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RESEARCH ARTICLE

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Low-dose heparin sodium as a protective factor against bronchiolitis obliterans formation after adenovirus infection

Li Peng[a], Lili Zhong[a], Rong Hu[b], Lei Cui[c], Silan Liu[a], Han Huang[a], Xiaofang Ding[a], Min Chen[a] and Lin Lin[a]

[a]Hunan Provincial Key Laboratory of Pediatric Respirology, Pediatric Medical Center, Hunan Provincial People's Hospital (the First Affiliated Hospital of Hunan Normal University), Changsha, P. R. China; [b]Department of Pediatrics, Changde First People's Hospital, Changde, P. R. China; [c]Department of Pediatrics, People's Hospital of Xiangxi Tujia-Miao Autonomous Prefecture, Jishou, P. R. China

ABSTRACT

Background: Adenovirus (ADV) pneumonia in children is a significant contributor to the occurrence of post-infectious bronchiolitis obliterans (BO). Heparin sodium has known anti-inflammatory, immunomodulatory, and tissue repair properties. However, its role in treating BO after ADV infection remains unclear.

Methods: A retrospective analysis was conducted on 793 children diagnosed with ADV pneumonia and hospitalized in the southern region from January 2019 to December 2019. Among them, 307 cases were classified as single ADV pneumonia. We utilized directed acyclic graphs to analyze the causal relationships between various variables, which further helped us identify the independent and confounding variables for constructing our regression model. Propensity score matching (PSM) was also employed to control for confounding variables that could not be intervened in this study, ensuring baseline level equilibrium and correction. We utilized univariate logistic regression analysis to explore the factors influencing BO development after ADV pneumonia.

Results: Among the 793 children diagnosed with ADV pneumonia, 86 cases (10.84%) progressed to BO. The proportion of heparin use was higher in the non-BO group than in the BO group after PSM. The univariate regression analysis revealed that acute respiratory failure, neurological involvement and fibrinogen (FIB) were risk factors for the development of BO in ADV pneumonia cases (OR > 1, $p<0.05$), but low-dose heparin sodium treatment and hemoglobin (OR < 1, $p<0.05$) exhibited protective effects against BO formation. Among the 307 children with single ADV pneumonia (excluding confounding factors), 33 cases (10.75%) developed BO. The univariate regression analysis further indicated that fever duration, acute respiratory failure and FIB were risk factors for the development of BO in single ADV pneumonia (OR > 1, $p<0.05$), while low-dose heparin sodium treatment (OR < 1, $p<0.05$) was protective against BO formation after a single ADV pneumonia.

Conclusion: Low-dose heparin sodium treatment may be a protective factor against the development of BO after ADV pneumonia infection.

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Adenovirus pneumonia; bronchiolitis obliterans; heparin sodium

1. Introduction

Bronchiolitis obliterans (BO) is a chronic irreversible obstructive lung disease manifested by recurrent or persistent dyspnea and airflow obstruction. It is often characterized by damage to the small airway epithelium and the partial or complete obstruction of bronchioles or alveolar tubules by granulation or fibrosis tissue [1]. Post-infection BO (PIBO), caused by respiratory tract infections, is the predominant type of BO observed in children [2]. Adenovirus (ADV) is a significant pathogen responsible for severe pneumonia in children and the most common cause of PIBO [3]. The interaction between pathogens and host factors affects the pathogenic process of ADV pneumonia. Studies have shown that various risk factors, such as viral load, host age, and the production of inflammatory factors, contribute to the occurrence and development of BO after ADV infection [4–6]. However, research on protective factors associated with PIBO is lacking.

CONTACT Lili Zhong zhonglili1@hunnu.edu.cn Hunan Provincial Key Laboratory of Pediatric Respirology, Pediatric Medical Center, Hunan Provincial People's Hospital (the First Affiliated Hospital of Hunan Normal University), 61 Jie-Fang West Road, Fu-Rong District, 410005, Changsha, P. R. China.

Heparin, a long-standing anticoagulant drug that has been used for many years [7] has been shown to possess various non-anticoagulant effects through further clinical research. These effects include anti-inflammatory properties, airway resistance reduction, immune regulation, and tissue repair [8]. Therefore, heparin sodium may potentially prevent the formation of granulation or fibrotic tissue in PIBO, leading to improved patient prognosis. This study aims to explore the relationship between heparin application and BO after ADV infection and its potential significance for BO prevention and treatment.

In observational studies, confounding factors can bias the relationship between exposure and outcome measures, and unmeasured confounders may potentially influence the results. In this study, we plan to employ directed acyclic graphs (DAGs) to assess the causal relationships between various variables, which will aid in selecting independent and confounding variables to build our regression model. We will use the propensity score matching (PSM) method to control for non-interventional confounding variables in this study, which ensures balance and correction at the baseline level. Furthermore, we will utilize univariate logistic regression models to analyze the factors influencing BO development after ADV pneumonia.

2. Materials and methods

2.1. Patient and data collection

The retrospective study involved 1014 children under the age of 5 with ADV pneumonia who were hospitalized in southern China between January 2019 and December 2019 with written informed consent obtained from their guardians. Patients with chronic lung disease, recurrent respiratory infections, recurrent wheezing or a history of asthma, bronchopulmonary dysplasia, immunosuppressive or immunosuppressive disorders, severe heart, liver, or kidney diseases, malignancies were excluded. Among these 1014 patients, data on clinical symptoms and signs were missing in 42 (4.14%) cases, incomplete imaging data were obtained in 38 (3.75%) cases, data on laboratory were missing in 59 (5.82%) cases, incomplete treatment data were obtained in 20 (1.97%) cases, follow-up data were missing for 62 (6.11%) patients. Finally, 793 patients were included in the analyses (Figure 1). These children were divided into two groups, the BO group and the non-BO group, based on whether they developed BO during the 2-year follow-up period. The cases of ADV infection included in this study completed at least five follow-ups after discharge. Specifically, a

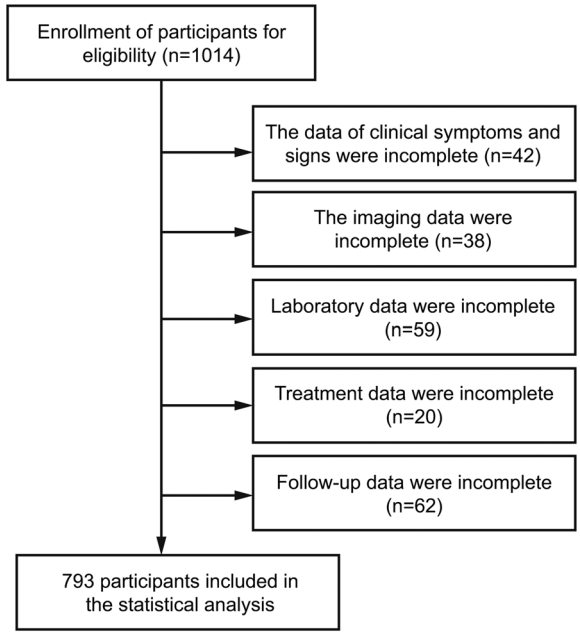

Figure 1. Flowchart of study participant selection for the final observational analysis.

clinic follow-up was conducted two weeks post-discharge. In addition, a chest CT scan and lung function test were rechecked within three months post-discharge. A respiratory specialist outpatient follow-up was conducted at 12 months. Subsequent follow-ups were conducted at 18 and 24 months, primarily through phone calls or WeChat. However, if the participant prefers, an in-person appointment could be scheduled at the outpatient clinic of the respiratory specialty. The scheduling of additional follow-up sessions was left to the doctor's discretion based on observed changes in the clinical symptoms and signs presented by the patient. These measures were taken to ascertain the occurrence of post-infectious BO. This study was approved by the Ethics Committee of Hunan Provincial People's Hospital (2020-07).

ADV pneumonia diagnosis meets the following criteria [9]: (i) Presence of acute lower respiratory symptoms; (ii) Evidence of pulmonary infiltration observed on chest X-ray or computed tomography (CT); (iii) Evidence of ADV infection through PCR detection of respiratory virus in nasopharyngeal swabs. The definition of single ADV pneumonia refers to identifying ADV infection only in nasopharyngeal specimens, with no detection of other pathogens.

Clinical diagnostic criteria for BO are as follows [10]: (i) Presence of continuous or repeated wheezing or coughing, shortness of breath, dyspnea, and exercise intolerance; (ii) Auscultation of extensive wheezing and moist rales in both lungs, lasting for more than 6 weeks, with poor response to bronchodilators;

(iii) High-resolution CT scan revealing BO-related changes (such as a mosaic sign, bronchiectasis, and bronchial wall thickening); (iv) Pulmonary function tests showing small airway obstructive ventilation dysfunction or mixed ventilation dysfunction, with mostly negative bronchial dilation test results. (v) Exclusion of cough and asthma caused by pulmonary cystic fibrosis, congenital bronchopulmonary dysplasia, and other diseases.

All patients who meet the diagnostic criteria for pneumonia can receive low-dose heparin sodium (10 IU/kg q6h), with the dosage being adjusted and determined based on the Diagnosis and Treatment Specification for Children with Adenovirus Pneumonia (2019 Edition) [11]. Low-dose heparin sodium should not be used concomitantly with drugs with compatibility contraindications. Additionally, we would avoid using this treatment in patients with a history of heparin allergy, bleeding tendencies, delayed blood clotting (such as hemophilia), ulcers, trauma, and severe liver dysfunction. Although strict blood monitoring is generally not required during the use of low-dose heparin sodium for intravenous injection, it could potentially exacerbate bleeding. Therefore, we do not usually use this treatment as a routine for patients with significant bleeding.

2.2. Data collection

Data on demographics, clinical features, and laboratory characteristics were analyzed. Demographic and clinical information includes age, gender, length of hospital stays, fever duration, gamma globulin use, glucocorticoids, heparin sodium, fiberoptic bronchoscopy and lavage, and X-ray or CT imaging reports. Laboratory specimens included blood, nasopharyngeal swabs, and sputum. Laboratory data were obtained within 24 h of hospitalization, such as white blood cell (WBC), hemoglobin (Hb), platelet (PLT), C-reactive protein (CRP), procalcitonin (PCT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), D-dimer (DD), alanine transaminase (ALT), creatine kinase MB (CK-MB), and fibrinogen (FIB).

2.3. Detection of mixed infections

Nasopharyngeal swabs were collected and tested for the presence of influenza A, influenza B, parainfluenza types 1, 2, 3, and respiratory syncytial virus antigens (respiratory virus detection kit, Diagnostic Hybrids, USA) using an immunofluorescence assay. The sections were examined under a fluorescence microscope at 200× magnification. Positive staining was confirmed when at least two intact cells displayed fluorescence specific to a certain virus type. Other microorganisms, such as typical bacteria, were detected by Gram staining of sputum samples and blood culture. Atypical bacteria, including Chlamydia pneumoniae and Mycoplasma pneumoniae, were tested using antibodies in blood samples.

2.4. Statistical analysis

The data analysis was conducted using SPSS 25.0 statistical software. Normally distributed were represented as mean ± standard deviation (x ± s). The comparison between the two groups was conducted using an independent sample t-test. Non-normally distributed data were represented as median, and the comparison between the two groups was analyzed using the Wilcoxon rank-sum test. A significance level of $p < 0.05$ was considered statistically significant. Count data were expressed as percentages (%), and the χ^2 test was used for comparison between groups. A DAG was constructed using DAGitty online software to select the independent and confounding variables for the regression model. Confounding variables were balanced between the two groups using the PSM method, with a caliper set at 0.2. Logistic regression analysis was conducted to identify the relevant risk factors for BO formation in children with ADV pneumonia. The variable selection criterion was set at $p < 0.05$, and the exclusion criterion was set at $p > 0.1$.

3. Results

3.1. Clinical features of patients with ADV pneumonia

In this study, there were 793 children with ADV pneumonia, of which 86 cases (10.84%) advanced to BO. The BO group consisted of 60 males (69.77%), which was higher than the non-BO group (61.53%), but this difference was not statistically significant ($p = 0.136$, Table 1). Children in the BO group were younger than those in the non-BO group, which was a statistically significant finding ($p = 0.019$, Table 1). BO patients experienced a longer duration of fever throughout the disease ($p < 0.001$, Table 1). In addition, the BO group displayed a higher incidence of extrapulmonary complications, including shortness of breath, lung rales and wheezing, mucus plugs, acute respiratory failure, cardiovascular dysfunction, urinary, neurological, and hemophilic syndromes, lung consolidation, and pleural effusion ($p < 0.05$, Table 1). Moreover, a higher proportion of patients requiring admission to PICU and ventilator-assisted ventilation was observed in the BO

Table 1. Comparing the baseline data characteristics before and after PSM in the non-BO group and BO group among all ADV pneumonia patients.

	Before PSM		After PSM	
Variables	non-BO group ($n=707$)	BO group ($n=86$)	non-BO group ($n=186$)	BO group ($n=77$)
Characteristic				
Gender (male)	435 (61.53)	60 (69.77)	118 (63.44)	54 (70.13)
Age [M (P25–P75)]/ (months)	24 (12–39)	16 (11–34) [a]	18 (11–35)	17 (11–34)
Congenital heart disease [*n* (%)]	34 (4.81)	11 (12.79) [a]	22 (11.83)	11 (14.29)
Signs and symptoms				
Fever duration [M (P25–P75)]/ (d)	7 (5–10)	11 (9–16) [a]	10 (7–15)	10 (8–16)
Shortness of breath [*n* (%)]	170 (24.05)	49 (56.98) [a]	95 (51.08)	41 (53.25)
Lung rales [*n* (%)]	481 (68.03)	72 (83.72) [a]	154 (82.80)	63 (81.82)
Lung wheezing [*n* (%)]	142 (20.08)	34 (39.53) [a]	68 (36.56)	30 (38.96)
Mucus plugs [*n* (%)]	4 (0.57)	10 (11.63) [a]	3(1.61)	3 (3.90)
Acute respiratory failure [*n* (%)]	91 (12.87)	58 (67.44) [a]	88 (47.31)	51 (66.23) [a]
Cardiovascular dysfunction [*n* (%)]	22 (3.11)	15 (17.44) [a]	15 (8.06)	12 (15.58)
Gastrointestinal disorder [*n* (%)]	222 (31.40)	35 (40.70)	60 (32.26)	30 (38.96)
Urinary system involvement [*n* (%)]	10 (1.41)	5 (5.81) [a]	4 (2.15)	53 (3.90)
Neurologic involvement [*n* (%)]	61 (8.63)	20 (23.26) [a]	24 (12.90)	19 (24.68) [a]
Hemophagocytic syndrome [*n* (%)]	24 (3.39)	13 (15.12) [a]	19 (10.22)	10 (12.99)
Mixed infection [*n* (%)]	316 (44.70)	48 (55.81)	103 (55.38)	41 (53.25)
Disease severity				
Admission to PICU [*n* (%)]	99 (14.00)	41 (47.67) [a]	69 (37.10)	36 (46.75)
Ventilator-assisted ventilation [*n* (%)]	91 (12.87)	31 (36.05) [a]	36 (19.35)	19 (24.68)
Laboratory characteristic				
WBC [M (P25–P75)]/$\times 10^9$ L^{-1}	6.99 (5.32–8.47)	6.57 (4.80–8.73)	6.30 (4.40–7.90)	6.50 (4.81–8.36)
Hb [x ± s]/g·L^{-1}	107.38 ± 16.94	97.59 ± 15.37 [a]	101.94 ± 15.58	98.27 ± 15.36 [a]
PLT [M (P25–P75)]/$\times 10^9$ L^{-1}	252 (203–341)	219 (143–274) [a]	233 (156–300)	210 (146–262)
CRP [M (P25–P75)]/mg·L^{-1}	10 (4–20)	15 (6–32) [a]	10 (3–19)	15 (5–29)
PCT [M (P25–P75)]/ng·mL^{-1}	0.399 (0.28–0.74)	0.86 (0.38–2.67) [a]	0.57(0.28–0.74)	0.82 (0.37–2.64)
LDH [M (P25–P75)]/U·L^{-1}	414 (405–552)	763 (415–1241) [a]	631 (414–1206)	753 (414–1234)
DD [M (P25–P75)]/mg·L^{-1}	0.85 (0.52–1.72)	2 (0.85–5.46) [a]	1.70 (0.85–4.60)	1.80 (0.85–5.45)
FIB [M (P25–P75)]/mg·$dL-^1$	2.76 ± 1.03	3.19 ± 0.85[a]	2.72 ± 0.94	3.04 ± 0.96 [a]
CK-MB [M (P25–P75)]/U·L^{-1}	29 (22–43)	37 (28–53) [a]	35 (26–52)	37 (28–48)
ALT [M (P25–P75)]/U·L^{-1}	18 (13–27)	24 (15–45) [a]	21 (15–33)	24 (15–45)
AST [M (P25–P75)]/U·L^{-1}	46 (36–66)	73 (52–112) [a]	66 (47–104)	71 (49–111)
Radiological characteristics				
Lung consolidation [*n* (%)]	251 (35.50)	64 (74.42) [a]	134 (72.04)	58 (75.32)
Pleural effusion [*n* (%)]	62 (8.77)	27 (31.40) [a]	45 (24.19)	23 (29.87)
Treatment				
Low-dose heparin [*n* (%)]	273 (38.61)	21 (24.42) [a]	79 (42.47)	17 (22.08) [a]
Heparin usage duration [M (P25–P75)]/ (d)	9 (6–12)	10 (9–15)	8 (6–11)	9 (8–13)
Corticosteroids [*n* (%)]	257 (36.35)	68 (79.07) [a]	134 (72.04)	60 (77.92)
Gamma globulin [*n* (%)]	319 (45.12)	58 (67.44) [a]	114 (61.29)	50 (64.94)
Fiberoptic bronchoscopy lavage [*n* (%)]	294 (41.58)	42 (48.84)	87 (46.77)	34 (44.16)

Abbreviations: PSM: propensity score matching; ADV: adenovirus; BO: bronchiolitis obliterans; PICU: Pediatric Intensive Care Unit; WBC: white blood cell; Hb: hemoglobin; PLT: platelet; CRP: C-reactive protein; PCT: procalcitonin; LDH: lactate dehydrogenase; DD: D-dimer; FIB: fibrinogen; CK-MB: creatine kinase MB; ALT: alanine transaminase; AST: aspartate aminotransferase.
Note: Compared with non-BO group, [a]$P<0.05$.

group than non-BO group ($p<0.05$, Table 1). In the laboratory examination results, the BO group showed significantly decreased levels of Hb and PLT, as well as increased levels of CRP, PCT, LDH, DD, FIB, CK-MB, ALT, and AST compared to the non-BO group ($p<0.05$, Table 1). There were no significant differences between the two groups regarding WBC count, mixed infection, heparin usage duration and proportion of patients requiring fiberoptic bronchoscopy lavage treatment. In addition, compared to the non-BO group, the BO group had a lower proportion of low-dose heparin sodium usage, but a significantly higher proportion of corticosteroids and gamma globulin usage ($p<0.05$, Table 1). In all our cases, no adverse effects were observed when using low-dose heparin sodium.

3.2. Clinical characteristics of BO and non-BO groups after a single ADV pneumonia infection

In this study, 307 children with single ADV pneumonia were diagnosed, of which 33 cases (10.75%) advanced to BO. In the BO group, there were 26 males (78.79%), which was higher than the non-BO group (62.77%), but this difference was not statistically significant ($p=0.069$, Table 2). The children in the BO group were younger than those in the non-BO group, but there was no statistically significant difference ($p=0.068$, Table 2). The duration of fever in BO patients was longer throughout the disease ($p<0.001$, Table 2). In addition, the BO group had a higher incidence of extrapulmonary complications, including shortness of breath, lung rales and wheezing, mucus

plugs, acute respiratory failure, cardiovascular dysfunction, hemophagocytic syndrome, lung consolidation, and pleural effusion ($p < 0.05$, Table 2). Moreover, a higher proportion of patients requiring admission to PICU was observed in the BO group than non-BO group ($p < 0.05$, Table 2). In terms of laboratory test results, compared to the non-BO group, the BO group had significantly decreased Hb and PLT levels and increased levels of CRP, PCT, LDH, DD, FIB, CK-MB, ALT, and AST ($p < 0.05$, Table 2). There were no significant differences in WBC count, heparin usage duration and proportion of patients requiring fiberoptic bronchoscopy lavage treatment between the two groups. Furthermore, compared to the non-BO group, a lower proportion of patients in the BO group received low-dose heparin sodium, but a significantly higher proportion of corticosteroids and gamma globulin treatment ($p < 0.05$, Table 2).

3.3. Results from PSM

According to the results of the DAG analysis (Figure 2), the PSM method was used to perform a 1:4 (BO: non-BO) nearest neighbor matching on the non-interventional confounding variables of children with ADV pneumonia (Table 1). After PSM, only acute respiratory failure, neurologic involvement, levels of Hb and FIB, and low-dose heparin treatment were statistically significant ($p < 0.05$, Table 1). After PSM of the data form children with a single ADV infection, significant associations were found for variables including fever duration, acute respiratory failure, FIB level, and low-dose heparin treatment ($p < 0.05$, Table 2).

Table 2. Comparison of the clinical characteristics of single ADV pneumonia in the non-BO group and BO group before and after PSM.

	Before PSM		After PSM	
Variables	non-BO group ($n = 274$)	BO group ($n = 33$)	non-BO group ($n = 83$)	BO group ($n = 36$)
Characteristic				
Gender (male)	172 (62.77)	26 (78.79)	56 (67.47)	28 (77.78)
Age [M (P25–P75)]/ (months)	24 (12–36)	15 (9–29)	17 (10–29)	14 (9–29)
Congenital heart disease [*n* (%)]	15 (5.47)	6 (18.18) [a]	10 (12.05)	6 (16.67)
Signs and symptoms				
Fever duration [M (P25–P75)]/ (d)	7 (5–9)	10 (9–13)[a]	9 (7–12)	10 (9–13)[a]
Shortness of breath [*n* (%)]	60 (21.90)	18 (54.55) [a]	35 (42.17)	20 (55.56)
Lung rales [*n* (%)]	165 (60.22)	30 (90.91) [a]	66 (79.51)	31 (86.11)
Lung wheezing [*n* (%)]	60 (21.90)	15 (45.45) [a]	27 (32.53)	14 (38.89)
Mucus plugs [*n* (%)]	1 (0.36)	4 (12.12) [a]	1 (1.20)	2 (5.56)
Acute respiratory failure [*n* (%)]	33 (12.04)	23 (69.70) [a]	35 (42.17)	25 (72.22) [a]
Cardiovascular dysfunction [*n* (%)]	9 (3.28)	4 (12.12) [a]	7 (8.43)	4 (11.11)
Gastrointestinal disorder [*n* (%)]	82 (29.93)	9 (27.27)	24 (28.92)	11 (30.56)
Urinary system involvement [*n* (%)]	5 (1.82)	2 (6.06)	2 (2.41)	1 (2.78)
Neurologic involvement [*n* (%)]	18(6.57)	4(12.12)	12 (14.46)	8 (22.22)
Hemophagocytic syndrome [*n* (%)]	5(1.82)	6(18.18) [a]	3 (3.61)	4 (11.11)
Disease severity				
Admission to PICU [*n* (%)]	35 (12.77)	10 (30.30) [a]	28 (33.73)	14 (38.89)
Ventilator-assisted ventilation [*n* (%)]	32 (11.68)	5 (15.15)	11 (13.25)	5 (13.89)
Laboratory characteristic				
WBC [M (P25–P75)]/$\times 10^9$ L^{-1}	7 (5.57–8.43)	7 (5–10)	7 (4.68–8.51)	7 .10 (5.10–9.96)
Hb [x ± s)]/g·L^{-1}	107.46 ± 17.76	97.48 ± 12.37[a]	104.84 ± 17.04	100.75 ± 12.58
PLT [M (P25–P75)]/$\times 10^9$ L^{-1}	295.43 ± 144.48	231.63 ± 114.71[a]	231 (157–280)	226 (159–268)
CRP [M (P25–P75)]/mg·L^{-1}	9.6 (4.21–15.51)	22 (6–34)[a]	9.6 (5.5–33.1)	16 (4.3–33)
PCT [M (P25–P75)]/ng·mL^{-1}	0.398 (0.13–0.52)	0.956 (0.39–1.69) [a]	0.44 (0.38–2.87)	0.73 (0.39–3.58)
LDH [M (P25–P75)]/U·L^{-1}	414 (396–489)	926 (457–1391) [a]	572 (414–946)	612 (414–1257)
DD [M (P25–P75)]/mg·L^{-1}	0.82 (0.42–1.21)	1.98 (0.82–3.84) [a]	1.25 (0.84–3.55)	1.72 (0.85–3.78)
FIB [M (P25–P75)]/mg·dL^{-1}	2.86 ± 1.19	3.16 ± 0.84 [a]	2.46 ± 1.07	3.02 ± 0.87 [a]
CK-MB [M (P25–P75)]/U·L^{-1}	28 (21–44)	38 (30–54) [a]	33 (27–50)	38 (29–52)
ALT [M (P25–P75)]/U·L^{-1}	17 (13–25)	24 (14–36) [a]	22 (16–29)	24 (14–44)
AST [M (P25–P75)]/U·L^{-1}	46 (34–62.75)	72 (45–115) [a]	63 (46–84)	68 (40–113)
Radiological characteristics				
Lung consolidation [*n* (%)]	93 (33.94)	25 (75.76) [a]	57 (68.68)	23 (63.89)
Pleural effusion [*n* (%)]	20 (7.30)	11 (33.33) [a]	17 (20.48)	11 (30.56)
Treatment				
Low-dose heparin [*n* (%)]	92 (33.58)	3 (9.09) [a]	39 (46.99)	9 (25) [a]
Heparin usage duration [M (P25–P75)]/ (d)	10 (7–13)	11 (8–15)	9 (7–13)	10 (9–14)
Corticosteroids [*n* (%)]	75 (27.37)	26 (78.79) [a]	54 (65.06)	29 (80.56)
Gamma globulin [*n* (%)]	114 (41.61)	20 (60.61) [a]	45 (54.22)	23 (63.89)
Fiberoptic bronchoscopy lavage [*n* (%)]	94 (34.31)	11 (33.33)	33 (39.76)	13 (36.11)

Abbreviations: PSM: propensity score matching; ADV: adenovirus; BO: bronchiolitis obliterans; PICU: Pediatric Intensive Care Unit; WBC: white blood cell; Hb: hemoglobin; PLT: platelet; CRP: C-reactive protein; PCT: procalcitonin; LDH: lactate dehydrogenase; DD: D-dimer; FIB: fibrinogen; CK-MB: creatine kinase MB; ALT: alanine transaminase; AST: aspartate aminotransferase.
Note: Compared with non-BO group, [a]$p < 0.05$.

3.4. Predictive risk factors for BO formation after ADV pneumonia

Univariate logistic regression analysis showed that acute respiratory failure (OR, 2.184 [95% CI, 1.256–3.798]), neurological involvement (OR, 2.211 [95% CI, 1.129–4.332]), FIB (OR, 2.490 [95% CI, 1.011–6.132]) were identified as risk factors for the formation of BO in children with ADV pneumonia ($p < 0.05$, Table 3). Additionally, low-dose heparin sodium (OR, 0.384 [95% CI, 0.2080.708]) and Hb (OR, 0.240 [95% CI, 0.054–0.896]) were found to be a protective factor against BO ($p < 0.05$, Table 3).

3.5. Predictive risk factors for BO formation after a single ADV pneumonia infection

Univariate logistic regression analysis demonstrated that acute respiratory failure (OR, 3.117 [95% CI, 1.356–7.164]), fever duration (OR, 2.613 [95% CI, 1.412–4.836]) and

FIB (OR 2.129 [95% CI, 1.053–4.304]) was identified as an independent risk factors for the formation of single ADV pneumonia BO in children ($p < 0.05$, Table 4). However, low-dose heparin sodium (OR 0.376 [95% CI, 0.1580.897]) was a protective factor against the occurrence of BO ($p < 0.05$, Table 4).

4. Discussion

The pathogenesis of BO after ADV infection is closely related to inflammation and abnormal immune response. The infection causes damage to the respiratory epithelial cells, leading to abnormal inflammatory response and fibrosis and impairing their repair function. Heparin sodium possesses anti-inflammatory, immunomodulatory, and tissue repair effects, which may confer protection against the occurrence and progression of BO. Our research results are consistent with the hypothesis. In this study, we evaluated the use of heparin sodium in both BO and non-BO patients, and observed a significantly lower proportion of heparin sodium usage in the BO group compared to the non-BO group. Even after adjusting for other variables, heparin sodium remained a protective factor against BO after ADV infection. The biological mechanism by which heparin sodium affects BO is not yet clear. Several potential reasons may explain its protective effect. Firstly, heparin has been shown to inhibit inflammatory response through multiple pathways [12]. Secondly, heparin can bind to various components of the complement system and modulate its excessive activation, thus preventing complement-mediated immunopathological damage and exerting an immunosuppressive role [13]. Lastly, heparin could promote tissue repair in traumatic tissues

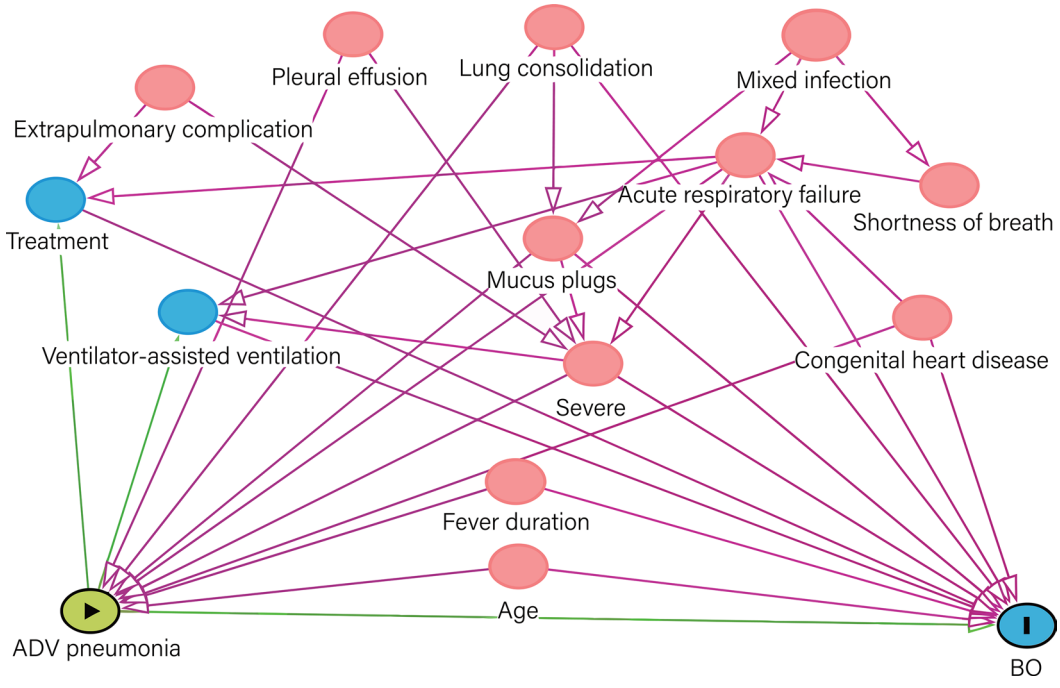

Figure 2. DAG of the causal relationship of each variable. Variables denoted in green with symbols represent exposure factors, while those in blue with symbols signify outcome factors. Blue variables without symbols denote ancestral factors of the outcome variable, and pink variables represent confounding variables. ADV: adenovirus; BO: bronchiolitis obliterans.

Table 3. Univariate logistic regression analysis of risk factors related to BO in ADV pneumonia.

Variable	Partial regression coefficient (β)	SEβ	Wald χ^2 value	*P* value	OR (95% CI)
Acute respiratory failure	0.781	0.282	7.666	0.006	2.184 [1.256–3.798]
Neurologic involvement	0.794	0.343	5.350	0.021	2.211 [1.129–4.332]
FIB	0.912	0.460	3.937	0.047	2.490 [1.011–6.132]
Hb	−1.427	0.672	4.510	0.034	0.240 [0.054–0.896]
Low-dose heparin	−0.958	0.312	9.773	0.002	0.384 [0.208–0.708]

Abbreviations: ADV: adenovirus; BO: bronchiolitis obliterans; FIB: fibrinogen; Hb: hemoglobin; SEβ standard error of β; OR: odds ratio; 95% CI: 95% confidence interval.

Table 4. Univariate logistic regression analysis of risk factors related to BO in single ADV pneumonia.

Variable	Partial regression coefficient (β)	SEβ	Wald χ^2 value	*P* value	OR (95% CI)
Fever duration	0.961	0.314	9.351	0.002	2.613 [1.412–4.836]
Acute respiratory failure	1.137	0.425	7,627	0.006	3.117 [1.356–7.164]
FIB	0.756	0.359	4.431	0.035	2.129 [1.053–4.304]
Low-dose heparin	−0.978	0.443	5.246	0.022	0.376 [0.158–0.897]

Abbreviations: ADV: adenovirus; BO: bronchiolitis obliterans; FIB: fibrinogen; Seβ: standard error of β; OR: odds ratio; 95% CI: 95% confidence interval.

[14]. Based on these mechanisms, heparin sodium may prevent the occurrence of BO in children with ADV pneumonia. In this study, we also confirmed the protective effect of heparin sodium in the univariate analysis of BO formation in a single ADV infection.

The most common method for analyzing influencing factors is the "single factor followed by multiple factors" approach for screening independent variables to construct regression models. However, previous research has shown that the factors affecting BO are numerous and complex. Using the traditional approach may overlook the impact of confounding and mediator variables, omitting important influencing factors from the multiple-factor model. DAG is a theoretically driven method for screening independent variables. It constructs a causal relationship network based on theoretical causality to identify suitable variables for the model. This method provides a more intuitive way to identify causal relationships and circumvent the limitations of traditional confounding factor judgment criteria. It serves as a guiding principle for independent variable selection in regression analysis, which is the primary task in analyzing factors that affect BO [15]. Therefore, this study applied a DAG to identify the causal relationships among influencing factors leading to BO and selected the appropriate independent variables and confounding factors for building the regression model. The confounding factors selected through the DAG included age, fever duration, congenital heart disease, mucus plugs, pleural effusion, lung consolidation, shortness of breath, mixed infection, severity, acute respiratory failure, and extrapulmonary complication.

PSM is a semi-parametric method used to balance baseline confounding factors. Balancing differences in baseline data between groups reduces confounding effects and reflects the true association between the study variable and the outcome variable [16]. In this study, multiple confounding variables and covariates were considered in the baseline data, and some were strongly correlated. Including all these variables in the regression model could result in poor model fit and biased results. Therefore, PSM was applied in this study to control for confounding variables between the two patient groups, thus correcting for baseline discrepancies and increasing comparability between groups. Using the occurrence of BO as the outcome variable, we found that after PSM, in ADV pneumonia only acute respiratory failure, neurologic involvement, FIB, Hb and low-dose heparin treatment showed differences between the BO and non-BO groups. Among children with single ADV pneumonia, only the fever duration, acute respiratory failure, FIB and low-dose heparin treatment showed differences between the BO and non-BO groups. Logistic regression analysis was then conducted to identify factors associated with BO in children with ADV infection, aiming to provide evidence-based evidence for clinical prevention and treatment of BO.

In the univariate logistic regression analysis, we not only found the protective effect of low-dose sodium heparin but also identified the fever duration, acute respiratory failure and FIB as independent risk factors for the occurrence of BO in children with single ADV infection. Single ADV pneumonia is characterized by continuous fever or intermittent fever. If the fever lasts for a long time, it indicates that the inflammation persists and is not effectively controlled. This high inflammatory state can lead to sustained high fever, ultimately contributing to the occurrence of BO. Prolonged duration of fever is a risk factor for the development of BO in ADV pneumonia, consistent with previous findings [6]. Acute respiratory failure indicates a more severe condition of ADV pneumonia, with an extended duration of illness and increased damage to the small airways, which in turn raises the likelihood of developing BO during the

recovery phase [17]. Therefore, acute respiratory failure is also a risk factor for BO in ADV pneumonia [18]. Under normal physiological conditions, the fibrinolytic system and hemolytic system of the body are in dynamic equilibrium. However, when the body is imbalanced, it fails to promptly eliminate FIB, leading to enhanced coagulation function. In infectious diseases, FIB is often used as an infection predictor [19]. FIB levels are significantly higher in the adenovirus pneumonia BO group compared to the non-BO group, and are a risk factor for BO formation. This may be related to the fact that children in this group often experience inflammatory responses such as high fever and respiratory failure, resulting in coagulation dysfunction. Furthermore, in all ADV pneumonia cases, extrapulmonary complications such as nervous system disorders serve as risk factors for BO, suggesting that children with ADV pneumonia in the BO group may suffer from more severe damage in other organ systems, particularly the nervous system, manifesting as symptoms such as poor mental state and toxic encephalopathy. On the other hand, Hb is a protective factor against BO in these cases. This may be because if the Hb content in the body is insufficient, the blood's oxygen-carrying capacity is low. Combined with severe lung infection and respiratory failure, their oxygen-uptake ability is impaired, resulting in severe hypoxia that cannot meet the body's normal needs, which is unfavorable for patient prognosis. Unfortunately, this protective effect is not significant in single ADV pneumonia cases and requires further investigation.

This study has some limitations that should be acknowledged. Firstly, the results may not be representative of all regions in China, suggesting the need for future multi-center studies to provide a more comprehensive perspective. Additionally, the potential confounding effects of other drugs were not accounted for in the analysis. In summary, our findings suggest that heparin sodium serves as a protective factor against the formation of BO in children with ADV pneumonia. These results highlight the promising clinical applications of heparin sodium in the treatment of BO.

Author contributions

LP: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing-original draft; LZ: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing-review & editing; RH, SL, HH, XD, MC, and LL: Data curation, Formal analysis, Visualization; LC: Investigation, Methodology, Formal analysis, Visualization, Writing-review & editing. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the subjects and their guardians who understood and participated in these experiments. The study was approved by the Ethics Committee of Hunan Provincial People's Hospital with judgment's reference number 2020-07. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Written informed consent for publication was obtained from children and their guardians.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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