized in the evaluation of infectious diseases. Hematologic markers such as white blood cells (WBC), lymphocytes (LYM), platelets (PLT), and eosinophils (EO) are widely used laboratory indicators in the clinical assessment of infections (3,4). C-reactive protein (CRP), an acute-phase reactant, can rise rapidly in response to acute inflammatory processes. Although it is well known to increase markedly in bacterial infections, elevated levels may also be observed in viral infections and can serve as an indicator of disease severity (5,6). Blood gas parameters—including pH, partial pressure of oxygen ($PO_2$), partial pressure of carbon dioxide ($PCO_2$), bicarbonate ($HCO_3$), and lactate—are essential in the assessment of respiratory failure, particularly in LRTIs. These parameters are also commonly used to evaluate and monitor the morbidity and potential mortality associated with the disease (7).

This study aimed to investigate the changes in laboratory parameters and their relationship with clinical outcomes in infections caused by common respiratory viruses—such as influenza virus (IFV), human respiratory syncytial virus (hRSV),



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human rhinovirus (hRV), human metapneumovirus (hMPV), and human bocavirus (hBoV)—in children without underlying chronic diseases.

## MATERIALS and METHODS

### Study Group and Study Design

This study was conducted on 983 children who presented to and were hospitalized at the Pediatric Clinics of Ankara Bilkent City Hospital Children's Hospital. The study population included children aged between one month and 18 years, admitted between January 2020 and December 2024, who underwent multiplex real-time PCR (mPCR) testing on nasopharyngeal swab samples and had no history of chronic disease. Children who presented to the hospital solely with respiratory infection symptoms, had an mPCR sample taken at admission, and had no comorbid conditions were included in the evaluation. Patients who tested positive for IFV, hBoV, hMPV, hRV, or hRSV via mPCR were retrospectively analyzed. Exclusion criteria included a history of chronic disease, improperly collected samples, detection of multiple viral agents on mPCR testing, hospitalization for reasons unrelated to respiratory distress, or detection of non-respiratory pathogens. Laboratory parameters are examined only when clinically indicated. In the study group, the laboratory parameters of patients who underwent these tests were evaluated.

### Data Collection

In this study, data were retrospectively obtained from the electronic health records system of Ankara Bilkent City Hospital. The analyzed variables included patient age, gender, initial admission location [Pediatric Intensive Care Unit (PICU) or non-intensive care unit (non-PICU)], type of respiratory support provided at admission [invasive mechanical ventilation (IMV), non-invasive ventilation (NIV), high-flow nasal cannula (HFNC), or mask oxygen therapy], and laboratory parameters. The evaluated laboratory parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), pH, partial pressure of carbon dioxide ($PCO_2$), partial pressure of oxygen ($PO_2$), bicarbonate ($HCO_3$), lactate (LAC), C-reactive protein (CRP), white blood cell count (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), eosinophils (EO), platelets (PLT), large unstained cells (LUC), delta neutrophil index (DNI), and neutrophil-to-lymphocyte ratio (NLR).

### Multiplex RT-PCR Analysis

Respiratory viruses were identified using the multiplex real-time PCR assay (Rotor-Gene Q, QIAGEN, Germantown, Maryland, United States). This technique facilitates the detection of various pathogens, including IFV, hRSV, hCoV (Corana 229E, OC43, NL63, HKU1, SARS-COV2), hPIV, hMPV, hRV, EV, hBoV, hAdV, and human parechovirus. Additionally, bacterial pathogens including *Mycoplasma pneumoniae*, *Bordetella pertussis, Chlamydophila pneumoniae, Haemophilus influenzae,* and *Streptococcus pneumoniae* were also detected.

### Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 23.0 (Chicago, Illinois, USA) was implemented for the statistical analysis. The Kolmogorov-Smirnov test and the examination of histograms were used to evaluate the compliance of numerical and continuous variables with normal distribution. Numerical data with a normal distribution were expressed as the mean and standard deviation, while data with a non-normal distribution were expressed as the median and interquartile range (IQR). Percentages (%) and numbers (n) were used to express categorical variables. In contrast, the Mann-Whitney U test was used to compare continuous variables that did not meet the normal distribution. The Kruskal-Wallis test evaluated continuous variables from many groups that did not fit into the normal distribution. Categorical variables were analyzed with the Pearson chi-square or Fisher's Exact Test. When comparing more than one group, p values were calculated using the Bonferroni correction. Binary logistic regression analysis was performed to evaluate the association between age and the likelihood of PICU admission. The significance level was established at $p<0.050$.

## RESULTS

This study was conducted on a total of 983 pediatric patients, with males comprising 62% (n=609) of the participants. The distribution of detected viral agents was as follows: IFV accounted for 31.7% (n=317), hRSV 22.5% (n=221), hRV 11.5% (n=113), hMPV 10.0% (n=98), and hBoV 24.3% (n=239). The median age across the study group was established as three (1–5) years. The median ages according to viral agents were as follows: five (2–9) years for IFV, two (1–3) years for hRSV, one (0.4–4) year for hRV, three (2–4) years for hMPV, and 2.5 (1–4) years for hBoV. A statistically significant difference in age was found among the viral agents ($p<0.001$), with IFV infections occurring in older age groups compared to other viruses ($p<0.001$), while hRV and hRSV infections were more frequently observed in younger children ($p<0.001$).

IFV infection was associated with a significant increase in AST and ALT levels compared to hRSV, hRV, hMPV, and hBoV infections ($p<0.001$). hBoV infections were characterized by higher CRP levels compared to IFV infections ($p<0.001$). Significant increases in $PCO_2$ and $HCO_3$ levels were observed in hRSV infections, and these parameters were significantly higher compared to hRV, hMPV, and hBoV infections ($p<0.001$). hRV infections were associated with noticeable increases in WBC, EO, and PLT levels ($p<0.001$). hMPV infections were particularly noted for increases in LDH and CRP levels ($p<0.001$). Among

**Table I: Laboratory biomarkers by viral respiratory pathogens in pediatric patients**

| Tests | IFV* | hRSV* | hRV* | hMPV* | hBoV* | Total* | p† |
|---|---|---|---|---|---|---|---|
| AST (U/L)* | 302; 43 (32.7-66) | 212; 37 (30-47.7) | 109; 34 (28-43) | 86; 38 (31.5-51) | 227; 33 (28-42) | 936; 37 (30-49) | <0.001 |
| ALT (U/L) | 302; 23 (17-36) | 212; 23 (17-29) | 109; 21 (16-27) | 87; 20 (15-28) | 231; 19 (15-25) | 941; 21 (16-30) | <0.001 |
| LDH (U/L) | 282; 329 (282.7-386.3) | 185; 330 (287.5-371.5) | 104; 316 (278.5-376.5) | 80; 363.5 (304-404) | 202; 337.5 (300.7-383) | 853; 333 (291-383) | 0.026 |
| pH | 194; 7.43 (7.40-7.47) | 176; 7.40 (7.36-7.44) | 82; 7.40 (7.35-7.43) | 68; 7.42 (7.39-7.44) | 193; 7.41 (7.37-7.46) | 713; 7.42 (7.37-7.45) | <0.001 |
| $PCO_2$ (mmHg) | 194; 31.9 (28.3-35.9) | 176; 36.1 (31.6-41.8) | 82; 33.8 (29.3-39) | 68; 33.8 (29.1-39.3) | 191; 30.7 (27.6-36) | 712; 33.3 (28.9-38.1) | <0.001 |
| $PO_2$ (mmHg) | 194; 44.7 (37.2-58.3) | 176; 47.2 (36.3-59.9) | 81; 48.2 (36.1-61.3) | 67; 43.8 (37.9-59.8) | 194; 49.2 (38.4-64.5) | 712; 47.1(37.4-60.8) | 0.610 |
| $HCO_3$ (mEq/L) | 193; 21.3 (19.3-22.9) | 176; 22.3 (20.1-25) | 82; 20.8[d] (18.2-22.9) | 68; 21.9 (19.1-25.1) | 191; 19.9[d] (17.8-21.9) | 710; 21.2 (18.9-23.3) | <0.001 |
| LAC (mmol/L) | 193; 1.7 (1.2-2.6) | 177; 2 (1.4-2.9) | 80; 2 (1.7-2.5) | 67; 1.9 (1.5-2.6) | 191; 1.8 (1.3-2.4) | 708; 1.9 (1.4–2.6) | 0.008 |
| CRP (mg/L) | 305; 9.1 (3.3-20.4) | 212; 4 (1-20) | 113; 4 (2-8) | 85; 10 (1-40) | 224; 11.3 (4-30.7) | 939; 8.5 (1.9–20.8) | <0.001 |
| WBC x$10^9$/L | 298; 7.04 (4.71-9.74) | 212; 8.85 (6.82-11.49) | 112; 11.08 (8.04-14.85) | 88; 9.99 (6.60-12.99) | 229; 10.69 (8.57-13.29) | 939; 9.10 (6.33–12.34) | <0.001 |
| ANC x$10^9$/L | 298; 3.75 (2.01-6.37) | 212; 3.24 (1.90-4.99) | 112; 5.73 (2.92-9.45) | 88; 4.38 (2.78-6.70) | 229; 6.59 (4.34-9.78) | 939; 4.44 (2.38–7.25) | <0.001 |
| ALC x$10^9$/L | 297; 1.67 (1.04-2820) | 212; 4.19 (2.92-5.48) | 112; 3.21 (1.76-5.06) | 88; 3.79 (2.11-5.63) | 229; 2.51 (1.55-3.94) | 938; 2.66 (1.50–4.42) | <0.001 |
| EO (/µL) | 298; 30 (10-70) | 212; 90 (30-230) | 112; 110 (40-280) | 88; 55 (12.5-177.5) | 228; 100 (40-257.5) | 938; 60 (20–180) | <0.001 |
| PLT (/µL) | 298; 266 (201-350) | 212; 427 (334-504) | 112; 430 (332-544) | 88; 366 (294-507) | 229; 355 (285-450) | 939; 347 (264–465) | <0.001 |
| LUC (/µL) | 298; 160 (100-270) | 212; 350 (230-477.5) | 112; 245 (142.5-380) | 87; 330 (210-460) | 228; 150 (40-280) | 937; 210 (90–360) | <0.001 |
| DNI | 247; 0.1 (0.1-0.5) | 212; 0.1 (0.1-0.1) | 112; 0.1 (0.1-0.1) | 87; 0.1 (0.1-0.1) | 222; 0.1 (0.1-0.1) | 880; 0.1 (0.1-0.1) | 0.161 |
| NLR | 298; 2.2 (0.9-4.3) | 177; 0.7 (0.3-1.4) | 112; 1.5 (0.6-1.5) | 55; 1 (0.4-2.2) | 217; 2.8 (1.2-5.4) | 859; 1.7 (0.7–4.1) | <0.001 |

***:** *n; median (min-max),* **†:** *Kruskal-Wallis test,* ***AST:*** *Aspartate Aminotransferase,* ***ALT:*** *Alanine Aminotransferase,* ***LDH:*** *Lactate Dehydrogenase,* ***CRP:*** *C-Reaktif Protein* ***WBC:*** *White Blood Cell,* ***ANC:*** *Absolute Neutrophil,* ***ALC:*** *Absolute Lymphocyte,* ***EO:*** *Eosinophil,* ***PLT:*** *Platelet,* ***LUC:*** *Large Unstained Cells,* ***DNI:*** *Delta Neutrophil Index,* ***NLR:*** *Neutrophil-to-Lymphocyte Ratio,* ***LAC:*** *Lactate*

all viral agents, hBoV infections showed the highest levels of CRP and WBC (p<0.001) (Table I).

A total of 84.5% (n=831) of the patients were admitted to non-PICU units, while 15.5% (n=152) were admitted to the PICU. The most common viral agent associated with intensive care admission was hRSV, followed by hMPV, hBoV, hRV, and IFV (p<0.001). When laboratory data were compared in relation to intensive care admissions, a decrease in pH levels and increases in $PCO_2$ and $HCO_3$ levels were significantly associated with the need for intensive care (p<0.001 for each). Additionally, elevated LDH levels (p=0.013) and higher LUC values (p=0.012) were also significantly associated with PICU admission. Higher rates of intensive care admission were observed in younger age groups, with the frequency decreasing as age increased. Each one-year increase in age was associated with a 14% decrease in the likelihood of PICU admission (Exp(B)=0.853; 95% CI: 0.794–0.915; p<0.001).

During the treatment process, 2.6% (n=26) of the patients received IMV, 14.2% (n=140) received NIV, 9.9% (n=97) received HFNC, and 73.2% (n=720) received oxygen support via mask. Among all biomarkers, pH and partial pressure of $PCO_2$ were the parameters that showed the most consistent and significant differences across the respiratory support groups. In patients who underwent IMV, pH levels were significantly lower compared to the NIV (p=0.003), HFNC (p<0.001), and mask (p<0.001) groups. Similarly, $PCO_2$ levels in the IMV group were significantly higher compared to the NIV (p=0.004), HFNC (p<0.001), and mask (p<0.001) groups. Although significant differences were observed in LDH, $HCO_3$, and LUC levels—particularly between the NIV and mask groups—these differences were not consistent across all groups. Parameters such as CRP, ANC, and PLT did not show statistically significant differences between the groups. Furthermore, no significant difference was found between patients treated with HFNC and those treated with a mask in terms of the biomarkers analyzed.

## DISCUSSION

Viral agents causing LRTIs may result in clinical presentations ranging from mild symptoms to severe morbidity and mortality. In this study, laboratory parameters associated with hospital admissions due to common viral agents were evaluated in a large cohort of children without underlying chronic diseases. Furthermore, differences in laboratory findings among various viral agents and their potential effects on the clinical course were investigated. The findings of this study contribute to the identification of virus-specific biomarkers and support improved risk stratification in the management of pediatric patients.

The literature indicates that viral agents are most commonly detected in children under the age of four (8–11). The median age findings in our study are consistent with these reports. It was found that the median age of children with IFV infection was higher compared to those with other viral agents, in line with previous studies (12,13). The median ages associated with other viral agents also showed similarities to previously reported data (2). The findings suggest that careful clinical evaluation is essential for children under the age of three in cases of hRV, hRSV, hMPV, and hBoV, and for older children in cases of IFV. In particular, when assessing hospitalized children without underlying conditions, clinicians should consider that viral agents may play a significant role in hospital admissions among those under five years of age. Therefore, special attention should be given during the initial clinical assessment of patients within this age group.

Increases in ALT and AST levels are important biomarkers indicating potential organ failure, while LDH reflects tissue damage and the presence of hypoxia (14). In the literature, some studies have reported elevated liver enzyme levels in 19.4% of patients, with the highest frequencies observed in infections caused by hRSV (50%) and IFV (35.8%) (15). Another study found that the highest prevalence of elevated ALT and AST levels occurred in IFV cases (33.7%), followed by hRSV infections (30.9%) (16). Consistent with these findings, the present study also observed the most frequent elevations of ALT and AST in IFV infections. LDH has been identified as a significant prognostic marker in infectious diseases such as COVID-19 and RSV in various studies (17,18). In adult patients, elevated LDH levels in pneumonia have been directly associated with increased mortality (19). In pediatric cases, LDH levels were found to be significantly higher in intensive care admissions related to COVID-19 (20). In a study involving infants, LDH levels were reported to be higher in human metapneumovirus (hMPV) infections compared to hRSV infections (21). In the present study, we observed the most notable LDH elevations in hMPV infections, followed by human bocavirus (hBoV). Although elevations in ALT and AST were not significantly associated with intensive care admissions, LDH levels, consistent with previous reports, showed a significant association. Therefore, assessing ALT and AST levels upon admission for evaluating the risk of organ failure, and monitoring LDH levels as a predictor of tissue damage and the potential need for intensive care, is crucial in clinical management. Patients with elevated biomarker levels, particularly LDH, should be closely monitored for morbidity and mortality.

In the literature, hRSV has been reported as the viral agent most frequently associated with intensive care admissions and the need for intubation (22,23). Consistently, in the present study, hRSV infections constituted the highest-risk group. Analysis of blood gas parameters revealed significant increases in partial pressure of $PCO_2$ and $HCO_3$ levels in hRSV infections. The bronchiolitis commonly observed in hRSV cases may reduce alveolar ventilation, leading to the development of hypercapnia. This condition is often accompanied by a metabolic compensation, which results in elevated bicarbonate levels. Additionally, the lowest pH values were observed in patients requiring intubation—particularly those with hRSV infections—rather than in patients with other viral agents. A decrease in pH and an increase in $PCO_2$ are considered important biomarkers for predicting respiratory failure (24,25). In the current study, these parameters showed the most pronounced differences in the intubation group relative to other patient groups. Therefore, rapid and careful assessment of pH and $PCO_2$ levels is critical in making decisions regarding intensive care admission or intubation, and may significantly enhance clinical management.

The literature indicates that CRP levels can serve as an important early biomarker for sepsis and mortality. In particular, CRP levels at the time of hospital admission are considered a key indicator for evaluating disease severity. Several studies have demonstrated statistically significant associations between CRP and other inflammatory markers with mortality (26,27). In the present study, the highest CRP levels were observed in infections caused by human bocavirus (hBoV) and human metapneumovirus (hMPV). Notably, the pronounced elevation

of CRP in hBoV infections suggests that this virus may trigger a systemic inflammatory response. Supporting this finding, previous reports have stated that hBoV infections may present with elevated CRP levels and thus mimic bacterial infections (28). Therefore, in cases with high CRP levels at admission, the exclusion of viral etiologies should be carefully considered prior to initiating antibiotic therapy, as part of the clinical decision-making process.

White blood cell (WBC) count is one of the most commonly used laboratory parameters in the evaluation of childhood infections. In the present study, significant increases in WBC, EO, and PLT levels were observed in infections caused by hRV and hBoV. The eosinophilia observed in hRV infections may reflect not only viral inflammation but also suggest that these infections occur more frequently or more severely in children with a history of atopy or asthma (29). In contrast, lower EO levels in hRSV and IFV infections support this distinction. Furthermore, previous studies have indicated that hRV may lead to a more severe clinical course in children with underlying allergic conditions (30). The increase in platelet count is generally interpreted as reactive thrombocytosis and is recognized as a marker of the systemic inflammatory response to infection (31,32). In our study, the highest platelet levels were observed in hRV and hRSV infections, suggesting that both viruses may elicit a strong inflammatory response. Lymphocyte subgroups, such as ANC and ALC, can provide valuable insights into the nature and progression of an infection. The finding that the highest ANC levels were observed in hBoV and hRV infections suggests that these viruses may be associated with neutrophil-dominant inflammation. Conversely, the highest ALC levels were detected in hRSV infections, indicating a predominantly lymphocytic response. In this context, evaluating both ANC and ALC levels in combination may serve as a useful clinical tool for distinguishing viral from bacterial infections.

The neutrophil-to-lymphocyte ratio (NLR) is commonly used as an indicator of systemic inflammation in adult populations (33). In pediatric cases, several studies have reported elevated NLR levels during severe viral infections (34,35). In the present study, the highest NLR levels were observed in infections caused by hBoV, suggesting that hBoV may be associated with a more aggressive inflammatory response. However, viral infections can present with varying hematological profiles, including not only neutrophilia and lymphopenia but also neutropenia accompanied by lymphopenia. These variations indicate that NLR may not consistently serve as a reliable standalone marker for assessing the severity of viral infections in children.

Some studies have reported that decreased levels of large unstained cells (LUC) are significantly associated with intensive care admissions, suggesting that an inadequate production of activated lymphocytes and a compromised immune response may contribute to severe clinical outcomes (34). Conversely, the present study found that increased LUC levels were significantly associated with both intubation and intensive care admissions. LUC represents immature cell populations and typically rises during viral infections. This elevation is particularly notable in cases where the immune system is strongly stimulated and may serve as a useful biomarker in clinical presentations resembling sepsis (36,37). Although the prognostic use of LUC levels in pediatric patients remains limited, it is considered to have potential for further investigation in future research.

While this study provides important findings, several limitations should be acknowledged. First, its single-center and retrospective design restricts the generalizability of the results to broader populations. Nevertheless, the inclusion of a large pediatric cohort and the exclusion of children with chronic diseases are notable strengths that enhance the study's internal validity. In addition, the study did not assess the impact of viral load, as detected by multiplex real-time PCR (mPCR), on clinical outcomes. This omission may limit the clinical interpretation of certain laboratory parameters. The absence of sputum cultures also complicates the exclusion of secondary bacterial infections. However, the mPCR assay covers several common pneumonia pathogens, and any patients in whom these pathogens were identified were excluded from the study.

This study demonstrates that common viral respiratory infections in pediatric patients are associated with distinct biomarker profiles. Notably, elevated AST and ALT levels in IFV infections, as well as the significant association of increased partial pressure of $PCO_2$ and $HCO_3$ levels in hRSV infections with the need for intensive care, are particularly striking. Human bocavirus (hBoV) infections exhibited the highest values in inflammatory markers such as CRP, WBC, and NLR, suggesting a more pronounced systemic inflammatory response. The findings indicate that certain biomarkers measured at the time of admission—such as pH, $PCO_2$, LDH, and CRP—may serve as valuable tools in predicting disease severity. Therefore, early evaluation of these biomarkers can significantly contribute to risk stratification and decision-making regarding intensive care needs in the clinical management of pediatric viral infections.

## Ethics Committee Approval

This study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethics Committee of Ankara Bilkent City Hospital (10.07.2024/TABED 2-24-299).

## Contribution of the Authors

***Kalaycı F :*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***Çelebier K:*** Taking responsibility in patient follow-up, collection of relevant biological materials,

data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Reviewing the article before submission scientifically besides spelling and grammar.


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### Conflict of interest

The authors declare that there is no conflict of interest.

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