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Methods in pediatric infectious diseases 2024

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Editorial: Methods in pediatric infectious diseases 2024

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Editorial on the Research Topic
Methods in pediatric infectious diseases 2024

As a continued Research Topic of the previous "*Methods in Pediatric Infectious Diseases 2022*" (1), this Research Topic received more attention and submissions, partially due to the influence of the global Covid-19 pandemic (2). The methods applied in understanding the immune mechanisms and clinical manifestations of pediatric infections continue to evolve, reflecting both the challenges and opportunities in advancing pediatric care. Within this topic, fourteen articles are presented that describe important and recent findings in pediatric infectious diseases.

During the COVID-19 pandemic, children were the least affected group, but those cases exhibited a wide range of clinical manifestations, ranging from asymptomatic to severe conditions. In order to better understand the impacts of SARS-CoV-2 Omicron, Sun et al. conducted an observational cohort study to describe the outcomes of severe and critically ill children infected in northeastern China and found that respiratory failure and COVID-19-associated neurological disorders were the most common complications. They pointed out that chest computed tomography (CT) score, the Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score, and serum aspartate aminotransferase (AST) were important indicators of poor outcomes in children. Takane-Cabrera et al. led a similar methodology aiming to describe the characteristics and risk factors associated with disease severity across six waves of COVID-19 in children in Mexico. They found that the most affected children were the 12–17-year-old group. However, the 0–2-year-old group had higher rates of hospitalization, ICU admission, and case fatality rate (CFR), meaning that children under two years of age had the worst outcomes.

Multisystem inflammatory syndrome in children (MIS-C) is a severe complication of the COVID-19 infection. In order to understand the effectiveness of biologic and target-synthetic therapies in preventing MIS-C development, Khoury et al. conducted a retrospective cohort study based on the major Israeli health organization. Children aged 0–18 years who tested positive for COVID-19 were included, and none of the individuals who received treatments developed MIS-C. These authors suggested a possible association between biological and target-synthetic therapies and reduced risk of MIS-C following COVID-19 infection in children.

Acute bronchiolitis is the most common respiratory infection and a major cause of hospitalization in infants. Cortazzo et al. evaluated the clinical relevance of bacterial and/or viral respiratory coinfection in infants younger than three months old

hospitalized with bronchiolitis in Rome. The statistical analyses revealed that there is a correlation between respiratory coinfection and a longer hospital stay and use of invasive mechanical ventilation. The same association was not seen in the viral mono-infection group. Additionally, premature infants were found to be at higher risk of respiratory coinfections compared to viral mono-infections.

Through the years, vaccination campaigns have been able to significantly decrease the incidence of many diseases, as exemplified by the Covid-19 pandemic (3). But what is the role of pediatricians when facing a vaccine-resistant family? Moorthy et al. answered this tough but important question and pointed out a possible solution. The idea was to compromise with the family to develop a vaccine schedule that was not complete, since partial immunity was better than no immunity. An adapted vaccine schedule should be proposed, and a more effective vaccine (4) would be more likely to be accepted by the family when factors such as disease severity and mortality risk, availability of treatment, vaccine risk profile, what diseases were actively spreading in the patient's community, and potential threats to public health were carefully considered (5). Likewise, Starshinova et al. presented a review on the history of the Bacillus Calmette–Guérin (BCG) vaccine and how the genetic alterations in BCG strains have evolved from the original variant. Besides the anti-tuberculosis effect, the BCG vaccine offered protection against infections involving mucous membranes showing how important it was to study the variety of protective benefits of this vaccine.

Mycoplasma pneumoniae (MP) is the major pathogen causing community-acquired pneumonia (CAP) in children 5 years old or older. Ding and Jiang showed in a recent review that some of the most common biomarkers in clinical practice, such as C reactive protein (CRP), procalcitonin (PCT), and serum amylase A (SAA), were good tools for clinical use because of their high sensitivity in the early diagnosis of MP pneumonia. Besides that, CRP and LDH measures were able to predict treatment courses and the patient's response. The authors concluded that the affordable and convenient blood work and cytokine-based markers, joined with these indicators, improved the accuracy of the diagnosis. Zhang et al. reported a rare case of a 9-year-old boy with refractory MP pneumonia complicated by bilateral pulmonary embolism and pulmonary infarction. Initially, the patient was treated with the conventional protocol for MP pneumonia, but after laboratory exams and chest CT, he met the diagnostic criteria for both severe MP and refractory MP pneumonia. The treatment was approached with a multidisciplinary protocol combining anti-infective agents, anti-inflammatory therapy, and adjusted anticoagulation. The patient had a rapid recovery with some residual sequelae.

Furthermore, one of the possible complications of MP pneumonia in children is plastic bronchitis (PB). Although rare, it is a severe condition that involves the formation of obstructions to the airways and can lead to respiratory failure if not treated promptly. In this retrospective cohort study, Lian et al. developed and validated a nomogram incorporating the CD4+/CD8+ ratio to predict PB in children who underwent bronchoscopy. Fever duration, presence of atelectasis, elevated D-dimer, and reduced CD4+/CD8+ ratio were identified as independent predictors of PB in MP pneumonia. Such a tool, together with other potential non-invasive imaging tools (6–8), could support bronchoscopy decision-making and optimize outcomes in pediatric cases of MP pneumonia. Another possible complication is the parapneumonic effusion (PPE), a pleural effusion caused by infectious and non-infectious disorders. Although the incidence of severe PPE has declined since the introduction of the 13-valent pneumococcal conjugate vaccines, immunological screening in children is of the most importance since early diagnosis and adequate treatment is directly connected to better outcomes. Rószai et al. investigated the immunological function of children with severe PPE during hospitalization and after full recovery through a prospective study. The duration of hospitalization was longer in the immunocompromised group when compared to the non-immunocompromised group. Within the first group, there were immunodeficiency virus infection, immunoglobulin A deficiency, mannose-binding lectin deficiency, and specific antibody deficiency.

The clinical use of biomarkers for diagnoses, especially for the early detection of infections in children, is extremely important and essential for positive prognoses. Yin et al. led a retrospective study to evaluate the most commonly used markers for neonatal late on-set sepsis (LOS). The markers analyzed included neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), CPR, platelet-to-neutrophil ratio (PNR), and procalcitonin. ROC analysis showed high levels of specificity and sensitivity, pointing to the potential of NLR and PLR as reliable biomarkers for LOS diagnosis. Additionally, the combination of NLR, PLR, and CRP further improved diagnostic accuracy.

Cytomegalovirus (CMV) is a virus part of the *Herpesviridae* family and is a leading cause of congenital infections, which have a high and damaging incidence in preterm infants. Li et al. described a case report of a 2-day-old child delivered at 36 + 2 gestation weeks who had scattered bleeding spots across the body, hemorrhagic diathesis, thrombocytopenia, positive blood CMV IgM, and high levels of CMV DNA in blood and urine. The infection caused CMV retinitis, which was a common result of CMV infection in patients with immunodeficiencies. The treatment chosen was an antiviral protocol with Ganciclovir.

Pulmonary echinococcosis is a parasitic infection associated with high morbidity and mortality rates and is especially relevant in endemic regions. China accounts for more than 70% of all recorded cases worldwide. Li et al. reported the case of a 13-year-old girl who was initially misdiagnosed with pulmonary tuberculosis. After surgical intervention and histopathological examination, the diagnosis of pulmonary echinococcosis was confirmed, and the patient received Albendazole treatment and symptomatic management, which led to full recovery.

It is well known that pediatric leukemia is an important health issue in childhood. Although the survival rates of pediatric leukemia are higher than 65%, infections may increase the morbidity and mortality in children. Li et al. analyzed patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) to determine the prognosis of different types of

acute infection in pediatric leukemia patients. The authors found that the incidence of pneumonia and sepsis is significantly higher in the AML group. Besides that, younger children in both groups had more favorable prognoses than older children. Nanomedicine or other factors could be a promising way to treat those patients (9, 10).

In conclusion, pediatric infectious diseases have caused a serious global public health problem. Therefore, to understand its latest diagnosis, prognosis, and treatment methods are of the utmost importance. Factors such as efficient clinical predictors for early diagnosis and adequate treatment must be continuously studied and widely disseminated.

Author contributions

IK: Conceptualization, Writing – review & editing. GM: Data curation, Formal analysis, Writing – original draft. ZS: Conceptualization, Supervision, Validation, Writing – review & editing.

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On compromising with vaccine-hesitant families

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Introduction

My patient was a smiling, unvaccinated 6-year-old boy. Halloween was around the corner, and we bonded over his Spider-Man costume. When we got to vaccines, however, the mood expectedly soured. They were "unnatural," his parents shared; the pharmaceutical industry was replete with liars and the CDC was bought. The only indication of progress since last visit's counseling was a new openness to the tetanus vaccine. They promised to think hard about it for next time but offered no explanation for why it was special. Perhaps they had a friend whose child fell sick with tetanus. I have often wondered if I should have dug more into it. What if I could have identified other vaccines that met their criteria?

How should pediatricians respond to vaccine hesitancy?

When it comes to persuading vaccine-hesitant families to vaccinate, pediatricians are understandably expected to do the legwork. Their offices administer vaccines, and the relationships they build with families, sometimes spanning generations, position them to address individual concerns and bridge to the broader medical establishment. The best option is for pediatricians to attempt to convince parents about the benefits of vaccination. There are evidence-based recommendations for how to most effectively communicate about vaccine recommendations, including using closed-ended statements, bundling discussions of multiple vaccines and giving regular affirmation as part of broader motivational interviewing (1, 2).

However, when best practices fail, some pediatricians consider dismissing vaccine-hesitant families from their practice. While this may be acceptable in extreme circumstances (3), it interrupts care continuity and arguably subverts professional values like tolerance that are central to caring for patients in a pluralistic society (4). Some have argued that child welfare services should become involved to engage the courts to compel immunization. However, vaccine refusal generally fails legal and ethical standards for overriding parental authority to make health-related decisions for their child.

Weighing reliable protection from serious illness against relatively low risks for adverse health events, vaccination is widely regarded to be in a child's best interest. However, failure to act to *maximally* promote a child's interest is not sufficient to justify state intervention according to most interpretations of the best-interests standard (5). Moreover, state investigations can be disruptive and traumatizing, and the result in

these cases is likely only to diminish trust in medical and governmental institutions. The harm principle and constrained parental autonomy model define stricter standards still (6, 7). They require that the vaccine be highly efficacious with low morbidity and that not receiving it put the patient at serious risk of imminent harm, or according to the constrained parental autonomy model, deprive a child of their basic needs. Partly due to herd immunity, which protects the individual unvaccinated child, the standards are not met. The pediatrician treating a child in a persistently vaccine-resistant family is left with few good options; they may eventually decide to ignore the topic altogether. But what if instead they offered to compromise with those families on when or even which vaccines their child receives, working with them to develop a schedule that integrates their values and preferences with pressing public health realities?

Is compromise ethical?

The idea of any compromise on childhood vaccination understandably invites backlash. The CDC schedule reflects decades of careful study into immune system development on the one hand and infection spread patterns on the other. We know vaccination per the CDC schedule is safe and effective, but we do not know that for unofficial schedules. Some countries have experimented with prioritizing vaccines through selective mandates in response to outbreaks, but testing the effectiveness of these interventions is challenging in the short term, and there may even be decreased uptake of other vaccines (8). Responding to concerns about the quantity of shots administered in a single day, some pediatricians have published their own versions of the vaccine schedule, correctly condemned by experts as the substitution of limited experience for mountains of data. To our knowledge, none of these has been thoroughly studied. However, if some vaccination provides more protection than no vaccination—which follows from any individual vaccine efficacy study demonstrating protection against the specific disease as a health benefit —then it seems in the best interest of the child to receive even a reduced number of vaccines.

Pediatricians have a responsibility to promote public health, but their principal responsibility is to the children they treat. So, after failing to convince parents to vaccinate per the standard CDC or catch-up schedule, a pediatrician might, for example, emphasize vaccination of the child living in an older home against tetanus, for which there can be no herd immunity because spread is not from person to person and spend less energy on vaccination against hepatitis A, which is associated with self-limited illness and low rates of mortality. In so doing, this pediatrician is not claiming they know better than the CDC. Rather, they are engaging in a form of harm reduction, a strategy that has proved extremely effective in other areas of public health concern, such as substance use treatment (9). Harm reduction is aimed at minimizing the consequences of health-adverse choices when eliminating them is not immediately possible.

For families who reject the CDC schedule and have not responded positively to counselling, compromising on when, which, and how many vaccines to receive, may lead to partial immunity. This benefits both the child and their peers and prevents further estrangement from social institutions. Compromise as an approach is humble and non-coercive. It establishes an open, non-judgmental atmosphere that preserves the possibility for complete immunization, perhaps according to the evidence-based schedule, down the road. Had I asked the family of that costumed 6-year-old why they were reconsidering tetanus vaccination, I might have learned they were receiving health information from a new source or that their new priest had offered a vaccine-friendlier interpretation of scripture. I might have learned their neighbor knew someone who had gotten tetanus and nearly died. This could have been an opening to build additional rapport or focus on specific scientific misconceptions. Revisiting the conversation at the next visit with these reference points might have made the family that much more amenable to accepting vaccination, tetanus or otherwise. Building this shared understanding might be even more crucial if there were a future local outbreak of a highly communicable disease.

Some families have more nonspecific concerns about vaccines, informed by a mix of historic distrust of medical institutions due to discriminatory treatment, cultural preferences for traditional healing, peer anecdotes, and online misinformation. Other vaccine-hesitant families may have more concrete pseudoscientific beliefs, e.g., concerns about heavy metals or autism, and they may request a vaccination schedule that accommodates these heterodox beliefs. We recognize that by accepting an alternate timeline, there is a risk of validating those pseudoscientific beliefs. For this reason, it is important to be clear during counseling—which will have first aimed to correct any scientific misconceptions—that any alternate schedule is a compromise for the sake of a commonly desired end and not simply one of many equally defensible options.

Discussion: what does ethical compromise look like, practically?

Primary care physicians must weigh several factors when customizing a vaccine schedule. One is the likelihood a disease will kill or seriously harm the patient, acknowledging that presentations can vary dramatically: sometimes polio is mild, and sometimes chicken pox is deadly. Another is the existence of treatment for the disease, and the patient's ability and willingness to access it, as well as the risks associated with treatment. Next, there is the risk profile of the vaccine itself. Then, there is the matter of what diseases are actively spreading in the patient's community. Finally, the physician must consider what pathogens would most threaten public health, including accounting for the patient's immunosuppressed contacts and how else they spend their time. For example, measles kills 2 in 1,000 children who catch it, has a 90 percent transmission rate, and is actively spreading across the United States.

Accurately performing this analysis for each patient is clearly a tall order – hence the CDC's schedule. However, with continued objection to vaccines, a physician should feel empowered to

promote vaccines the family is likely to agree to, based on their stated objections, and recommend vaccines according to their good-faith estimate of medical usefulness. Pediatricians cannot imply that these customized vaccination suggestions are a substitute for evidence-based schedules. They must be offered as last-resort compromises, intended to avoid further alienating vaccine-hesitant families. Pediatricians who prefer not to accommodate an alternate schedule risk a missed opportunity to partially vaccinate a child, and parents might shop for more accommodating, but overall less scrupulous, providers.

Tailored vaccine counseling will require more time, energy, and expertise than most physicians have. There may be a unique role for the pediatric infectious disease specialist and local epidemiologists within this framework. Whether independently or as part of local public health data monitoring committees, they might be needed in consultation to support complex individual assessments and to compile and publish data about local outbreaks, community demographic and comorbidity data, and rates of vaccine uptake. This data could be integrated into an interactive tool to estimate semi-individualized likelihoods of illness susceptibility that could subsequently aid physicians making these complex compromises. The tool could be compared to a hospital antibiogram, which summarizes the susceptibility of bacteria cultured from patients to different antibiotics to inform antibiotic selection.

There is evidence to suggest some pediatricians already feel a level of comfort using an alternative immunization schedule upon parent request. Many also prioritize certain shots, like DTaP (diphtheria-tetanus toxoids-acellular pertussis) and the pneumococcal conjugate vaccine, both of which prevent diseases associated with serious morbidity and mortality in childhood, over the COVID-19 or influenza vaccines (10). Our hope is that expert infectious disease advice will help bring as much evidence as is reasonably possible to clinician *ad hoc* judgments and allow willing pediatricians to take a more active role in shaping a vaccine-schedule compromise, building and maintaining the durable, trusting connections with families that are the precondition for delivering high quality, mutually satisfactory healthcare. Safeguarding public health in the 21st century requires combatting vaccine hesitancy. This may require the flexibility for harm reduction, which safeguards autonomy while preserving a commitment to good health values.

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Epidemiological characterization of COVID-19 in children under 18 years old in Mexico: an analysis of the pandemic

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Objective: The study aimed to describe the characteristics and risk factors associated with disease severity across six waves of COVID-19 in the pediatric population in Mexico.
Methods: A cohort study was conducted using data from the Mexican Ministry of Health, covering the period from March 2020 to March 2023. The dataset included patients under 18 years of age with confirmed SARS-CoV-2 infection. Univariate, bivariate, and logistic regression analyses were performed to determine demographic and clinical characteristics, mortality across waves, and age group distributions.
Results: Of the total cohort, 9.5% were children, with 497,428 confirmed cases. Among these, 50% were male, 4.4% required hospitalization, and there were 1,447 (0.03%) deaths. The highest prevalence was observed in the 12–17-year age group (52%), followed by the 5–11-year age group (32%), with incidence rates peaking towards the end of 2021 and the early 2022. Although the 0–2-year age group represented 9.6% of cases, it had higher hospitalization (40%), ICU admission (58%), and case fatality rate (CFR) (44%). Cardiovascular disease, hypertension, diabetes and immunosuppression were identified as risk factors for severe outcomes. The initial wave displayed the highest CFR (OR 5.28) especially in children aged 0–2 years.
Conclusions: Children were less affected during the pandemic compared to adults; however, children under two years-old experienced more severe outcomes. Currently, with 95% of the population estimated to be immune due to vaccination and/or prior infection, children under 2 years of age are now at higher risk of severe disease and should be evaluated for vaccination as a public health policy.

KEYWORDS

COVID-19, SARS-CoV-2, children, Mexico, pandemic, incidence, waves, mortality

1 Introduction

Globally, since the emergence of the SARS-CoV-2 (COVID-19) pandemic in China, early 2020 reports indicated that the adult population was disproportionately affected, with 98% of cases occurring in individuals over 18 years of age and only 2% in the pediatric population (1, 2).

Clinical manifestations of SARS-CoV2 infection in children vary, from asymptomatic cases to severe conditions such as pneumonia or Pediatric Inflammatory Multisystemic syndrome (PIMS) (3), which may require hospitalization and can lead to complications including death or long COVID-19 as a post-infectious syndrome. A systematic review of COVID-19 including patient series from China, Italy, Spain, and the United States reported that 5%–21% of cases in children were asymptomatic, while only 2% exhibited symptoms typical of upper respiratory tract infections (4, 5). Another systematic review found that 12% of infected children with SARS-CoV-2 showed symptoms, with a prevalence of 25% for cough, 9% for fatigue and 33% for fever, and 4% required intensive care (6).

In Mexico, from February 28, 2020, to March 31st, 2023, 497,428 confirmed cases (6.5%) (7) have been reported in individuals under 18 years old. Six waves of COVID-19 occurred, each associated with a distinct viral variant resulting from mutations in the genetic material of the virus, including Wuhan-HU1, B.1.1.159, Delta, Omicron, BA.4/BA.5 and XBB.1.5. These mutations affected the infectivity, pathogenicity, and immune evasion capabilities of the virus, contributing to outbreaks or more severe clinical presentations (8, 9).

The initial symptoms of COVID-19 documented in the pediatric population in Mexico included cough (53%), headache (53%), fever (47%), sore throat (33%), runny nose (29%), myalgia (28%), general discomfort (27%), arthralgia (23%), chills (21%), irritability (19%), diarrhea (17%), dyspnea (14%), chest pain (12%), abdominal pain (12%), conjunctivitis (10%), vomiting (7%), tachypnea (7%), and cyanosis (3%), with a case fatality rate CFR) of 1.3% (10).

The risk of infection was lower among children under 10 years of age and in school settings compared to adults, while adolescents in community and high schools' environments had a comparable risk (11).

Despite being the last age group to receive access to COVID-19 vaccination in Mexico (only children aged 5–17 years), the hospitalization and mortality rates in this population remained lower than those in adults (3.8 per 100,000 inhabitants). However, national data on the clinical characteristics and demographics of SARS-CoV-2 infection in children and adolescents are limited (12).

This study aimed to provide a descriptive analysis of the incidence, clinical and demographic characteristics, and risk factors associated with mortality in the pediatric population during the different COVID-19 waves in Mexico.

2 Materials and methods

2.1 National COVID-19 database

This study was conducted by analyzing a national database provided by the Ministry of Health (13), covering the period from March 2020 to March 2023. The dataset was filtered to focus on pediatric patients aged 18 years or younger with a probable diagnosis of SARS-CoV-2 infection. Variables associated with demographic characteristics and risk factors were extracted from the database, which recorded the presence of diabetes mellitus, hypertension, obesity, asthma, immunosuppression, tobacco exposure, cardiovascular diseases, chronic kidney disease, and chronic obstructive pulmonary diseases (COPD) as co-morbidities.

2.1.1 Definition of co-morbidities included in the database

Diabetes was defined as a history of diabetes characterized by fasting plasma glucose >100 mg/dl or postprandial glucose <140 mg/dl (14). Hypertension was defined as a history of hypertension characterized by a blood pressure higher than 140/90 in two different time points (15). Obesity was defined by a body mass index of 30 kg/m^2 or higher (16). Asthma was defined as a history of asthma characterized by wheezing, shortness of breath, coughing and chest tightness (17). Immunosuppression was defined as history of an impairment of the immune response resulting from conditions or factors intrinsic or extrinsic to the immune system because of malnutrition, metabolic disorders, use of immunosuppressive medications, chronic infections, malignancies, and severe trauma (18). Tobacco exposure was defined as history of an involuntary household exposure to tobacco smoke (19). Cardiovascular disease was defined as a history of cardiac impairment such as arrythmias, chronic cardiac failure, ischemic cardiomyopathy, among others (20). Chronic kidney disease was defined as a history of chronic kidney disease characterized by glomerular filtration rate of less than 60 ml/min per 1.73 m^2, or markers of kidney damage, or both, of at least 3 months duration, regardless of the underlying cause (21). Chronic obstructive pulmonary disease (COPD) was defined as a history of COPD characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases (22).

2.1.2 Subjects included in the analysis

Initially, a COVID-19 database comprising 25,963,617 individuals was filtered, resulting in a subset of 2,470,045 patients younger than 18 years old. This subset was further filtered to include only those with SARS-CoV-2 infection (Figure 1). A confirmed case was defined as individuals with a positive polymerase chain reaction test (PCR) for SARS-CoV-2, a positive antigen tests, or a positive result determined through clinical presentation and epidemiological association, or confirmation by

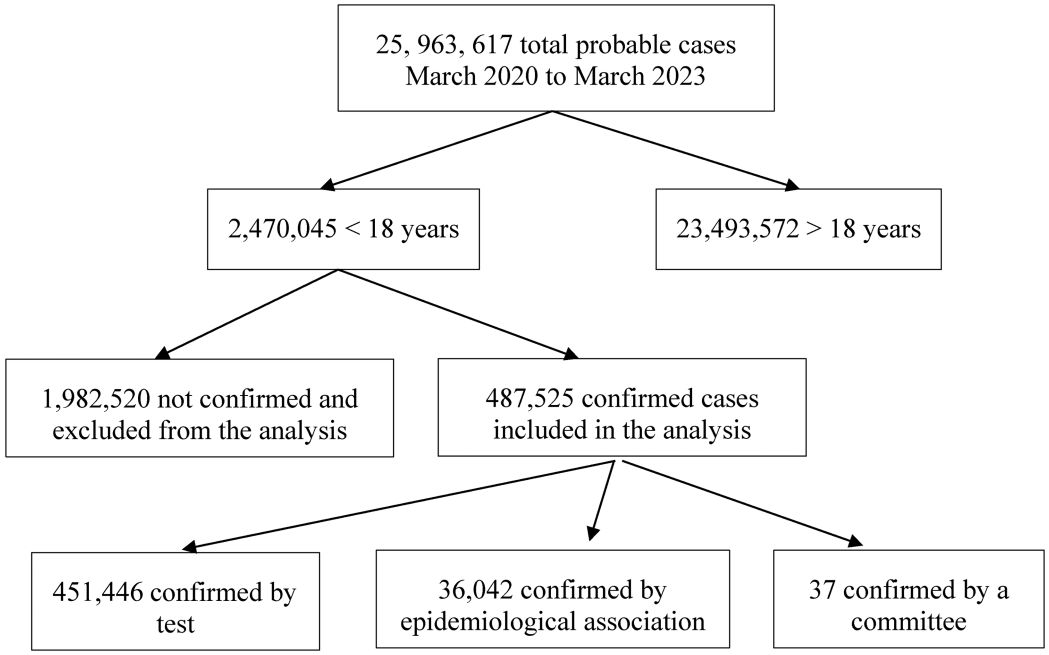

FIGURE 1
Flowchart of patients included in the study.

a review committee (23, 24). During the pandemic, PCR or antigen testing for SARS-CoV-2 was not widely available throughout the country, especially at the onset. Consequently, the government classified patients as COVID-19 cases based on suggestive symptoms and known contact with a confirmed case (epidemiological contact), or in deceased cases, on clinical history assessed by a review committee, even in the absence of a test (23, 24).

2.2 Criteria for variables definition

Patients were grouped by age into four categories: 0–2 years, 3–4 years, 5–11 years, and 12–17 years. This classification was based on the national vaccination strategy, which prioritized the 12–17 age group followed by the 5–11 age group. Furthermore, the highest mortality rates had previously been observed in infants aged 0–2 years (10).

The clinical severity of the disease was categorized into three levels: those who developed pneumonia, those who required intubation, and those who died, according to the database definitions.

Suspected Case: An individual of any age who, within the past 7 days, has presented with two or more signs and symptoms (such as cough, fever, or headache), accompanied by at least one of the following symptoms (dyspnea as a severity indicator, arthralgia, myalgia, sore throat, rhinorrhea, conjunctivitis, or chest pain).

Severe Acute Respiratory Infection Case: Any individual who meets the criteria for a suspected case of mild respiratory illness and additionally presents with difficulty breathing and is hospitalized.

Confirmed Case: An individual who meets the operational definition of a suspected case and has a laboratory-confirmed PCR diagnosis, a positive antigen tests, or a positive result determined through epidemiological association, clinical presentation, or confirmation by a review committee.

Pneumonia: patients who were clinically diagnosed with pneumonia.

Intubation: patients who required mechanical ventilation and were intubated (13, 23).

2.3 Definition of COVID-19 waves during the pandemic in Mexico

An additional classification stratified patients according to the COVID-19 pandemic waves, considering the sample collection and/or medical appointment dates. This stratification was aligned with national epidemiological reports to ensure accurate incidence reporting.

An open database from the General Directorate of Epidemiology of the Ministry of Health was utilized to delineate the timeline of each wave. The intervals between each wave were adjusted to facilitate objective analysis, with each wave linked to the predominant circulating variant, based on data from GISAID and the COVIGEN (acronym for the Spanish name, Mexican Consortium of Genomic Vigilance) (25). The following wave periods were defined: Wave 1 (Wuhan-HU1) from March 1st, 2020, to September 23rd, 2020; Wave 2 (B.1.1.519) from September 24th, 2020, to May 15th, 2021; Wave 3 (Delta variant) from May 16th, 2021, to December 15th, 2021; Wave 4 (Omicron variant) from December 16th, 2021, to May 15th,

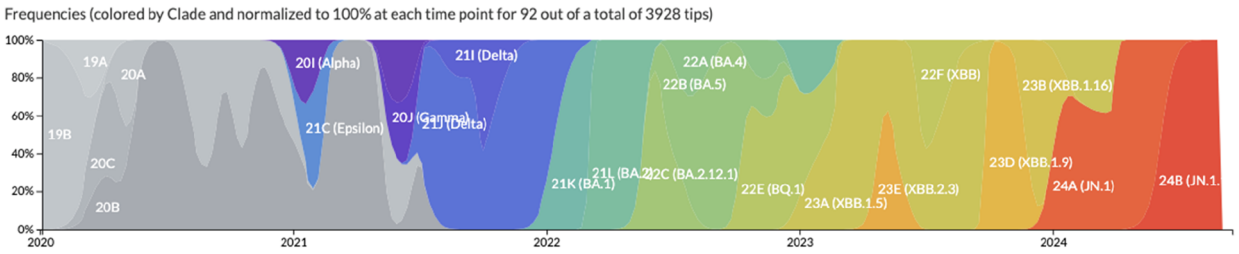

FIGURE 2
COVID-19 variants in Mexico. Downloaded from https://nextstrain.org/ncov/gisaid/global/all-time?f_country=Mexico under the The CC-BY-4.0 (accessed on September, 2024).

2022; Wave 5 (BA.4 and BA.5 variants) from May 16th, 2022, to October 15th, 2022; and Wave 6 (XBB 1.5 variant) from October 16th, 2022, to March 6th, 2023 (Figure 2).

2.4 Statistical analysis

Descriptive and bivariate statistics were used to analyze the variables, with percentages also calculated. Analyses were performed using the Statistical Package for Social Sciences IBM® software (SPSS version 25). Categorical variables were compared using the χ^2 test or Fisher's exact test, as appropriate, while continuous variables were compared using the student's *t*-test. A *p*-value <0.05 was considered statistically significant. Logistic regression was conducted to assess lethality across different waves. The case fatality rate of each wave was compared to the wave with the lowest lethality.

Additionally, an analysis of comorbidities associated with SARS-CoV-2 case fatality rate was performed for each wave.

COVID-19 incidence was calculated by dividing the number of positive cases (diagnosed through testing and/or clinical criteria) per semester by the total population of children in each age group, then multiplying by 100,000. Lethality was calculated similarly, using the number of deaths.

2.5 Ethical considerations

The study was approved by the Research and Ethics Committees of the Faculty of Medicine, Universidad Nacional Autónoma de México (FM/DI/093/2020). Informed consent was not required because the work was done with a secondary database analysis where the identity of the subjects was not available.

3 Results

3.1 COVID-19 confirmed cases included in the analysis

The COVID-19 case registry from the Mexican Ministry of Health, covering the period from March 2020 to March 2023, included a total of 25,963,617 individuals. Among these, 2,470,045 were children under 18 years of age (9.5%), with 487,525 children having a clinical and/or laboratory-confirmed COVID-19 diagnosis and included in the analysis (Figure 1). Of the confirmed cases, 451,446 (92.6%) were verified by a diagnostic test, with 113,298 (22.7%) confirmed by PCR and 374,227 (77.3%) by antigen testing. An additional 36,042 cases (7.4%) were identified based on clinical symptoms and epidemiological association (contact with a confirmed test-positive case), while 37 cases (0.000076%) were confirmed by a committee.

3.2 Incidence according to different variables

The states most affected, with the highest proportion of pediatric cases, were Mexico City (34.5%), Mexico state (9.2%), Guanajuato (5.6%), Nuevo León (4.5%), Tabasco (3.5%), San Luis Potosí (3.4%), with the remaining states each less than 2% of cases (Figure 3).

Of this population, 246,855 (50.6%) were male and 240,670 (49.4%) were female.

Based on age classification, the group with the highest prevalence was those aged 12–17 years, accounting for 52.1% of cases, followed by the 5–11-year-old group with 32.4% cases, the 0–2-year-old group with 9.6%, and the 3–4-year-old group with 5.9% (Table 1). The highest incidence rate for the 12–17-year-old group was observed in the second half of 2021, with an incidence of 584.01 per 100,000 people. In contrast, incidence rates for the 3–4 year and 5–11year groups were 223.35 and 369.22 per 100,000 people, respectively, peaking in the first half of 2022. The peak incidence in the 0–2-year-old group, at 200.3 per 100,000 people, was the last to occur, reaching its maximum in the second half of 2022 (Figure 4A).

Contrary to incidence patterns, mortality rates were higher in the 0–2-year-old group, particularly during the second half of 2020, which aligns with the circulation of the B.1.1.519 variant, and in the second half of 2021, coinciding with the Omicron variantś prevalence. Mortality peaked again in the first half of 2022 with the circulation of BA.4/BA.5 variants (Figure 4B).

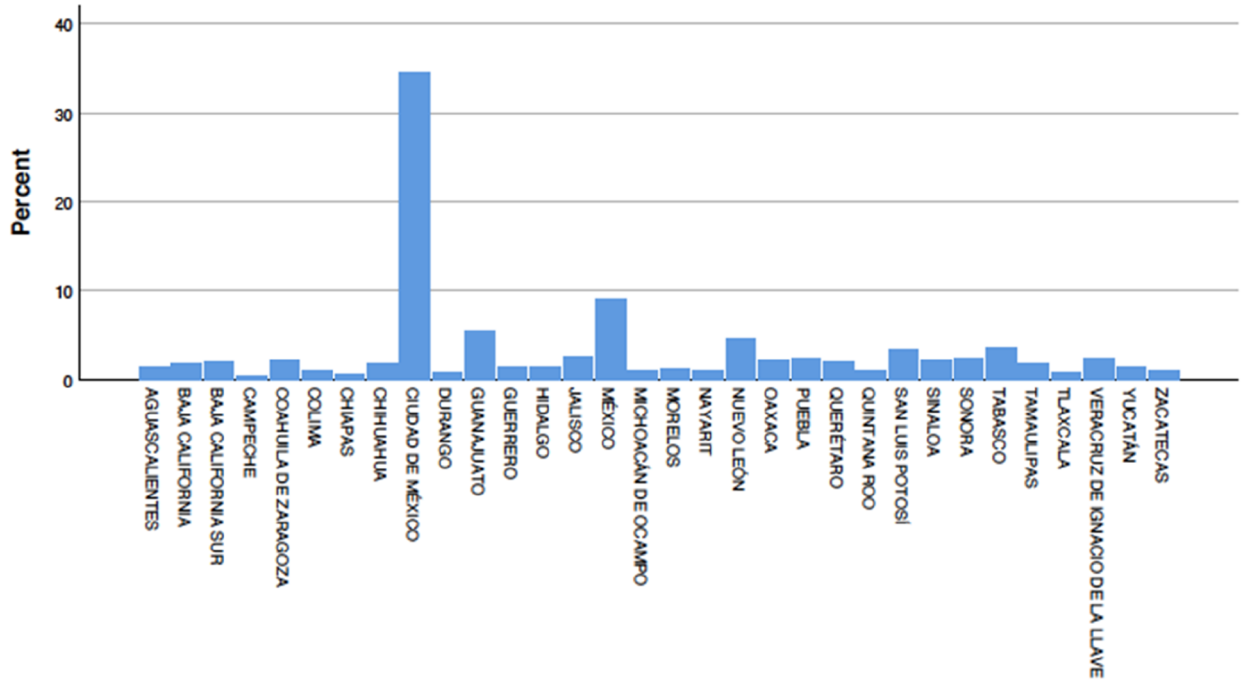

FIGURE 3
Distribution of pediatric COVID-19 cases by state.

TABLE 1 Demographic characteristics of children with confirmed COVID-19 diagnosis.

Characteristics	0–2 years *n* (%)	3–4 years *n* (%)	5–11 years *n* (%)	12–17 years *n* (%)	Total *n* (%)	*p*
Confirmed cases	46,715 (9.6)	28,877 (5.90)	157,970 (32.40)	253,963 (52.10)	*n* = 487,525	
Country region						
North	10,858 (23.2)	5,818 (20.1)	28,529 (18.1)	49,050 (19.3)	94,255 (19.3)	<0.001
% age/North	11.50	6.20	30.30	52.00		
Center	18,632 (39.9)	13,340 (46.2)	81,327 (51.5)	127,530 (50.2)	240,829 (49.4)	
% age/Center	7.70	5.50	33.80	53.00		
West	10,881 (23.3)	6,241 (21.6)	28,871 (18.3)	45,244 (17.8)	91,237 (18.7)	
% age/West	11.90	6.80	31.60	49.60		
South	6,344 (13.6)	3,478 (12.0)	19,243 (12.2)	32,139 (12.7)	61,204 (12.6)	
% age/South	10.40	5.70	31.40	52.50		
Gender						
Female	21,575 (46.2)	13,463 (46.6)	75,743 (47.9)	129,889 (51.1)	240,670 (49.4)	<0.001
% female/age	9.00	5.60	31.50	54.00		
Male	25,140 (53.8)	15,414 (53.4)	82,227 (52.1)	124,074 (48.9)	246,855 (50.6)	
% age/male	10.20	6.20	33.30	50.30		
Hospital area						
Ambulatory	38,181 (81.7)	26,880 (93.1)	152,856 (96.8)	248,298 (97.8)	466,215 (95.6)	<0.001
% age/ambulatory	8.20	5.80	32.80	53.30		
Hospitalization	8,534 (18.3))	1,997 (6.9)	5,114 (3.2)	5,665 (2.2)	21,310 (4.4)	
% age/hospitalization	40.00	9.40	24.00	26.60		
ICU	971 (2.1)	89 (0.3)	244 (0.2)	372 (0.1)	1,676 (0.3)	<0.001
% age/IC	57.9	5.3	14.6	22.2		
Indigenous	465 (15.2)	173 (5.6)	823 (26.9)	1,601 (52.3)	3,062 (100)	

The vertical columns represent the number and percentage of children in each age group for the corresponding characteristic. The percentages within each characteristic (horizontal rows) represent the proportion of children in the different age groups who possess that characteristic.
A *p*-value of <0.05 is considered statistically significant.

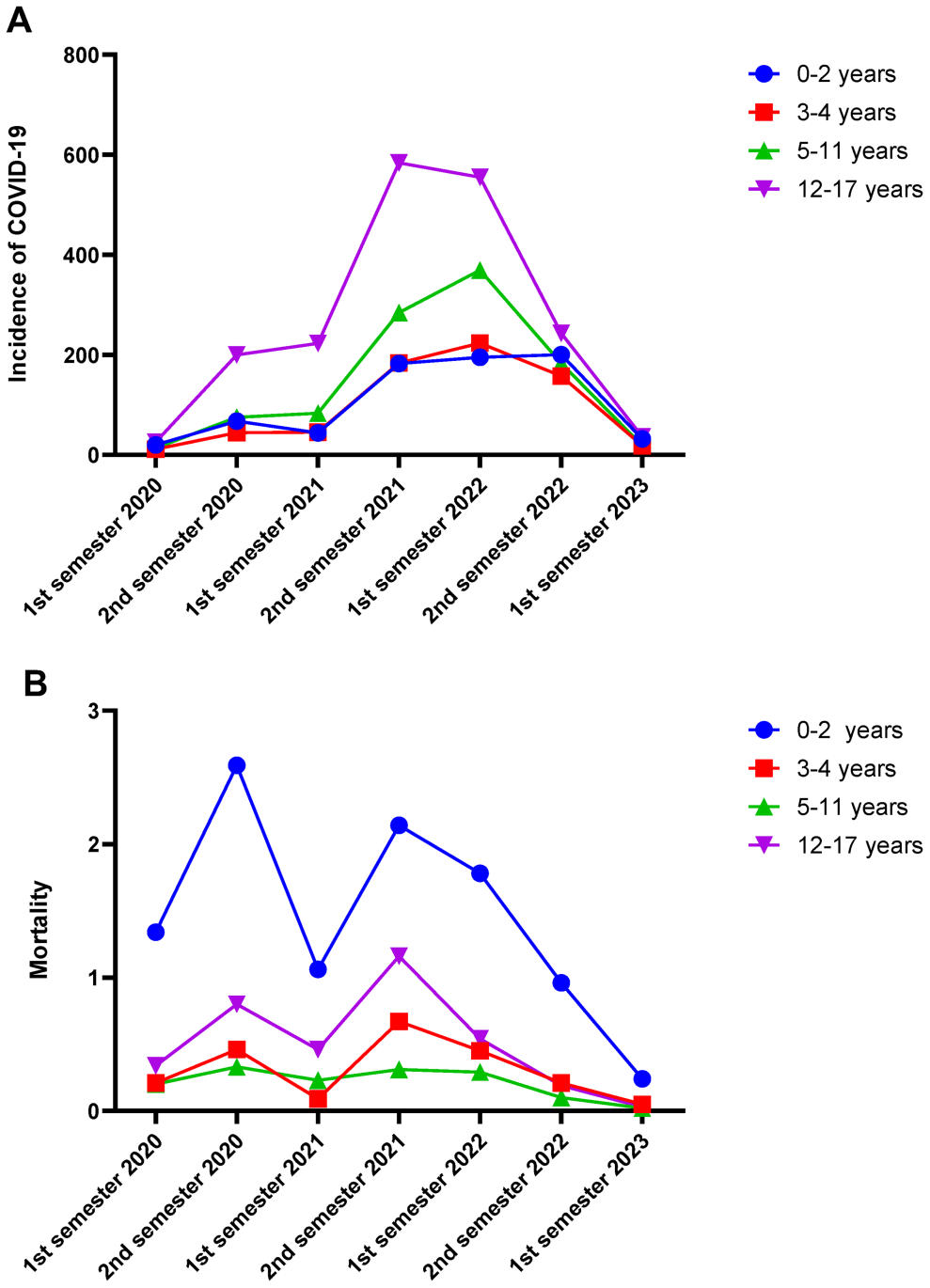

FIGURE 4
Incidence of COVID-19 cases and deaths in Mexican children during the SARS-CoV2 pandemic. The figure shows the semiannual distribution of the incidence (A) and mortality rate (B) of SARS-CoV-2 cases per 100,000 children under 18 years of age, from 2020 to 2023, categorized by age groups in Mexican children with COVID-19. The first semester of 2023 includes only from January to March 2023. The incidence and mortality rate are cases per 100,000 children.

There were two peaks in monthly death counts: January 2022 and August 2021, corresponding to the circulation of the Omicron and Delta variants, respectively (Figure 5).

There were 3,062 (0.7%) indigenous children with COVID-19, with 1,510 (49%) female and 1,552 (50.7%) males, the highest proportion being in the 12–17 years age group with 1,601 individuals (52.3%), followed by the 5–11 years group with 823 (26.9%), the 0–2 years group with 465 (15.2%), and the 3–4 years group with 173 (5.6%) ($p < 0.001$) (Table 1). Geographically, from the indigenous children 443 (14.5%) were from the North region, 720 (23.5%) from the Center, 582 (19%) from the West, and 1,316 (43%) from the South.

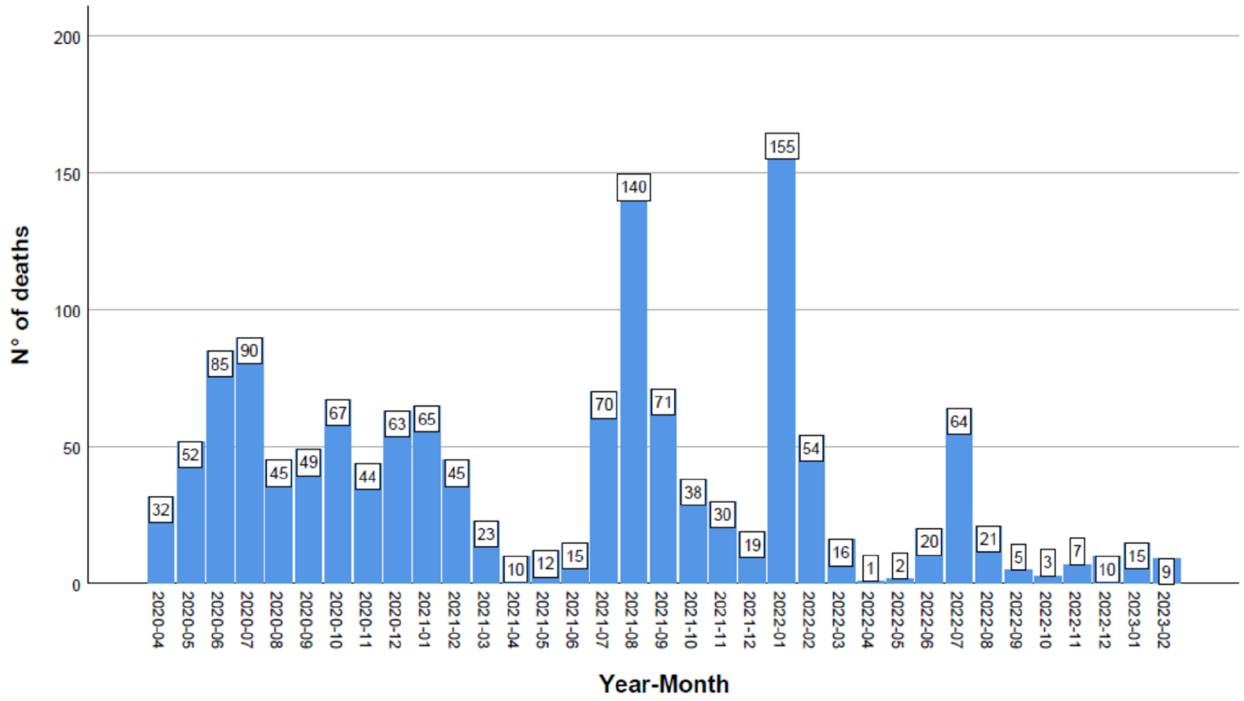

FIGURE 5
Number of deaths in the pediatric population in Mexico during the COVID-19 pandemic. The graph illustrates the number of deaths per month and per year, with the highest monthly peaks occurring during the predominance of the Delta and Omicron waves.

Geographically, most cases were concentrated in the central region of the country (49.4%). Of the total cases, 466,215 patients (95.6%) were ambulatory, while 21,310 (4.4%) required hospitalization. Notably, the 0–2 years age group exhibited the highest hospitalization rate, accounting for 8,534 cases (18.3%), and 2% of ICU admissions (representing 58% of all admissions across age groups) (Table 1).

3.3 Risk factors

Among patients with SARS-CoV-2 infection, asthma and obesity were the most prevalent comorbidities, accounting for 2.4% and 2.2% cases, respectively. Immunodeficiency was the third most common risk factor, present in 0.5% of cases, with the 0–2-year and 3–4-year groups showing the highest rates (both 0.7%). Tobacco smoke was notably high in the 12–17 age group, with 1,039 cases (0.4%), and obesity was most common in this group as well, accounting for 3% (70% of cases across all age groups) (Table 2).

Disease severity was classified into two groups: patients who developed pneumonia (9,916 cases, 2.0%) and those who required intubation (1,294 cases, 0.3%). The highest proportion of pneumonia and intubation cases occurred in the 0–2-year age group, representing 7.2% (3,333 cases) and 1.4% (677 cases) of all cases, respectively. A total of 1,447 deaths (0.03%) were recorded, with the 0–2 age group accounting for the highest proportion of deaths (44.8% across age groups, 1.4% of all the cohort), followed by the 12–17 age group with 33.1% of deaths across ages (0.2% of all the cohort) (Table 3).

A comprehensive analysis of the disease severity across SARS-CoV-2 variants revealed that the highest percentage of deaths occurred during the first wave (Wuhan-HU1), especially in children aged 0–2 years (164 cases, 0.7%), followed by the 12–17-year age group (99 cases, 0.42%). Throughout the COVID-19 pandemic, the CFR decreased consistently across all age groups, except during the XBB.1.5 wave, which exhibited a slight increase, especially among the 0–2 age group (tripling the lethality observed during BA.4/BA.5). The lethality rate was significantly higher in males than females. Geographically, the central region recorded the highest number of deaths across all variant's waves (Table 4).

The results indicated that cardiovascular disease (OR: 2.56) was the strongest predictor of mortality, followed by hypertension (OR: 1.99), diabetes (OR: 1.60), and immunosuppression (OR: 1.57) particularly during the first wave. Notably, asthma appeared to be a protective factor (Table 5).

Significant differences were observed across waves, with the first wave showing the highest lethality (OR: 5.28), which subsequently decreased over time (Table 6).

In this study, indigenous population was used as a proxy for low socioeconomic status. Indigenous status was identified as a statistically significant risk factor for pneumonia (OR: 3.2, 95% CI: 2.7–3.7), intubation (OR: 6.2, 95% CI: 4.6–8.2), ICU admission (OR: 4.6, 95% CI: 3.4–6.1), and death (OR: 4.3, 95% CI: 3.1–5.9).

TABLE 2 Risk factors.

Risk factors	0–2 years *n* = 46,715 *n* (%)	3–4 years *n* = 28,877 *n* (%)	5–11 years *n* = 157,970 *n* (%)	12–17 years *n* = 253,963 *n* (%)	Total *n* = 487,525 *n* (%)	*p*
Exposure to tobacco smoke						
Yes	146 (0.3)	31 (0.1)	185 (0.1)	1,039 (0.4)	1,401 (0.3)	<0.001
% in each age group	10.40	2.20	13.20	74.20		
Comorbidities						
Asthma	256 (0.5)	415 (1.4)	3,922 (2.5)	6,889 (2.7)	11,482 (2.4)	<0.001
% age/asthma	2.20	3.60	34.20	60		
Obesity	398 (0.9)	129 (0.4)	2,613 (1.7)	7,494 (3.0)	10,634	<0.001
% age/obesity	3.70	1.20	24.60	70.50	2.2	
Immunosuppression	327 (0.7)	201 (0.7)	874 (0.6)	947 (0.4)	2,349 (0.5)	<0.001
% age/ immunosuppression	13.90	8.60	37.20	40.30		
Diabetes	258 (0.6)	63 (0.2)	391 (0.2)	1,079 (0.4)	1,791 (0.4)	<0.001
% age/diabetes	14.40	3.50	21.80	60.20		
Cardiovascular disease	469 (0.1)	111 (0.4)	470 (0.3)	721 (0.3)	1,771 (0.4)	<0.001
% age/CVD	26.50	6.30	26.50	40.70		
Hypertension	333 (0.7)	60 (0.2)	276 (0.2)	748 (0.3)	1,417 (0.3)	<0.001
% age/hypertension	23.50	4.20	19.5	52.80		
Chronic Kidney Disease	69 (0.1)	32 (0.1)	226 (0.1)	529 (0.2)	856 (0.2)	<0.001
% age/CKD	8.10	3.70	26.40	61.80		
COPD	56 (0.1)	16 (0.1)	85 (0.1)	176 (0.1)	333 (0.1)	<0.001
% age/COPD	16.80	4.80	25.50	52.90		

The vertical columns represent the number and percentage of children in each age group with the corresponding risk factor. The percentages within each characteristic (horizontal rows) represent the proportion of children across different age groups who exhibit that risk factor.
A *p*-value of <0.05 is considered statistically significant.

TABLE 3 Clinical status of severity.

Clinical Status	0–2 years *n* = 46,715 *n* (%)	3–4 years *n* = 28,877 *n* (%)	5–11 years *n* = 157,970 *n* (%)	12–17 years *n* = 253,963 *n* (%)	Total *n* = 487,525 *n* (%)
Condition					
Pneumonia	3,333 (7.2)	739 (2.6)	2,283 (1.5)	3,561 (1.4)	9,916 (2.0)
Intubation	677 (1.4)	80 (0.3)	219 (0.1)	318 (0.1)	1,294 (0.3)
Clinical outcome					
Death	648 (1.4)	92 (0.3)	228 (0.1)	479 (0.2)	1,447 (0.3)
% age/death	44.80	6.40	15.80	33.10	100

This table presents the clinical status of severe COVID-19 among each age group. The "*n*" and "%" represent the number and percentage of the total sample in each age group. The "% age/death" corresponds to the percentage of deaths within each age group.

4 Discussion

This study delineated the incidence, demographic, and clinical characteristics of COVID-19 among children in Mexico, while identifying and evaluating risk factors associated with disease lethality, thereby providing a comprehensive understanding of COVID-19´s the progression in the pediatric population during the pandemic.

Although COVID-19 was initially reported to primarily impact the adult population, children have also been affected. However, due to the lower proportion of pediatric cases, children have been considered at reduced risk for severe outcomes and fatalities, resulting in limited studies on this age group (26). In countries such as the United States, the United Kingdom, and China, a higher hospitalization rate has been noted among children aged 1–4 years (27). In alignment with these findings, over 95% of cases in this study were managed on an outpatient basis, with only 0.3% requiring intensive care and 4.4% requiring hospitalization. Among hospitalized cases, 40% were children aged 0–2 years, followed by the 3–4-year-old group, confirming that while pediatric cases are typically mild or asymptomatic, disease severity may increase in early infancy.

While children under 4 years are particularly vulnerable to severe outcomes, other age groups remain relevant. Throughout the pandemic, the highest number of cases were among adolescents aged 12–17 years. However, despite the lower number of cases, the 0–2-year age group had the highest percentage of recorded deaths (44.8% across age groups), consistent with findings from Most et al. (28).

This discrepancy may be attributed to two factors: the immune system immaturity in very young patients, which may increase their susceptibility to severe diseases and the presence of chronic

TABLE 4 Prevalence of lethality by age, sex and region during the different waves.

Predominant variant	Wuhan-HU1 *n* = 23,274 *n* (%)	B.1.1.519 *n* = 67,495 *n* (%)	Delta *n* = 144,308 *n* (%)	Omicron *n* = 123,262 (%)	BA.4 BA.5 *n* = 111,458 *n* (%)	XBB 1.5 *n* = 17,728 *n* (%)	*p*
Age							
0–2 years	164 (0.7)	157 (0.2)	138 (0.09)	104 (0.08)	58 (0.05)	27 (0.15)	<0.001
3–4 years	21 (0.09)	13 (0.01)	27 (0.01)	18 (0.014)	10 (0.008)	3 (0.05)	<0.001
5–11 years	62 (0.2)	62 (0.09)	49 (0.03)	45 (0.03)	16 (0.014)	5 (0.02)	<0.001
12–17 years	99 (0.42)	99 (0.14)	161 (0.11)	72 (0.05)	28 (0.02)	9 (0.05)	<0.001
Sex							
Female	159 (0.68)	146 (0.21)	186 (0.12)	109 (0.08)	65 (0.05)	19 (0.1)	<0.001
Male	187 (0.8)	185 (0.27)	189 (0.13)	130 (0.1)	47 (0.04)	25 (0.14)	<0.001
Region							
North	82 (0.35)	90 (0.13)	94 (0.06)	60 (0.0.04)	21 (0.01)	5 (0.02)	<0.001
Center	121 (0.51)	126 (0.33)	114 (0.07)	67 (0.05)	33 (0.02)	13 (0.07)	<0.001
West	55 (0.23)	74 (0.1)	73 (0.05)	65 (0.05)	30 (0.02)	16 (0.09)	<0.001
South	88 (2.4)	41 (0.06)	94 (0.06)	47 (0.03)	28 (0.02)	10 (0.05)	<0.001

The number and proportion of deaths in each age, sex, and region group across the different waves of the pandemic were calculated. A *p*-value of <0.05 was considered statistically significant.

TABLE 5 Risk factors for lethality associated with different waves of the pandemic.

Risk factors	Wuhan-HU1 *n* (%)	B.1.1.519 *n* (%)	DELTA *n* (%)	OMICRON *n* (%)	BA.4/BA.5 *n* (%)	XBB 1.5 *n* (%)	*p*	95% CI	OR
Domestic smoking	3 (1.6)	4 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.094	(0.216–1.255)	0.521
Diabetes	17 (10.1)	23 (6.1)	15 (3)	5 (1.4)	0 (0)	1 (1.4)	<0.001	(1.157–2.216)	1.602*
COPD	0 (0.0)	3 (4.3)	0 (0.0)	1 (1.3)	0 (0.0)	1 (9.1)	0.056	–	–
Asthma	4 (0.5)	8 (0.4)	4 (0.1)	2 (0.1)	2 (0.1)	0 (0)	0.007	(0.277–0.714)	0.445*
Immunosuppression	41 (9.6)	28 (7.9)	27 (7.0)	22 (4.0)	14 (2.9)	6 (4.2)	<0.001	(1.285–1.920)	1.571*
Hypertension	14 (9.6)	21 (6.9)	19 (5.1)	4 (1.4)	1 (0.4)	0 (0.0)	<0.001	(1.407–2.826)	1.994*
Cardiovascular diseases	22 (10.7)	23 (7.3)	26 (6.8)	20 (4.7)	5 (1.5)	4 (3.9)	<0.001	(2.007–3.284)	2.567*
Obesity	23 (2.3)	26 (1.2)	41 (1.2)	3 (0.1)	3 (0.2)	2 (0.8)	<0.001	(1.097–1.793)	1.402*
Chronic kidney disease	14 (12.8)	18 (11.5)	13 (6.3)	10 (5.3)	3 (1.8)	2 (5.9)	0.003	(1.908–3.715)	2.663*
Intubated	169 (46.2)	115 (45.8)	125 (45.8)	78 (42.2)	51 (37.8)	21 (25.0)	0.007	(0.985–1.006)	0.995

The number and percentage of each risk factor were calculated from the total sample for each wave. COPD, Chronic obstructive pulmonary disease; OR, odds ratio; CI, confidence Interval. **p*-value <0.05 is considered statistically significant.

TABLE 6 Lethality of the different waves of the COVID-19 pandemic in Mexico.

	OR	95% CI	*p*
Wuhan-HU1	5.285	(3.799–7.354)	<0.001
B.1.1.519	4.802	(3.449–6.686)	<0.001
Delta	3.176	(2.289–4.406)	<0.001
Omicron	2.438	(1.744–3.407)	<0.001
BA.4/BA.5	1.373	(0.957–1.971)	0.086

Logistic regression analysis results for lethality among different waves compared to the sixth wave (XBB.1.5). OR, odds ratio; CI, confidence interval.
p-value <0.05 statistically significant.

diseases and other risk factors among older patients, which may contribute to disease progression and fatal outcomes.

In this study, cardiovascular diseases, hypertension, diabetes, immunosuppression, and obesity emerged as primary risk factors associated with lethality. A cross-sectional analysis involving 43,465 patients aged 18 or younger with COVID-19 similarly identified type 1 diabetes, congenital heart and cardiovascular anomalies, obesity, hypertension, epilepsy, neuropsychiatric disorders, and chronic diseases, as risk factors for hospitalization or severe disease (29).

Asthma emerged as notable condition in our study. Initially, the Mexican Clinical Guide for the COVID-19 treatment identified asthma as a risk factor for severe COVID-19 in both children and adults (30), based on the heightened susceptibility of asthma patients to viral respiratory infections (31).

However, subsequent studies in Mexico and other countries did not find a significant association between asthma and hospital admission (32), suggesting that asthma might serve as a protective factor. Indeed, allergic asthma (33), has been associated with lower severity and reduced ACE2 receptor expression (29), which is crucial for the virus's entry into the host cell (34). Our study aligns with these findings, identifying asthma as a protective factor.

From a Public Health perspective, the pandemic underscored social and economic disparities in Mexico (35, 36), including social inequitable healthcare access. In Mexico, states with high marginalization indices are primarily located in the southern region (37). This study included fewer participants from these areas than from other regions yet observed higher lethality during the first wave (2.4% vs. 0.35% in the North, 0.51% in the Center, and 0.23% in the West). In this study, indigenous population served as a proxy for low socioeconomic status and was identified as a risk factor for pneumonia, intubation, and death. The highest proportion of the indigenous population was in the South, which is consistent with the region's highest mortality and lowest reporting rates. This trend is consistent with many authors highlighting the association between poverty and a worse disease outcome (38, 39). In this context, it is possible that the lower number of reported cases was due to the limited access to diagnosis and health services in these marginalized regions. Another observation is that most cases are concentrated in the central region of the country. This is due to Mexico City's large population, which, along with its metropolitan area, totals approximately 22,281,442 people (18% of the total population). Consequently, a high number of COVID-19 cases were recorded in this central region. Additionally, greater access to healthcare services and higher testing rates contributed to the increased case detection in this area.

Lastly, this study examined the lethality of different COVID-19 variants over time in Mexico, revealing significant differences in lethality across pandemic waves and in the demographic characteristics of deceased patients. Other studies in Mexico have noted that the frequency of pediatric cases of COVID-19 in different waves correlates with school closures and social distancing measures.

We observed that the first two waves exhibited higher lethality and lower incidence, whereas subsequent waves showed increased incidence in the pediatric population due to the transition from confinement to schools reopening in the third and fourth waves (36). Additionally, lower lethality was observed as new variants of SARS-CoV-2 emerged, consistent with our findings. This shift may reflect the progressive increase in vaccination coverage during the second and third waves, the development of natural immunity in the population, and the establishment of hybrid and herd immunity (38).

One study limitation was the use of a secondary database, which included significant underreporting. Nevertheless, this dataset represents the only available national information.

During the pandemic, adults, especially older adults, were prioritized for vaccination due to higher severe disease risk (39). However, as 95% of the population now has immunity through vaccination and/or infection (40), infants under 2 years are increasingly susceptible and more prone to severe disease and mortality and may benefit from vaccination as a public policy measure.

5 Conclusion

This study evaluated the impact of the COVID-19 pandemic on the pediatric population in Mexico, with the highest lethality and risk observed in children under two years. Despite an overall increase in cases over time, a decrease in severe cases and fatalities was observed, likely due to natural immunity and adult vaccination, both of which have altered the disease trajectory. However, chronic diseases remain critical; cardiovascular diseases, hypertension, and diabetes were the primary risk factors, with the greatest lethality peaking during the first wave, which was associated with the Wuhan-HU1 variant. Prevention strategies targeting susceptible individuals, particularly children without immunity to SARS-CoV2, should be considered in public policy through vaccination efforts.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Research and Ethics Committees of the Faculty of Medicine, Universidad Nacional Autónoma de México (FM/DI/093/2020). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

IT-C: Formal Analysis, Investigation, Data curation, Writing – original draft. FO-V: Formal Analysis, Investigation, Data curation, Writing – original draft. ID-T: Data curation, Formal Analysis, Investigation, Writing – original draft. AH-G: Formal Analysis, Investigation, Data curation, Writing – original draft. AV: Formal Analysis, Investigation, Methodology, Writing – review & editing, Data curation. MG-L: Investigation, Writing – review & editing. PB-C: Investigation, Writing – review & editing. MP-S: Investigation, Writing – review & editing, Data curation. LC-M: Data curation, Writing – review & editing. JD-R: Writing – review & editing, Data curation. RW-C: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

JD-R is a member of the Becton Dickinson, Merck Sharp & Dohme and Sanofi Pasteur speaker's bureau and served on the advisory board for Sanofi Pasteur. RW-C is a member of the Seegene, Reckitt, Asofarma, AstraZeneca and Sanofi Pasteur speaker's bureau and served on the advisory board for Sanofi Pasteur, Asofarma and AstraZeneca.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The early diagnostic value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in neonatal late-onset sepsis

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Purpose: The purpose of this study is to investigate the early diagnostic value of the neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and C-reactive protein (CRP) in neonatal late-onset sepsis (LOS), as well as to evaluate the combined diagnostic utility of these markers for the early detection of neonatal LOS.
Methods: The late-onset sepsis of newborns admitted to the neonatal intensive care unit of our hospital were retrospectively collected. 142 children with Late-Onset Sepsis (LOS) were selected as the LOS group, 50 neonates with systemic infection were selected as the systemic infection group, 50 neonates who underwent physical examination were selected as the non-systemic infection group. The differences of NLR, PLR, platelet-to-neutrophil ratio (PNR), and C-reactive protein (CRP), Procalcitonin among the three groups were compared.
Results: The levels of NLR and PLR in LOS group were significantly higher than those in systemic infection group and non-systemic infection group. The Receiver Operating Characteristic (ROC) curve result revealed that the area under ROC (AUC, Area Under Curve) of NLR for the diagnosis of LOS was 0.903. When the optimal cut-off value was 1.30, the sensitivity and specificity were 89.4% and 81.0%. The AUC of PLR for the diagnosis of LOS was 0.833. When the optimal truncation value was 57.86, the sensitivity and specificity were 92.3% and 68.0%. The AUC of CRP for the diagnosis of LOS was 0.876, and the sensitivity and specificity were 76.8% and 87.0% when the optimal cut-off value was 10.21 mg/dl. When NLR, PLR, and CRP were combined to diagnosis LOS, The AUC was 0.942, the sensitivity and specificity were 90.8% and 86.0%.
Conclusions: The levels of NLR and PLR in the LOS were higher, which have certain value in the early diagnosis of LOS, and combined with CRP can improve the diagnostic efficiency.

KEYWORDS

neonatal, diagnostic, LOS, NLR, PLR

1 Introduction

Neonatal sepsis (NS) is a systemic infection in newborns caused by pathogenic microorganisms entering the bloodstream through various routes (1, 2). When it occurs after 72 h of birth, it is classified as late-onset sepsis (LOS). NS is common in neonatal intensive care units (NICUs) and is a leading contributor to neonatal mortality. A 2018 epidemiological study across 12 countries reported an incidence of 2.2%, with a

mortality rate ranging from 11% to 19% (3, 4). This highlights NS as a critical global public health concern.

Neonatal sepsis (NS) can affect multiple organ systems, including the digestive, respiratory, circulatory, and hematologic systems, often presenting with nonspecific symptoms. This complexity makes early identification based on clinical signs challenging. Moreover, neonates' underdeveloped immune systems and immature organ structures contribute to their heightened susceptibility to infections. Without timely intervention, the infection can rapidly disseminate, progressing from asymptomatic to septic shock, disseminated intravascular coagulation, or even death. Therefore, early detection, accurate diagnosis, and prompt treatment are critical to reducing NS-related mortality. Although blood cultures are essential for detecting infection, sepsis is primarily a clinical diagnosis based on a life-threatening response to infection (5). Early detection of sepsis can be challenging, as blood cultures alone cannot confirm the presence of sepsis, and the diagnostic process relies on clinical criteria and biomarkers. Commonly used nonspecific markers, such as white blood cell count, offer suboptimal sensitivity and specificity. Additionally, emerging inflammatory markers like Interleukin 6 (IL-6), serum amyloid A, and CD64 face clinical limitations due to high costs and restricted detection conditions (6). Thus, the search for rapid, reliable, and specific biomarkers for NS diagnosis remains essential for improving clinical outcomes.

Recently, the NLR and PLR have been widely reported as reliable markers for various infectious diseases such as pneumonia and appendicitis. They have demonstrated value in the diagnosis, severity assessment, and prognosis of these diseases (7, 8). There have been reports indicating that NLR and PLR have good predictive roles in the diagnosis and assessment of adult sepsis (9). This suggests that NLR and PLR may also serve as predictive indicators for NS, providing a reference for early clinical diagnosis. In this study, we conducted a retrospective analysis of clinical data from 242 neonates, comparing the levels of NLR and PLR between those with LOS and those with systemic infections or non-infectious diseases. Moreover, these ratios were compared with the commonly used clinical marker CRP. The aim was to explore the potential application value of NLR and PLR in the early diagnosis of LOS.

2 Materials and methods

2.1 Sample collection

Retrospective data were collected from January 1, 2017, to December 31, 2020, at the Neonatal Intensive Care Unit of Lianyungang Hospital affiliated with Xuzhou Medical University. A total of 142 full-term neonates with LOS admitted during this period were selected as the LOS group. Additionally, 50 newborns with systemic infections admitted during the same period (presenting with signs of infection upon admission, with sepsis diagnosis excluded during hospitalization, including 38 cases of neonatal pneumonia and 12 cases of neonatal omphalitis) were chosen as the systemic infection group. Furthermore, 50 newborns undergoing outpatient examinations (either in neonatal outpatient clinics or pediatric health check-up clinics) were included as the non-systemic infection group.

2.1.1 Inclusion criteria

(1) LOS Group: Neonates aged between 7 and 28 days, diagnosed according to the diagnostic criteria for neonatal LOS established by the Neonatology Group of the Chinese Pediatric Society in 2019. The diagnosis requires clinical manifestations and positive blood culture results. If the blood culture identifies pathogenic bacteria, it must meet the criteria of two consecutive cultures yielding the same pathogenic strain or a single positive culture with elevated inflammatory markers, along with antibiotic treatment for 5 days or more. The pathogenic bacteria include, but are not limited to, Coagulase-negative staphylococci (CoNS), Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and other commonly recognized neonatal pathogens.
(2) Systemic infection Group: Neonates aged between 7 and 28 days with signs of infection upon admission, with sepsis diagnosis excluded during hospitalization.
(3) Non-systemic infection Group: Neonates aged between 7 and 28 days undergoing outpatient examinations at our hospital's neonatal outpatient clinics or pediatric health check-up clinics.
(4) Blood samples from all patients were collected on the day of admission, before the initiation of antimicrobial treatment, under aseptic conditions. Clinical data were complete.

2.1.2 Exclusion criteria

(1) Gestational age less than 37 weeks, age less than 7 days, or greater than 28 days.
(2) Newborns with genetic metabolic disorders, chromosomal diseases, or congenital developmental abnormalities.
(3) Newborns with concomitant immune system disorders, hematologic disorders, or impaired liver or kidney function.
(4) Newborns who received antimicrobial or antiplatelet drug therapy before blood sampling.
(5) Newborns with a history of maternal transfusion during delivery or postnatal transfusion.
(6) Positive blood culture without clinical evidence of sepsis, considered as specimen contamination in newborns.

2.2 Sample information

We collected general clinical data for all newborns during hospitalization, including age, gestational age, gender, birth weight, delivery method, and Apgar scores at 1 and 5 min. Laboratory test results, including neutrophil count, lymphocyte

Abbreviations
NS, NS; LOS, late-onset sepsis; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; CPR, C-reactive protein; PCT, procalcitonin; PNR, platelet-to-neutrophil ratio.

count, platelet count, CRP, and PCT, were obtained for all three groups of newborns. The NLR, PLR, and PNR were calculated. All blood samples for testing were collected within 30 min of admission, prior to the initiation of antimicrobial treatment. Additionally, blood samples for blood culture, complete blood count, CRP, and PCT tests were collected simultaneously and sent for analysis.

2.3 Statistical analysis

Statistical analysis was performed using SPSS 24.0 software. Normally distributed quantitative data are expressed as mean ± standard deviation (±s), while non-normally distributed data are presented as median (interquartile range) [M(P25, P75)]. For normally distributed data with homogeneity of variance among the three groups, one-way ANOVA with LSD *post-hoc* tests was used for pairwise comparisons. For data without homogeneity of variance, Welch's ANOVA was applied, followed by Games-Howell *post-hoc* comparisons. Non-normally distributed data were analyzed using the Kruskal–Wallis test for overall comparison, with pairwise comparisons conducted via the Bonferroni method. Categorical data are presented as [number (%)] and compared using appropriate statistical tests. Multiple logistic regression was employed to identify independent risk factors for neonatal late-onset sepsis (LOS). The diagnostic value of each marker for neonatal LOS was assessed using receiver operating characteristic (ROC) curves. A significance level of $P < 0.05$ was considered statistically significant.

3 Results

3.1 Comparison of general clinical data among the three groups of newborns

In the comparative analysis of newborns, various factors including age, gestational age, birth weight, gender, delivery method, 1 min Apgar score, and 5 min Apgar score were scrutinized across three groups: the LOS group, the systemic infection group, and the non-systemic infection group. The examination yielded no statistically significant differences in these parameters ($P > 0.05$), as meticulously outlined in Table 1.

3.2 The NLR, PLR, PNR, CRP, PCT among three groups

Examining the data presented in Table 2, the comparison among Neonatal LOS group, systemic infection group, and non-systemic infection group newborns revealed noteworthy trends. Specifically, NLR, PLR, PNR, CRP, and PCT exhibited higher values in the LOS group in comparison to both the systemic infection and non-systemic infection groups. Furthermore, these parameters were elevated in the systemic infection group compared to the non-systemic infection group, with these differences proving statistically significant ($P < 0.05$).

Pairwise comparisons further showed that PNR values were lower in the LOS group than in both the systemic infection and non-systemic infection groups, with these differences also statistically significant ($P < 0.05$). However, no significant difference was found between the systemic infection and non-systemic infection groups ($P > 0.05$), as shown in Table 3.

Further analysis of PCT highlighted statistically significant variances between the LOS group and the non-systemic infection group, as well as between the systemic infection group and the non-systemic infection group ($P < 0.05$). Interestingly, no statistically significant difference was identified between the LOS group and the systemic infection group ($P > 0.05$), as depicted in Figure 1.

3.3 Logistic regression analysis

To ascertain independent factors influencing neonatal LOS, a multiple logistic regression analysis was conducted. The results revealed that NLR, PLR, and CRP were independent risk factors for the occurrence of neonatal LOS, while PNR and PCT were not independent influencing factors for neonatal LOS (Table 4). These results contribute valuable insights into the specific markers that independently contribute to the risk profile of neonatal LOS, enhancing our understanding of the underlying factors associated with this condition.

TABLE 1 Comparison of general clinical data among the three groups of newborns.

Indicator	LOS group ($n = 142$)	Systemic infection group ($n = 50$)	Non-systemic infection group ($n = 50$)	χ^2	P
Age (Day)	10.89 ± 3.19	11.18 ± 2.16	10.34 ± 3.32	1.009	0.366
Gestational age (Week)	39.10 ± 1.11	39.39 ± 0.99	39.18 ± 1.04	1.301	0.274
Birth weight, (kg)	3.44 ± 0.44	3.52 ± 0.45	3.46 ± 0.57	0.568	0.567
Male [Number (%)]	84 (59.15)	30 (60.00)	26 (52.00)	0.896	0.639
Vaginal Delivery [Number (%)]	117 (82.39)	40 (80.00)	42 (84.00)	0.280	0.869
1 min Apgar score	9 (9,9)	9 (9,9)	9 (9,9)	3.549	0.170
5 min Apgar score	10 (10,10)	10 (10,10)	10 (10,10)	0.915	0.633
Oxygen saturation (%)	97.85 ± 1.65	98.02 ± 1.27	97.80 ± 1.50	0.304	0.738
Systolic blood pressure (mmHg)	75.32 ± 6.56	74.98 ± 5.47	76.82 ± 4.41	1.465	0.233
Diastolic blood pressure (mmHg)	46.65 ± 4.52	45.38 ± 4.64	46.10 ± 4.74	1.586	0.207
Heart rate (beats/min)	137.66 ± 10.37	139.58 ± 10.78	136.48 ± 8.01	1.237	0.292

TABLE 2 Comparison of NLR, PLR, PNR, CRP, and PCT among three groups.

Indicator	LOS group (n = 142)	Systemic infection group (n = 50)	Non-systemic infection group (n = 50)	F/H	P
NLR	2.41 ± 0.95	1.14 ± 0.83	0.69 ± 0.43	95.545	<0.001
PLR	76.30 ± 17.55	58.39 ± 18.97	47.38 ± 18.03	54.676	<0.001
PNR	31.74 (26.23,40.08)	64.91 (37.26,99.32)	72.73 (53.16,103.85)	77.501	<0.001
CRP	12.55 (10.32,25.45)	9.63 (7.42,10.65)	0.27 (0.12,0.52)	133.709	<0.001
PCT	0.50 (0.18,1.13)	0.35 (0.16,0.60)	0.10 (0.68,0.15)	76.745	<0.001

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein (mg/dl); PNR, platelet-to-neutrophil ratio; PCT, procalcitonin (ng/ml).

TABLE 3 Pairwise comparisons of NLR, PLR, PNR, CRP, and PCT among three groups.

Gropup	NLR	PLR	PNR	CRP	PCT
LOS group	2.41 ± 0.95	76.30 ± 17.55	31.74 (26.23,40.08)	12.55 (10.32,25.45)	0.50 (0.18,1.13)
Systemic infection group	1.14 ± 0.83	58.39 ± 18.97	64.91 (37.26,99.32)	9.63 (7.42,10.65)	0.35 (0.16,0.60)
P	<0.001	<0.001	<0.001	<0.001	0.131
LOS group	2.41 ± 0.95	76.30 ± 17.55	31.74 (26.23,40.08)	12.55 (10.32,25.45)	0.50 (0.18,1.13)
None infection group	0.69 ± 0.43	47.38 ± 18.03	72.73 (53.16,103.85)	0.27 (0.12,0.52)	0.10 (0.68,0.15)
P	<0.001	<0.001	<0.001	<0.001	<0.001
Systemic infection group	1.14 ± 0.83	58.39 ± 18.97	64.91 (37.26,99.32)	9.63 (7.42,10.65)	0.35 (0.16,0.60)
None infection group	0.69 ± 0.43	47.38 ± 18.03	72.73 (53.16,103.85)	0.27 (0.12,0.52)	0.10 (0.68,0.15)
P	0.008	0.002	0.363	<0.001	<0.001

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein (mg/dl); PNR, platelet-to-neutrophil ratio; PCT, procalcitonin (ng/ml).

3.4 The diagnostic value of NLR, PLR, CRP, and combined indicators for neonatal LOS

Based on the results of the multiple regression analysis, Receiver Operating Characteristic (ROC) curves were constructed (Figure 2) to assess the predictive value of markers for neonatal late-onset sepsis (LOS). The Area Under the Curve (AUC) for NLR was 0.903, with an optimal cutoff of 1.30, achieving a sensitivity of 89.4% and specificity of 81.0%. For PLR, the AUC was 0.833, with an optimal cutoff of 57.86, yielding a sensitivity of 92.3% and specificity of 68.0%. CRP demonstrated an AUC of 0.876, with an optimal cutoff of 10.21 mg/L, resulting in a sensitivity of 76.8% and specificity of 87.0%. When combining NLR, PLR, and CRP, the AUC increased to 0.942, with sensitivity of 90.8% and specificity of 86.0%, as shown in Table 5.

4 Discussion

Neonatal sepsis, particularly late-onset sepsis, continues to be a leading cause of morbidity and mortality in neonates, despite advances in neonatal care. Early diagnosis remains a critical challenge, and biomarkers such as the NLR and PLR have emerged as potential diagnostic tools. In this study, we focused on the diagnostic value of NLR and PLR in neonatal LOS, demonstrating their role as independent risk factors and providing new insights into their utility when combined with traditional inflammatory markers like CRP and PCT.

Elevated NLR has been identified as a significant marker in various forms of neonatal sepsis, with several studies pointing to its potential as an early diagnostic tool. Our study further supports this conclusion, finding that NLR was significantly higher in the LOS group compared to both systemic infection and non-systemic infection groups ($P < 0.05$). This reflects heightened neutrophil activity and lymphocyte depletion, both indicative of a robust inflammatory response in the context of neonatal sepsis. Our findings align with prior studies, such as those by C.D. Russell et al. in adult sepsis and E. Tamelytė et al. in pediatric sepsis, where elevated NLR was shown to correlate with disease severity and prognosis (10, 11).

A key contribution of our study is the specific focus on neonatal LOS, an area that has received less attention in the literature compared to early-onset sepsis (EOS). By examining the role of NLR in the diagnosis of LOS, we provide new evidence to support its diagnostic potential in this distinct population. Our results demonstrate that NLR is not only an early diagnostic marker but also an independent risk factor for neonatal LOS, as confirmed by multiple regression analysis. The ROC analysis, showing an AUC of 0.903 with sensitivity of 89.4% and specificity of 81.0%, further highlights NLR's strong diagnostic performance, comparable to findings in other studies on sepsis.

Interestingly, changes in NLR have also been reported in other co-infectious conditions, such as viral hepatitis. Xiao-Mao Li et al. have demonstrated that in patients with viral hepatitis, both NLR and PLR can also reflect the degree of inflammation and immune response (12, 13). In viral hepatitis, elevated NLR is often associated with more severe disease and worse prognosis, particularly in the setting of acute liver failure or chronic hepatitis. These findings underscore the broader relevance of NLR and PLR in various inflammatory and infectious diseases, further supporting their potential as universal markers of inflammation and immune dysfunction. However, the diagnostic

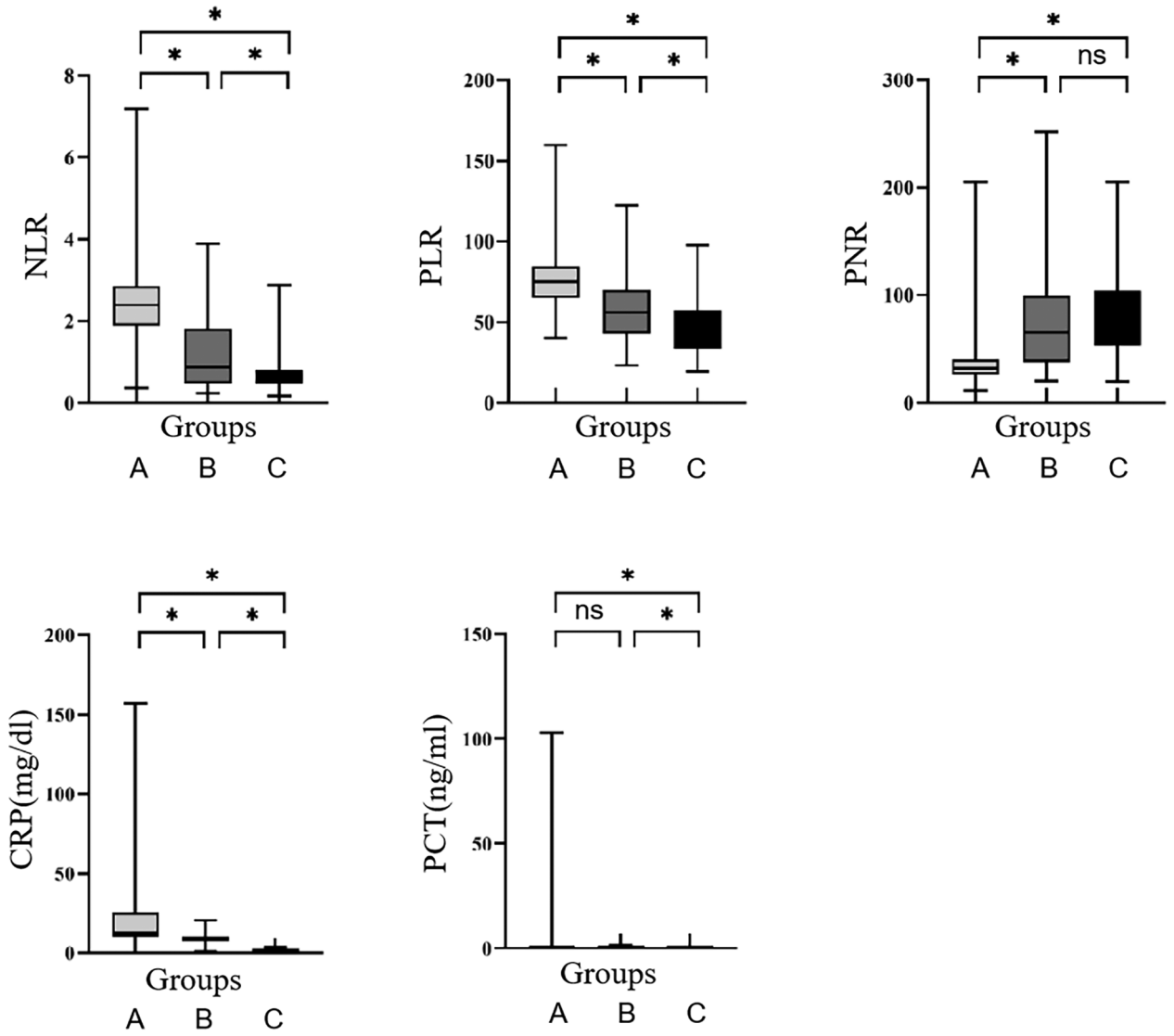

FIGURE 1
Comparison of NLR, PLR, PNR, CRP, and PCT among the three groups. **(A)** LOS group; **(B)** systemic infection group; **(C)** non-systemic infection group. *$P < 0.05$, ns, no significance.

TABLE 4 Multiple logistic regression analysis of the independent factors influencing the occurrence of neonatal LOS.

Independent variable	*β*	Wald	OR value	95%*CI*	*P*
NLR	1.431	9.022	4.184	1.644–10.647	0.003
PLR	0.036	5.352	1.037	1.006–1.069	0.021
CRP	0.173	17.055	1.189	1.095–1.291	<0.001
PNR	0.003	0.173	1.003	0.988–1.020	0.678
PCT	0.605	1.363	1.832	0.663–5.057	0.243

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein (mg/dl); PNR, platelet-to-neutrophil ratio; PCT, procalcitonin (ng/ml).

thresholds and their specific roles in viral infections may differ from those in bacterial sepsis, as viral infections typically involve more complex immune responses, including altered lymphocyte subsets and cytokine profiles.

In addition to NLR, we explored the diagnostic utility of PLR in neonatal sepsis. Our study found that PLR was significantly elevated in the LOS group compared to the systemic infection and non-systemic infection groups ($P < 0.05$), suggesting its potential role as an inflammatory marker. PLR has been previously linked to disease severity in adult sepsis and has shown promise in predicting outcomes (14–17). In our study,

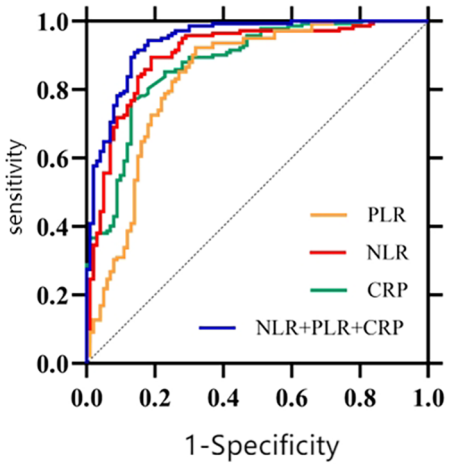

FIGURE 2
ROC curves for diagnosing neonatal LOS using NLR, PLR, CRP, and combined indicators.

logistic regression analysis identified PLR as an independent risk factor for neonatal LOS, with an AUC of 0.833, sensitivity of 92.3%, and specificity of 68.0%.However, while PLR showed promising diagnostic potential, our study also found that PNR did not serve as an independent risk factor for neonatal LOS, indicating its limited utility in this context. This suggests that, although platelet activation is central to inflammation and coagulation, PNR may not offer significant added value in the diagnosis of neonatal LOS, at least compared to NLR.

One of the major strengths of our study is the integrated approach of combining traditional inflammatory markers such as CRP and PCT with NLR and PLR. While CRP and PCT are widely used in clinical practice, they have limitations in early diagnostic accuracy, particularly for neonatal sepsis. Our study found that CRP was significantly elevated in the LOS group compared to the systemic infection and non-systemic infection groups ($P < 0.05$), confirming its diagnostic value as an independent risk factor for neonatal LOS (AUC = 0.876). PCT also showed elevated levels in the LOS and systemic infection groups compared to non-infected neonates ($P < 0.05$), though no significant difference was observed between the LOS and systemic infection groups.

Logistic regression further indicated that PCT was not an independent factor for neonatal LOS, highlighting the limitations of PCT as a sole diagnostic tool. However, when CRP was combined with NLR and PLR, the diagnostic efficacy was significantly enhanced, with an AUC of 0.942. This combined approach offers a novel contribution to the field, as it improves upon the diagnostic performance of individual markers. The integration of multiple biomarkers could provide a more robust tool for early and accurate detection of neonatal LOS, which is essential for improving clinical outcomes.

An important aspect of our study is the comparison of diagnostic criteria for neonatal sepsis between China and international standards. Our study followed the criteria established by the Neonatology Group of the Chinese Pediatric Society, which primarily relies on clinical manifestations and common inflammatory markers like CRP and PCT. However, international guidelines, such as the Phoenix Consensus, adopt a more comprehensive approach, incorporating multi-dimensional assessments including clinical scoring systems (e.g., pSOFA), organ dysfunction, and additional biomarkers like IL-6, IL-8, and PCT (5).

While our study demonstrated the significant diagnostic potential of NLR and PLR under the Chinese criteria, the broader, more dynamic diagnostic framework of the Phoenix Consensus may result in different performance for these markers. The international guidelines place greater emphasis on early organ dysfunction and inflammation beyond just neutrophil and platelet counts. This could potentially affect the relative weight and diagnostic thresholds of NLR and PLR in different clinical settings.

Despite the promising results of NLR and PLR, recent advancements in sepsis diagnostics have introduced a variety of novel biomarkers that could complement or even enhance these traditional indicators. New screening tools such as Presepsin (sCD14-ST), PTX3, nCD64, and Monocyte Distribution Width (MDW) have shown potential in improving sepsis detection (18–21). Among these, the LIP score, which incorporates biomarkers like lymphocyte count, INR, and PCT, is particularly noteworthy. Although the LIP score has shown promise in adult populations, its application to neonatal sepsis remains to be validated (22). The inclusion of these novel biomarkers alongside traditional ones could provide a more comprehensive diagnostic approach, offering both sensitivity and specificity in neonatal sepsis diagnosis (23). Future studies comparing the diagnostic performance of the LIP score and other emerging tools with the markers explored in our study would be beneficial in refining neonatal sepsis diagnostic protocols and improving clinical decision-making.

In conclusion, our study provides novel insights into the diagnostic value of NLR and PLR in neonatal LOS. These markers, when combined with traditional inflammatory

TABLE 5 Diagnostic value of NLR, PLR, CRP, and combined indicators for neonatal LOS.

Indictor	AUC	Optimal cutoff	Sensitivity (%)	Specificity (%)	95% *CI*	*P*
NLR	0.903	1.30	89.4	81.0	0.862–0.944	<0.001
PLR	0.833	57.86	92.3	68.0	0.776–0.889	<0.001
CRP	0.876	10.21	76.8	87.0	0.831–0.920	<0.001
NLR + PLR + CRP	0.942	-	90.8	86.0	0.913–0.971	<0.001

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein (mg/dl); PNR, platelet-to-neutrophil ratio; PCT, procalcitonin (ng/ml).

markers like CRP and PCT, offer a promising approach to early and accurate diagnosis, which is critical for improving clinical outcomes. Our findings specifically address neonatal LOS, an area with less extensive research compared to early-onset sepsis. We believe that the integrated use of NLR, PLR, and traditional markers enhances diagnostic efficacy and may ultimately lead to more targeted interventions for neonates with suspected sepsis. As the field of sepsis diagnostics continues to evolve, future research will be essential to explore the role of emerging biomarkers and refine diagnostic protocols for neonatal sepsis.

5 Limitations

Despite the promising results, this study has several limitations. Firstly, it was a single-center, retrospective study, which may limit the generalizability of the findings to broader populations. Future multi-center, prospective studies with larger sample sizes are needed to validate these results and assess their applicability across different neonatal care settings. Secondly, while we focused on a range of biomarkers, there may be other inflammatory markers or biomarkers that could further enhance diagnostic accuracy but were not included in this study. Additionally, the lack of follow-up data on long-term outcomes in neonates with LOS means that we could not evaluate the prognostic value of these markers in predicting clinical outcomes such as survival rates or neurological development. Lastly, although NLR and PLR were identified as independent risk factors for LOS, the underlying biological mechanisms contributing to these changes were not fully explored, and further research into the pathophysiological role of these markers is warranted.

6 Conclusions

This study highlights the significant diagnostic value of the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in the early detection of neonatal late-onset sepsis (LOS). The ROC analysis revealed that NLR had an AUC of 0.903, with a sensitivity of 89.4% and specificity of 81.0%, while PLR demonstrated an AUC of 0.833, with a sensitivity of 92.3% and specificity of 68.0%. These results underscore the potential of NLR and PLR as reliable biomarkers for LOS diagnosis. Additionally, the combination of NLR, PLR, and CRP further improved diagnostic performance, yielding an AUC of 0.942. This combination may enhance clinical decision-making in identifying neonatal LOS at an early stage.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by Lianyungang Clinical Medical College of Nanjing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

QY: Conceptualization, Data curation, Investigation, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. JY: Conceptualization, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. LS: Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. QZ: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. WX: Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Supplementary material

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Biomarkers associated with the diagnosis and prognosis of *Mycoplasma pneumoniae* pneumonia in children: a review

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Community-acquired pneumonia (CAP) is a major cause of death in children, and *Mycoplasma pneumoniae* (MP) is the main pathogen of CAP in children in China. Although *Mycoplasma pneumoniae* pneumonia (MPP) is usually a self-limiting disease, many children develop multiple complications due to drug resistance or untimely diagnosis and treatment, and may even progress to severe MPP or refractory MPP with a poor prognosis. It is important to explore the value of biomarkers that can be used in clinical practice to assess the severity of pneumonia and assist in clinical decision making. In this article, we searched the literature in the last four years to review the roles of various types of biomarkers in MPP and the associated clinical predictive models, with the aim of helping pediatricians to understand the evaluation indexes related to MPP in children other than microbiology.

KEYWORDS

biomarker, predictive model, *Mycoplasma pneumoniae*, child, diagnosis, prognosis

1 Introduction

Pneumonia is a serious global child health problem and the leading cause of death in children. *Mycoplasma pneumoniae* (MP) is the main pathogen causing community-acquired pneumonia in children aged 5 years and over (National Health Commission of the People's Republic of China, 2023). According to statistics, the incidence of community-acquired pneumonia in children caused by MP infection in China is 15%-37%, with the highest incidence in the 5-10 age group (Tsai et al., 2021). Especially in the two years following the COVID-19 outbreak, the incidence of MPP has shown a clear upward trend in many countries (Dumke, 2024; Dungu et al., 2024). *Mycoplasma pneumoniae* pneumonia (MPP) possesses a degree of self-limiting characteristics, and the prognosis for treatment is typically favorable. However, some children experience a poor response to standard treatment and develop into refractory MPP (RMPP). Particularly following the COVID-19 pandemic, macrolide antibiotic-resistant MP infections have become more prevalent, resulting in an annual increase in the incidence of RMPP. Such children are

prone to a combination of multiple intrapulmonary or extrapulmonary conditions, which may even lead to death in severe cases (Xu et al., 2024). Therefore, the early diagnosis of MPP and the accurate understanding of the condition are particularly important. The initial clinical symptoms in children with MPP are nonspecific. MP culture is the gold standard for diagnosis of the disease, with demanding and time-consuming conditions, while MP nucleic acid and antibody tests are more commonly used in clinical practice. However, there is a lack of sensitive markers for assessing the severity and prognosis of the disease. As our understanding of MPP has deepened in recent years, many biomarkers have been considered that are associated with the early recognition, determination of severity, and prognostic assessment of MPP. Moreover, with the advancement of Internet-based information technology, a range of predictive models has emerged. The application of these biomarkers can assist in optimizing clinical treatment decisions and reducing the incidence of poor prognoses. In this paper, we conducted a search in both English databases (such as PubMed) and Chinese databases (such as CNKI) using the keywords ("*Mycoplasma pneumoniae*" or "*Mycoplasma pneumoniae* infection" or "*Mycoplasma pneumoniae* pneumonia") and ("children" or "adolescents" or "pediatrics") and ("diagnosis" or "prognosis" or "severity" or "differentiation" or "value" or "biomarker"). We focused on biomarker literature related to MPP published between January 2021 and December 2024, carefully screening and organizing the results. Some important related articles published before the screening time limit were also included. This article mainly discusses 6 aspects: cell-based markers, protein-based markers, cytokine-based markers, nucleic acid-based markers, imaging-based markers, and multi-indicator joint predictive models.

2 Cell-based markers

2.1 Lymphocyte-associated ratio

In MPP children without bacterial infection, white blood cell (WBC) levels usually do not change significantly, mostly manifested as an increase in the percentage and count of neutrophils and monocytes, and a decrease in the percentage and count of lymphocytes (Zheng and Zhuo, 2021). Platelet is also involved in the immune response of the body after MP infection, resulting in increase in quantity and volume. In recent years, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), monocyte-lymphocyte ratio (MLR), mean platelet volume-to-lymphocyte ratio (MPVLR) have been recognized as important markers to reflect the immune-inflammatory status of the body. They are related to the prognosis of various types of tumors, cardiovascular and cerebral vascular diseases, and diabetes mellitus (Yang et al., 2021; Zhang et al., 2021; Sun and Li, 2022; Li et al., 2024b). In children with MPP, the levels of NLR, PLR, MLR, and MPVLR were significantly elevated, which were positively correlated with the severity of the disease and the pathological changes of lungs (He et al., 2024; Pei and Luo, 2024; Qiu, 2024; Wang and Shen, 2024; Wang et al., 2024). The high levels of NLR, as well as PLR, MLR, and MPVLR, suggested strong inflammatory reactions and platelet activation. In clinical practice, these indicators can be combined and observed to assist in the diagnosis of MPP and to determine the severity of the disease. In the identification of pathogens, Chen et al (Chen et al., 2024). compared a series of peripheral blood parameters between children with MPP and influenza. They found that PLR levels were significantly higher in children with influenza than in children with MPP. This suggests that PLR may have certain value in differentiating MPP from influenza. In addition, Chu et al (Chu et al., 2024). found that lymphocyte percentage (Lym%) (cut-off value: 22.1%) and neutrophil percentage (Neu%) (cut-off value: 65.2%) were effective in differentiating MPP and influenza A infections, and platelet distribution width (PDW) was effective in differentiating MPP and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, with a threshold of 15%. In terms of the prognosis of MPP, NLR, PLR and MPVLR are independent predictors of poor prognosis such as severe MPP (SMPP) and RMPP (Li et al., 2023; Jiang and Liu, 2024; Mu et al., 2024; Wang and Shen, 2024). For children with MPP over 6 years old, children with NLR >3.92 or MPVLR >5.29 are more likely to progress to RMPP, and the accuracy of prediction is higher than that of C reactive protein (CRP) (Ling et al., 2021).

In conclusion, as a basic test program, blood routine related indexes provide a new basis for early differential diagnosis of MPP and identification of children with high-risk prognosis. However, the use of test results at what point in the course of the disease as a reference and the associated thresholds need to be further investigated to determine. In particular, it is not known whether infants and preschool children, whose Neu% and Lym% vary from time to time, have different thresholds than older children.

2.2 Lymphocyte subset

Lymphocyte subsets are mainly related to the immune response of the body after MP infection, which is mainly characterized by a decrease in the levels of $CD3^+$ and $CD4^+$ as well as an increase in the levels of $CD8^+$ and $CD19^+$. Moreover, the levels of $CD3^+$, $CD4^+$, and CD4+/CD8+ were shown to be lower in the acute phase than in the recovery phase, and lower in severe than in mild of MPP (Jin et al., 2021; Jiang et al., 2022; Kong, 2022; Song, 2022; Wang and Gong, 2024). For early identification of RMPP, the predictive value of $CD4^+$ count was superior to $CD3^+$, $CD19^+$, $CD56^+$ and $CD4^+/CD8^+$, but the specific diagnostic cut-off value varied in different studies. In the study of Li et al (Li et al., 2019), $CD4^+ < 599.89$ cells/μL has a high predictive value for RMPP, with sensitivity as high as 90% and an areas under curve (AUC) of 0.900 [95% confidence interval (95%CI): 0.852-0.948]. The cut-off value of $CD4^+$ obtained by Yao et al (Yao et al., 2023). was relatively high, which was 1370 cells/μL, and the sensitivity was 85.94%. The specific and reliable cut-off value needs to be further studied and confirmed. Among T lymphocytes, both T helper cell 17 (Th17) and regulatory T cells (Treg) are differentiated from $CD4^+$ T lymphocytes, the former mainly exerts pro-

inflammatory effects through the secretion of IL-17, while the latter mainly exerts immunosuppressive effects to reduce the body's immunity to MP through IL-10. With the progression of MPP, Th17/Treg, $CD3^+CD56^+$ showed a significant increase (Cao et al., 2022). In addition, children with MPP usually have varying degrees of decreased lung function. In infants and young children, there is a risk of developing asthma if not treated timely (Wang, 2023; Ha et al., 2024). It was shown that the levels of forced expiratory volume in one second (FEV1) and forced expiratory volume in one second/forced vital capacity (FEV1/FVC) in children with MPP were positively correlated with $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and negatively correlated with $CD3^-CD19^+$, $CD19^+CD23^+$. When $CD3^+$ <50.38%, $CD4^+$ <41.78%, or $CD8^+$ >28.60%, children with RMPP had a higher probability of developing plasticoid bronchiolitis (PB), and the sensitivity of combining these three indicators to diagnose PB in children with RMPP was 87.50% (Wang and Wang, 2022). It can be seen that lymphocyte subsets are closely related to the onset and progression of MPP, and the severity of MPP children can be effectively evaluated by monitoring specific lymphocyte levels (Figure 1).

3 Protein-based markers

3.1 Acute phase protein

CRP, serum ferritin (SF), and serum amylase A (SAA) are all acute phase proteins originating from the liver. When the body is stimulated by MP infection, activated immune cells (such as macrophage and monocyte) release interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), and these inflammatory factors reach the liver through blood circulation. IL-6 can bind to hepatocyte membrane receptors, activate the signal transducer and activator of transcription 3 pathway, and promote the expression of CRP and SAA genes. IL-1β and TNF-α can activate the nuclear factor kappa-light-chain-enhancer of activated B cells pathway to promote the expression of SAA gene. In addition, IL-6 and IL-1β can not only directly induce ferritin synthesis in hepatocytes, but also elevate intracellular ferritin levels by regulating iron metabolism (Mosquera-Sulbaran et al., 2021; Gehrer et al., 2023; Chang et al., 2025).

Among the three, SAA rises the most earliest, usually within 3-6 h, and the magnitude of the rise is larger than that of CRP. And it can be rapidly reduced to normal levels after the antigen was cleared. This makes it a sensitive indicator to reflect the infection and recovery of the organism (Sack, 2020). In children with pneumonia due to infection by different pathogens, the elevation of SAA levels in those with MP infection falls between that of viral and bacterial infections; furthermore, using 247.56 mg/L as the diagnostic threshold for MPP yields a sensitivity of up to 90.0% (Pan et al., 2024). In children with MP infection, changes in SAA levels positively correlate with $CD8^+$ and negatively correlate with $CD4^+$. SAA >203.56 mg/L had a sensitivity of 86.7% and a specificity of 83.3% for diagnosing MP infection, with an AUC of up to 0.924 (Jiang et al., 2022).

CRP generally begins to increase 6-8 h after infection, peaks within 48 h, and lasts longer than SAA during the inflammatory process (Sproston and Ashworth, 2018). "The guideline for the diagnosis and treatment of MPP in children (2023)" pointed out that CRP began to increase significantly after 3 days of fever in SMPP, and the degree of increase was positively correlated with the severity of the disease (National Health Commission of the People's Republic of China, 2023). It was found that the level of CRP was lower in the MP group than in the bacterial group (Zhu and Guo, 2024). CRP =16.91 mg/L can be used as a cut-off value to differentiate MPP from bacterial pneumonia with a sensitivity of

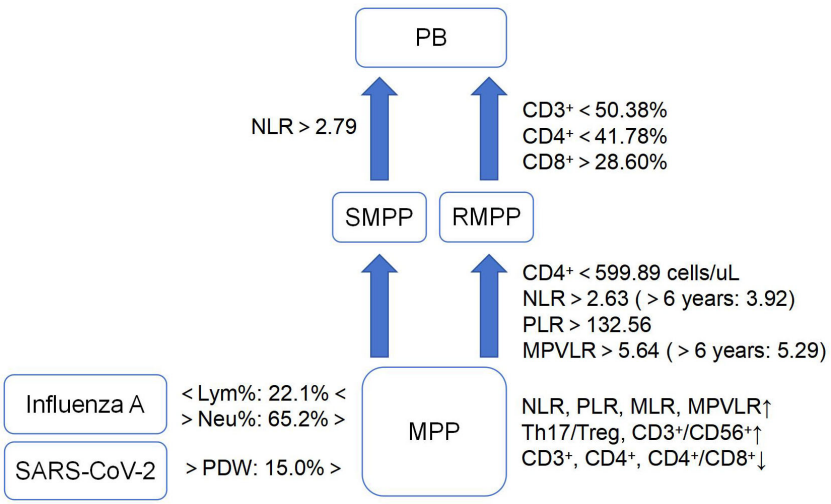

FIGURE 1
Characteristics of cell-based markers in MPP and its different prognoses.(MPP, *Mycoplasma pneumoniae* pneumonia; SMPP, severe MPP; RMPP, refractory MPP; PB, plasticoid bronchiolitis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; MLR, monocyte-lymphocyte ratio; MPVLR, mean platelet volume-to-lymphocyte ratio; Lym%, lymphocyte percentage; Neu%, neutrophil percentage; PDW, platelet distribution width; Th17/Treg, T helper cell 17/regulatory T cell). ↑, increase; ↓, decrease.

75.8% and a specificity of 85.7% (Luo and He, 2024). However, it is difficult to perform a definitive identification of pathogens by applying CRP alone. The time of CRP elevation maybe advance and the magnitude of the elevation increases when MPP is combined with bacterial infection (Tan et al., 2024). Chen et al (Chen et al., 2024; Wang et al., 2024). found that CRP was a biomarker for predicting RMPP and SMPP, with cut-off values of 39.34 mg/L and 33.56 mg/L, respectively. Wang et al (Wang et al., 2024). divided the children with SMPP into two groups according to whether lung tissue necrosis occurred. They found that CRP >67.5 mg/L on days 6-10 of the disease course could identify the subtype of pulmonary necrosis early, with a sensitivity of 96% and a specificity of 89%. A study investigated the regression time of imaging lesions of 399 children with lobar pneumonia caused by MP infection, and found that the regression time of children with CRP ≥25.92 mg/L often exceeded 2 months (Zheng et al., 2024). In addition, CRP ≥12.27 mg/L and 76.73 mg/L were also independent risk factors for mucus plug formation in children with MPP and for pulmonary thrombosis in children with SMPP, respectively (Zhang et al., 2021; Yu et al., 2024). Meanwhile, CRP ≥137 mg/L can be used to predict the occurrence of PB in children with RMPP (Liu et al., 2024). In summary, CRP has limited value in identifying MP from pathogens such as bacteria and viruses, but has good value in assessing and predicting the severity of MPP.

Compared to CRP and SAA, SF has a delayed response, generally rising 24-48 h after infection and lasting for a long time. Wei et al (Wei et al., 2024). showed that SF was positively correlated with the severity of MPP. The increase in SF of children with RMPP is greater than that of children with general MPP (GMPP), and it can reflect the degree of pulmonary inflammation and tissue damage (Fu et al., 2022). Wen et al (Wen et al., 2021). showed that SF >329.01 ng/mL predicted RMPP with a specificity of 93.13% and an AUC of 0.90 (Figure 2).

3.2 Lactic dehydrogenase

LDH is a glycolytic enzyme that exists in almost all major organs of the body. When inflammatory damage occurs in lung tissue due to MP infection, LDH enters the blood with cell division or cell damage, resulting in an increase in the level of LDH (Yan et al., 2021). The magnitude of the increase in LDH is positively correlated with the severity of MPP (Wang et al., 2023a). LDH >354 U/L can predict the occurrence of SMPP in children (Qiu et al., 2022). LDH >379 U/L as the diagnostic cut-off value for RMPP has a sensitivity of 66.67% and a specificity of 93.91% (Chen et al., 2024). Wen et al (Wen et al., 2021). also achieved similar results. The regression of lung lesions in such children often took longer, which may take more than 8 weeks (Zheng et al., 2024). For children with macrolide-resistant mutations in macrolide-unresponsive MPP (MUMPP), LDH ≥399 U/L suggested that the risk of the children progressing to RMPP will increase significantly (Cheng et al., 2024). In terms of complications in MPP, LDH of 393.0 U/L can predict the occurrence of necrotizing pneumonia (NP) in children with MPP, with sensitivity and

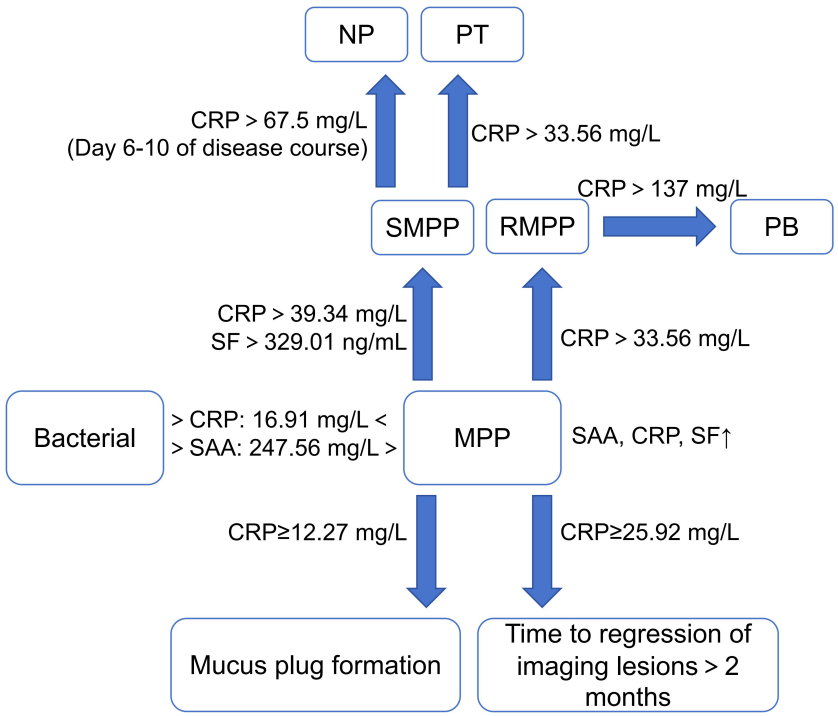

FIGURE 2
Characteristics of acute phase protein in MPP and its different prognoses. (MPP, Mycoplasma pneumoniae pneumonia; SMPP, severe MPP; RMPP, refractory MPP; NP, necrotizing pneumonia; PT, pulmonary thrombotic; PB, plasticoid bronchiolitis; SAA, serum amylase A; CRP, C reactive protein; SF, serum ferritin). ↑, increase.

specificity of 68.3% and 86.2%, respectively. And as the LDH continued to rise, the risk of NP in children with MPP will continue to increase (Ren et al., 2024). LDH >462.65 U/L (Zhang et al., 2021) and >482 U/L (Zhong et al., 2021) were independent risk factors for mucus plug formation and PB in children with MPP, respectively. In addition, a high level of LDH is also an independent risk factor for SMPP combined with bronchiolitis obliterans (BO) (Zheng et al., 2022). In terms of treatment, LDH is significantly increased in children with RMPP requiring steroid hormone therapy, and the LDH level at the time of the child's visit can be used to preliminarily determine whether the child with MPP needs to apply steroid hormones and the optimal dose (Wei et al., 2024). At the same time, when the child's LDH reaches 1,114 U/L, it also prompts that the child may require oxygen inhalation at present, and the child's vital signs need to be evaluated in time (Lee and Choi, 2022). It can be seen that similar to CRP, LDH has good value and high sensitivity in predicting severe cases and serious complications of MPP as well as guiding clinical treatment.

3.3 D-dimer

As a specific degradation product of fibrin, D-dimer mainly reflects the function of fibrinolytic system. When MP infects the organism, local tissue ischemia and hypoxia cause damage to vascular endothelial cell and collagen exposure, which will also activate the human fibrinolytic system and complement system, resulting in an increase in D-dimer. The clinical symptoms of MPP in children with high D-dimer levels are more serious and require a longer treatment cycle, which may be closely related to the severity of lung inflammation after MP infection (Zheng et al., 2021). Qiu et al. (2022) showed that D-dimer was a better predictor of SMPP than CRP and LDH in a study of 786 children with MPP. Children with D-dimer >0.31 mg/L (although it may be within the normal range) are more likely to have pleural effusion. And when D-dimer >0.40 mg/L, there is a high risk of SMPP. In addition, D-dimer >2.10 mg/L also has a clear diagnostic value for RMPP, with a specificity of 81.86% (Wen et al., 2021). Another study also found that D-dimer >7.33 mg/L was significantly related to MP-positive in pleural effusion. The clinical symptoms of such children are more serious than those with MP-negative in pleural effusion (Li et al., 2024).

In terms of complications in MPP, children with SMPP and RMPP should be alert to thrombosis if D-dimer is significantly increased (Fu et al., 2023). The further study showed that D-dimer >3.98 mg/L can be used as an independent predictor for pulmonary thrombotic (PT) in children with SMPP, with sensitivity and specificity higher than 90% and an AUC of 0.95 (Yu et al., 2024). "The guideline for the diagnosis and treatment of MPP in children (2023)" also indicated that chest pain and/or hemoptysis in children with MPP accompanied by D-dimer ≥5 mg/L will help to diagnose PT (National Health Commission of the People's Republic of China, 2023). In addition, the significantly increased D-dimer correlates with NP in children with SMPP. D-dimer >3.705 mg/L can independently predict MPP combined with NP, with an AUC of 0.865. And the time to regression of imaging lesions in such children may be >3 months (Li et al., 2023; Luo et al., 2023). In conclusion, it can be seen that in children with MPP, D-dimer is mainly used for the prediction of PE and the assessment of the severity of MPP.

3.4 Procalcitonin

PCT is a calcitonin peptide substance secreted by thyroid C cells, consisting of 116 amino acid factors, and is a common systemic inflammatory biomarker. Severe pneumonia caused by bacteria usually has a significant increase in WBC, CRP and PCT in the early stage of the disease (Yin and Mo, 2022). Thus PCT serves as a specific biomarker of bacterial infection, whereas it is commonly used to identify bacterial and nonbacterial infections. Ruan et al (Ruan et al., 2024). compared the clinical characteristics of 506 children with MPP and 311 children with *Streptococcus pneumoniae* pneumonia (SPP), both MPP and SPP children had an increase in PCT. And the increase in children with SPP was greater. The reason may be that MP has lipopolysaccharide and endotoxin effects similar to Gram-negative bacteria, which induce macrophages and monocytes to secrete PCT. Some scholars also compared the level of PCT in children with pneumonia caused by bacteria, MP, and virus, and found that the magnitude of the elevation in the three groups was bacterial > MP > virus. PCT >0.605 ng/mL can effectively diagnose MPP (Pan et al., 2024). Among children with MPP, Jiang et al (Jiang et al., 2022). found that the level of PCT in the acute phase was higher than that in the recovery phase and in healthy children. And the sensitivity and specificity of 1.12 ng/mL as the diagnostic cut-off value of MP infection were higher than 70%. Meanwhile, PCT is also positively correlated with the severity of MPP, and severe cases than mild cases (Weng et al., 2022; Wang et al., 2024). As a common inflammatory marker, it can be seen that the main value of PCT in MPP is the early differential diagnosis of MPP, but the diagnostic thresholds of identifying pathogens need to be determined by further studies with large sample sizes.

In addition, albumin (Alb) and prealbumin (PA) have also been shown to be associated with the progression of MPP. Both are mainly produced by the liver. In addition to maintaining pH and colloid osmolality, Alb also has anti-inflammatory, antioxidant, and anticoagulant effects. Compared with Alb, the half-life of PA is relatively short and it mainly transports thyroxine and vitamin A. The sensitivity of PA to predict infection is higher than that of Alb (Ranasinghe et al., 2022). Deng et al (Deng et al., 2023). found that the level of Alb levels in children with MPP was negatively correlated with MP-DNA, IL-6, IL-10, TNF-α and INF-γ. PA ≤144.5 mg/L in children with MPP is an independent predictor of mucus plug formation (Zhang et al., 2021) (Figure 3).

4 Cytokine-based markers

4.1 Interleukin

IL is a group of small signal molecules secreted by activated immune cells and some non-immune cells. They are an important

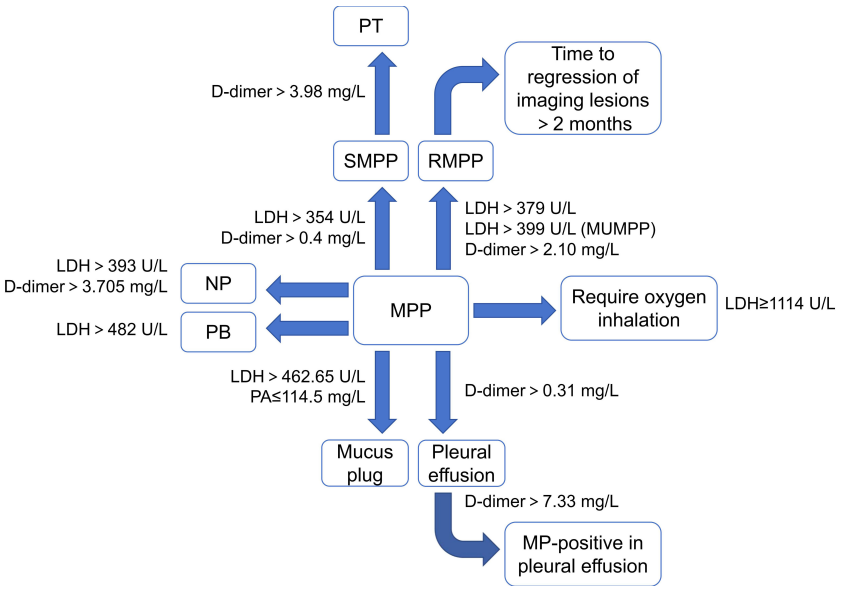

FIGURE 3
Characteristics of other protein-based markers in MPP and its different prognoses. (MPP, Mycoplasma pneumoniae pneumonia; SMPP, severe MPP; RMPP, refractory MPP; NP, necrotizing pneumonia; PT, pulmonary thrombotic; PB, plasticoid bronchiolitis; LDH, lactic dehydrogenase; PA, prealbumin).

part of humoral immunity and cellular immunity. After MP infection, they exert their biological effects by binding to specific receptors on the membrane of target cells and are closely related to the synthesis of biomarkers such as CRP (Linge et al., 2022). Abnormally elevated IL-25 and IL-33 were present in children with MPP, and both were negatively correlated with the children's lung function-related indices such as tidal volume and time to peak tidal expiratory flow/total expiratory time ratio (Wang et al., 2022). Children with SMPP tend to have lower levels of IL-2 and higher levels of IL-6, IL-8, IL-10, and IL-17 than children with GMPP (Han et al., 2024; Leerach et al., 2024; Wang et al., 2024). The levels of IL-6 and IL-10 in bronchoalveolar lavage fluid (BALF) were also significantly higher in children with a high MP-DNA load than in those with a low MP-DNA load (Deng et al., 2023). In the comparison of macrolide-resistant MPP (MRMPP) and macrolide-sensitive MPP (MSMPP), the levels of IL-13 and IL-33 in the MRMPP group were several times higher than those of MSMPP (Wu et al., 2021). Studies have shown that IL-2, IL-6, and IL-10 are independent risk factors for SMPP (Han et al., 2024; Wang et al., 2024). IL-2 >57 pg/mL and IL-6 >55.835 pg/mL as the diagnostic cut-off values for SMPP had high sensitivity and specificity, with AUC of 0.923 and 0.874, respectively (Han et al., 2024). IL-17α ≥13.4 pg/mL and IL-18 ≥472.0 pg/mL could also independently predicted RMPP, with AUC of 0.81 and 0.85, respectively (Gao et al., 2023). IL-8 >2721.33 pg/mL can independently predict the occurrence of PB in children with MPP (Zhong et al., 2021). In addition, MP infection can cause airway hyperresponsiveness, which carries the risk of developing into bronchial asthma if the treatment is not timely. Such children often have higher levels of IL-6 (Shi et al., 2022).

MP combined with viral infection is also common in clinical practice. A study showed that IL-5, IL-6, and IL-10 in serum and BALF of children with adenovirus-infected pneumonia are higher than those of children with MPP (Wei et al., 2024). In MPP combined with adenoviral infection, IL-4, IL-6, IL-8, and IL-10 were significantly increased, with more pronounced elevations in severely ill children. The AUC of IL-6, IL-8, IL-10, and IL-17α combined to predict co-infection with adenovirus in children with SMPP was 0.802 (Yi et al., 2024). A significant increase in IL-2 and a significant decrease in IL-12 occur in MP combined with Epstein Barr virus (EBV) infection, while high IL-2 and low IL-12 are independent risk factors for poor prognosis in such children. The AUC for IL-2 >3.265 ng/L and IL-12 <13.895 ng/L combined to predict a poor prognosis for these children was up to 0.915 (Hao, 2024). However, the trend of IL-2 changes in this study seems to be inconsistent with other studies (Han et al., 2024). Therefore, the level characteristics of IL-2 in children with MPP still need further research to be verified (Figure 4).

4.2 Chemokine

The process of inflammatory response in MPP is closely related to the migration and recruitment of immune cells (such as T-lymphocytes, neutrophils, and eosinophils) to the inflammation site. It is inseparable from chemokines. Chemokines mainly include two categories: receptors and ligands. Chemokine ligands associated with MP infection include eotaxin, Regulate and activate normal T cell expression and secretion factors (RANTES), C-C motif chemokine ligand 17 (CCL17), C-C motif chemokine ligand 2

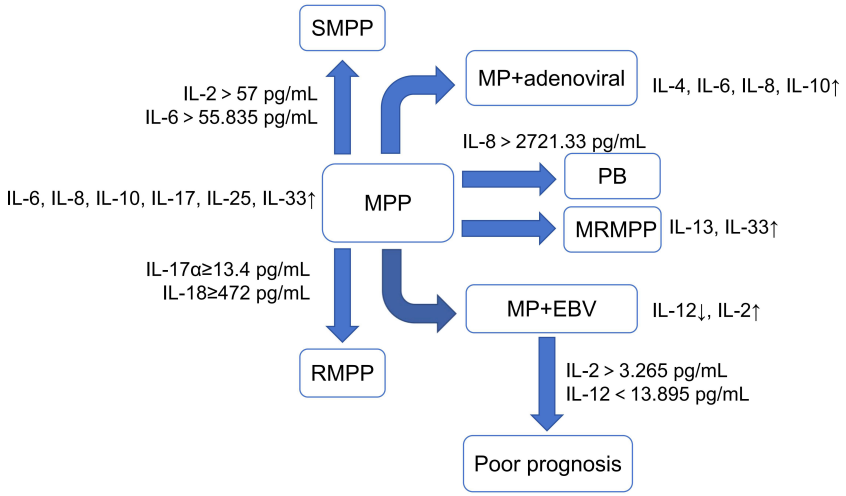

FIGURE 4
Characteristics of Interleukin in MPP and its different prognoses. (MPP, Mycoplasma pneumoniae pneumonia; SMPP, severe MPP; RMPP, refractory MPP; PB, plasticoid bronchiolitis; MRMPP, macrolide-resistant MPP; EBV, Epstein Barr virus). ↑, increase; ↓, decrease.

(CCL2). Among them, eotaxin mainly tends to bring eosinophils closer to the inflammation site and release eosinophil cationic protein. It will not only directly damage the airway epithelial cells and cause airway hyperresponsiveness, but also induces the release of cysteinyl leukotrienes. Then it triggers airway smooth muscle contraction, increases vascular permeability, and ultimately leads to airway remodeling (Maskey et al., 2022). A study showed that the level of eotaxin in children with MPP was abnormally elevated and the lung function-related indicators of children decreased with the increase of eotaxin (Wang et al., 2022). RANTES can bind to receptors on the surface of WBC (such as T lymphocytes, monocytes) to achieve activation and thus regulate immune responses. MP can induce the production and secretion of RANTES on small airways, which is closely related to asthma due to MP infection (Dakhama et al., 2003). And the degree of elevated RANTES is positively correlated with the severity of the disease (Xu et al., 2024). Zhang et al (Zhang et al., 2023). found that RANTES was an independent risk factor for RMPP, and that 3.74 ng/mL as the diagnostic threshold was able to predict the development of RMPP. CCL17 mainly selectively activates Th2 cells. Zhao et al (Zhao et al., 2024). found that the higher the level of CCL17, the more severe the condition of children with MP infection. The sensitivity and specificity of CCL17>53.51ng/L as the diagnostic cut-off value for MP infection were 69.01% and 87.50%, respectively, with an AUC of 0.813. CCL2 is similar to RANTES in that both chemotaxis a variety of white blood cells to recruit to sites of inflammation. A study showed that CCL2 in serum generally does not change significantly in children with MPP, but it was significantly elevated in BALF. And 0.645 ng/mL as the diagnostic threshold for CCL2 in BALF to predict RMPP had an AUC of 0.94 (Zhu et al., 2023).

The main chemokine receptor associated with MP infection is C-X-C chemokine receptor type 2 (CXCR2), which is mainly expressed on the surface of neutrophils and macrophages. It has been demonstrated that the mean fluorescence intensity of CXCR2 in children with MP is significantly higher than that in healthy children. And it is positively correlated with the severity of the disease (Chen et al., 2022). However, no research has yielded a specific diagnostic cut-off value.

4.3 Hepatocyte growth factor

Currently, HGF has been confirmed as a protective factor of lung tissue, mainly derived from endothelial cells, alveolar macrophages, fibroblasts, and activated lung neutrophils, with important roles in lung tissue development and lung injury repair (Sang and Qiao, 2024). Previous studies (Liu et al., 2020; Yuan et al., 2021) have shown that HGF is an independent risk factor for SMPP. The sensitivity and specificity of HGF ≥1169.20 pg/mL to predict SMPP were 88.46% and 61.22%, respectively. And through the dynamic monitoring of HGF, it was found that HGF can rapidly decrease to the normal range when the disease was relieved. So it can be used to evaluate the effect of clinical treatment in order to adjust the treatment plan timely. However, there are fewer correlation studies between HGF and MPP. And the sample sizes involved in these two studies are limited. The specific diagnostic cut-off value still needs to be confirmed by further research.

4.4 Autotaxin

Autotaxin is a secretory glycoprotein originating from liver tissue with lysophospholipase D activity, which is mainly involved in cell proliferation, cell migration, lipid metabolism, angiogenesis and inflammation (Nikitopoulou et al., 2021). The study showed that the level of autotaxin in serum and BALF of children with RMPP was positively correlated with the degree of inflammation. The level of autotaxin in children with RMPP is significantly higher than that of GMPP, and showed a trend of being significantly higher

in the acute phase than in the recovery phase. The sensitivity and specificity of diagnosing RMPP were >80% with 14.25 mg/L and 10.86 mg/L as the diagnostic thresholds of serum autotaxin and BALF autotaxin respectively (Fu et al., 2022).

5 Nucleic acid-based markers

5.1 microRNA

miRNA is non-coding RNA with a length of 19-25 nucleotides. Studies have confirmed that miRNA is involved in almost all biological processes of cellular activities, including cell proliferation, migration, inflammation, differentiation and apoptosis (Gan et al., 2023). Li et al (Li et al., 2024a). showed that miR-34a was highly expressed in children with MPP, especially in SMPP. And it was a risk factor for poor recovery and its AUCs for recognizing MPP, distinguishing between mild and severe disease, and predicting poor recovery were 0.873, 0.788, and 0.821, respectively. miR-492, miR-223, miR-155, miR-1323, and miR-23a are also significantly upregulated in children with MPP. Among them, the expression levels of miR-223, miR-155, and miR-1323 are positively correlated with the severity of the disease. Overexpression of miR-492 can induce macrophages to secrete IL-6, thus playing a role in the inflammatory response of MPP. Overexpression of miR-23a is closely related to airway hyperresponsiveness caused by MP infection, while miR-1323 is an independent risk factor for RMPP complicated with mixed respiratory virus infection. The AUCs of miR-23a, miR-223, and miR-155 for predicting MP infection are 0.728, 0.772, and 0.850, respectively, with high sensitivity and specificity (Shi et al., 2022; Jia et al., 2023; Ren et al., 2023; Xu et al., 2024; Zhao et al., 2024). This suggests that inhibiting the expression of miR-34a, miR-492, and other miRNAs may serve as a clinical means to alleviate disease progression in severe or refractory cases of MPP. Conversely, serum levels of miR-29c and miR-146a are significantly downregulated in children with MPP. The combined diagnosis of MPP using these two miRNAs yields an AUC of 0.966, with a sensitivity of 94.6% and a specificity of 89.2% (Wang et al., 2023). Furthermore, overexpression of miR-146a in alveolar macrophages has been shown to reduce levels of proinflammatory cytokines. The high level of miR-146a is associated with the suppression of inflammatory cascade reactions after lung infection and improved survival rates (Yoshikawa et al., 2024; Zhao et al., 2024). Additionally, miR-146a can serve as a biomarker for predicting treatment response (Wang et al., 2024).

5.2 Genetic polymorphism

Recent researches have found that the genetic polymorphisms of various immune and inflammation-related factors are associated with the susceptibility and prognosis of MP. Orosomucoid 1-like 3 (ORMDL3) is a gene located on chromosome 17, containing 2 introns and 3 exons. It encodes a transmembrane protein located on the endoplasmic reticulum membrane, regulating Ca^{2+} concentration, which leads to the unfolded protein response and subsequently induces inflammatory reactions (Guo et al., 2022). Liu et al (Liu et al., 2024). reported that the GG genotype at the rs4794820 locus and the TT genotype at the rs7216389 locus of the ORMDL3 gene may be potential factors contributing to severe MP infection-induced asthma. The apolipoprotein E (ApoE) gene, located on chromosome 19, encodes a protein consisting of 299 amino acid residues. Besides its involvement in lipid metabolism and cholesterol transport, ApoE is also associated with immune response, antioxidation, and other processes (Khalil et al., 2021). The IL-8 gene, located on chromosome 4, harbors multiple single nucleotide polymorphism loci (Hodeib et al., 2021). According to research conducted by Ni et al (Ni et al., 2023), both the CC genotype and allele C of the ApoE gene at the rs429358 locus, as well as the AA genotype and allele A of the IL-8 gene at the rs4073 locus, can influence the susceptibility to MPP and its prognosis by regulating protein expression.

6 Imaging-based markers

MPP often manifests as mild symptoms accompanied by severe lung imaging changes. Therefore, more intuitive lung imaging data also serves as a crucial biomarker for diagnosing MPP and assessing its severity. The length and area of lung parenchymal lesions measured by lung ultrasound can be used to assess the extent of lesions in children with MPP (Guo et al., 2023). Furthermore, there is a correlation between lung imaging findings and serological indicators. According to one study (Wang et al., 2021), children with MPP exhibiting interstitial pneumonia on imaging tend to have more pronounced changes in CRP, while those with lung consolidation show a more significant increase in D-dimer and fibrinogen levels. Huang et al (Huang et al., 2024). analyzed the clinical manifestations and outcomes of MPP children with different imaging features, finding that compared to bronchopneumonia, children with consolidation/atelectasis often exhibit severe abnormalities in clinical manifestations and laboratory indicators. These children also tend to have poorer outcomes, being more prone to developing RMPP, NP, and BO. Additionally, MPP children who have already developed NP may retain imaging manifestations of atelectasis even after treatment (Hou et al., 2025). Besides conventional imaging, CT radiomics has begun to be studied by many scholars. This approach involves extracting a large amount of high-throughput information from medical images through deep analysis, which radiologists often cannot identify or quantify visually. Both Wang et al (Wang et al., 2022). and Li et al (Li et al., 2025). employed methods like SelectKBest and Lasso to screen radiomic features. These radiomic features, when combined, demonstrate high accuracy in distinguishing between bacterial pneumonia and MPP. Moreover, the CT score represents a unique way of presenting CT image results. Lu et al (Lu et al., 2024). determined the CT score based on the degree of ground-glass opacity changes in the lung lobes, assigning specific weight ratios to different CT findings (such as ground-glass opacity, paving stone sign, and consolidation). The

results indicate that the CT score has a certain predictive value for RMPP, albeit with limited sensitivity and specificity. Another study utilized AI to quantify lung lesion volumes on CT images, which has been initially proven as a potential biomarker for RMPP (Qian et al., 2024). Among various CT image presentation methods, the CT score is easy to implement, whereas CT radiomics and AI-based CT image quantification are more challenging to promote in clinical settings due to their complex processing workflows.

Furthermore, as previously mentioned, children with MPP, especially infants, often exhibit airway hyperreactivity and varying degrees of lung function alterations. Lung ventilation imaging stands as an effective tool to assess the pulmonary function of these patients. There are three primary methods of lung ventilation imaging: radionuclide lung ventilation imaging, computed tomography lung ventilation imaging, and MRI lung ventilation imaging. Compared to the other two methods, MRI lung ventilation imaging offers advantages such as higher spatial resolution and the absence of radiation harm (Peiffer et al., 2024). In December 2022, the Food and Drug Administration approved the use of hyperpolarized (HP) ^{129}Xe gas as the first group of HP MRI contrast agents for lung ventilation imaging. However, the slow production speed, high cost, and incompatibility with low-field MRI scanners of HP ^{129}Xe gas have hindered its widespread use, driving the development and validation of a series of proton-HP gases. Currently, validated and feasible options include HP butane gas (Ariyasingha et al., 2024b) and hyperpolarized diethyl ether gas (Ariyasingha et al., 2024a). Although they are not yet widely used in clinical settings, their characteristics of mass producibility, affordability, and compatibility with any MRI scanner suggest that they will facilitate the application of magnetic resonance lung ventilation imaging in MPP in the future.

7 Multi-indicator joint predictive models

Although some indicators have demonstrated good applicability, the combined use of multiple indicators in clinical practice can significantly improve diagnostic sensitivity and specificity (Table 1), enabling a more accurate understanding of the patient's condition. Clinical prediction models based on big data and multiple algorithms (such as logistic regression and random forests) further assist medical professionals in making better clinical decisions (Table 2).

7.1 Early differential diagnosis models for MPP

The study on early differential diagnosis model of MPP primarily focuses on distinguishing it from viral infections. Lin et al (Lin et al., 2025). conducted a retrospective review of 264 children with MPP and 72 children with viral pneumonia. Through least absolute shrinkage and selection operator (Lasso) regression analysis and Logistic regression analysis, they identified four factors: TNF-α/IL-10, age, IL-8, and PCT, to construct a predictive model. After analysis, the predictive model demonstrated a good accuracy in distinguishing between viral pneumonia and MPP, with a C-index of 0.878 and an AUC of 0.875. Guo et al (Guo et al., 2023). primarily used peripheral blood parameters as variables, incorporating five independent predictors: age, fever, PCT, WBC, Lym, and eosinophil (Eos). Both the training and validation sets achieved an AUC greater than 0.8. Zeng et al (Zeng et al., 2024). developed a predictive model based on age, gender, erythrocyte sedimentation rate (ESR), and D-dimer, which also demonstrated excellent performance in terms of discrimination, calibration, and clinical application value. Some scholars have further refined the scope of differentiation by constructing differential diagnosis models between MP and adenovirus, influenza virus, and COVID-19. Among them, the prediction model with age, severe pneumonia, bilateral pneumonia, ground-glass attenuation, consolidation, atelectasis, CRP, and LDH as the main variables achieved an AUC of 0.866 (95%CI: 0.831-0.901) for distinguishing adenovirus from MP (Zhang et al., 2022). The prediction model using lymphocyte count (Lym#), platelet count (Pla#), eosinophil percentage (Eos%), monocyte percentage (Mon%), high fluorescence intensity cells, and PLR as the primary variables yielded an AUC of 0.995 for discriminating between influenza virus and MP (Chen et al., 2024). And after normalization, this model has the best efficacy among all models. Lastly, the prediction model focusing on age, CRP, IL-6, and PCT as key variables resulted in an AUC of 0.80 (95%CI: 0.69-0.91) for differentiating COVID-19 from MP (Zhou et al., 2024). In addition, the clinical definition of MUMPP is that those who show no improvement or even further deterioration in their condition after 72 h of regular treatment with macrolide antibiotics. By assessing such patients upon admission using a predictive model, early identification of MUMPP can be achieved, which is beneficial for improving early treatment efficacy. Rao et al (Rao et al., 2024). developed a MUMPP prediction model based on the highest temperature before admission, pleural effusion, neutrophil count (Neu#), CRP, and PCT, which demonstrated excellent predictive performance.

7.2 Predictive models for SMPP

Based on MP-IgM, Eos%, eosinophil count (Eos#), ESR, and PA, Chang et al (Chang et al., 2022). developed a nomograph prediction model with an AUC of 0.777 for predicting SMPP. Zhang et al (Zhang et al., 2024). constructed a prediction model with relatively better predictive performance (AUC=0.867) based on age, albumin to globulin ratio (AGR), NLR, CRP, ESR, mean platelet volume (MPV), comorbid infections, pleural effusion, primary disease, duration of fever, and wheezing. Additionally, they established an online dynamic nomogram to facilitate clinical application. Li et al (Li et al., 2024). built a nomogram prediction model for SMPP based on the age, decreased breath sounds, respiratory rate, duration of fever, hospital stay, incidence of mixed infection, SF, and LDH of 1332 MPP children. The predictive performance of this model was similar to that established by Zhang et al (Zhang et al., 2024).

TABLE 1 Diagnostic efficacy of multi-indicator joint application.

Reference	Case number	Indicators	Diagnosis	AUC	95%CI	Sensitivity	Specificity
(He et al., 2024)	248	SAA, CRP, NLR, PLR	SMPP	0.874	0.801-0.928	82.61%	79.73%
(Wang and Gong, 2024)	320	$CD3^+$, $CD4^+$, $CD8^+$, $CD4^+$/$CD8^+$, IL-8, IL-10, IL-13, IFN-*y*	MPP	0.924	0.935-0.972	94.76%	90.48%
(Yao et al., 2023)	128	MP-DNA, $CD4^+$	RMPP	0.828	0.752-0.904	96.88%	68.75%
(Wang and Wang, 2022)	129	$CD3^+$, $CD4^+$, $CD8^+$	PB	0.862	0.774-0.950	87.50%	71.43%
(Shi et al., 2022)	106	miR-23a, FeNO, IL-6	MPP with airway hyperresponsiveness	0.840	0.771-0.904	86.84%	72.06%
(Yi et al., 2024)	201	CRP, PCT, LDH, Neu%, D-dimer	SMPP	0.977	0.955-0.999	96.00%	93.00%
(Yi et al., 2024)	201	IL-6, IL-8, IL-10, IL-17a	SMPP	0.802	0.719-0.885	89.80%	47.30%
(Hao, 2024)	495	IL-2, IL-12	poor prognosis	0.915	0.858-0.971	85.10%	84.30%
(Yuan et al., 2021)	105	LDH, D-dimer, HGF	SMPP	0.941	0.886-0.997	80.77%	97.96%
(Chen et al., 2022)	118	CD162, CDCR2, CDCR4	SMPP	0.881	0.801-0.961	88.90%	83.30%
(Zhang et al., 2023)	70	IL-6, RANTES	RMPP	0.824	0.688-0.959	79.41%	83.33%
(Zhao et al., 2024)	143	miR-223, miR-155, CCL17	MPP	0.941	0.889-0.974	88.73%	88.89%
(Ren et al., 2023)	100	miR-1323, IL-6	MP+viral infection	0.902	0.826-0.952	86.00%	86.00%
(Wang et al., 2023)	93	miR-29c, miR-146a	MPP	0.966	–	94.60%	89.20%
(Liu et al., 2024)	172	CX3CL1, CD40L, TGF-β1	poor prognosis	0.900	0.812-0.956	80.77%	87.04%

7.3 Predictive models for RMPP

Cheng et al (Cheng et al., 2020). used the LASSO regression model to determine optimal predictors (LDH, Alb, Neu%, hyperthermia) and constructed a nomogram predicting RMPP with an AUC of 0.884 (95%CI: 0.823-0.945). Shen et al (Shen and Sun, 2024). found that days of fever, pleural effusion, and levels of WBC, Neu#, LDH, CRP, NLR, and serum uric acid (SUA) were independent predictors of RMPP. Based on the criteria of fever lasting over 10.5 days, presence of pleural effusion, WBC $>10.13\times10^9$ cells/L, Neu# $>6.43\times10^9$ cells/L, CRP >29.45 mg/L, LDH >370.50 U/L, NLR >3.47, and SUA <170.5 μmol/mL, they constructed a prediction model for RMPP. The average AUC of the nomogram was 0.956 (95%CI: 0.937-0.974). Age, duration of fever, Lym#, D-dimer, and lung imaging score were used as variables to construct a predictive nomogram, and the AUC for predicting RMPP was 0.907 (Li et al., 2023). Based on this, Pei et al (Pei and Luo, 2024). incorporated additional indicators such as body mass index (BMI), WBC, Neu#, Pla#, NLR, PLR, CRP, and PCT, which increased the AUC of the model for predicting RMPP to 0.963 (95% CI: 0.946–0.981). Combining clinical practice, literature research, and regression analysis, Shen et al (Shen et al., 2022). constructed a predictive model using CRP, LDH, and D-dimer as predictors, achieving an AUC of 0.881 (95%CI: 0.843–0.918) for predicting RMPP. Liu et al (Liu et al., 2022). based on CRP and LDH, integrated lung ultrasound-related indicators such as pleural effusion and consolidation size/body surface area (BSA), resulting in an AUC of 0.955 (95%CI: 0.919–0.978) for predicting RMPP.

7.4 Predictive models for severe comorbidities in MPP

The formation of mucus plugs in the airways of children with MPP is closely associated with RMPP. Luan et al (Luan et al., 2023). developed a nomogram prediction model based on age, pleural effusion, D-dimer, and plasma IFN-*y*, which can accurately predict the formation of bronchial mucus plugs, facilitating timely removal via bronchoscopy. Without timely intervention, it is prone to progress into bronchiolitis obliterans (BO). A regression model constructed with variables such as hospital stay, duration of fever, atelectasis, Neu%, peak LDH, peak CRP, SF, and PaO_2/FiO_2, has an AUC of 0.904 for predicting the occurrence of BO, with a sensitivity and specificity of 88% and 83%, respectively (Liu et al., 2024). The accumulation of mucous plugs can also lead to the occurrence of PB. For the prediction of PB, Zhao et al (Zhao et al., 2022). identified 6 variables through Lasso regression, including peak body temperature, Neu%, PLT, IL-6, LDH, and atelectasis, as important predictors for constructing a nomogram. The average AUC of this nomogram was 0.813 (95%CI: 0.769-0.856). Zhang et al (Zhang et al., 2023). achieved a higher predictive performance (AUC=0.944) with a nomogram constructed based on pre-

TABLE 2 Predictive models for joint application of multiple indicators.

Reference	Online predictive models web site	Target for projections	Indicators	Case number	training set		validation set	
					AUC	95% CI	AUC	95% CI
(Guo et al., 2023)	https://zhxylxy0160128.shinyapps.io/Nomogram/	Virus vs MP	age, fever, PCT, WBC, Lym#, Eos#	792	0.859	0.764-0.954	0.820	0.671-0.969
(Zeng et al., 2024)	–	COVID-19 vs MP	age, gender, ESR, D-dimer	590	0.858	0.827-0.888	0.794	0.729-0.859
(Chen et al., 2024)	https://dxonline.deepwise.com/prediction/index.html?baseUrl=%2Fapi%2F&id=42468&topicName=undefined&from=share&platformType=wisdom	Influenza vs MP	Lym#, Eos, HFC, PLT, PLR, Mon%	423	0.995	–	0.893	–
(Rao et al., 2024)	–	MUMPP	the highest temperature before admission, Neu#, CRP, PCT, pleural effusion	224	0.825	0.755-0.894	0.828	0.729-0.928
(Zhang et al., 2024)	https://ertongyiyuanliexiantu.shinyapps.io/SMPP/	SMPP	age, AGR, NLR, CRP, ESR, MPV, coinfection, pleural effusion, primary disease, fever days ≥ 7, wheeze	526	0.876	0.840-0.913	0.839	0.755-0.924
(Li et al., 2024)	–	SMPP	age, decreased sounds of breathing, SF, LDH, incidence of co-infection, respiratory rate, fever duration, days of hospital-stay	1332	0.862	0.839-0.886	–	–
(Shen et al., 2022)	–	SMPP	CRP, LDH, D-dimer	299	0.881	0.843-0.918	0.777	0.661-0.893
(Shen and Sun, 2024)	–	RMPP	fever duration, pleural effusion, WBC, NEP, CRP, LDH, NLR, SUA	369	0.956	0.937-0.974	–	–
(Liu et al., 2022)	–	RMPP	CRP, LDH, pleural effusion, consolidation size/BSA	90	0.955	0.919-0.978	0.916	0.838-0.964
(Cheng et al., 2020)	–	RMPP	LDH, Alb, Neu%, hyperthermia	219	0.884	0.823-0.945	0.881	0.807-0.955
(Li et al., 2023)	–	RMPP	age, fever duration, Lym#, D-dimer, and radiological imaging change	517	0.907	–	0.964	–
(Pei and Luo, 2024)	–	RMPP	BMI, fever duration, WBC, Neu#, CRP, NLR, PLR	338	0.963	0.946-0.981	–	–
(Luan et al., 2023)	–	airway mucus plug	age, pleural effusion, D-dimer, Plasma IFN-γ	263	0.817	0.747-0.889	–	–
(Liu et al., 2024)	–	BO	days of hospital-stay, fever duration, pulmonary hypotension, Neu%, highest LDH, SF, highest CRP, PaO_2, PaO_2/FiO_2, pleural effusion, et al.	116	0.904	0.874-0.936	0.823	0.776-0.878

(Continued)

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TABLE 2 Continued

Reference	Online predictive models web site	Target for projections	Indicators	Case number	training set		validation set	
					AUC	95% CI	AUC	95% CI
(Zhang et al., 2023)	–	PB	cough duration, presence of fever before bronchoscopy, extrapulmonary complications, pleural effusion, LDH	120	0.944	0.779-0.962	–	–
(Zhao et al., 2022)	–	PB	peak body temperature, Neu%, PLT, IL-6, LDH and pulmonary atelectasis	547	0.813	0.769-0.856	0.895	0.847-0.943
(Jia et al., 2024)	https://ertong.shinyapps.io/DynNomapp/	pulmonary consolidation	age, fever duration, Lym#, CRP, SF, $CD8^+$ T lym%, CD^{4+} T lym%	491	0.902	0.871-0.933	0.883	0.809-0.956
(Xie et al., 2024)	–	PT	NLR, IL-6, D-dimer, pleural effusion, NP	175	0.912	0.871-0.952	–	–
(Luo and Wang, 2023)	–	NP	MP + bacterial infection, chest pain, LDH, CRP, D-dimer, fever	252	0.870	0.813-0.927	0.843	0.757-0.930
(Wang et al., 2023b)	–	Whether BAL treatment is needed	fever duration, CRP, D-dimer, pleural effusion	202	0.915	0.827-0.938	0.983	0.912-0.996

bronchoscopy fever, extrapulmonary complications, pleural effusion, cough duration, and LDH. Children with MPP who are older, have a long duration of fever, decreased Lym#, elevated CRP, elevated SF, increased percentage of $CD8^+$ T lymphocytes, and decreased percentage of $CD4^+$ T lymphocytes are more likely to develop pulmonary consolidation. A dynamic nomogram model constructed using these variables to predict pulmonary consolidation has an AUC of 0.902 (95%CI: 0.871-0.933) (Jia et al., 2024). Pulmonary necrosis and pulmonary embolism are both serious complications of MPP. Luo et al (Luo and Wang, 2023). constructed a predictive nomogram for pulmonary necrosis using variables such as MP combined with bacterial infection, chest pain, LDH, CRP, duration of fever, and D-dimer. The validation of this model has demonstrated good clinical applicability. Xie et al (Xie et al., 2024). through multivariate logistic regression analysis, found that NLR, IL-6, CRP, LDH, D-dimer, pulmonary necrosis, pleural effusion, and pericardial effusion are all risk factors for embolism in children with RMPP. The nomogram prediction model based on these factors has high accuracy in predicting the risk of embolism.

7.5 Predictive models for early bronchoscopic intervention in MPP

"The guideline for the diagnosis and treatment of MPP in children (2023)" stated that bronchial alveolar lavage (BAL) should be performed as early as possible for MPP children suspected of having mucus plug obstruction and PB to reduce the occurrence of complications (National Health Commission of the People's Republic of China, 2023). Early BAL can help prevent MPP from progressing to SMPP or reduce the severity of SMPP (Wu et al., 2023). Li et al (Li et al., 2024). found that fever duration ≥6.5 days before bronchoscopy, CRP ≥20.94 mg/L, LDH ≥461.5 U/L, and pleural effusion are risk factors for MPP children requiring bronchoscopic intervention. By assigning scores to different factors, when the total score is ≥6 out of 10, the tendency for bronchoscopic intervention is >80%. The higher the score, the greater the likelihood of bronchoscopic intervention. Currently, clinicians often rely solely on imaging to determine whether MPP children should undergo BAL. Atelectasis is a strong indicator for bronchoscopic intervention, but many MPP children with bronchial mucus plugs show lung consolidation on imaging. Wang et al (Wang et al., 2023b). developed a predictive model that assesses the probability of BAL for MPP children with lung consolidation based on fever duration, CRP, D-dimer, and pleural effusion. The external validation AUC is as high as 0.983 (95%CI: 0.912-0.996).

To more accurately and objectively compare the effectiveness of different prediction models, we standardized the case numbers across the 20 prediction models included in this study. Specifically, we converted the case numbers for each model into relative proportions (the percentage of each model's case number relative to the total case number across all models). Since the AUC is already a standardized value, it can be used directly. Considering that clinical prediction models need to balance both sample

TABLE 3 Ranking of the effectiveness of different prediction models.

Reference	Target for projections	case number	AUC	Standardized case number	Comprehensive score (weighting 5:5)	ranking
(Chen et al., 2024)	Influenza vs MP	423	0.995	5.36%	0.5243	1
(Li et al., 2024)	SMPP	1332	0.862	16.89%	0.5155	2
(Pei and Luo, 2024)	RMPP	338	0.963	4.29%	0.5029	3
(Shen and Sun, 2024)	RMPP	369	0.956	4.68%	0.5014	4
(Li et al., 2023)	RMPP	517	0.907	6.56%	0.4863	5
(Liu et al., 2022)	RMPP	90	0.955	1.14%	0.4832	6
(Luo and Wang, 2023)	pulmonary consolidation	491	0.902	6.23%	0.4821	7
(Guo et al., 2023)	Virus vs MP	792	0.859	10.04%	0.4797	8
(Zhang et al., 2023)	PB	120	0.944	1.52%	0.4796	9
(Zhang et al., 2024)	SMPP	526	0.876	6.67%	0.4714	10
(Wang et al., 2023b)	Whether BAL treatment is needed	202	0.915	2.56%	0.4703	11
(Xie et al., 2024)	PT	175	0.912	2.22%	0.4671	12
(Zeng et al., 2024)	COVID-19 vs MP	590	0.858	7.48%	0.4664	13
(Shen et al., 2022)	SMPP	299	0.881	3.79%	0.4595	14
(Liu et al., 2024)	BO	116	0.904	1.47%	0.4594	15
(Cheng et al., 2020)	RMPP	219	0.884	2.78%	0.4559	16
(Luo and Wang, 2023)	NP	252	0.870	3.20%	0.4510	17
(Zhao et al., 2022)	PB	547	0.813	6.94%	0.4412	18
(Rao et al., 2024)	MUMPP	224	0.825	2.84%	0.4267	19
(Luan et al., 2023)	airway mucus plug	263	0.817	3.34%	0.4252	20

representativeness and model discrimination ability, we set the weighting ratio to 5:5. We then calculated a comprehensive score for each model (comprehensive score = 50% × AUC + 50% × standardized case number) and ranked them accordingly (Table 3). In a study conducted by Shi et al (Shi et al., 2022), they precisely evaluated the effectiveness of a biomarker by comparing the 95%CI of biomarker means between the observation and control groups. Adopting a similar approach, we utilized the 95%CI of the AUC from prediction models to graphically present the top 5 models with higher comprehensive scores (Figure 5). Instead of using the variables employed in model construction, we opted for this method because model variables often possess multiple dimensions, such as percentages (e.g., neutrophil percentage), hundreds (e.g., platelet count), and qualitative indicators (e.g., the presence of pleural effusion). These variables might not be suitable for simultaneous representation on a single chart, as demonstrated by Shi et al (Shi et al., 2022). However, by using the AUC and its 95%CI, we can also more accurately compare the AUC levels of different prediction models that aggregate numerous biomarkers, rather than focusing on individual biomarkers.

Nevertheless, we have also identified some shortcomings. Firstly, most current research on MPP-related biomarkers is

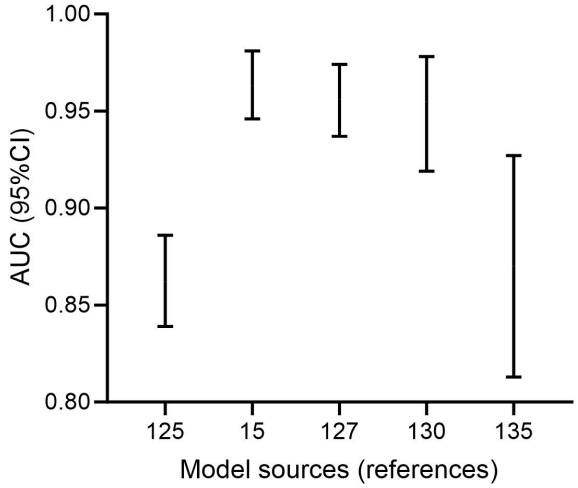

FIGURE 5
Five predictive models with excellent comprehensive scores. (The two ends of the error bar represent the 95%CI of AUC. The horizontal coordinate decreases from left to right in the rankings of comprehensive scores. As the top-ranked and fifth-ranked models did not provide the corresponding 95%CI for AUC, we included the sixth-ranked and seventh-ranked models in the plot for a supplement.).

limited to single-center retrospective studies with a narrow sample scope and limited sample size. Some studies only include a few tens of cases, leading to diagnostic cut-off values that may not have good universality. Secondly, after comparing the effectiveness of these prediction models by assigning appropriate weights to the number of cases and AUC, and displaying the results graphically, we found that the AUC level of the second-ranked model is significantly lower than that of the third, fourth, and sixth-ranked models. Additionally, despite some models having high AUC values, their effectiveness remains relatively low due to inadequate sample sizes (poor sample representativeness). Finally, the established prediction models primarily incorporate variables such as peripheral blood parameters and clinical symptoms/signs, covering a relatively limited range of variable types. Furthermore, some prediction models have not undergone a comprehensive internal development and external validation process (Luan et al., 2023; Zhang et al., 2023; Li et al., 2024; Pei and Luo, 2024; Shen and Sun, 2024; Xie et al., 2024), which may impact the reliability and generalizability of the models to some extent. Future research should aim to enrich prediction models by incorporating additional variables strongly associated with the predicted outcomes, while also including a larger number of cases to enhance sample representativeness. Adequate validation is essential to ensure the usability of the models. By adopting an online prediction model approach, the utilization process of the models will not be complicated due to the appropriate increase in variables, rather than relying solely on a static nomogram format. Finally, the use of new technologies (such as magnetic resonance pulmonary ventilation imaging) may also be able to help the clinical management of MPP. Given the complex clinical detection processes associated with gene-related biomarkers, there is potential to design targeted therapeutic drugs based on the functions of these genes, which may find promising applications in severe and refractory pediatric cases.

8 Conclusions

In summary, these involved indicators all have a certain correlation with the severity of MPP. Among them, CRP, PCT, and SAA, which are the most common indicators in clinical practice, exhibit high sensitivity in the early differential diagnosis of MPP. Meanwhile, CRP and LDH can, to some extent, predict the treatment measures that should be taken for children as well as their response to treatment. Affordable and convenient blood routine, and cytokine-based markers detected using enzyme-linked immunosorbent assay, can be used alongside other aforementioned indicators to jointly assess the condition. For detecting complex and expensive biomarkers (such as nucleic acid-based markers), they can be utilized in areas like new drug development for MPP to maximize their application value. However, as we all know, it is difficult to accurately achieve the purposes of diagnosis, prediction, and evaluation by relying on a single indicator alone. The combined detection of multiple indicators can greatly improve the sensitivity and specificity of diagnosis, maximizing its clinical application value. Some studies have established joint prediction models based on multiple indicators, and some scholars have even designed an online prediction model, vastly improving the application efficiency of these models and greatly assisting pediatricians.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Congenital cytomegalovirus retinitis of prematurity: a case report and literature review

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Introduction: Ophthalmopathy induced by cytomegalovirus (CMV) infection is most common in immunodeficient patients without other congenital infections. This paper reports a clinical case of retinitis due to congenital CMV infection in a preterm infant and reviews the relevant literature.
Case presentation: A 2-day-old female infant at 36^{+2} gestation weeks presented with a 2-day history of scattered bleeding spots across the body, hemorrhagic diathesis, thrombocytopenia, positive blood CMV IgM, and blood and urine CMV DNA levels significantly above the detection limit by PCR analysis. Maternal serological examination indicated blood CMV IgM positivity. The laboratory test results, CMV IgM positivity in the mother's blood was used to confirm a diagnosis of congenital CMV infection. Later, antiviral treatment with ganciclovir was provided for 3 weeks. Fundus examination indicated a few white exudates along the peripheral retina in both eyes, with a white sheath of peripheral retinal vessels above the temporal plane in the right eye. A diagnosis of CMV retinitis was considered after obtaining abnormal fluorescein fundus angiography results. Ganciclovir was administered at 0.5 mg weekly into the vitreous cavities of the eyes for 3 weeks, and the vascular white sheaths disappeared. Retinitis recurred at 6 months of age, and antiviral treatment was recommended. However, the family rejected it.
Conclusion: Congenital cytomegalovirus retinitis carries substantial risks. For infants suspected of this condition, early initiation of antiviral therapy is crucial to enable timely intervention, improve prognosis, and enhance the child's quality of life.

KEYWORDS

congenital, cytomegalovirus infection, retinitis, prematurity, ganciclovir

1 Introduction

Human cytomegalovirus (HCMV) is a DNA virus from the *Herpesviridae* family that only infects humans. It is mainly transmitted through the placenta, breastfeeding, sexual contact, blood transfusion, solid organ transplantation, or hematopoietic stem cell transplantation (1). HCMV is a major cause of congenital infections worldwide, with a prevalence of nearly 0.2%–6.1% in live births (2).

Cytomegalovirus (CMV) infections can present with ocular involvement, severely affecting visual function, and ocular abnormalities manifesting as retinitis, corneal endotheliitis, and anterior uveitis, with CMV retinitis being the most common (3). CMV retinitis is observed in patients with immunodeficiencies, such as acquired immunodeficiency syndrome (1). However, its onset in preterm infants with congenital CMV infections is rare. This article analyzes the clinical data of a preterm infant with congenital CMV infection associated with retinitis. The clinical characteristics, diagnosis, treatment, and prognosis of retinitis in preterm infants with CMV infections are discussed to improve early recognition and overall disease outcomes.

2 Methods

This study retrospectively analyzed the clinical data of a preterm infant with ocular involvement after congenital CMV infection admitted to the neonatal ward of the Affiliated Children's Hospital of Soochow University. The Ethics Committee of the Children's Hospital of Soochow University approved the study. Written informed consent was obtained from the patient's family.

In the literature review, the keywords "congenital," "cytomegalovirus retinitis," "newborn," and "infant" were used to search foreign databases, such as PubMed and Elsevier, from database inception to April 2024. Immunocompromised patients were excluded. Articles were screened according to the following criteria: English language and full-text availability, and whether the search involved articles on clinical manifestations, diagnostic tests, or treatments of patients. This study was a single case report and the literature review and was not designed as a systematic review. Therefore, its methodological quality could not be assessed.

3 Case presentation

A 2-day-old female infant at 36 + 2 weeks of gestation presented with tachypnea present since birth and scattered petechiae over the body for 2 days, accompanied by hemorrhagic diathesis, thrombocytopenia, positive CMV IgM serology, and high CMV DNA viral loads exceeding the detection limit in both blood and urine specimens by PCR analysis. The child was the result of the mother's first pregnancy and first delivery (G1P1), delivered by cesarean section in an outside hospital because of reduced blood flow in the umbilical artery at a gestational age of 36 ± 2 weeks, with a birth weight of 2,750 g. The mother's screening test was positive for CMV IgM. However, she did not receive treatment and had no other complications during her pregnancy. She had one episode of elevated blood glucose, which became normal after dietary modifications. The child experienced shortness of breath and exhibited poor crying post-birth. This was accompanied by slight face and limb bruising and scattered pinpoint-sized hemorrhages on the trunk and facial skin. After admission, paired blood culture sets (two bottles per set) were conducted, and the results were negative after 5 days of incubation. The blood was positive for CMV IgM. Chest x-ray showed thickening and blurring of the lung texture, and patchy or diffuse exudative shadows were seen, suggesting inflammation in both lungs. The patient was given hooded oxygen, cephalosporin, adenosine monophosphate, and intravenous gammaglobulin infusion. For further management, the patient was transferred to our hospital's Neonatal Intensive Care Unit. On admission, the examination revealed a temperature (T) of 36.5°C, pulse (P) of 135 beats/min, respiration (R) of 65 breaths/min, weight (Wt) of 2.65 kg, and percutaneous arterial oxygen saturation (SPO_2) of 94% (box oxygen). The patient demonstrated poor mental response, irritability, and occasional limb tremors. Scattered petechiae could be seen on the skin, with ecchymosis observed around the mouth and feet. The skin appeared dry, and pinpoint-sized bleeding spots gradually spread across the body. The fontanelle was flat and soft, about 1.0 cm × 1.0 cm. The neck was soft, and slight shortness of breath was observed because of the inspiratory triple concave sign. Neither dry nor wet rales were heard, the rhythm was synchronous, the precordial heart rhythm was regular, and the heart sounds were moderate. However, a grade 2/6 murmur was heard in the precordial region. The abdomen was dilated, the liver was 3 cm below the ribs, the spleen could not be detected below the ribs, the intestinal sounds were audible, the limb muscle tone was slightly high, the limb ends were warm, and primitive reflexes were poorly elicited.

Upon admission, CMV and hearing examinations indicated left and right hearing thresholds at 100 dB Sound Pressure Level. No abnormalities were seen in the ophthalmological screening for retinopathy of prematurity. The patient was treated with latamoxef (40 mg/kg, twice daily) for infection, along with filtered white platelets (0.5 units) to prevent hemorrhage and compound glycyrrhizin combined with adenosine monophosphate for liver protection. Additionally, intravenous ganciclovir was administered (5 mg/kg/dose every 12 h for 2 weeks). Maintenance treatment was provided for a week, during which the ophthalmological examination showed no significant abnormalities. Cranial magnetic resonance imaging revealed bilateral lateral ventricle hemorrhage, subarachnoid hemorrhage, and brain alterations characteristic of preterm infants. The spontaneous hemorrhage was likely attributed to thrombocytopenia, and the prior treatment regimen was continued for two additional days. Platelet levels stabilized at 42×10^9/L, skin hemorrhagic spots diminished, liver function normalized, and vital signs were stable. Hence, the patient was transferred to the general ward. In the general ward, the patient received latamoxef (40 mg/kg, twice daily) and ganciclovir (5 mg/kg/dose, every 12 h). The ganciclovir dose was reduced to 5 mg/kg/d followed a 17-day induction. CMV DNA viral loads were quantified by quantitative PCR in paired urine and plasma specimens. The fundoscopic examination indicated stage I retinopathy in zone III of the right eye. Antiviral maintenance therapy was continued, and subsequent ophthalmological screening established stage I retinopathy within the same region. Another CMV examination was repeated, followed by the discontinuation of ganciclovir.

Following 34 days of therapeutic intervention, the pediatric patient was discharged in an afebrile state with intact mental status and age-appropriate responsiveness. No significant bleeding spots were observed on the skin or mucous membranes. The liver was 2 cm below the ribs, with the spleen not palpable. The platelet count was recorded at 177×10^9/L. Five weeks after birth, the patient underwent a follow-up fundus examination in the ophthalmology clinic of our hospital. There was a slight white exudation in the vascular distribution of the peripheral retina in both eyes and a white sheath of the peripheral retinal vessels above the temporal surface of the right eye (Figure 1A). Fundus fluorescence angiography indicated blurred edges and fluorescein leakage in the peripheral retinal vessels at an early stage above the temporal plane of the right eye (Figure 1B), which worsened later (Figure 1C). A positive CMV DNA test or positive rapid virus isolation in urine, saliva, and/or blood samples within the first 3 weeks of life indicates congenital infection. In contrast, if

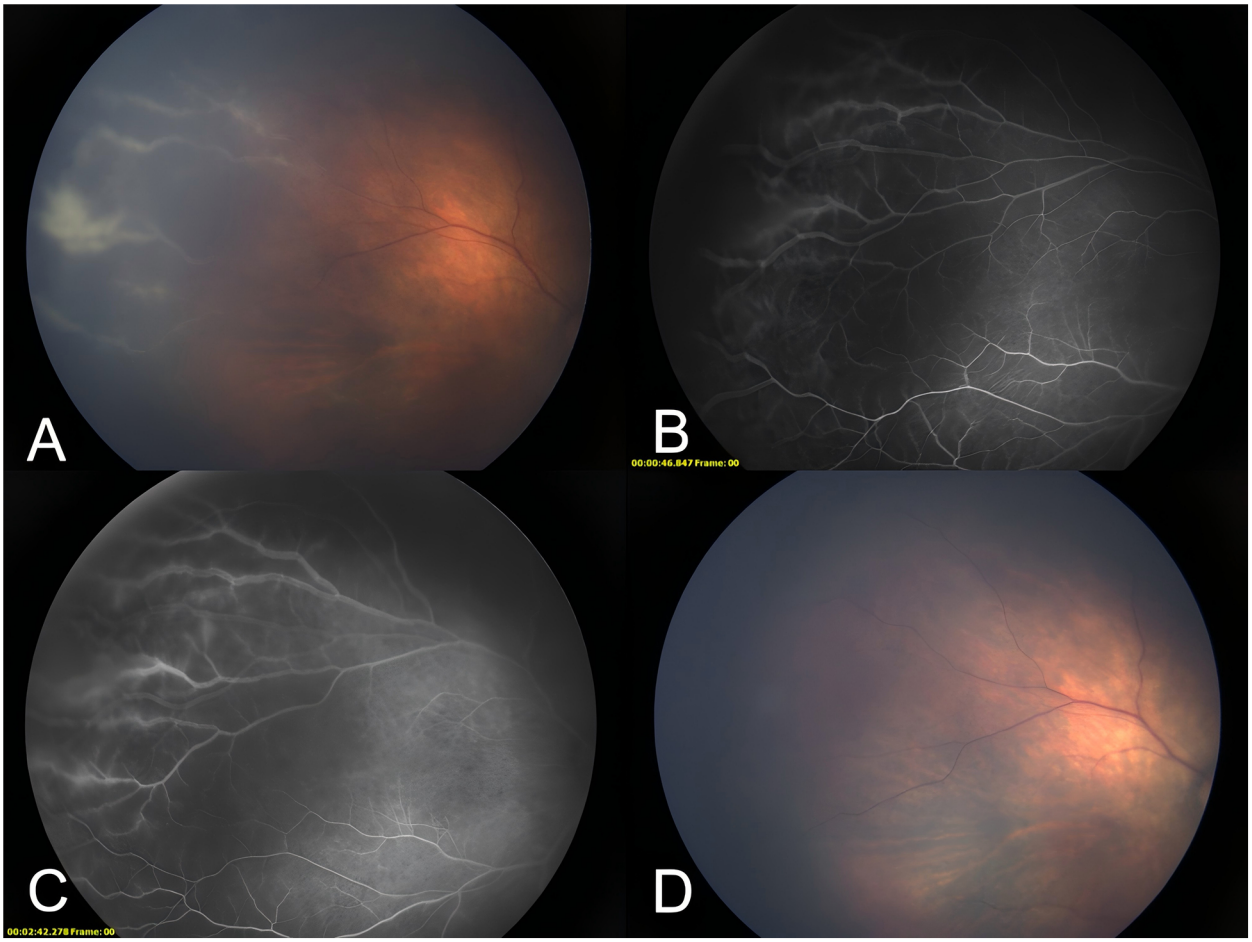

FIGURE 1
A photograph of the fundus of the patient. **(A)** The white sheath of peripheral retinal vessels above the temporal plane of the right eye. **(B)** Early blurring of peripheral retinal vessels above the temporal plane of the right eye, with fluorescein leakage. **(C)** Late stage of the peripheral retinal vessels above the temporal plane of the right eye with worsening fluorescein leakage. **(D)** The white sheath of peripheral retinal vessels above the temporal plane of the right eye disappeared after three treatments with vitreous cavity ganciclovir injections.

the pathogen detection results are negative during the first 3 weeks after birth but become positive for CMV DNA or rapid virus isolation in urine, saliva, and/or blood samples after 3 weeks, this suggests an acquired infection (4). Based on the child's medical history and the clinical data, the ocular lesions were identified as CMV retinitis. Weekly injections of 0.5 mg ganciclovir were given to both eyes for 3 weeks, inducing the disappearance of the vascular white sheaths (Figure 1D). Retinitis recurred at 6 months of age, and the patient was followed up by telephone. However, the parents were not compliant and voluntarily abandoned the treatment. This case included routine blood examinations (Table 1) and CMV load results (Table 2).

4 Literature review and discussion

CMV retinitis occurs in immunocompromised patients. However, disease onset in premature infants with congenital CMV infections has been rarely reported. Congenital CMV infection is associated with a high mortality rate, and survivors tend to recover from non-neurological damage. However, neurological damage can be irreversible. The incidence is high and damaging in preterm infants. Moreover, congenital CMV infection affects multiple organs in the body. Still, no clear treatment guidelines have been developed, and adult treatment protocols are used. The current literature search identified 11 relevant papers involving 11 children with incomplete case information. A summary of the reported cases is represented in Table 3 (5–15). Gestational age was recorded in 10 cases, among which six were preterm. However, the early diagnosis and treatment of preterm infants did not focus on congenital CMV retinitis, resulting in untimely treatment. Therefore, preterm infants with maternal CMV and a CMV infection history must undergo a timely ophthalmological assessment to exclude this pathology.

Our patient (Case 12) is consistent with previously reported congenital CMV retinitis cases in core diagnostic methods (including serological IgM positivity and blood or urine CMV DNA PCR testing) and first-line treatment strategies (intravenous ganciclovir combined with intravitreal injection). Both cases face challenges of retinitis recurrence. The subsequent use of intravitreal

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TABLE 1 The child's laboratory blood test results.

Date	1.19	1.21	1.25	1.28	1.29	1.31	2.03	2.12	2.16	2.20
WBC count ($\times10^9$/L)	7.99	7.35	10.35	6.91	9.5	6.45	7.51	–	8.48	13.73
LY percentage (%)	72.1	69.8	74.6	84.9	87.1	85.7	76.7	–	87.0	61.5
HB (g/L)	172	152	154	140	141	132	121	–	96	92
RBC count ($\times10^{12}$/L)	5.04	4.51	4.72	4.38	4.37	3.94	3.72	–	3.56	2.89
PLT count ($\times10^9$/L)	15	116	41	42	45	63	121	–	177	225
NE percentage (%)	17.4	11.7	16.5	11.2	9.2	11.4	22.1	–	12.6	26.6
CRP (mg/L)	23.94	3.17	1.58	1.32	0.86	0.62	1.25	–	0.67	–
ALT (U/L)	10.5	–	52.2	34.7	–	–	32.9	159.4	92.4	63.8
AST (U/L)	132.1	–	172.3	87.4	–	–	62.2	180.3	75.3	59.4
LDH (U/L)	1,563.7	–	669.1	621.7	–	–	363.5	411.9	266.5	414.1
TBIL (μmol/L)	64.7	–	21.8	16.1	–	–	10.1	6.9	6.3	4.7
DBIL (μmol/L)	20.8	–	10.95	8.64	–	–	6.3	4.4	3.64	2.5
IBIL (μmol/L)	43.9	–	10.85	7.46	–	–	3.8	2.5	2.66	2.2
GGT (U/L)	89.1	–	358.9	462.3	–	–	502.1	289	216.5	146.9
ALP (U/L)	363.7	–	228	241	–	–	247	257	268	267.7
TP (g/L)	75.4	–	65.5	64.1	–	–	61.2	57.2	58	62.6
ALB (g/L)	32.3	–	31.1	31	–	–	31.4	32.1	33.9	36.4

WBC, white blood cell; LY, lymphocyte; HB, hemoglobin; RBC, red blood cell; PLT, platelet; NE, neutrophil; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; TBIIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin.

TABLE 2 Cytomegalovirus laboratory findings in the patient.

Date	Cytomegalovirus DNA (urine)	Cytomegalovirus DNA (blood)
Day 3 of life	5.27×10^7 copies/ml	2.57×10^4 copies/ml
Day 21 of life	2.93×10^7 copies/ml	1.40×10^3 copies/ml
Day 36 of life	2.41×10^7 copies/ml	1.45×10^3 copies/ml

injections leads to high drug concentrations in the retina and vitreous cavity, suppressing viral replication and controlling local infection. This prevents progression to the macula and irreversible vision loss. We did not perform drug resistance gene testing (e.g., UL97/UL54 mutation analysis) because of the risk of systemic toxicity and the patient's existing thrombocytopenia, delaying treatment adjustment. Additionally, poor family adherence (abandoning treatment post-recurrence) directly contributed to disease deterioration. This was in contrast with most cases in which the prognosis improved from the use of foscarnet, valganciclovir, or laser interventions. Unlike typical intrauterine infection cases (e.g., Cases 1, 5, and 6), this case may depict a perinatal infection (later mother-to-child transmission), similar to Case 8, but without fatal involvement of the central nervous system. In this case, the MRI findings of intraventricular hemorrhage in the preterm infant, potentially associated with placental hypoperfusion and perinatal asphyxia, suggest that MRI serves as a promising tool for detecting fetal brain injury (16). Despite no reported deaths, the poor prognosis underscores the crucial role of resistance monitoring, long-term follow-up, and parental education while managing CMV retinitis. This case also highlights the multisystem involvement characteristic of perinatal asphyxia, emphasizing the importance of combining early imaging with biomarker analysis for comprehensive evaluation (17–19).

CMV infection of retinal pericytes primarily presents as a lytic infection. It damages the proximal region of the inner blood-retinal barrier (IBRB), significantly losing these supportive cells (20). In premature infants, the structural integrity of the IBRB is significantly decreased because of vascular dysplasia (e.g., persistent avascular zones) (21). This facilitates the hematogenous dissemination of CMV into retinal tissue. Studies have demonstrated that hereditary NOS2 gene homozygous frameshift mutations, resulting in loss of nitric oxide synthase 2 function, significantly impair anti-CMV immunity and predispose to fatal infections (22). Furthermore, abnormalities in tetrahydrobiopterin (BH4) metabolism may exacerbate oxidative stress and indirectly compromise immune responses, suggesting that genetic mutations modulate CMV susceptibility through distinct pathways (23). Similarly, CMV can become a lifelong latent infection in immunocompromised individuals via immune evasion mechanisms. These infections can be reactivated by external stimuli, leading to a dynamic cycle of "infection-latency-activation," (24) which explains the recurrence seen in our case. Typical CMV retinitis presentations involve explosive/hemorrhagic (necrosis and hemorrhage resembling "pizza pie" within the posterior pole) and granular forms (granular lesions in the peripheral retina) (25, 26). In this case, the patient exhibited yellow-white granular necrotic lesions with edema and vascular sheathing within the superior temporal peripheral retina (Zone III). This matched the granular lesion characteristics in the literature without hemorrhagic manifestations. The patient presented with thrombocytopenia (15×10^9/L), systemic petechiae, and retinitis (fluorescein leakage and vascular sheathing), which are consistent with typical congenital CMV infection features (e.g., hepatosplenomegaly, thrombocytopenia, and ocular involvement) (27). Microcephaly was not reported; however, the rapid progression and recurrence of retinitis indicate systemic and multi-organ involvement of CMV infections. The literature suggests that about 13% of newborns with congenital CMV infections demonstrate identifiable symptoms at birth. The clinical manifestations in Case 12 support this ratio, highlighting the requirement for screening asymptomatic or atypical cases (e.g., 87%

TABLE 3 Clinical characteristics of 12 patients with congenital cytomegalovirus retinitis (including our case).

Patient	Gender	Gestational age	Onset time	Diagnostic methods	Serum CMV antibodies in the mother	Clinical manifestation	Treatment	Outcome
Patient 1	Female	37 weeks	At birth	Serum CMV IgM-positive + urine CMV isolation + clinical features (ventriculomegaly and thrombocytopenia)	Positive CMV IgG and negative IgM in late pregnancy serum	Thrombocytopenia, hearing impairment, hepatitis, hepatomegaly, and uveitis	Intravenous ganciclovir (5 mg/kg three times daily for 3 weeks) + CMV immunoglobulin	Platelet count recovered, enlarged liver and spleen/ventricles resolved, and neuromotor activity was normal after 5 months
Patient 2	Female	41 weeks	Four weeks of age	Elevated CMV IgG/IgM + blood/urine CMV DNA positivity (PCR)	The titers of CMV IgG and IgM were elevated	Hepatitis, retinitis, and protein-losing gastrointestinal disease	intravenous ganciclovir (5 mg/kg twice daily for 2 weeks) + CMV immunoglobulin (2 weeks)	Diarrhea and uveitis resolved, and urinary CMV DNA remained positive at 8 months but without sequelae
Patient 3	Unknown	35 weeks	One day after birth	Urine CMV culture + PCR positive	The antibody status of the mother was not clearly described	Retinitis, microcephaly, and intracranial calcification	Intravitreal ganciclovir sodium injection (12 times)	Retinitis improved briefly but there was a recurrence without systemic complications
Patient 4	Male	unknown	Seven months of age	Blood/urine CMV DNA high load (PCR) + serology (hypergammaglobulinemia)	The antibody status of the mother was not clearly described	Hepatomegaly, retinitis with dystrophic intraocular calcification, and bone marrow suppression	Ganciclovir (5 mg/kg/day) + IVIG (discontinued because of neutropenia), followed by uncontrolled recurrence	Died of disseminated CMV infection (respiratory failure)
Patient 5	Female	32 weeks	19th day of life	Maternal serum CMV positive + infant urine CMV positive + fundus examination (frosted glass vitreitis)	IgG was positive, IgM was negative	Retinitis	Intravenous ganciclovir (6 mg/kg/day for 42 days) + intravitreal foscarnet injection	Retinitis resolved completely after repeated episodes, leaving a macular scar
Patient 6	Female	36 weeks	4th day of life	Aqueous humor CMV DNA positive (PCR) + serology (maternal CMV IgM positive)	Both CMV IgG and IgM were positive	Hepatomegaly, retinitis, microcephaly	Oral valganciclovir (6 weeks)	Vasodilation subsided, leaving choroidal retinal atrophy
Patient 7	Unknown	36 weeks	Present from birth	Vitreous/serum CMV DNA positive + UL97 gene testing (ganciclovir resistance)	The antibody status of the mother was not clearly described	Retinitis	Ganciclovir (6 mg/kg twice daily for 62 days) + foscarnet + laser photocoagulation	Viral load decreased and retinitis subsided, followed by oral valganciclovir prophylaxis
Patient 8	Male	37 weeks	Four months of age	Aqueous humor/cerebrospinal fluid CMV DNA positive (PCR) + maternal serum IgG positive (IgM negative)	CMV IgG was positive and IgM was negative	Retinitis and central nervous system involvement	Intravenous ganciclovir (10 mg/kg/day) + intravitreal injection (1 mg/0.1 cc twice weekly)	Death from CMV encephalitis (central nervous system involvement)
Patient 9	Unknown	26 weeks	Gestational age 39 weeks	Serum CMV IgM/IgG positive + urine CMV DNA (PCR)	The antibody status of the mother was not clearly described	Thrombocytopenia and retinitis	FA-guided laser treatment for ROP + oral valganciclovir (5 ml daily)	Retinal inflammation healed and vision normalized
Patient 10	Female	unknown	Four weeks of age	Serum CMV IgM/IgG positive + confirmed by PCR	The antibody status of the mother was not clearly described	Retinitis	Intravenous ganciclovir (10 mg/kg/day for 4 weeks) → oral valganciclovir (4 weeks)	Choroidal retinitis healed and general condition improved
Patient 11	Unknown	38 weeks	One day after birth	Urine CMV DNA high viral load (PCR) + UL97 gene mutation (A594P, ganciclovir resistant)	CMV IgM was positive, but IgG was not mentioned	Thrombocytopenia, hearing impairment, microcephaly, uveitis	Ganciclovir (5 mg/kg every 12 h for 16 days) + phosphonoformate combination → oral valganciclovir	Retinitis subsided and CMV viral load stabilized
Patient 12	Female	36 weeks	One day after birth	Mother CMV IgM-positive + infant blood/urine CMV DNA-positive (PCR) + fundus examination (vascular pallor and fluorescein leakage)	CMV IgM was positive, but IgG was not mentioned.	Thrombocytopenia, hearing impairment, hepatitis, hepatomegaly, and retinitis	Intravenous ganciclovir (17 days) → intravitreal ganciclovir injection (3 weeks)	Treatment was abandoned after retinitis recurrence, and the disease was uncontrolled at 6 months

CMV, cytomegalovirus; PCR, polymerase chain reaction; IVIG, intravenous immunoglobulin; IgG, immunoglobulin G; IgM, immunoglobulin M.

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of asymptomatic infected infants in a Spanish study were diagnosed by screening) (28). Regular neonatal CMV screening enhances the detection rates for asymptomatic or mildly symptomatic infections (29). In this case, the mother tested positive for CMV IgM late during her pregnancy but did not receive prenatal intervention. This induced an undetected perinatal infection, causing retinitis. When universal screening of saliva or dried blood spots has been implemented, similar cases were diagnosed earlier, enabling timely antiviral treatment (30). This can reduce the risk of retinitis and neurological sequelae. Retinopathy of prematurity involves abnormal retinal vascular development. It typically presents with vascular tortuosity, neovascularization, and staged progression (I–V) (31) but lacks systemic symptoms. Diagnosis depends on fundus examination and preterm birth history. CMV retinitis is caused by either maternal vertical transmission or immunodeficiency (such as human immunodeficiency virus infection). It is characterized by peripheral retinal necrotic lesions with yellow-white exudates and hemorrhage. Moreover, it is accompanied by multi-system manifestations, including hepatosplenomegaly, thrombocytopenia, or encephalitis (27). The key diagnostic tests involve CMV DNA PCR analysis (from blood, urine, or aqueous humor) or positive CMV IgM antibodies. Due to the potential visual impairment induced by congenital CMV infection, infants having ocular-related clinical manifestations at birth must undergo at least one eye examination every year (32).

Ganciclovir, an antiviral drug approved by the Food and Drug Administration in 1989, has been a cornerstone of CMV retinitis treatment and prevention. It inhibits viral DNA polymerase, effectively blocking CMV replication (33). Ganciclovir is administered systemically or through intravitreal injection. Long-term antiviral therapy has a dual purpose in managing congenital CMV infections. It controls acute infections while potentially reducing the risk of distant recurrences. Vicente et al. revealed that valacyclovir administration during pregnancy significantly improved the proportion of asymptomatic newborns (79.4%). However, 32.35% of these children developed long-term sequelae. This includes sensorineural hearing loss, suggesting that short-term prenatal treatment may not suppress all complications. Therefore, extended treatment or postnatal maintenance therapy is needed (34). Intravitreal ganciclovir injections bypass the blood-retinal barrier, providing therapeutic concentrations directly within the retina and reducing systemic side effects, such as neutropenia. This route has demonstrated enhanced efficacy in treating CMV retinitis. The results were evidenced in Case 3 in this paper, where the retinal vascular sheath was removed entirely from the right eye of the patient after 3 weeks of intravitreal ganciclovir injections. Dheyab et al. observed that long-term oral valganciclovir (≥450 mg twice daily) treatment of CMV-associated anterior uveitis effectively controlled intraocular pressure and inflammation without recurrence within a follow-up period of 12–108 months, signifying the benefits of prolonged high-dose therapy (35). Additionally, Tranos et al. demonstrated that the recurrence of CMV anterior uveitis was closely associated with treatment duration and specific biomarkers. About 61.4% of patients required oral and topical medications for up to 44 months to manage recurrences, highlighting the necessity for individualized and long-term treatment regimens (36). While surgical interventions like vitrectomy with silicone oil injections are typically utilized for advanced complications, such as retinal detachment (37), long-term antiviral therapy may reduce the need for such procedures. In one study, valganciclovir treatment enabled 35 out of 40 patients with uveitis to avoid surgery, with only one case requiring filtering surgery because of endothelial inflammation. This demonstrates the potential of antiviral therapy in preventing structural damage (35). However, further investigation of the role of antiviral therapy in preventing recurrences during the long-term management of congenital CMV infection is needed.

CMV infections in pregnant women are classified into latent and active infections. Pre-pregnancy CMV IgG-positive/IgM-negative status indicates a latent infection, which rarely causes intrauterine infection and does not adversely affect the fetus. In contrast, an active CMV infection during pregnancy is associated with active viral replication and is divided into primary and non-primary infections (such as reactivated and reinfected infections). Research suggests that the risk of vertical transmission is higher during primary infections (38, 39) and increases with gestational age. Moreover, transmission rates reach approximately 75% during late gestation (40). Maternal primary cytomegalovirus infection co-occurring with severe concurrent infections significantly elevates maternal-fetal risks. Multiple infections may synergistically exacerbate placental barrier damage, leading to higher intrauterine transmission rates, fetal multi-organ involvement, and long-term neurodevelopmental impairments (41, 42). Observational cohort studies found that raising immunoglobulin levels could be a promising intervention to prevent mother-to-child transmission of primary infections and significantly decrease this risk (43). Additionally, aggressive antiviral therapy has been shown to reduce vertical transmission by 70% in maternal primary infection during pregnancy or early gestation (44). Gene and vaccine therapy are novel CMV disease treatment methods that are currently being investigated in animal experiments and require further observation for efficacy (40). Due to the lack of an applicable vaccine, susceptible pregnant women must avoid contact with secretions from known patients. If patient contact occurs, disinfection must be done to minimize transmission risk. This reduces the CMV infection rates of pregnant mothers to decrease congenital CMV infection. Several studies have recommended universal neonatal HCMV screening for the early detection of all CMV infants so that children with uveitis and other sequelae can be identified early for swift intervention and better outcomes (45).

Breastfeeding can reduce the risk of retinopathy of prematurity (46), but it is also a primary route through which neonates can contract postnatal CMV infections (47). Studies demonstrated that fresh breast milk from CMV-positive mothers led to a 19% [95% confidence interval (CI): 11%–32%] prevalence of CMV infection in very low birth weight infants, with 10% showing clinical signs and 4% developing sepsis-like syndrome. However, the infection rate dropped to 4.4% (95% CI: 2.4%–8.2%) when frozen breast milk was used, although the severe disease rate was unchanged (48). Extremely low birth weight (ELBW) infants (birth weight <1,000 g) remain at higher risk. A study indicated that 26% of ELBW infants aged 23–26 weeks were infected via breast milk, all exhibiting clinical symptoms (49). The risk of infection correlates strongly with factors

like viral load in breast milk, breastfeeding frequency, and infant comorbidities (50). Currently, four primary feeding strategies have been suggested for the preterm infants of CMV-positive mothers: freeze-thawed breast milk, pasteurized breast milk, direct breastfeeding, and formula. Freezing decreases virus viability without eliminating transmission risk (51). Traditional pasteurization (heating at 62.5°C for 30 min) inactivates CMV and destroys the bioactive components of breast milk (52). Short-term pasteurization (heating at 62.5°C for 5 s) partially decreases the infection risk, but transmission remains possible (53). Importantly, maternal immunity (e.g., serum-neutralizing antibodies or lactoferrin in breast milk) does not provide protective immunity for preterm infants (54, 55). Therefore, clinical decisions must weigh the benefits of breast milk against the risk of CMV infection. However, standardized prevention guidelines are still lacking. Prioritizing pasteurized breast milk is recommended for high-risk groups like ELBW infants, and infection indicators must be dynamically monitored for better-individualized management.

5 Strengths and limitations

1. Retrospective analysis of the clinical data and regression of congenital cytomegalovirus-infected retinitis in a premature infant.
2. Our literature review involved a thorough search of congenital cytomegalovirus retinitis in preterm infants born before May 30, 2024, in foreign databases, and the results are discussed.
3. Due to the rarity of the disease, the number of cases was limited. Moreover, the patient's family abandoned treatment at a later stage. This restricted us from performing long-term post-treatment follow-ups.

6 Conclusion

This case, as well as the literature review, suggest that congenital CMV retinitis, although rare in preterm infants, poses a significant disability risk. The immaturity of the preterm immune system, viral reactivation, and poor treatment compliance are key factors leading to recurrence. Early combined systemic and local antiviral therapy can effectively manage acute infections. Still, vigilance involving drug resistance and the necessity for long-term maintenance therapy is needed. Parental education and dynamic monitoring (such as viral load and fundoscopy assessments) are critical for improving outcomes. Future efforts must focus on developing standardized clinical pathways for congenital CMV infections while exploring prenatal screening and perinatal intervention strategies that can decrease the risks of vertical transmission and long-term sequelae.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Medical Ethics Committee of the Children's Hospital of Soochow University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this case report.

Author contributions

YL: Data curation, Writing – original draft, Writing – review & editing. WS: Data curation, Writing – original draft, Writing – review & editing. XJ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. LZ: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – review & editing. XZ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Pulmonary echinococcosis mimicking tuberculosis in a child from a dual-endemic region: a case report

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Background: Pulmonary echinococcosis represents a significant health challenge, particularly in endemic regions, and is associated with substantial morbidity and mortality. Its nonspecific clinical presentation and radiological diversity often lead to misdiagnosis. Here, we report a case of pulmonary echinococcosis initially misdiagnosed as pulmonary tuberculosis.
Case presentation: We report a case of a 13-year-old girl from a region endemic for both echinococcosis and tuberculosis. She initially presented with recurrent cough, hemoptysis, night sweats, and a pulmonary cystic lesion and was diagnosed with pulmonary tuberculosis. However, her condition progressively deteriorated despite antituberculosis therapy. Ultimately, surgical intervention and histopathological examination confirmed pulmonary echinococcosis, and the patient achieved complete recovery after therapy.
Conclusion: For patients from regions endemic for both tuberculosis and echinococcosis who present with cough, hemoptysis, or pulmonary cystic or cavitary lesions, it is crucial to differentiate pulmonary echinococcosis from pulmonary tuberculosis. The final diagnosis should be supported by other microbiological-serological and/or histopathological tests.

KEYWORDS

pulmonary echinococcosis, pulmonary tuberculosis, children, pulmonary cysts, surgery

1 Introduction

Pulmonary echinococcosis, a zoonotic parasitic disease caused by the larval stage of cestodes of the genus *Echinococcus*, poses a significant public health burden, particularly in pastoral regions. It not only leads to economic losses in the livestock industry but also causes severe health complications and even death in humans (1, 2). China has been reported to have the highest number of echinococcosis cases, accounting for 73.55% of all recorded cases worldwide (3). The clinical manifestations of pulmonary cystic echinococcosis depend on the cyst volume. Small pulmonary echinococcosis lesions are often asymptomatic, whereas large cysts can result in acute, life-threatening complications (4, 5). The most common symptoms were cough (53%–62%), chest pain (49%–91%), dyspnea (10%–70%) and hemoptysis (12%–21%), and other less frequently symptoms include dyspnea, malaise, nausea, and vomiting and thoracic deformations (6). Due to its non-specific symptoms and diverse radiological manifestations, pulmonary echinococcosis has a broad differential diagnosis, making its diagnosis challenging and prone to misdiagnosis (7). When a pulmonary cystic lesion ruptures and forms a cavity, it is challenging to distinguish it from pulmonary tuberculosis (6).

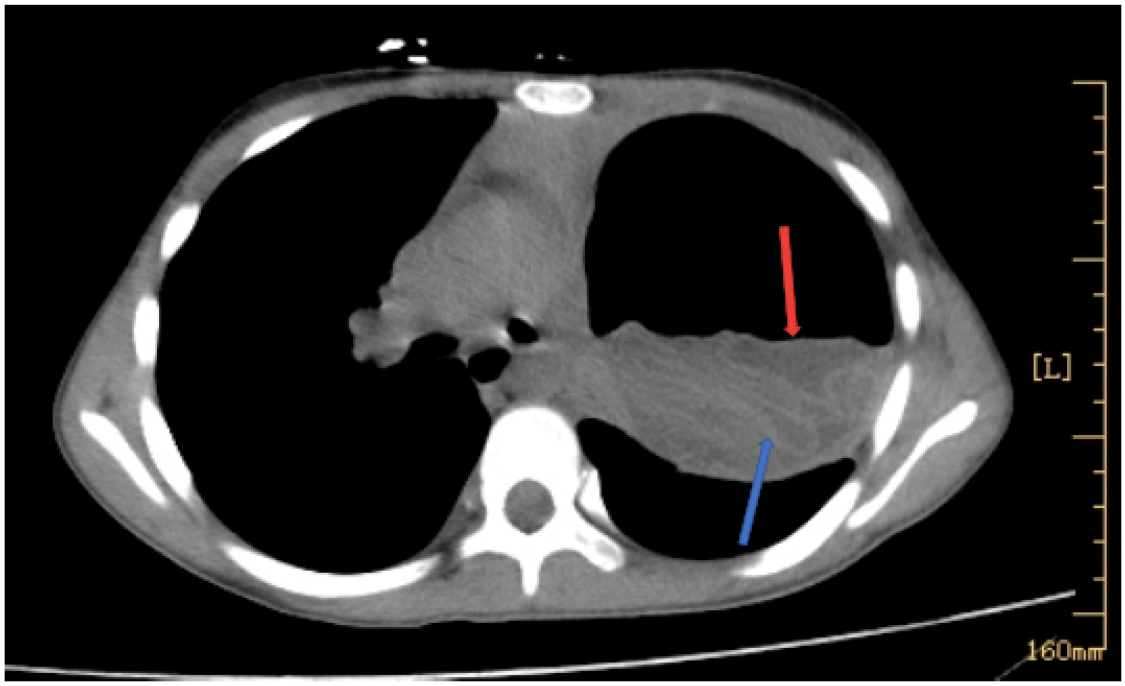

FIGURE 1
Image of the chest CT. The images revealed cystic liquid shadow (the red arrow), and whirl sign or a ribbon sign (the blue arrow).

Here, we present a case of pulmonary echinococcosis in a child who was initially misdiagnosed with pulmonary tuberculosis, aiming to raise awareness of pulmonary echinococcosis and reduce misdiagnosis.

2 Case presentation

A 13-year-old female patient from a rural region of Tibet presented with a one-year history of recurrent cough, hemoptysis and night sweats, without fever. She had been diagnosed with pulmonary tuberculosis at a local hospital following chest x-ray and computed tomography (CT) findings. The patient or her family were unable to provide information on whether any microbiological tests for tuberculosis were performed. Following eight months of antituberculosis treatment, her symptoms of cough and hemoptysis partially improved, but she developed exertional dyspnea and failed to gain weight. Four days prior to admission, the patient experienced severe paroxysmal coughing episodes with hemoptysis, shortness of breath, and profuse sweating. Her maternal uncle had a history of tuberculosis during childhood, though they had no prolonged contact. She consumed undercooked beef and lived in an environment where cattle and sheep were raised.

On admission, her physical examination revealed: heart rate of 92 beats per min, respiratory rate of 29 breaths per min, and a blood pressure of 93/61 mmHg. The patient appeared emaciated, with restricted left-sided respiratory movement and diminished breath sounds in the left lower lung. Crackles were audible. Heart rhythm was regular, but heart sounds were slightly muffled. No other abnormalities were noted on examination. There was no visible Bacille Calmette-Guérin scar on her arms. Bloodwork showed elevated eosinophils (1.17×10^9/L), and normal white blood cell count (4.07×10^9/L) and C-creative protein. Chest CT revealed a left lung gas-fluid level with pleural effusion, mediastinal shift, and whirl sign or a ribbon sign, suggestive of hydro-pneumothorax (Figure 1). The tests on admission for tuberculosis, including purified protein derivative (PPD) skin test, interferon-gamma release assay (IGRA), and sputum acid-fast bacilli smear, were negative.

On the second day of admission, due to progressive respiratory distress, she underwent surgery, during which the lesion and surrounding tissues were completely excised. Intraoperative finding showed that extensive fibrous adhesions on the chest wall and lung surface, with purulent debris and scattered hemorrhagic foci. After removal of the fibrous membrane, a few ruptures were identified, with bubbles emanating from them. The left upper lobe exhibited massive cystic changes, with significant fibrosis and thickening of the cyst wall. The cyst was filled with gas, purulent fluid, and dark brown, granular, melanin-like material, and the inner cyst wall was smooth (Figure 2). The left lower lobe was collapsed due to compression, and there was significant mediastinal shift towards the right side. The microbiological analysis of respiratory secretions yielded negative results for bacterial, fungal, and mycobacterium tuberculosis cultures.

Abbreviations
CT, computed tomography; PPD, Purified protein derivative; IGRA, interferon-gamma release assay.

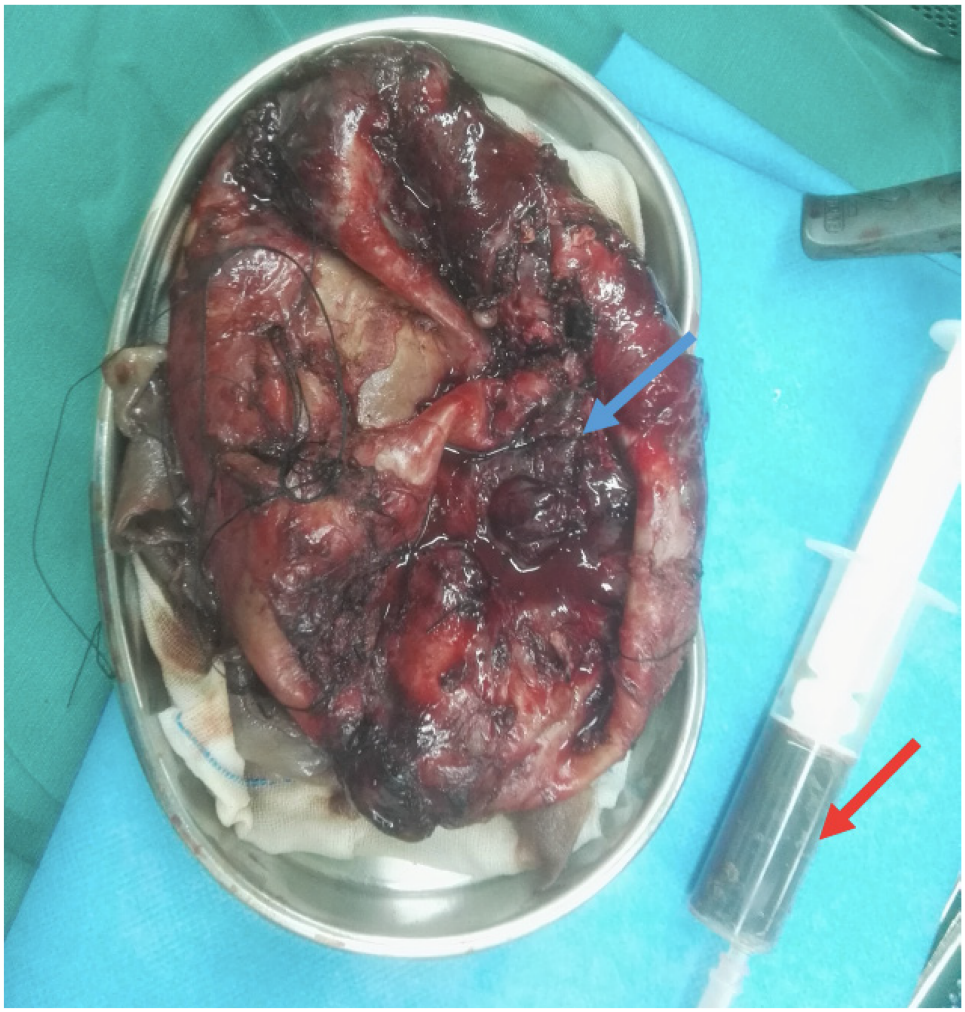

FIGURE 2
Macroscopic appearance. The left upper lobe exhibits massive cystic changes (the blue arrow), with significant fibrosis and thickening of the cyst wall. The cyst is filled with gas, purulent fluid, and dark brown, granular, melanin-like material (the red arrow).

Histopathological examination of the pulmonary lesions revealed granulomatous inflammation with the presence of parasitic structures, morphologically consistent with *Echinococcus granuloses* infection (cystic echinococcosis). Postoperative treatment included one month of albendazole, along with symptomatic management, and no complications were observed. Approximately six months post-discharge, the pediatric patient reported no cough, hemoptysis, night sweats or dyspea. Thereafter, the patient did not return for further clinical evaluation or participate in any telephone follow-up assessments.

3 Discussion

Hydatid cyst disease, caused by *Echinococcus granulosus,* is a significant public health concern in pastoral regions, where it is endemic (1, 8). The lung is the second most commonly affected organ after the liver (9, 10). The clinical presentation of pulmonary hydatid cysts largely depends on the cyst's size and location. Small, intact cysts are often asymptomatic and may be incidentally detected during imaging studies. In contrast, larger or ruptured cysts can produce significant symptoms such as, cough, hemoptysis, dyspnea and recurrent pneumonia, mimicking pulmonary tuberculosis (4, 11). Additional complications of cyst rupture may include acute respiratory distress syndrome and secondary infections, which can be life-threatening (4). This overlap in symptoms makes distinguishing hydatid cysts from tuberculosis particularly challenging in regions endemic to both disease, as demonstrated in this case. The patient, a 13-year-old girl from a dual-endemic region, presented with recurrent cough, hemoptysis, and night sweats, leading to an initial misdiagnosis of tuberculosis. The temporary improvement of cough and hemoptysis following antituberculosis therapy delayed further investigations, highlighting the diagnostic challenge posed by the nonspecific nature of the symptoms (Table 1). For patients undergoing empirical anti-tuberculosis

TABLE 1 Evidence of pulmonary tuberculosis and echinococcosis.

Contents	Pulmonary tuberculosis	Echinococcosis
Recurrent cough	Yes	Yes
Hemoptysis	Yes	Yes
Without fever	Uncertain	Yes
Exposure to TB	Yes	No
Exposure to sheep and cattle	No	Yes
Negative PPD test, IGRA	No	Uncertain
Negative tuberculosis microbiology	No	Uncertain
CT revealing ribbon sign	No	Yes
Granuloma and parasitic body in pathology	No	Yes

Yes, supportive evidence; No, non-supportive evidence; Ucertain, not sure.

therapy, if there is no significant clinical improvement after two months, and both acid-fast bacilli smear microscopy and mycobacterium tuberculosis culture yield negative results, it is imperative to re-evaluate the diagnosis of tuberculosis. Particularly in patients with a history of contact with cattle or sheep, the possibility of pulmonary hydatid disease should be strongly suspected and thoroughly investigated.

Imaging findings are critical for the diagnosis of hydatid cysts. Typical pulmonary hydatid cysts appear on CT scans as well-defined, homogeneous lesions with low density and smooth walls (12). However, rupture can produce characteristic signs such as the air crescent sign, inverse crescent sign, and air bubble sign. Complete rupture may present with imaging findings such as the cumbo sign, whirl sign, waterlily sign, or rising sun sign, which are key indicators for differentiating hydatid cysts from tuberculosis (12, 13). Although imaging plays a central role, overlapping radiological features with tuberculosis, such as cavity formation, may still confound the diagnosis, particularly in complicated cases. However, pulmonary imaging diagnostic technologies are continuously advancing. High-resolution ventilation proton MRI using hyperpolarized propane gas has been demonstrated to achieve both high resolution and ultrafast scan speeds (14, 15). Inhaled diethyl ether, as a gaseous contrast agent, has been validated for its applicability in MRI examinations of a wide range of pulmonary diseases (16). The future application of more advanced imaging technologies may further facilitate the differential diagnosis of diseases.

The presence of pulmonary lesions in a patient with a history of exposure to sheep and cattle, as in this case, should raise suspicion for hydatid cyst disease. The integration of imaging characteristic with serological detection of IgG antibodies against *Echinococcus* can significantly improve diagnostic accuracy. For instance, negative results from both the PPD test and IGRA can effectively rule out tuberculosis (8). Furthermore, direct bronchoscopic visualization combined with biopsy can expedite diagnostic clarification, facilitating timely and effective therapeutic interventions (17). In this case, the negative results for PPD and IGRA tests were useful in revisiting the initial diagnosis of tuberculosis and proceeding with further diagnostic workup.

Surgical intervention is the preferred treatment for pulmonary cystic echinococcosis, particularly in complicated cases involving cyst rupture or secondary infection (18). Complete cyst removal is essential to prevent recurrence and avoid complications such as chronic fistula formation (19). Albendazole therapy is often used postoperatively to prevent recurrence. In this case, postoperative albendazole therapy proved effective, and the patient recovered without recurrence or significant sequelae.

This case highlights several important considerations for clinicians managing patients with pulmonary lesions in endemic regions. Firstly, hydatid cyst disease should always be included in the differential diagnosis for pulmonary cavities, especially in children from dual-endemic regions. Secondly, recognizing typical imaging characteristic and incorporating relevant laboratory tests of PPD, IGRA, and serological detection of IgG antibodies against *Echinococcus* can improve diagnostic accuracy. Thirdly, the clinical symptoms of tuberculosis and pulmonary hydatid disease may overlap, leading to diagnostic confusion. Differential diagnosis should be established through comprehensive evaluation, including microbiological assays for *Mycobacterium tuberculosis*, assessment of prior tuberculosis exposure or animal contact history, serological testing for *Echinococcus-specific* IgG antibodies, analysis of pulmonary imaging features, and, when indicated, histopathological examination of affected tissues. Finally, multidisciplinary management, including timely surgical intervention and postoperative pharmacological therapy, is crucial for ensuring favorable outcomes.

4 Conclusion

The clinical presentation of echinococcosis is non-specific, and its CT imaging features are diverse, making the diagnosis of pulmonary echinococcosis challenging. When a patient presents with cough, hemoptysis, dyspnea, and pulmonary cavities, distinguishing it from pulmonary tuberculosis can be difficult. Epidemiological history (such as exposure to tuberculosis, contact with dogs or sheep), PPD skin test, IGRA, tuberculosis microbiological tests, serological testing for *Echinococcus-specific* IgG antibodies, and characteristic imaging signs of echinococcosis, can assist in diagnosis. Surgical resection and histopathological examination are crucial for confirming the diagnosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was obtained from the Ethical Board of the West China Second Hospital. Written informed consent to

participate in this study was not required from the participants or the participants' legal guardians in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the minor(s)' legal guardian for the publication of any potentially identifiable images or data included in this article.

Author contributions

QG: Writing – review & editing. YL: Writing – original draft. YL: Data curation, Writing – original draft.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Characteristics and outcomes in severe and critically ill children with first wave SARS-CoV-2 Omicron infection in Northeast China

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Aim: To describe the characteristics of severe and critically ill children with first-wave SARS-CoV-2 Omicron infection admitted to the pediatric intensive care unit (PICU) at the National Children's Regional Medical Center in Northeast China and to explore factors associated with poor outcomes.

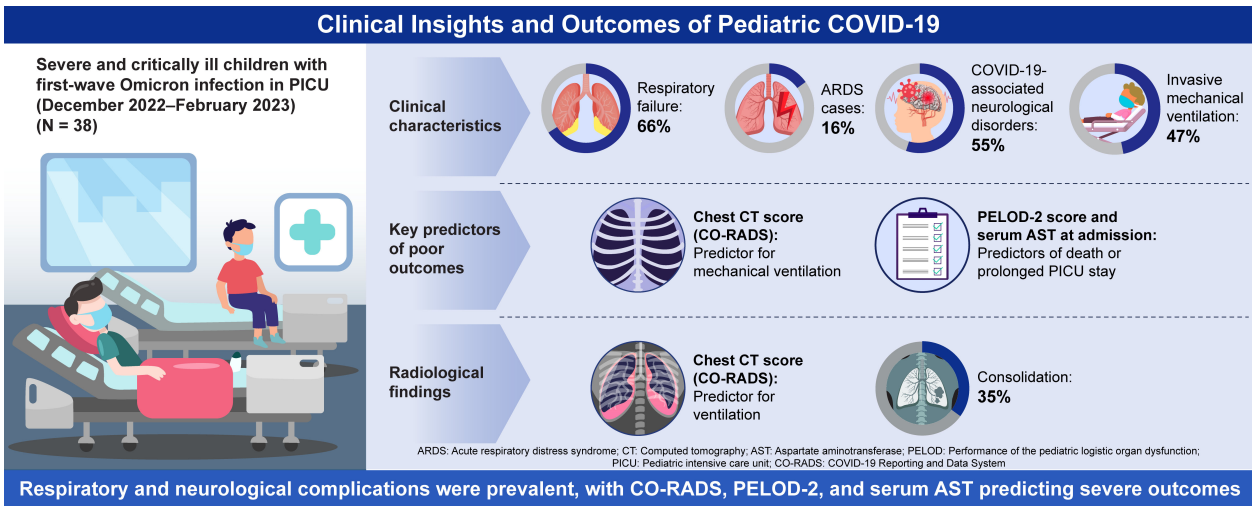
Methods: This observational cohort study was conducted in a PICU in northeastern China and included children under 18 years of age who were severely and critically ill due to SARS-CoV-2 Omicron infection between December 2022 and February 2023. Patients were categorized into two groups: the invasive mechanical ventilation (IMV) group and the non-IMV group. The primary outcome measured was the need for IMV, while secondary outcomes included death or prolonged PICU stay. Univariate and multivariate logistic regression analyses were performed to identify risk factors for poor outcomes.

Results: A total of 38 severe and critically ill children were included in the study. Of these, 25 (66%) were diagnosed with respiratory failure, and four (16%) developed acute respiratory distress syndrome. Additionally, 21 (55%) were diagnosed with COVID-19-associated neurological disorders, and 18 (47%) received IMV. Multivariate logistic regression analysis identified the chest computed tomography (CT) score, based on the COVID-19 Risk Assessment and Diagnosis System (CO-RADS), was statistically significant as an independent predictor for IMV in severe and critically ill children (odds ratio [OR]: 2.781 [95% confidence interval (CI): 1.021–7.571]). Furthermore, the Pediatric Logistic Organ Dysfunction-2 (PELOD-2) score and serum aspartate aminotransferase (AST) levels at admission were found to be independent predictors of death or prolonged PICU stay.

Conclusions: Respiratory failure and COVID-19-associated neurological disorders were the most common complications among severe and critically ill children with first-wave SARS-CoV-2 Omicron infection. Chest CT score, PELOD-2 score, and serum AST levels may serve as important indicators of poor outcomes in this patient population.

KEYWORDS

COVID-19, Omicron variant, PICU, respiratory failure, neurological disorder



GRAPHICAL ABSTRACT
Characteristics and outcomes in severe and critically Ill children with first wave SARS-CoV-2 omicron infection.

1 Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has spread globally since its emergence in late 2019 (Baker et al., 2022). As the virus continues to mutate, different COVID-19 variants have emerged with varying characteristics. The Omicron variant (B.1.1.529), first identified in South Africa in November 2021, became the predominant strain during the fourth wave of the global COVID-19 pandemic (World Health Organization, 2021). The Chinese government implemented strict epidemic control measures, including the "zero COVID" policy, which remained in effect until December 2022. After the zero-tolerance policy on COVID-19 ended in 2022, China experienced a widespread COVID-19 epidemic driven by the Omicron variant (Chinese Center for Disease Control and Prevention, 2023). Between December 2022 and February 2023, the cumulative number of confirmed cases increased by approximately 89.4 million, bringing the cumulative total to 99 million in China (World Health Organization, 2023a). During this period, all locally transmitted SARS-CoV-2 cases were confirmed to be caused by the Omicron variant according to the Chinese Center for Disease Control and Prevention.

Studies have shown that while the Omicron variant is more transmissible than previous variants, it is generally associated with lower disease severity and a reduced incidence of multisystem inflammatory syndrome in children (MIS-C) (Tian et al., 2022; Wang et al., 2022; Whittaker et al., 2022; Wolter et al., 2022; Choi et al., 2023; Ross et al., 2023; Wang et al., 2023). However, extra-pulmonary organ involvement, the need for invasive mechanical ventilation (IMV), and mortality rates in severe and critical cases have not significantly decreased (Corriero et al., 2022; de Prost et al., 2022). The high transmissibility of Omicron led to an increased medical burden on both adult and pediatric healthcare systems. Studies have reported a rapid rise in pediatric SARS-CoV-2 infections and hospitalizations during the first Omicron wave (Cloete et al., 2022; Menni et al., 2022). Although most pediatric cases are mild to moderate, some children develop severe illness requiring intensive care unit (ICU) admission (Bhalala et al., 2022; Uka et al., 2022; Zhang et al., 2023). There is limited research on children who have experienced severe or critical illness due to first-time infection with the Omicron variant.

In this study, we investigated children with severe and critical COVID-19 who were admitted to the pediatric ICU (PICU) of the National Regional Medical Center in Northeast China during the first Omicron epidemic and explored factors associated with poor outcomes.

2 Methods

2.1 Study design and population

In this retrospective study, we investigated children aged <18 years who were admitted to the largest PICU at Shengjing Hospital of China Medical University, which serves as the National Children's Regional Medical Center in Northeast China. The study was approved by the institutional ethics committee (Ethics No. 2023PS30K). Medical records from the electronic medical record system were reviewed for patients admitted to the PICU between December 1, 2022, and February 28, 2023. All enrolled

Abbreviations: PELOD-2, Pediatric Logistic Organ Dysfunction 2 score; AST, Aspartate Aminotransferase; IMV, Invasive Mechanical Ventilation; CT, Computed Tomography; CO-RADS, COVID-19 Reporting and Data System; ARDS, Acute Respiratory Distress Syndrome; mRS, Modified Rankin Scale; PICU, Pediatric Intensive Care Unit; CRP, C-Reactive Protein; IL-6, Interleukin 6; CK-MB, Creatine Kinase-MB isoenzyme; PCT, Procalcitonin; NIV, Non-invasive Ventilation; HFNO, High-Flow Nasal Oxygen; INR, International Normalized Ratio; D-dimer, Fragment of fibrinogen produced when blood clots break down; ADV, Adenovirus; MV, Mechanical Ventilation.

children had laboratory-confirmed COVID-19 infection, confirmed by real-time reverse-transcriptase polymerase chain reaction of nasopharyngeal swab samples. The Chinese Center for Disease Control and Prevention tested hospitalized patients to determine the COVID-19 strain during the epidemic, confirming that all cases were caused by the Omicron variant.

The inclusion criteria were: (a) Compliance with the diagnostic criteria for severe and critical cases as defined by the National Health Commission of the People's Republic Of China (NHCC) (Sun et al., 2025) (b) Availability of clinical data.

The exclusion criteria were (a) Refusal to participate (b) Discontinuation of therapy, including self-discharge against medical advice.

2.2 Criteria for diagnosis and definition

According to the guidelines on the diagnosis and treatment of new coronavirus pneumonia (version 10), issued by the NHCC on January 5, 2023 (Sun et al., 2025), all cases were classified into four severity groups: mild, moderate, severe, and critical. Severe cases were defined as meeting at least one of the following conditions: Persistent high fever > 3 days, shortness of breath, SpO2 ≤ 93% on room air at rest, alar flapping, the presence of three concave signs or wheezing, unconsciousness, convulsions, or difficulty feeding with signs of dehydration. Critical cases were defined as meeting at least one of the following criteria: Respiratory failure requiring mechanical ventilation, shock, and ICU admission for other organ dysfunctions. Details of the diagnostic criteria and differences from the WHO guidelines (World Health Organization, 2023b) are provided in Supplementary Table 1. Only severe and critical cases were included in this study.

Data extracted from medical records included: Pediatric Critical Illness Score (PCIS) at admission (Zhang et al., 2025), Pediatric Logistic Organ Dysfunction (PELOD-2) score at admission (Leteurtre et al., 2013), and modified Rankin Scale (mRS) score at discharge (Quinn et al., 2009). The diagnostic criteria for pediatric acute respiratory distress syndrome (ARDS) were based on the guidelines established by the Second Pediatric Acute Lung Injury Consensus Conference (PALICC-2) group in 2023 (Emeriaud et al., 2023). The chest CT score was based on the COVID-19 Risk Assessment and Diagnosis System (CO-RADS) (Prokop et al., 2020), which was employed to evaluate the severity of lung involvement in COVID-19 pneumonia. Furthermore, we assessed the mRS score for several patients 90 days post-discharge.

2.3 Data collection and classification

Age, sex, preexisting medical conditions, clinical data (including symptoms, time of onset, clinical diagnosis, and presence of complications), laboratory data, radiological findings (X-ray, computed tomography [CT], and magnetic resonance imaging [MRI] characteristics), treatment strategies, and patient outcomes were collected. Data were verified directly from the electronic medical records by at least two members of the research group to reduce information and transcription biases.

Patients were categorized into two groups based on the use of IMV. A comprehensive analysis was conducted on demographic information, clinical characteristics, laboratory and imaging results, treatment, outcomes, and risk factors associated with poor outcomes in both groups. Primary outcomes were defined as the need for IMV, while secondary outcomes included death or prolonged PICU stay (defined as ≥ 14 days).

2.4 Statistical analysis

The data in this study did not follow a normal distribution. Therefore, quantitative data were presented as the median and interquartile range (IQR), while categorical variables were reported as numbers (percentages). Cases in which corresponding tests or examinations were not performed were excluded, and the results were expressed as the number of positive outcomes divided by the total number tested. The Wilcoxon signed-rank test was used to compare continuous variables, while the corrected chi-square test or Fisher's exact test was applied for categorical variables. Bivariate regression analyses were conducted to screen variables for inclusion in multivariate regression models, which aimed to identify factors associated with the need for IMV and factors influencing prolonged PICU stay or death. Backward stepwise regression was applied in the multivariable analysis. Statistical significance was set at $p < 0.05$. Variables included in the final multivariate model were reported with odds ratios (ORs) and their 95% confidence intervals (CIs). All statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp.).

3 Results

3.1 Demographic data and clinical features

A total of 38 severe and critically ill children admitted to the PICU were included in this study, of whom 26 (68%) were classified as critical cases. Among these patients, 20 (53%) were male. The ages of the patients ranged from 30 days to under 17 years, with a median age of 25.5 (IQR: 12.5, 79.75) months. Children aged < 3 years accounted for 61% of the cases. Among the study population, 15 (39%) had received at least one dose of an inactivated COVID-19 vaccine (provided by Sinopharm CO.Ltd in Beijing or Wuhan, China and Kexing Biotch CO.Ltd in Shenzhen, China). Additionally, nine (24%) patients had pre-existing medical conditions. A total of 18 (47%) patients received IMV. Five of 38 patients (13%) developed cardiopulmonary arrest post-procedure, and four of five cases (80%) were successfully resuscitated.

Baseline characteristics and complications of the study population are summarized in Table 1. Several categorical variables, including fever, dyspnea, respiratory failure, laryngeal obstruction, ARDS, and the number of complications, were significantly associated with IMV. Among continuous variables,

TABLE 1 Baseline information of 38 children with severe or critical COVID-19.

Item	Total (N=38)	Non-IMV (n=20)	IMV (n=18)	*p*- value
Male n (%)	20 (53)	9 (45)	11 (61)	0.352
Age Group n (%)				0.757
<1 y	9 (24)	6 (30)	3 (17)	0.454
1–2 yr	14 (37)	6 (30)	8 (44)	0.503
3–6 yr	7 (18)	4 (20)	3 (17)	1.0
7–17 yr	8 (21)	4 (20)	4 (22)	1.0
Age (months) median (Q1,Q3)	25.5 (12.5,79.75)	25 (5,85.25)	26 (15,84)	0.988
Disease severity classification **n (%)**				**0.000**
Severe disease	12 (32)	12 (60)	0	
Critical disease	26 (68)	8 (40)	18 (100)	
Vaccine n (%)	15 (39)	8 (40)	7 (39)	0.604
Pre-existing medical conditions n (%)	9 (24)	4 (15)	5 (22)	0.709
BPD	3 (8)	2 (10)	1 (6)	
Congenital atrial septal defect	1 (3)	–	1 (6)	-
Methylmalonic acidemia	1 (3)	–	1 (6)	-
Aplastic anemia	1 (3)	–	1 (6)	-
Diabetes	1 (3)	1 (5)	–	-
Epilepsy	1 (3)	1 (5)	–	-
Congenital hypothyroidism	1 (3)	–	1 (6)	-
Symptoms n (%)				
Fever	24 (63)	9 (45)	15 (83)	**0.02**
Cough	26 (68)	15 (75)	11 (61)	0.489
Dyspnea	27 (71)	9 (45)	18 (100)	**0.000**
Altered consciousness	26 (68)	15 (75)	11 (61)	0.489
Convulsion/seizures	15 (39)	10 (50)	5 (28)	0.198
Convulsive status	3 (8)	2 (10)	1 (6)	1.0
Nausea/Vomiting	6 (16)	3 (15)	3 (17)	1.0
Diarrhea	5 (13)	1 (5)	4 (22)	0.17
Complications n (%)				
Respiratory failure	25 (66)	7 (35)	18 (100)	**0.000**
Central nervous system	21 (55)	12 (60)	9 (50)	0.745
Myocardial damage	9 (24)	2 (10)	7 (39)	0.058
Laryngeal obstruction	6 (16)	0	6 (33)	**0.007**
DIC	3 (8)	0	3 (17)	0.097
ARDS	4 (11)	0	4 (22)	**0.041**
Number of complications	3 (2,5)	2 (1,4.5)	5 (3,7.5)	**0.001**
Coinfection n (%)	15 (39)	5 (25)	10 (56)	0.096
Mycoplasma pneumoniae	11 (29)	4 (20)	7 (39)	0.288

(Continued)

TABLE 1 Continued

Item	Total (N=38)	Non-IMV (n=20)	IMV (n=18)	*p*- value
EB virus	2 (5)	0	2 (11)	0.218
Herpes simplex virus	2 (5)	1 (5)	1 (6)	1.0
PCIS median (Q1,Q3)	92 (88,98)	96 (92,100)	90 (81,93)	**0.001**
PELOD2 median (Q1,Q3)	1.5 (0,3)	0 (0,1)	2 (2,8.25)	**0.000**
P/F median (Q1,Q3)	268 (158,380)	456 (224,520)	260 (154,310)	0.057

Data are presented as the median (interquartile range) or number of patients (percentage) unless otherwise indicated. BPD, Bronchopulmonary dysplasia; PCIS, Pediatric Clinical Illness Score; PELOD2, pediatric logistic organ dysfunction-2 score.
P-values < .05 are in bold type.
P/F, PaO_2/FiO_2.

the PCIS, the PELOD-2, and the chest CT score (CO-RADS) were all significantly higher in the IMV group.

Figure 1 illustrates the clinical manifestations and complications in both IMV and non-IMV groups. The most common symptom was respiratory distress, followed by cough, altered mental state, and fever. The most significant complication was respiratory failure, followed by acute brain dysfunction.

3.2 Biochemical and radiological findings

Several continuous variables derived from laboratory data and the chest CT score based on CO-RADS were associated with the requirement for IMV support. Patients in the IMV group had lower mean platelet volume (MPV) levels and higher levels of creatine kinase (CK) and CK-MB isoenzyme. Additional laboratory findings are detailed in Table 2.

Chest CT was performed in 34 (89%) patients, all of whom exhibited inflammatory lesions, with 27 (79%) showing bilateral lung involvement. Consolidation was present in 12 (35%) cases (Supplementary Table 2). The chest CT scans of four children with ARDS with Omicron infections are shown in Figure 2. As depicted in the figure, the image revealed ground-glass opacity and large inflammation areas with partial consolidation, with two cases showing pneumothorax. Additional chest CT scans are provided in Supplementary Figure 1.

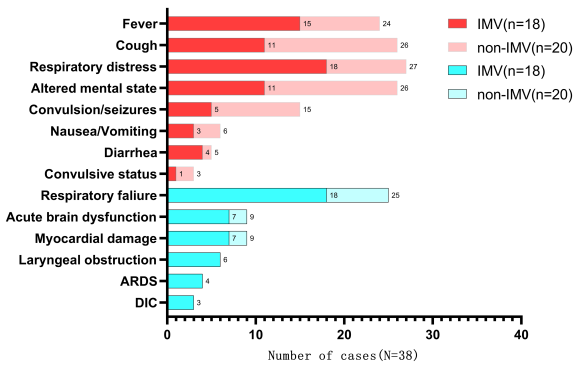


FIGURE 1
Main clinical manifestations and complications of severe or critically ill children with Omicron infection between IMV group and non-IMV group. The red part indicates clinical manifestations and the blue part indicates complications. CNS, central nervous system; DIC, disseminated intravascular coagulation.

Eighteen of 21 children (86%) with COVID-19-linked neurological disorders received head MRI, of whom 12 (67%) showed abnormalities. The most frequent clinical manifestations in these patients were unconsciousness and convulsions. MRI findings indicated that multiple brain regions were affected, either focally or diffusely. Figure 3 displays typical MRI findings from five critically ill children with neurological disorders. Nine of the 12 (75%) patients with MRI abnormalities had bilateral brain involvement, with the most commonly affected regions being the paraventricular white matter and the frontal and occipital lobes. These abnormalities were associated with higher mRS scores at discharge. Detailed descriptions of MRI findings are available in Supplementary Table 3. Additionally, 17 (81%) patients received lumbar puncture for cerebrospinal fluid (CSF) analysis, but none tested positive for the new coronavirus in the CSF. Details are described in Supplementary Table 2.

3.3 Therapeutic and outcome data

Details on medications, respiratory support, and advanced therapies administered to patients, along with their outcomes, are summarized in Table 3. Children in the IMV group had significantly longer PICU stays ($p < 0.001$). In this group, two patients (5%) died: one patient succumbed to acute necrotizing encephalopathy, while the other died due to ARDS, lung air leak, and cutaneous emphysema. Meanwhile, eight patients had mRS scores ≥ 3 at discharge, including the two deceased patients. At 90-day follow-up, mRS scores ≥ 3 persisted in two patients.

3.4 Analysis of factors associated with poor outcomes

Based on univariate analysis, sex and age were excluded from the subsequent multivariate analysis. In the multivariable model, the results showed that the chest CT score was the only independent predictor of IMV requirement in severe and critically ill children (OR 2.781 [95% CI 1.021–7.571]) (Table 4).

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TABLE 2 Comparison of the laboratory indices between non-IMV and IMV groups.

Item	Total (N=38)	Non-IMV (n=20)	IMV (n=18)	*p*-value
Blood routine test Median (Q1,Q3)				
WBC counts cell (*10^9/L)	6.2 (5.1,8.43)	6.045 (5.15,8.8)	6.3 (4.99,8.13)	0.953
Neutrophil counts cell (*10^9/L)	3.55 (1.9,5.63)	3.5 (1.5,4.6)	3.95 (2.375,6.275)	0.365
Lymphocyte counts cell (*10^9/L)	1.35 (0.9,3.23)	1.4 (0.925,3.475)	1.3 (0.85,3.225)	0.539
Hemoglobin levels (mg/L)	117 (101.5,128.5)	116.6 (99.25,127.25)	118 (105.5,129)	0.831
Platelet counts cell (*10^9/L)	179.5 (127,276.5)	174.5 (138,257)	185.5 (101.25,386.25)	0.569
Eosinopenia counts cell (*10^9/L)	0.15 (0,0.6)	0.35 (0,0.9)	0.1 (0,0.525)	0.367
MPV fL	8.45 (7.875,9.6)	9.1 (8.025,9.8)	8.25 (7.475,8.875)	**0.035**
NLR	2.29 (0.71,5.91)	1.61 (0.55,4.88)	3.194 (0.958,9.202)	0.219
PLR	123.32 (73.96,218.33)	88.35 (53.95,207.77)	149.09 (93.807,225.865)	0.169
SII	349.82 (131.68,1168.08)	247.31 (83.76,737.68)	559.15 (248,1370.88)	0.108
Cardiac biomarkers Median (Q1,Q3)				
CK (U/L)	188 (90.75,801.75)	118 (75.25,240.75)	294.5 (129.5,1117.75)	**0.044**
CKMB (U/L)	38.5 (19.68,51.25)	41.5 (20.5,47.75)	33 (16.6,90)	0.953
Troponin T (ng/L)	0.0155 (0.005,0.1183)	0.013 (0.00425,0.0835)	0.0165 (0.008,0.182)	0.497
Troponin I (ug/L)	0.0274 (0.0046,0.1340)	0.0061 (0.0039,0.1746)	0.0355 (0.0088,0.2088)	0.317
pro-BNP (pg/mL)	749.45 (223.25,1995.5)	545 (317.25,7502.75)	925.45 (182.5,1501)	0.671
CK - MB isoenzyme (ug/L)	5.3 (1.55,12.65)	2.6 (1.3,5.9)	9.45 (2.175,27.375)	**0.045**
LDH (U/L)	382.5 (316.25,837.25)	401 (308,1215.75)	365.5 (308.25,2034.25)	0.792
Liver function markers Median (Q1,Q3)				
ALT (U/L)	31.5 (17,81.5)	34 (17.5,73.25)	26.5 (17,121)	0.93
AST (U/L)	52.5 (42.75,174)	49 (39.75,174)	62.5 (43,215.5)	0.511
Albumin (g/L)	38.1 (34.3,41.65)	37.4 (34.2,41.5)	38.95 (33.825,43.2)	0.331
Kidney function Median (Q1,Q3)				
Cr (μmol/L)	24.7 (19.9,40.35)	23.45 (18.6,43.35)	27 (21.35,39.9)	0.604
Inflammation markers Median (Q1,Q3)				
CRP (mg/L)	6.6 (1.19,21)	6.6 (1.1775,17.4)	7.2 (1.55,24.98)	0.538
IL-6 (pg/mL)	17.15 (6.69,47.76)	16.17 (7.59,29.95)	22.99 (3.88,245.93)	0.233
PCT (ng/mL)	0.495 (0.195,2.35)	0.408 (0.231,1.56)	0.834 (0.15,2.905)	0.692
Coagulation function Median (Q1,Q3)				
PT (s)	12.8 (11.1,14.6)	12.2 (10.65,14.2)	13.3 (11.25,15.1)	0.409
APTT (s)	34 (30,43)	34.9 (32,43)	34 (28,45)	0.741
INR	1.2 (1.0,1.3)	1.1 (0.95,1.25)	1.25 (1.0,1.425)	0.157
Fib (g/L)	2.1 (1.5,2.6)	2 (1.455,2.515)	2.3 (1.475,2.725)	0.457
D-dimer (DDU)	314 (211,1242)	287 (208,1173)	376 (220,2180)	0.428
Myoglobin (ug/L) Median (Q1,Q3)	34.65 (16.58,150.68)	17.25 (14.48,36.35)	78.65 (22.5,364.38)	**0.012**
Lactate (mmol/L) Median (Q1,Q3)	1.7 (1.1,2.4)	1.3 (1.0,1.72)	2.05 (1.1,3.65)	0.111

(Continued)

TABLE 2 Continued

Item	Total (N=38)	Non-IMV (n=20)	IMV (n=18)	*p*-value
LAR	0.0414 (0.0295,0.0701)	0.0390 (0.0265,0.0574)	0.0582 (0.0309,0.0809)	0.181
FAR	0.0550 (0.0369,0.0661)	0.0550 (0.0402,0.0655)	0.0562 (0.0367,0.0783)	0.717
CAR	0.1570 (0.0315,0.6274)	0.1570 (0.0295,0.5673)	0.1921 (0.0412,0.6860)	0.649
LDH/Alb	9.6062 (8.1433,24.9026)	9.7608 (8.2740,36.4935)	9.4517 (6.6339,15.1263)	0.622
Glu (mmol/L) Median (Q1,Q3)	5.6 (4.74,7.05)	5.32 (4.31,6.62)	6.2 (4.95,8.65)	0.223
Immunoglobulin (g/l) Median (Q1,Q3)				
IgA	0.4285 (0.1525,1.1465)	0.466 (0.08,1.25)	0.296 (0.154,1.183)	0.692
IgM	0.7935 (0.4953,1.1375)	0.787 (0.489,1.17)	0.8 (0.473,1.165)	0.925
IgG	6.35 (4.2875,9.0425)	7.33 (4.97,14.9)	5.23 (3.475,8.19)	0.168
T-lymphocyte subsets cell (/ul) Median (Q1,Q3)				
T-lymphocyte count	656 (510,1822.5)	868 (464.5,2159)	645 (528,1133)	0.63
Th count	313 (219,830)	435 (212.5,1570.5)	435 (212.5,1570)	0.324
Ts count	266 (177.5,518.5)	266 (154,590.5)	249.5 (192,465.75)	0.809
Natural killer cells count	185.5 (87,273)	213 (107,389.5)	145 (67,243)	0.222
B-lymphocyte count	480 (287,785.5)	712 (208.5,1381)	479 (360,526)	0.475
Ts/Th	1.21 (0.945,1.615)	1.3 (0.955,2.29)	1.1 (0.708,1.48)	0.293
Chest CT Score	2.5 (1,4.25)	1 (1,3)	4 (1.75,5)	**0.008**

Data are presented as the median (interquartile range) or number of patients (percentage) unless otherwise indicated. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BNP, Brain natriuretic peptide; IL-6, Interleukin-6; CRP, C-reactive protein; LDH, Lactate dehydrogenase; LAR, Lactate/Albumin; FAR, Fibrinogen/Albumin; CAR, CRP/Albumin; NLR, neutrophil-lymphocyte ratio; PLR, Platelet-Lymphocyte Ratio; SII, systemic immune inflammation index; MLR, monocyte-lymphocyte ratio.
P-values < .05 are in bold type.

For secondary outcomes, multivariate analysis identified PELOD-2 (OR 2.717 [95% CI 1.011–7.299]) and serum AST levels (OR 14.766 [95% CI 1.395–156.308]) at admission as predictors of poor outcomes (death or prolonged PICU stay) in all severe and critically ill children with SARS-CoV-2 Omicron infection (Table 5).

4 Discussion

In this study, we described the clinical characteristics of severe and critically ill children diagnosed with the first wave of SARS-CoV-2 Omicron infection who were admitted to the PICU of the National Regional Children's Medical Center in Northeast China. Our findings indicate that chest CT score based on CO-RADS, serum AST levels at admission, and the PELOD-2 score may predict adverse outcomes in this specific population.

Our data showed that 61% of severely and critically ill children were younger than 3 years old. A statistical analysis of children in France with acute COVID-19 infections in the PICU found that 45.1% of patients were aged <2 years (excluding those under 1 month), while 60.7% were aged <5 years (Recher et al., 2023). Similarly, a British study reported that 61% (36/59) of PICU patients were aged <5 years (Ward et al., 2023). With the emergence of the Omicron variant, mathematical modeling and quantitative analyses of empirical data predict that first-time infections may occur at younger ages due to rising population immunity from prior Omicron infections and vaccination efforts (Koelle et al., 2022). A plausible explanation for this trend is that children aged <3 years remain unvaccinated (Tachikawa et al., 2025), making them more vulnerable to severe initial infections and increasing likelihood of requiring PICU admission.

Comorbidities have been consistently associated with severe SARS-CoV-2 infection in pediatric populations, with studies reporting that 15.6% to 83% of pediatric patients have at least one pre-existing condition across various cohorts (Ungar et al., 2023; Kulkarni et al., 2024). Our study aligns with this range, revealing that 24% of patients had comorbidities, with 13% requiring IMV and 8% receiving non-IMV. Although the clinical symptoms of Omicron infection are mild in the vast majority of healthy children, those with comorbidities may experience further deterioration of underlying conditions and a decline in quality of life (Dryden et al., 2022; Jassat et al., 2023; Calcaterra et al., 2024). Compared to the Alpha variant, the Omicron group had a higher prevalence of comorbidities, worse initial laboratory data, and higher in-hospital mortality rates (40.6% vs 15.2%, $p = 0.004$). The Charlson Comorbidity Index was identified as an independent risk factor for in-hospital mortality (Cheng et al., 2025).

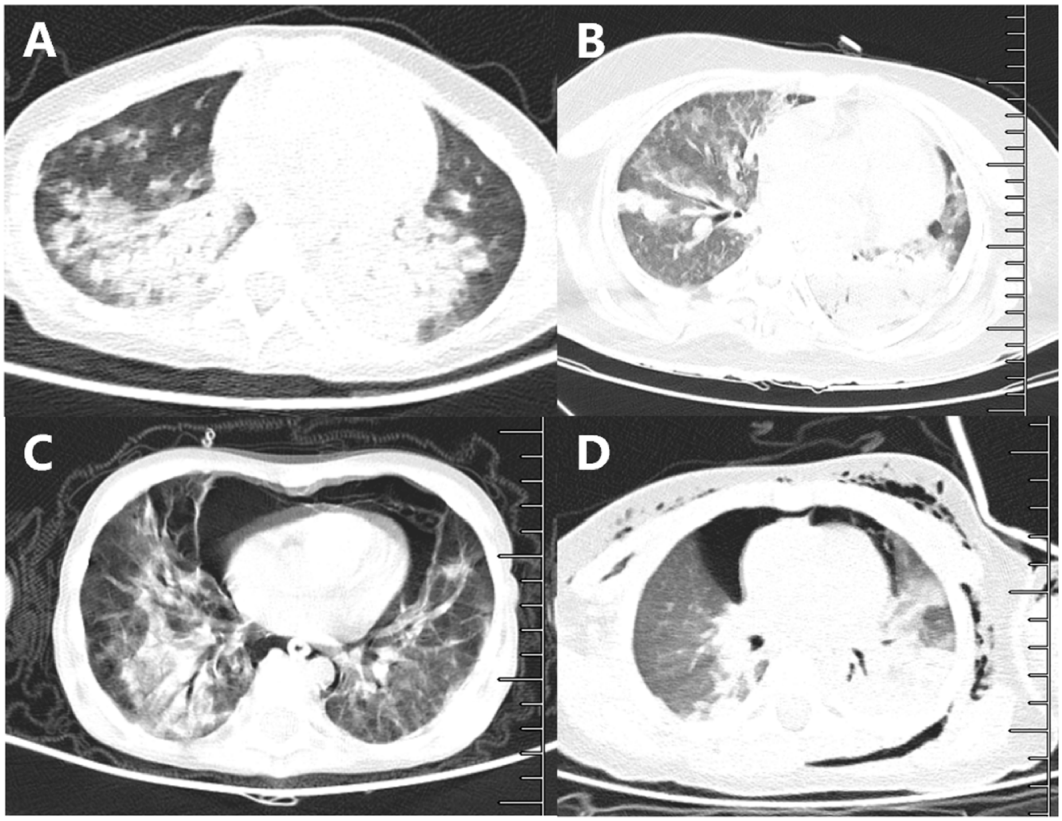

FIGURE 2
Chest CT findings of acute respiratory distress syndrome children with Omicron variant infection. Chest CT images of 4 critically ill children with ARDS: **(A)** a 4-year-old girl: ground-glass opacity and large areas of inflammation with partial consolidation; **(B)** a 16-year-old boy with methylmalonic acidemia; **(C)** a 6-year-old girl with mediastinal emphysema; **(D)** a 2-year-old boy with pneumothorax: partial effusion, who was coinfected with EBV.

These findings highlight the heightened risk of adverse outcomes in pediatric patients with comorbidities, emphasizing the need for closer monitoring and targeted interventions.

At admission, 25 children (66%) were diagnosed with respiratory failure, and four (16%) developed ARDS. A previous study in southern China (Guangdong) supports our findings (Lin et al., 2024b). Similarly, research on critically ill adults and children in the northeastern United States (New York) found that 90% of patients who developed ARDS initially presented with dyspnea (Derespina et al., 2020). Accurate identification of these presentations, combined with the early administration of respiratory support, may improve patient outcomes by minimizing disease progression and alleviating stress in healthcare facilities (Mizuno et al., 2023). Initial studies during the pandemic linked respiratory system impairment at hospital admission to adverse outcomes with ARDS, serving as an independent predictor of disease severity (Ni et al., 2024). Some research suggests that COVID-19 comorbidities increase with latitude, but we found no evidence to confirm an association between geographic location and complications or comorbidities in children.

COVID-19 is known to cause neurological complications (Bhalala et al., 2022). Studies report that approximately one-third of hospitalized children with Omicron infection experience neurological disorders, such as convulsions (Choi et al., 2023; Tang L. et al., 2024), with higher incidence rates in severe cases (LaRovere et al., 2023). A study by Lin et al. found that 70% of critically ill children with SARS-CoV-2 Omicron infection (Lin et al., 2024b) developed encephalopathy. Among the cohort, 21 (55%) had neurological complications, with convulsion being the most common manifestation (39%). However, we could not determine whether SARS-CoV-2 infection was the primary cause of these neurological symptoms. There are some potential pathogenic mechanisms of SARS-CoV-2 infection affecting the central nervous system (CNS): direct invasion of the CNS, excessive release of pro-inflammatory cytokines, and the immune escape effect of the virus (Viviani et al., 2003; Hautala et al., 2021; Kurd et al., 2021; Shi et al., 2023).

In our study, 12 patients had abnormal head MRI findings. A single-center study conducted in the United States found that the most prevalent imaging findings among adults were nonspecific white matter microangiopathy (55.4%), chronic infarcts (19.4%), acute or subacute ischemic infarcts (5.4%), and acute hemorrhage (4.5%) (Radmanesh et al., 2020). Additionally, a study focusing on ICU patients in France reported that 23% (6/26) of patients experienced cerebrovascular events (Cleret de Langavant et al., 2021). Research in South China involving children supports our findings, indicating that all cases exhibited bilateral involvement, particularly in the thalamus and basal ganglia (Lin et al., 2024a).

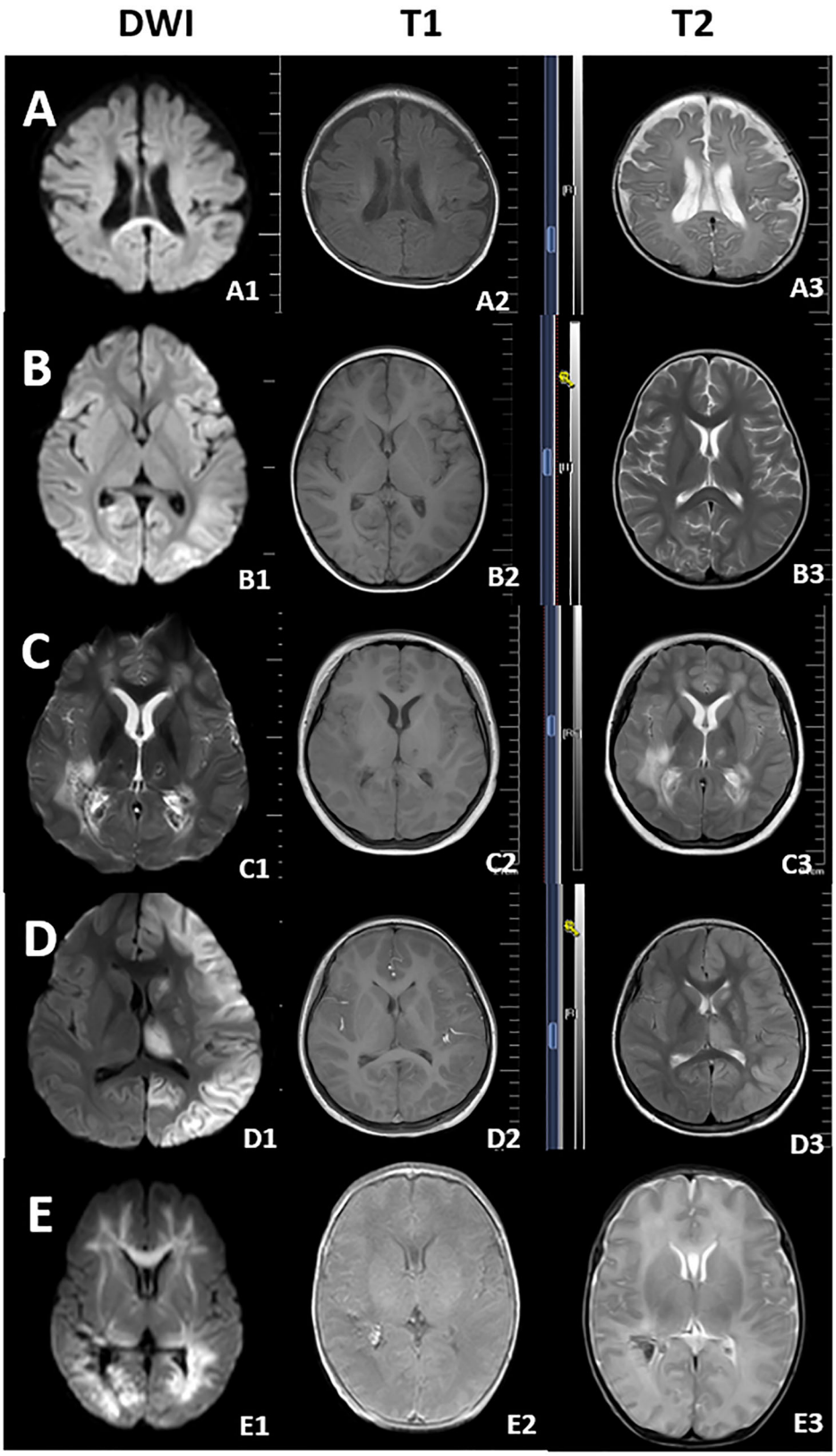

FIGURE 3
HEAD MRI of critically ill children infected with Omicron variant. **(A)** A 2-month-old boy with febrile seizures (For lung CT, see Supplementary Figures D1, D2). Cytotoxic edema was seen in the splenium of the corpus callosum. **(B)** A 28-month-old girl with febrile seizures. She had no obvious symptoms of respiratory system. Multiple cytotoxic edema lesions was seen in the brain. **(C)** A 11-year-old girl with febrile seizures. **(D)** A 9-year-old girl with status convulsion. She underwent shock and tracheal intubation and was discharged on the 8th day. **(E)** A 1-month-old boy, was admitted to the PICU at 39 weeks PMA. Head MRI shows extensive lesions in the brain.

TABLE 3 Treatment and outcomes of IMV and non-IMV groups with Omicron variant infection.

Item	Total (N=38)	Non-IMV (n=20)	IMV (n=18)	*p*-value
Treatment n (%)				
Antibiotics	34 (89)	16 (80)	18 (100)	0.107
Corticosteroids	31 (82)	13 (65)	18 (100)	**0.009**
Dexamethasone	16 (42)	4 (20)	12 (67)	**0.008**
Methylprednisolone	17 (45)	9 (45)	8 (44)	1.0
IVIG	15 (39)	7 (35)	8 (44)	0.741
Mannitol	13 (34)	7 (35)	6 (33)	1.0
Albumin	7 (18)	1 (5)	6 (33)	**0.038**
Antiviral drugs	3 (8)	0	3 (17)	0.097
Azvudine	1 (3)	0	1 (6)	-
Remdesivir	1 (3)	0	1 (6)	-
Monoclonal antibodies	1 (3)	0	1 (6)	-
Alprostadil	10 (26)	4 (20)	6 (33)	0.468
Adrenaline	3 (8)	0	3 (17)	-
Breathing support n (%)				
IMV≥96 h	16 (42)	0	16 (89)	**0.000**
IMV<96 h	2 (5)	0	2 (11)	-
Duration of IMV (h)	103.5 (0,170)	0	103.5 (0,170)	**<0.001**
High frequency ventilation	1 (3)	0	1 (6)	0.474
Inhaled nitric oxide therapy	2 (5)	0	2 (11)	0.218
Non-invasive assisted ventilation	11 (29)	5 (25)	6 (33)	0.724
Nasal cannula oxygen therapy	7 (18)	3 (15)	4 (22)	0.687
Advanced therapies n (%)				
Plasma exchange	7 (18)	4 (20)	3 (17)	1.0
CRRT	1 (3)	0	1 (6)	–
Tracheotomy	1 (3)	0	1 (6)	–
LOS (days) Median (Q1,Q3)	11.5 (7,18.25)	7.5 (5,11.75)	17 (12.25,22.5)	**0.000**
Outcomes n (%)				
LOS < 14 days and discharge	22 (58)	17 (85)	5 (28)	**0.001**
LOS ≥ 14 days and discharge	14 (37)	3 (15)	11 (61)	**0.006**
Death	2 (5)	0	2 (11)	0.218
mRS score at discharge Median (Q1,Q3)	0 (0.1.25)	0 (0,0)	0 (0,4)	0.298

Data are presented as the median (interquartile range) or number of patients (percentage). IVIG, Intravenous immunoglobulin; IMV, Invasive Mechanical Ventilation; CRRT, Continuous Renal Replacement Therapy; LOS, Length of hospital stays; IQR, interquartile range; mRS score, Modified Rankin Scale.
P-values < .05 are in bold type.

Possible reasons for these differences in brain involvement or clinical manifestations may relate to age and cardiovascular risk factors (Lu et al., 2024), or the likelihood of children developing acute necrotizing encephalopathy may be higher (Karami et al., 2023). Multicenter cohort studies on the adverse neurological functional outcomes and exploring factors are needed.

Our research shows that chest CT score is associated with the need for IMV in severe and critically ill children. Li et al. did not calculate chest CT scores but described CT findings of consolidation, linear opacities, crazy-paving pattern, bronchial wall thickening, high CT scores, and extrapulmonary lesions as features of severe or critical COVID-19 pneumonia (Li et al., 2020). A study conducted in Brazil

TABLE 4 Predictors of invasive mechanical ventilation.

Item	Non-IMV (n=20)	IMV (n=18)	OR (95%CI)	*p*-value
Univariable logistic regression analyses				
Disease severity classification (Critical)	8 (40)	18 (100)	–	0.998
PCIS ≤ 80	1 (5)	4 (22)	-	0.149
Number of complications≥3	8 (40)	16 (89)	12 (2.147-67.067)	**0.005**
Chest CT Score based on CO-RADS	1 (1,3)	4 (1.75,5)	2.028 (1.135,3.624)	**0.017**
CK≥188 (U/L)	4 (20)	9 (50)	4.0 (0.954,16.769)	0.058
CK-MB isoenzyme (ug/L) ≥10	1 (5)	9 (50)	14 (1.507,130.099)	**0.02**
Myoglobin (ug/L)≥34.65	1 (5)	6 (33.3)	6.75 (0.662-68.779)	0.107
IL-6≥20 pg/mL	4 (20)	10 (56)	5 (1.188-21.039)	**0.028**
Multivariable logistic regression analysis				
Number of complications≥3	8 (40)	16 (89)	4.919 (0.307-78.74)	0.26
CK-MB isoenzyme (ug/L) ≥10	1 (5)	9 (50)	-	0.999
Chest CT Score based on CO-RADS	1 (1,3)	4 (1.75,5)	2.781 (1.021-7.571)	**0.045**
IL-6≥20 pg/mL	4 (20)	10 (56)	4.045 (0.212-77.011)	0.353

Data are presented as the median (interquartile range) or number of patients (percentage). *P*-values < .05 are in bold type.

on non-invasive respiratory support and IMV concluded that alternating non-invasive respiratory support with HFNO increased IMV rates, regardless of comorbidities and chest CT scores among patients with COVID-19. The chest CT scores were significantly different among all groups (da Cruz et al., 2024). For immunocompromised patients, the total chest CT score was associated with longer hospitalization and ICU admission (Ghadery et al., 2024). Compared with prior studies, the results in our cohort accurately predicted the need for IMV in severe and critically ill children with first-time Omicron infection. Our findings further support the predictive value of chest CT scores for IMV requirements, which could be enhanced by combining CT analysis with other clinical parameters (Orlandi et al., 2021). The results above suggest that the chest CT score based on CO-RADS could be an

TABLE 5 Predictors of death or PICU stay ≥ 14 days.

Variables	Univariable		Multivariable	
Univariable logistic regression analyses	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Number of complications≥3	21.667 (2.412,194.649)	**0.006**	–	–
mRS score	2.01 (1.173,3.445)	**0.011**	-	–
PELOD2≥3	23.8 (3.99,141.963)	**0.001**	2.717 (1.011,7.299)	**0.047**
Neutrophil counts cell ≥3.55 × 10^9/L	4.714 (1.178,18.861)	**0.028**	–	-
CK≥188 (U/L)	4.714 (1.178,18.861)	**0.028**	–	-
Troponin I (ug/L) ≥0.0274	4.4 (1.041,18.599)	**0.044**	–	–
AST≥52.5 (U/L)	11.556 (2.411,55.392)	**0.002**	14.766 (1.395,156.308)	**0.025**
IL-6≥20 pg/mL	8.25 (1.65,41.247)	**0.008**	–	–
PCT (ng/mL)≥0.5	6.5 (1.467,28.804)	**0.014**	–	-
PT≥12.8 (s)	12.133 (2.405,61.202)	**0.003**	–	–
INR≥1.2	12.133 (2.405,61.202)	**0.003**	–	–
D-dimer (DDU)≥314	12.133 (2.405,61.202)	**0.003**	4.51 (0.494,41.169)	0.182
Lactate≥1.7 (mmol/l)	5.28 (1.196,23.317)	**0.028**	–	–

P-values < .05 are in bold type.

important factor in predicting poor outcomes for severe and critically ill children.

The study showed that PELOD-2 and serum AST levels ≥52.5 U/L at admission were associated with increased mortality or prolonged PICU stays. A multi-center survey on prolonged mechanical ventilation (MV) showed that the use of vasoactive agents and higher PELOD-2 scores at the time of the prolonged MV diagnosis were significantly associated with an increased risk of prolonged MV-related death. Meanwhile, early rehabilitation intervention was identified as crucial for improving patient outcomes (Zhang et al., 2024). Several studies used the PELOD-2 to predict mortality and prognosis (El-Nawawy et al., 2017; Schlapbach et al., 2018; Lee et al., 2022). These findings suggest that the predictive power of PELOD-2 may vary across specific subpopulations.

The results of this study indicated that abnormalities in various laboratory indicators were more pronounced in critically ill children within the IMV group. Notably, serum AST levels were a predictor of PICU duration. Other indicators included CK-MB isoenzyme (μg/L) ≥10, IL-6 ≥20 pg/mL, and coagulation indices (PT, INR, D-dimer). Early Omicron infection may induce subclinical cholangiocyte damage through a multifactorial and complex pathogenic process, differing from earlier strains of SARS-CoV-2 (Iheanacho and Enechukwu, 2022). Cao et al. (2024) observed that patients with abnormal liver enzyme levels exhibited significantly elevated inflammatory markers, including PCT, IL-6, and CRP. IL-6, as an inflammatory factor, may be attributed to stress responses in the liver, characterized by the release of major acute-phase cytokines in response to Omicron infection (Dufour et al., 2022). A study on adenovirus (ADV) infection found that AST levels were also associated with longer hospital stays in children (Tang S. et al., 2024). Based on these findings, serum AST levels can be considered a reliable indicator of prolonged PICU stays.

This study has some limitations. First, the relatively small sample size might limit the generalizability of our conclusions to patients in other regions. Second, since this was a retrospective study, potential biases and confounding factors might have influenced the results. Additionally, long-term follow-up data were not collected. Finally, standardized scores were not used to assess disease severity in patients, which should be considered in future research.

5 Conclusions

Respiratory failure and COVID-19-associated neurological disorders are major complications in severe and critically ill children with first-wave SARS-CoV-2 Omicron infection. Chest CT score based on CO-RADS may predict severe and critically ill children with SARS-CoV-2 Omicron infection requiring intensive mechanical ventilation. Additionally, serum AST levels at admission and the PELOD-2 score may predict prolonged PICU stays and mortality, helping to avert the development of potentially life-threatening complications.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by The ethics committee of Shengjing Hospital(Ethics No. 2023PS30K). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

TS: Validation, Writing – original draft, Writing – review & editing. YH: Validation, Writing – original draft, Writing – review & editing. LW: Supervision, Validation, Conceptualization, Methodology, Writing – review & editing. ZW: Data curation, Methodology, Writing – review & editing. CL: Supervision, Validation, Data curation, Methodology, Writing – review & editing. WX: Conceptualization, Formal Analysis, Methodology, Supervision, Validation, Writing – review & editing. KY: Conceptualization, Funding acquisition, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2025.1495783/full#supplementary-material

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Prognosis of different types of acute infection in the first episode of childhood acute leukemia

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Objective: The aim of the present study was to determine the prognosis of different types of acute infection in pediatric leukemia patients.
Methods: A retrospective study was carried out on pediatric leukemia patients with acute infections admitted to the Second Affiliated Hospital of Harbin Medical University between 1 September 2004 and 31 August 2022. Clinical characteristics, diagnostic findings, and prognostic outcomes were extracted from the eligible cases and analyzed.
Results: There were 36 cases of acute myeloid leukemia (AML) and 72 cases of acute lymphoblastic leukemia (ALL) that met the inclusion criteria. There were significant differences in the incidence of pneumonia (47.2% vs. 27.8%, $p = 0.045$) and sepsis (19.4% vs. 2.8%, $p = 0.006$) between the AML and ALL groups. There were 10 cases with a poor prognosis and 26 cases with a favorable prognosis in the AML group. There were no significant differences between the poor prognosis and the favorable prognosis groups except for age (14.2 ± 1.2 years vs. 9.6 ± 4.3 years, $p = 0.003$). There were 14 cases with a poor prognosis and 58 cases with a favorable prognosis in the ALL group. There were no significant differences between the poor prognosis and favorable prognosis groups except for age (13.4 ± 2.7 years vs. 9.2 ± 4.7 years, $p = 0.002$).
Conclusions: There were significantly more incidence of pneumonia and sepsis in children with AML. Younger AML and ALL children with acute infections have more favorable prognoses than older children.

KEYWORDS

prognosis, acute infection, acute pediatric leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, children

1 Introduction

Acute pediatric leukemia is a serious condition in childhood. In 2020, there were 67,008 new reported cases of pediatric leukemia globally, with male patients accounting for 57.85% of all cases (1). The survival rates of pediatric acute lymphoblastic leukemia (ALL) have been reported to be in the range of 83%–94% (2), compared to 65%–70% for pediatric acute myeloid leukemia (AML) (3). Infections may increase morbidity and mortality in patients with acute pediatric leukemia who have a much higher risk of infection, potentially due to their malfunctioning immune system, as well as the burden of their therapies, which could lead to repeated, prolonged, and complicated deterioration of immune cells (4). Infections in acute pediatric leukemia patients,

besides causing a higher mortality, also lead to prolonged hospitalizations, disrupt chemotherapy schedules, negatively affect patients' quality of life, and escalate the demand for extra healthcare resources and costs (5). This multifaceted impact underscores the need for vigilant infection management to optimize treatment outcomes and patient wellbeing.

In this study, we analyzed the prognosis of different types of acute infection in patients with pediatric leukemia and showed the most relevant factors that could affect the prognosis.

2 Methods

This retrospective study was conducted in pediatric leukemia patients with acute infections admitted to the Second Affiliated Hospital of Harbin Medical University between 1 September 2004 and 31 August 2022. The inclusion criteria were as follows: (1) patients aged under 18 years; (2) patients diagnosed with acute leukemia according to the established criteria (6, 7); (3) patients experiencing a first episode of leukemia with no prior treatment; (4) patients diagnosed with acute infection based on clinical symptoms and signs, supported by laboratory test results (8); (5) patients with signed informed consent to participate in medical research upon admission. The exclusion criteria were as follows: (1) patients with chronic infectious diseases; (2) patients with other types of cancer; (3) patients with COVID-19 infection, confirmed by nucleic acid amplification tests (NAATs) of nasal swab samples (9, 10); (4) patients with acute conditions needing surgical treatments; and (5) incomplete data.

Clinical characteristics such as age, gender, hospital stay, and different types of acute infections, including upper respiratory tract infection, pneumonia, pleurisy, sepsis, bronchitis, urinary tract infection, oral infection, perianal infection, acute enteritis, herpetic angina, skin infection, and soft tissue infection, were abstracted and analyzed.

Poor prognoses referred to uncontrolled infection or death before discharge from hospital. Favorable prognoses referred to controlled infection or cure of infection before discharge from the hospital.

2.1 Statistical analysis

Clinical data were extracted and summarized in an Excel file, then analyzed using SPSS 25.0. The *t*-test was used for continuous data and the chi-square test was used for categorical data. Fisher's exact test was used when the number in the group was less than five.

The level of significance was set at $p < 0.05$ (two sided).

3 Results

There were 36 cases of AML and 72 cases of ALL that met the inclusion criteria. There were no differences between the AML and ALL groups in terms of patient age, gender, hospital stay, upper respiratory tract infection, pleurisy, bronchitis, urinary tract infection, oral infection, perianal infection, acute enteritis, herpetic angina, skin infection, soft tissue infection, or poor prognosis ($p > 0.05$ for all comparisons). There were significant differences in the incidence of pneumonia (47.2% vs. 27.8%, $p = 0.045$) and sepsis (19.4% vs. 2.8%, $p = 0.006$) between the AML and ALL groups (Table 1).

TABLE 1 Clinical characteristics of patients in the AML and ALL groups.

Items	AML + ALL ($n = 108$)	AML ($n = 36$)	ALL ($n = 72$)	*p*
Age (years)	10.3 ± 4.6	10.9 ± 4.2[a]	10.0 ± 4.7[a]	0.337[b]
Male, *n* (%)	74 (68.5%)	24 (66.7%)	50 (69.4%)	0.770[c]
Hospital stay (days)	25.1 ± 16.2	28.6 ± 15.1[a]	23.3 ± 16.4[a]	0.109[b]
Upper respiratory tract infection	50 (46.3%)	12 (33.3%)	38 (52.8%)	0.056[c]
Pneumonia	37 (34.3%)	17 (47.2%)	20 (27.8%)	**0.045**[*c]
Pleurisy	1 (0.9%)	0 (0)	1 (1.4%)	1[d]
Sepsis	9 (8.3%)	7 (19.4%)	2 (2.8%)	**0.006**[*d]
Bronchitis	5 (4.6%)	2 (5.6%)	3 (4.2%)	1[d]
Urinary tract infection	2 (1.9%)	1 (2.8%)	1 (1.4%)	1[d]
Oral infection	8 (7.4%)	4 (11.1%)	4 (5.6%)	0.437[d]
Perianal infection	1 (0.9%)	1 (2.8%)	0 (0)	0.333[d]
Acute enteritis	4 (3.7%)	1 (2.8%)	3 (4.2%)	1[d]
Herpetic angina	1 (0.9%)	0 (0)	1 (1.4%)	1[d]
Skin infection	1 (0.9%)	0 (0)	1 (1.4%)	1[d]
Soft tissue infection	1 (0.9%)	1 (2.8%)	0 (0)	0.333[d]
Poor prognosis	24 (22.2%)	10 (27.8%)	14 (19.4%)	0.326[c]

Poor prognosis referred to uncontrolled infection or death. AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia.
Bold values represented $p < 0.05$.
[a]Mean ± standard deviation.
[b]*t*-test.
[c]Chi-square test.
[d]Fisher's exact test.
[*]$p < 0.05$ between AML and ALL.

Patients in the AML or ALL groups were further analyzed based on their prognosis at discharge. There were 10 cases with a poor prognosis and 26 cases with a favorable prognosis in the AML group. There were no significant differences between the poor prognosis and favorable prognosis groups except for age (14.2 ± 1.2 years vs. 9.6 ± 4.3 years, $p = 0.003$) (Table 2).

In the same token, there were 14 cases with a poor prognosis and 58 cases with a favorable prognosis in the ALL group. There were no significant differences between the poor prognosis and favorable prognosis groups except for age (13.4 ± 2.7 years vs. 9.2 ± 4.7 years, $p = 0.002$) (Table 3).

The age distribution of prognosis is shown in Table 4. A decreasing trend in favorable prognosis was observed with increasing age in participants in both the ALL and AML groups. There was a significant difference in prognosis between participants aged <12 years and those aged ≥12 years in both cohorts (ALL: $p = 0.0021$; AML: $p = 0.0004$).

4 Discussion

In another study, ALL was reported as the most common type of pediatric acute leukemia, accounting for approximately 75%–80% of cases (11). In our study, 66.7% of pediatric leukemia patients were

TABLE 2 Clinical characteristics of patients in the AML group.

Items	Poor prognosis ($n = 10$)	Favorable prognosis ($n = 26$)	p
Age (years)	14.2 ± 1.2[a]	9.6 ± 4.3[a]	**0.003**[b]*
Male	5 (50%)	19 (73.1%)	0.987[c]
Hospital stay (days)	33.2 ± 12.3[a]	26.8 ± 15.7[a]	0.270[b]
Upper respiratory tract infection	4 (40%)	8 (30.8%)	0.700[d]
Pneumonia	7 (70%)	11 (42.3%)	0.137[c]
Sepsis	4 (40%)	3 (11.5%)	0.076*[d]
Bronchitis	0 (0)	2 (7.7%)	1[d]
Urinary tract infection	1 (10%)	0 (0)	0.278[d]
Oral infection	2 (20%)	2 (7.7%)	0.305[d]
Perianal infection	0 (0)	1 (3.8%)	1[d]
Acute enteritis	1 (10%)	0 (0)	0.278[d]
Soft tissue infection	1 (10%)	0 (0)	0.278[d]

Poor prognosis referred to uncontrolled infection or death before discharged from hospital. Favorable prognosis referred to controlled infection or cure before discharged from hospital. AML, acute myeloid leukemia.
Bold values represented $p < 0.05$.
[a]Mean ± standard deviation.
[b]t-test.
[c]Chi-square test.
[d]Fisher's exact test.
*$p < 0.05$.

TABLE 3 Clinical characteristics of patients in the ALL group.

Items	Poor prognosis ($n = 14$)	Favorable prognosis ($n = 58$)	p
Age (years)	13.4 ± 2.7[a]	9.2 ± 4.7[a]	**0.002**[b]*
Male	9 (64.3%)	41 (70.7%)	0.641[c]
Hospital stay (days)	26.8 ± 14.9[a]	22.4 ± 16.6[a]	0.380[b]
Upper respiratory tract infection	6 (42.9%)	32 (55.2%)	0.407[c]
Pneumonia	3 (21.4%)	17 (29.3%)	0.744[d]
Pleurisy	0 (0)	1 (1.7%)	1[d]
Sepsis	0 (0)	2 (3.4%)	1[d]
Bronchitis	0 (0)	3 (5.2%)	1[d]
Urinary tract infection	0 (0)	1 (1.7%)	1[d]
Oral infection	1 (7.1%)	3 (5.2%)	1[d]
Acute enteritis	1 (7.1%)	2 (3.4%)	0.483[d]
Herpetic angina	0 (0)	1 (1.7%)	1[d]
Skin infection	0 (0)	1 (1.7%)	1[d]

ALL, acute lymphocytic leukemia.
Bold values represented $p < 0.05$.
[a]Mean ± standard deviation.
[b]t-test.
[c]Chi-square test.
[d]Fisher's exact test.
*$p < 0.05$.

diagnosed with ALL. This discrepancy might be due to differences in the inclusion and exclusion criteria, as well as variations related to environmental factors and racial demographics.

The most common acute infection observed among the enrolled acute leukemia patients in our study were upper respiratory tract infection (46.3%), followed by pneumonia (34.3%), sepsis (8.3%), and oral infections (7.4%). Neutropenia emerged as the leading risk factor for infection development (12). In addition, several other factors contribute to increased infection risk, including compromised cellular or humoral immunity, disruption of natural barriers (such as skin and mucous membranes), and the use of medical devices, such as vascular access catheters (13). Often, patients are affected by a combination of these risk factors, which can further elevate their susceptibility to infections and the risk of adverse outcomes.

We found that pneumonia (47.2% vs. 27.8%, $p = 0.045$) and sepsis (19.4% vs. 2.8%, $p = 0.006$) were significantly more common in AML than in ALL cases. This can be explained by the differing immunological deficits associated with each leukemia type. In AML, deficits in neutrophilic granulocytes lead to a higher incidence of bacterial and fungal infections, whereas in ALL, deficits in lymphocytes result in hypogammaglobulinemia, leading to reduced cell-mediated immunity (14).

During treatment for ALL and AML, different agents and schedules might be employed, leading to differences in prognosis (14, 15). Both the disease and its therapies place a heavy burden on the developing immune system. In acute leukemia, normal production of blood cells in the bone marrow is disrupted (16, 17) and leukemia cells crowd out healthy white blood cells, including lymphocytes and granulocytes, which are both essential for immune defense. This results in children experiencing frequent infections and fevers, as the body struggles to mount an effective response against pathogens.

Treatment for acute leukemia, primarily via chemotherapy, further exacerbates immune dysfunction (18–21). Chemotherapy targets rapidly dividing cells, affecting not only cancer cells but also healthy immune cells, leading to suppression of the immune system. This increases children's susceptibility to infections both during and after treatment. The effects of chemotherapy on the immune system can persist even after treatment completion, as evidenced by persistent abnormalities in immune parameters such as lymphocyte subsets and natural killer cell function. In the context of AML, children often experience multiple episodes of infection during intensive treatment, with sepsis being the most common (15). Infection-related mortality rates are in the range of 5.4%–7.3%. In ALL, the induction and consolidation phases pose significant risks for infections due to severe neutropenia. Infection-related mortality in ALL is generally lower, in the range of 2%–4%; however, infections remain a primary cause of treatment-related mortality. Understanding these risks is crucial for developing effective strategies to manage and prevent infections during leukemia treatment.

It is also interesting to note that a previous study in children and adolescents with ALL showed there were no significant associations between sex, race, age, and the development of acute respiratory infections (22). Another study investigating childhood AML with infections showed that age above 16 years was a factor associated with infection-related mortality (23). In general, independent of acute infections, the survival rate of ALL is highest when children diagnosed at 1–4 years of age, with a decline observed in older age groups. Infants aged under 1 year have the lowest survival rate in both ALL and AML (24). In our study, children aged under 12 years demonstrated a more favorable prognosis in both ALL and AML populations. The underlying pathophysiological mechanisms need further investigation and might be related to deficiencies of key factors in metabolism (25, 26).

TABLE 4 Age distribution of prognosis.

Age (years)	ALL				AML			
	Good (*n*)	Poor (*n*)	Total (*n*)	Good (%)	Good (*n*)	Poor (*n*)	Total (*n*)	Good (%)
1	0	0	0	NA	1	0	1	100
2	4	0	4	100	1	0	1	100
3	6	0	6	100	2	0	2	100
4	4	0	4	100	1	0	1	100
5	3	0	3	100	1	0	1	100
6	6	0	6	100	0	0	0	NA
7	2	1	3	66.7	0	0	0	NA
8	1	1	2	50	2	0	2	100
9	2	0	2	100	2	0	2	100
10	2	0	2	100	4	0	4	100
11	6	0	6	100	3	0	3	100
12	3	2	5	60	2	1	3	66.7
13	5	1	6	83.3	2	2	4	50
14	3	3	6	50	2	2	4	50
15	7	4	11	63.6	2	4	6	33.3
16	3	1	4	75	0	1	1	0
17	1	1	2	50	1	0	1	100
Total	58	14	72	80.6	26	10	36	72.2
<12	36	2			17	0		
≥12	22	12			9	10		
p^a	**0.0021**				**0.0004**			

Bold values represented $p < 0.05$.
[a]Fisher's exact test of prognosis difference between children aged <12 years and those aged ≥12 years.

The present study has some limitations. Notably, the sample sizes in certain subgroups—particularly when comparing infection type and age—were relatively small. Therefore, the conclusions drawn need to be validated in more robust studies, such as meta-analyses or large-scale studies.

5 Conclusion

In this study, by analyzing clinical data collected over an 18-year period at our hospital, we found a significantly higher incidence of pneumonia and sepsis in children diagnosed with AML compared to those with ALL. Younger children with AML or ALL who developed acute infections tended to have a better prognosis than older children.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical Committee of the Second Affiliated Hospital of Harbin Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

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Clinical relevance of bacterial and/or viral coinfection in acute bronchiolitis in an Italian neonatal unit during the 2021–2023 seasons

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Background: Acute bronchiolitis is a leading cause of hospitalization in young children worldwide, and literature reports conflicting data regarding the role of coinfections.
Objective: To evaluate the possible clinical relevance of bacterial and/or viral respiratory coinfection in a cohort of newborns/infants hospitalized for bronchiolitis.
Methods: Neonates and infants younger than three months admitted to neonatal units from October 2021 to March 2023 because of acute bronchiolitis were included in this retrospective study. Analyses were performed with Stata 11.1 ($p < 0.05$). Data were summarized as medians (IQR) or counts (%). Appropriate tests were used based on data type and distribution, with Benjamini–Hochberg correction for multiple comparisons. Odd Ratios (ORs) were unadjusted.
Results: In a cohort of 240 patients, respiratory coinfection was associated with a longer hospital stay ($p < 0.001$) and the need for invasive mechanical ventilation ($p < 0.001$) compared to viral mono-infection, highlighting a potential role in patient outcome. Moreover, we observed that premature patients are more likely to contract a respiratory coinfection than a viral mono-infection ($p = 0.011$).
Conclusion: Coinfections increased the clinical severity of bronchiolitis more than simple viral mono-infection in our cohort, contributing to a longer hospital stay and the need for invasive mechanical ventilation.

KEYWORDS

newborns, bronchiolitis, respiratory infections, coinfections, infants, viral monoinfection, RSV, rhinovirus

1 Introduction

Respiratory tract infections represent a significant public health problem due to limited therapeutic arsenal and emerging therapeutic resistance. Among respiratory infections, acute bronchiolitis is a common lung infection and a major cause of hospitalization in young children (1). In high-income countries, bronchiolitis accounts for up to 15%–17% of all hospitalizations in children younger than 2 years and 15% of emergency department presentations in infants (2).

Typically, signs of upper respiratory tract infection occur after an incubation period of 4–6 days. Subsequently, lower respiratory tract involvement becomes evident with variable degrees of breathing difficulty, crackles, and bilateral wheezing upon chest examination (2–4). The variable clinical presentation and the potential for sudden deterioration of the clinical conditions require close monitoring by healthcare professionals (5).

Respiratory syncytial virus (RSV) is the infective organism reported to be the most common cause of bronchiolitis (6). As viral detection methods are improving and their use is expanding, multiple viruses are increasingly found in infants with bronchiolitis. Indeed, other viruses associated with bronchiolitis, which often occur as coinfections, include rhinovirus, human metapneumovirus, adenovirus, parainfluenza viruses (PIVs 1–4), influenza viruses (Flu A/B), human bocavirus, enterovirus and human coronaviruses (7).

Non-viral coinfections with *Bordetella pertussis* and other atypical bacteria (i.e., *Mycoplasma pneumoniae*, as well as *Chlamydia pneumoniae* and *Chlamydia trachomatis*) or other bacteria are occasionally reported (2).

Despite growing interest, the clinical significance of viral and/or bacterial coinfections in bronchiolitis remains unclear, with conflicting evidence on their impact on disease severity, clinical course, and healthcare resource utilization (8–12). Therefore, this study aims to evaluate the clinical relevance of viral and bacterial coinfections in a cohort of newborns and infants hospitalized for bronchiolitis, to better understand their potential influence on patient management and outcomes.

2 Methods

2.1 Study design

We carried out a retrospective, observational study on newborns/infants under three months admitted to the Neonatal Intensive and Sub-Intensive Care Unit of Bambino Gesù Children's Hospital in Rome (Italy) for bronchiolitis from October 2021 to March 2023. Data were collected from electronic medical records, including demographic characteristics, laboratory findings, clinical outcomes, and treatments. Disease severity was classified based on respiratory support requirements. The diagnosis of bronchiolitis was based on clinical evaluation, according to national and international guidelines (1, 2). Typical criteria included signs of viral upper respiratory infection with increased respiratory effort (tachypnea, nasal flaring, chest retractions), wheezing, and/or crackles on auscultation in infants under 12 months. Disease severity was classified based on the level of respiratory support required, categorized as mild (no oxygen requirement), moderate (non-invasive oxygen support), or severe (invasive mechanical ventilation), consistent with current literature and clinical practice (1, 2).

2.2 Study population

This study included 240 neonates and infants (<3 months old) hospitalized with a clinical diagnosis of bronchiolitis. Mild cases not requiring hospitalization were excluded. Patients with congenital anomalies, primary immunodeficiencies, or other severe comorbidities were also excluded to ensure a more homogeneous study population.

2.3 Microbiology testing

All newborns/infants underwent standardized sample collection upon admission to the Emergency Department, and a microbiological investigation was carried out on samples from the upper respiratory tract for etiological diagnosis. The samples were processed and managed according to the standardized protocols of the Microbiology Laboratory.

Viral respiratory infection was defined by the detection of one or more viral etiological agents on any of the respiratory specimens, using molecular Polymerase Chain Reaction (PCR) methods (i.e., Allplex Respiratory Panel Assays [Seegene, Seoul, South Korea] or BioFire FilmArray Respiratory 2.1 Panel [BioMérieux Clinical Diagnostics, Salt Lake City, Utah, United States]). Bacterial infection was defined by a positive PCR result or a positive microbiological culture of the respiratory samples.

2.4 Ethics statements

The authors assert that all procedures of the study comply with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (13). Personal data were restricted to essential information and were treated in order to guarantee the respect of the privacy of the involved patients, as specifically stated by Italian Law D. Lgs. n.196 of 2003 about personal data protection. Written informed consent was not required, as the study is retrospective and has no patient-identifiable information. Despite this, our Scientific Directorate validated the study before submission to the journal, as in our hospital, all studies performed have to be approved by this office. In addition, parents or legal guardians of patients provided consent to use personal data for diagnosis, treatment, and related research purposes at the time of hospitalization.

2.5 Statistical analysis

Stata software version 11.1 (StataCorp, College Station, TX, USA) was used for statistical analysis, with significance set at $p < 0.05$. Descriptive statistics are expressed as median values and interquartile range (IQR) for continuous data and number (percentage) for categorical data. For categorical variables, significant differences were assessed by the Chi-square test (when all expected cell counts were ≥25) or Fisher Exact Test (if at least one cell had an expected count <25). For continuous variables, normality was assessed: if the data followed a normal distribution, comparisons between groups were conducted using the Student's *t*-test, otherwise the Mann–Whitney *U* test was applied. To adjust the significance for multiple comparisons and confirm potential associations, a Benjamini–Hochberg correction was applied. For odds ratio (OR) calculations, no logistic regression was used to adjust for potential confounders.

3 Results

3.1 Patients

From October 2021 to March 2023, 242 neonates and infants were admitted for bronchiolitis. The final study population consisted of 240 newborns/infants; two patients with no detected etiological agents were excluded, as shown in Figure 1.

Table 1 compares the demographic and clinical characteristics and diagnosis of the study population's monomicrobial or polymicrobial respiratory infections. In addition, the values representing the strength of the association between some independent variables and the presence of viral and/or bacterial coinfection (odds ratio) are shown in Table 2. All considerations regarding coinfection were independent of the etiology of the coinfection itself, whether with virus/virus or virus/bacteria detected, probably attributable to the cohort of patients analyzed in this study.

3.2 Microbiology, laboratory and clinical results

One hundred and fifty (62.5%) patients had viral mono-infection, and 90 had coinfections (37.5%), as detailed: 58 (64.4%) had only viral coinfections (virus/virus), and 32 (35.6%) had both bacterial and viral coinfections (virus/bacteria). Bacterial coinfection in hospitalized patients was reported in 13.3% (32/240); in particular, the presence of a single bacterium was detected in 19 coinfections, and the presence of ≥2 bacteria in 13 coinfections.

Among 150 patients with viral respiratory mono-infection, the median age was 22.5 days (IQR, 15–30.8 days), and 74 were (49.3%) males. Among 90 patients with respiratory coinfection (virus/virus or virus/bacteria), the median age was 29 days (IQR, 21–41 days), and 36 were (40%) males. Patients with coinfections were significantly older than those with viral mono-infections (median age 29 vs. 22 days, $p < 0.001$), suggesting increased susceptibility with age.

Premature patients had a significantly lower probability (73% reduction) of mono-infection than term patients, suggesting a higher susceptibility to respiratory coinfections. Patients who received invasive mechanical ventilation were significantly less likely (96.4% reduction) to have had a respiratory mono-infection. The two confidence intervals do not include 1, so these associations are statistically significant (Figure 2).

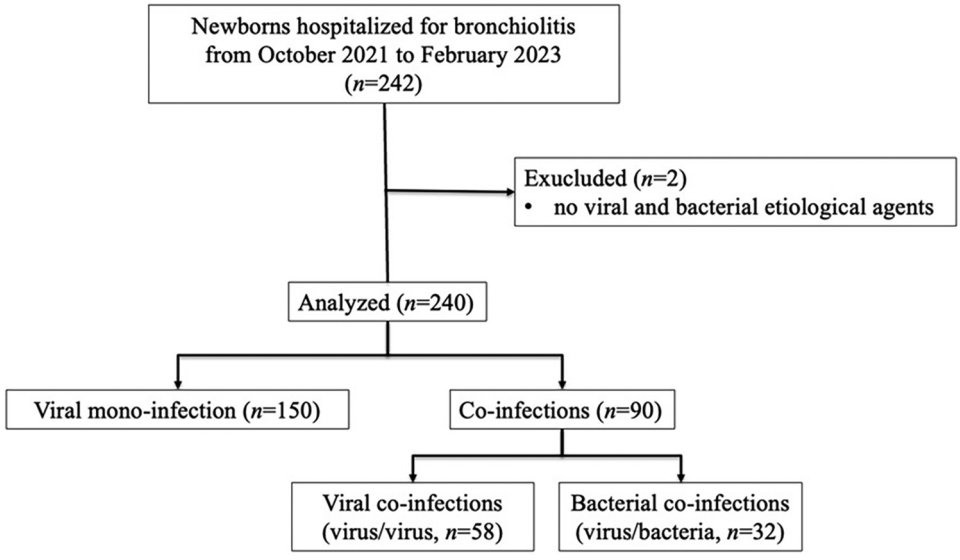

FIGURE 1
Flow-chart of patient inclusion.

TABLE 1 Comparison of demographic and clinical characteristics of newborns/infants hospitalized with monomicrobial or polymicrobial respiratory infections.

	Mono-infections	Coinfections	*p* value
240 newborns/infants [no. (%)]	150 (62.5)	90 (37.5)	
Age (days) [median (IQR)]	22.5 (15–30.8)	29 (21–41)	**<0.001***
Male [no. (%)]	74 (49.3)	36 (40)	0.182
Breastfeeding [no. (%)]	128 (85.3)	74 (82.2)	0.585
Sick relatives at home [no. (%)]	102 (68.0)	66 (73.3)	0.467
Length of hospital stay (days) [median (IQR)]	5 (3–8)	7 (5–12)	**<0.001#**
Prematurity/late preterm [no. (%)]	6 (4.0)	12 (13.3)	**0.011***
Previous Palivizumab prophylaxis [no. (%)]	1 (0.7)	3 (3.3)	0.150
Other pre-existing co-morbidity [no. (%)]	5 (3.3)	3 (3.3)	1.000
Fever [no. (%)]	26 (17.3)	18 (20.0)	0.609
Invasive mechanical ventilation [no. (%)]	1 (0.7)	14 (15.6)	**<0.001#**
No-invasive ventilation [no. (%)]	124 (82.7)	75 (83.3)	1.000
Duration of respiratory assistance (days) [median (IQR)]	5 (3–7)	5 (3–9)	0.072
Laboratory findings at time of diagnosis [median (IQR)]			
Procalcitonin level (ng/ml) [median (IQR)]	0.16 (0.12–0.34)	0.22 (0.14–0.22)	0.486
C-reactive protein level (mg/dl) [median (IQR)]	0.46 (0.1–1.43)	0.56 (0.20–1.70)	0.375
White blood cell count/mm3 [median (IQR)]	10,010 (8,060–12,410)	10,260 (7,925–12,383)	0.422
% Neutrophil [median (IQR)]	34.7 (23.9–46.2)	37.3 (26.8–47.08)	0.149
% Lymphocyte [median (IQR)]	46 (36.1–55)	43.5 (35.1–53.2)	0.135
Episodes with concurrent bacteremia [no. (%)]	3 (2.0)	3 (3.3)	0.674
Episodes with concurrent non-invasive bacterial infections [no. (%)]	17 (11.3)	15 (16.7)	0.246

Data are expressed as *n* (%) or median (IQR).
*Two-sided *p*-values were calculated by Chi-square test, or Mann–Whitney test, as appropriate.
#*p*-values that remain significant even after the Benjamini-Hochberg correction. Bold values are statistically significant differences ($p < 0.05$).

TABLE 2 Values representing the strength of the association between independent variables and the presence of viral and/or bacterial coinfection.

	Monoinfections	Coinfections	*p* value	OR (95% CI)
Male [no. (%)]	74 (49.3)	36 (40)	0.182	1.461 (0.860–2.480)
Breastfeeding	128 (85.3)	74 (82.2)	0.585	1.258 (0.622–2.545)
Sick relatives at home	102 (68.0)	66 (73.3)	0.467	0.773 (0.433–1.380)
Prematurity/late preterm [no. (%)]	6 (4.0)	12 (13.3)	**0.011***	0.271 (0.098–0.750)
Other pre-existing co-morbidity [no. (%)]	5 (3.3)	3 (3.3)	1.000	1.000 (0.233–4.288)
Fever [no. (%)]	26 (17.3)	18 (20.0)	0.609	0.839 (0.430–1.635)
Invasive mechanical ventilation [no. (%)]	1 (0.7)	14 (15.6)	**<0.001#**	0.036 (0.005–0.282)
No-invasive ventilation [no. (%)]	124 (82.7)	75 (83.3)	1.000	0.954 (0.475–1.915)
Episodes with concurrent bacteremia [no. (%)]	3 (2.0)	3 (3.3)	0.674	0.592 (0.117–2.997)
Episodes with concurrent non-invasive bacterial infections [no. (%)]	17 (11.3)	15 (16.7)	0.246	0.639 (0.302–1.353)

Data are expressed as *n* (%).
* Two-sided *p*-values were calculated by Chi-square test.
#*p*-values that remain significant even after the Benjamini-Hochberg correction.
Bold values are statistically significant differences ($p < 0.05$).

Infectious agents identified in all newborns/infants with bronchiolitis are reported in Table 3. As expected, RSV was the most common viral etiological agent identified in our study (82.5%), while rhinovirus was the second most common virus detected (23.8%). In all 240 samples analyzed, a total of 48 bacteria were detected (20%). *Haemophilus influenzae* (7.9%), *Moraxella catarrhalis* (3.8%), and *Streptococcus pneumoniae* (3.3%) were the three most commonly identified bacteria.

Analyzing demographic and clinical characteristics of newborns/infants in case of viral or bacterial coinfections, we found a significantly lower neutrophil percentage and higher lymphocyte count in patients with viral coinfections ($p = 0.038$ and $p = 0.029$, respectively) (Table 4).

4 Discussion

Bronchiolitis can be a severe cause of respiratory failure in newborns (14). Compared to viral mono-infection, patients with coinfection were older ($p < 0.001$), highlighting a greater risk of contracting polymicrobial respiratory infections than in those younger. The explanation could be attributable to the persistence of maternal antibodies that protect newborns from infections in the first weeks of life (15). Indeed, it is conceivable that passive immunity to (at least some) respiratory pathogens can be transferred to neonates by transplacental maternal antigen-specific IgG antibodies induced by maternal colonization or vaccination (16, 17).

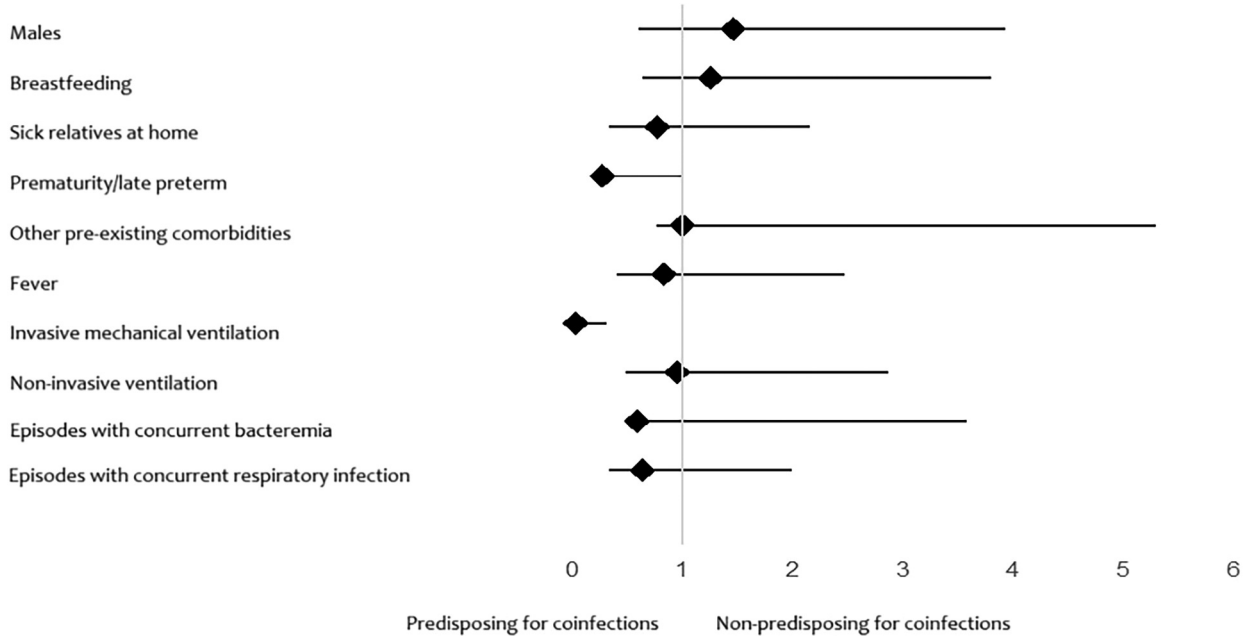

FIGURE 2
Associations with the risk of having a mono-infection and coinfection.

TABLE 3 Infectious agents identified in all patients ($n = 240$) with bronchiolitis during 2021–2023.

Infectious agent	Respiratory monoinfection	Respiratory coinfections (viral and/or bacterial)	Overall (%)	*p* value
Viruses				
RSV (no.)[a]	119	79	198 (82.5%)	**<0.001***
Rhinovirus (no.)	11	46	57 (23.8%)	**<0.001***
Human Metapneumovirus (no.)	8	7	15 (6.3%)	0.794
Parainfluenza (no.)	5	7	12 (5%)	0.771
Coronavirus OC43/229° (no.)	5	6	11 (4.6%)	1.000
Influenza A (no.)	2	6	8 (3.3%)	0.285
Adenovirus (no.)	0	3	3 (1.3%)	0.248
Enterovirus (no.)	0	3	3 (1.3%)	0.248
SARS-CoV-2 (no.)	0	2	2 (0.8%)	0.499
Bacteria				
Haemophilus influenzae (no.)	0	19	19 (7.9%)	/
Moraxella catarrhalis (no.)	0	9	9 (3.8%)	/
Streptococcus pneumoniae (no.)	0	8	8 (3.3%)	/
Staphylococcus aureus (no.)	0	7	7 (2.9%)	/
Bordetella parapertussis (no.)	0	2	2 (0.8%)	/
Escherichia coli (no.)	0	1	1 (0.4%)	/
Pseudomonas aeruginosa (no.)	0	1	1 (0.4%)	/
Klebsiella pneumoniae (no.)	0	1	1 (0.4%)	/

[a]Among 198 RSV detected, 14 were typed as RSV–A, 41 as RSV–B, 64 were not typed.
Data are expressed as *n* (%).
*Two-sided *p*-values were calculated by Chi-square test.
#*p*-values that remain significant even after the Benjamini–Hochberg correction.
Bold values are statistically significant differences ($p < 0.05$).

While the typical length of pregnancy is between 39 and 41 completed weeks of gestation, annually, 15 million children are stillborn preterm (a condition defined as delivery before 37 weeks) (18). The risks of adverse outcomes for preterm newborns increase significantly with a decrease in gestational age (19). In our cohort, those newborns/infants requiring hospitalization with coinfection were mostly preterm born ($p = 0.011$). Indeed, the most consistently identified risk factor associated with progression to severe bronchiolitis includes a gestational age of less than 37 weeks (2). This suggests that prematurity may also be a risk factor for contracting a polymicrobial respiratory infection.

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TABLE 4 Demographic and clinical characteristics of newborns/infants in case of viral or bacterial coinfections.

	Viral coinfections	Bacterial coinfections	*p* value
240 newborns/infants [no. (%)]	58 (64.4)	32 (35.6)	
Age (days) [median (IQR)]	28 (20.3–45.5)	31.5 (21–37.5)	0.243
Male [no. (%)]	26 (44.8)	10 (31.3)	0.263
Breastfeeding [no. (%)]	48 (82.8)	26 (81.3)	1.000
Sick relatives at home [no. (%)]	41 (70.7)	25 (78.1)	0.619
Length of hospital stay (days) [median (IQR)]	6 (4–8.8)	9.5 (6.8–12.2)	0.086
Prematurity/late preterm [no. (%)]	6 (10.3)	6 (18.8)	0.334
Previous Palivizumab prophylaxis [no. (%)]	2 (3.4)	1 (3.1)	1.000
Other pre-existing co-morbidity [no. (%)]	3 (5.2)	0 (0)	0.550
Fever [no. (%)]	11 (19.0)	7 (21.9)	0.787
Invasive mechanical ventilation [no. (%)]	6 (10.3)	8 (25.0)	0.077
No-invasive ventilation [no. (%)]	47 (81.0)	28 (87.5)	0.560
Duration of respiratory assistance (days) [median (IQR)]	5 (3–9)	6 (4–8)	0.164
Laboratory findings at time of diagnosis [median (IQR)]			
Procalcitonin level (ng/ml) [median (IQR)]	0.24 (0.16–0.32)	0.17 (0.13–0.2)	0.189
C-reactive protein level (mg/dl) [median (IQR)]	0.44 (0.19–1.19)	0.9 (0.31–2.28)	0.080
White blood cell count/mm3 [median (IQR)]	10,190 (7,843–12,358)	10,425 (8,020–12,748)	0.133
% Neutrophil [median (IQR)]	35.4 (24.05–46.65)	41.2 (32.9–48.7)	**0.038***
% Lymphocyte [median (IQR)]	44.6 (36.05–54.88)	38.15 (33.55–46.18)	**0.029***
Episodes with concurrent bacteremia [no. (%)]	1 (1.7)	2 (6.3)	0.287
Episodes with concurrent non-invasive bacterial infections [no. (%)]	9 (15.5)	6 (18.8)	0.771

Data are expressed as *n* (%) or median (IQR).

*Two-sided *p*-values were calculated by Chi-square test, or Mann–Whitney test, as appropriate.

None of the *p*-values remained significant even after the Benjamini–Hochberg correction. Bold values are statistically significant differences ($p < 0.05$).

In all our patients, respiratory coinfection was associated with a longer hospital stay ($p < 0.001$) and invasive mechanical ventilation ($p < 0.001$) compared to viral mono-infection, highlighting a potential role in patient outcome. As previously described, coinfection is associated with more severe disease (9), and, in addition, in our study, the severity of coinfections was independent of the etiology of the coinfection itself.

No significant differences were found considering other demographic and clinical characteristics, as shown in Table 1.

Furthermore, our findings highlighted, as demonstrated by other studies, that serious bacterial infections (i.e., bacteremia and meningitis) or other non-invasive concomitant bacterial infections are extremely rare in children with bronchiolitis, thus confirming the current guidelines that do not support the routine use of antibiotics in these patients (20).

We observed that RSV was mostly found in mono-infections ($p < 0.001$), while Rhinovirus was more frequently detected in coinfections ($p < 0.001$). The reasons why some viruses cause mono-infection or coinfection remain still unclear; one hypothesis is that the mono- vs. coinfection is associated with competitive interactions at the host-pathogen level (21). All viral associations found in our study were non-significant, without recurring associations. This result is different from what Mandelia and colleagues reported, namely that direct or indirect interactions occur between specific viral pathogens (21). This difference could be associated with the selected cohort of patients analyzed and does not exclude that specific associations can be found in other clinical conditions. Indeed, in another recent publication of our group, some specific viral associations have been found in patients with other clinical characteristics (22).

All infants diagnosed with bacterial coinfection received antibiotic therapy. However, the administration of antibiotics did not significantly correlate with a reduction in the duration of hospitalization or the need for mechanical ventilation. These findings suggest that while antibiotics were given for treating bacterial infections, their impact on overall disease severity in bronchiolitis cases remained limited.

Finally, the retrospective nature of the study design and the potential for selection or information bias may have influenced the results and their interpretation, representing a limitation of the study. As this was an observational study, we could only establish associations, not causal relationships, between coinfections and disease severity. Unmeasured confounders, such as viral load or specific host immune responses, may have influenced our findings.

Given the inherent limitations of standard microbiological and clinical assessments in rapidly distinguishing viral from bacterial involvement and in quantifying disease burden, there is a clear need for more precise, non-invasive diagnostic tools. In this context, emerging molecular imaging approaches hold considerable promise. Innovative agents—such as enzyme-activatable near-infrared fluorescent probes and pathogen-targeted radiotracers—have demonstrated the ability to non-invasively visualize and differentiate between viral and bacterial lung infections, as well as to monitor therapeutic response in real time (23–25). While these technologies remain largely in preclinical or early clinical stages, their future integration into routine practice could significantly enhance diagnostic accuracy and support personalized management strategies for vulnerable pediatric populations.

5 Conclusion

In our cohort, approximately one-third of newborns/infants hospitalized with bronchiolitis (37.5%) had a coinfection.

While viruses have been identified as the predominant infectious agents, it is important to recognize bacteria's role in respiratory coinfections. RSV (82.5%) and *Haemophilus influenzae* (7.9%) were the most common viral and bacterial etiological agents identified, respectively. Rhinovirus was the second most common viral agent in 23.8% of the cases. We observed that the increased detection of RSV and Rhinovirus in mono-infections and coinfections, respectively, was significant.

Coinfection increased the clinical severity of bronchiolitis more than simple viral mono-infection, contributing to a longer hospital stay and the need for invasive mechanical ventilation, especially in these young patients.

Finally, a significant association has been identified between prematurity and the presence of respiratory coinfections, considering prematurity as a risk factor.

Our results may be useful in understanding the prevalence and distribution of infectious agents in neonatal and pediatric respiratory infections to guide diagnostic and therapeutic strategies, including appropriate antibiotic management. Prospective studies would be needed to confirm our findings and deepen the understanding of factors influencing susceptibility to respiratory infections in newborns and infants.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies involving humans because of the local legislation and institutional requirements. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements because Parents or legal guardians of patients provided consent to use personal data for diagnosis, treatment, and related research purposes at the time of hospitalization.

Author contributions

VC: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. MA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. DD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. VF: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. VD: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. GV: Data curation, Investigation, Writing – review & editing. MR: Data curation, Investigation, Writing – review & editing. BL: Data curation, Investigation, Writing – review & editing. SR: Data curation, Investigation, Writing – review & editing. AS: Data curation, Investigation, Writing – review & editing. CR: Data curation, Investigation, Writing – review & editing. AB: Data curation, Investigation, Writing – review & editing. MR: Data curation, Investigation, Writing – review & editing. AD: Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. CP: Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. PB: Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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Case Report: Refractory *Mycoplasma pneumoniae* pneumonia complicated by pulmonary embolism and infarction in a child

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Mycoplasma pneumoniae (MP) is a significant pathogen of community-acquired pneumonia in children, typically following a benign course. However, some cases may progress to severe or refractory MP pneumonia (SMPP or RMPP) and lead to thromboembolic complications. This report describes a rare case of a 9-year-old boy with RMPP complicated by bilateral pulmonary embolism (PE) and pulmonary infarction. The patient initially presented with a fever and cough. Despite 24 days of prior treatment at another hospital, including macrolide, carbapenem, and tetracycline antibiotics and corticosteroids, he remained febrile with persistent wheezing when transferred to our institution. Through some laboratory findings and contrast-enhanced chest computed tomography, he fulfilled the diagnostic criteria for both SMPP and RMPP, accompanied by a PE with pulmonary infarction. A multidisciplinary therapeutic approach combining anti-infective agents (linezolid and moxifloxacin), anti-inflammatory therapy (methylprednisolone), and adjusted anticoagulation (low-molecular-weight heparin followed by rivaroxaban) led to rapid clinical improvement and normalization of inflammatory/coagulation markers. Complete resolution of the PE was further demonstrated by 3-month follow-up imaging. Residual focal necrosis in the right lower lobe was observed. This case highlights the potential for severe thromboembolic events in pediatric RMPP and underscores the importance of early recognition of imaging features (e.g., vascular filling defects and wedge-shaped infarcts) and integrated multidisciplinary management to optimize patient outcomes.

KEYWORDS

refractory *Mycoplasma pneumoniae* pneumonia, severe *Mycoplasma pneumoniae* pneumonia, pulmonary embolism, children, infection

1 Introduction

Mycoplasma pneumoniae (MP) is one of the most common pathogens of community-acquired pneumonia (CAP) in children. An MP infection follows a largely benign course, while pneumonia is the most prominent clinical manifestation. *M. pneumoniae* pneumonia (MPP) accounts for up to 40% of CAP cases in children, with 18% of children requiring hospitalization (1). Children with MPP have been reported to have a high risk of blood coagulation and thrombosis (2–4). The mechanism of thrombosis

caused by MPP is still unknown, but it is probably related to immune modulation (5). A pulmonary embolism (PE), the most common thromboembolism in patients with MPP, is a significant cause of residual atelectasis, organizing pneumonia, and pulmonary necrosis (6). Pediatric patients with a PE caused by MPP have rarely been reported. Herein, we report a case of a 9-year-old patient with refractory MPP (RMPP) who gradually developed a PE, and an etiological examination confirmed a *Haemophilus influenzae* superinfection. Following the administration of anticoagulation treatment, bronchoscopic interventional therapy, and systemic corticosteroid and antibiotic therapy, the patient demonstrated a favorable prognosis. This study aimed to contribute to the clinical understanding and management of such diseases by sharing practical diagnostic and therapeutic experiences.

2 Patient presentation

A 9-year-old boy with no significant family history, specifically nothing suggesting thromboembolic disease, presented with a fever and cough but no symptoms of hemoptysis, fatigue, or syncope within the previous 24 days. The chest computed tomography (CT) at approximately 2 weeks of the disease revealed wedge-shaped consolidations with pleural-based, hilar-pointing configurations (Figure 1). Outpatient antibiotic therapy (azithromycin and erythromycin) for 10 days, followed by inpatient dual broad-spectrum antibiotic therapy (minocycline and meropenem) for 13 days and immunosuppressive therapy [methylprednisolone (12 days) and intravenous immunoglobulin (3 days)] were initiated at the other hospital. However, the patient still presented with a persistent fever, persistent paroxysmal coughing, and wheezing. Thus, he was transferred to our hospital on 30 November 2023. At admission, *Mycoplasma pneumoniae*-polymerase chain reaction (MP-PCR) was positive, accompanied by elevated specific IgM of 48.8 (<20) and IgG >300. Moreover, the laboratory findings were white blood cell (WBC) level of 22.76×10^9/L, neutrophil ratio (NEUT%) of 74.2% (the neutrophil count was 13.7×10^9/L), procalcitonin (PCT) level of 0.83 ng/ml, and C-reactive protein (CPR) and serum amyloid A (SAA) levels of 75.05 and 232.3 mg/L, respectively. Moreover, the coagulation analysis revealed an elevated D-dimer level (5.18 mg/L) and elevated fibrin degradation products (FDP) (10.35 mg/L). Therefore, considering a double bacterial infection, he was intravenously administered linezolid (30 mg/kg/day, q8h) combined with oral moxifloxacin (10 mg/kg/day) as an anti-infection therapy, along with methylprednisolone (2 mg/kg/day, q12h) as an anti-inflammatory treatment and low-molecular-weight heparin (LMWH, 3,000 IU/day, qd) as an anticoagulation therapy (Table 1). Within the first 4 days after admission, his fever had gone, but the cough was still persistent. In addition, microbiological testing of sputum, bronchoalveolar lavage fluid, and blood samples for bacteria, fungi, and common respiratory viruses (including respiratory syncytial virus, adenovirus, influenza virus, rhinovirus, and human metapneumovirus) yielded negative results. On day 5, chest contrast-enhanced CT showed multiple filling defects in the bilateral lower lobe pulmonary arteries, involving the initial segments of some basal segmental arteries and their distal branches (Figure 2). Based on the above finding, bilateral PE with bilateral lower lung infarction was suspected. Thus, his dosage of low-molecular-weight heparin was adjusted to 6,000 IU/day, administered every 12 h. However, remarkably, coagulation examinations revealed a decrease in D-dimer level (0.97 mg/L) and fibrinogen (FIB) (4.35 g/L). On day 7, Gram-positive (G^+) bacteria were found in sputum culture. After 15 days of hospitalization, the condition of the patient was stable. In addition, the posteroanterior and lateral chest radiographs indicated that the inflammation in the lungs had been partially reduced compared to before. The anticoagulation regimen was transitioned from low-molecular-weight heparin calcium to oral rivaroxaban (15 mg/day). He was discharged after an 18-day stay, with normalized inflammatory markers and clinical improvement. His oral rivaroxaban (15 mg/day) treatment was continued for 3 months. At the 3-month follow-up, he was

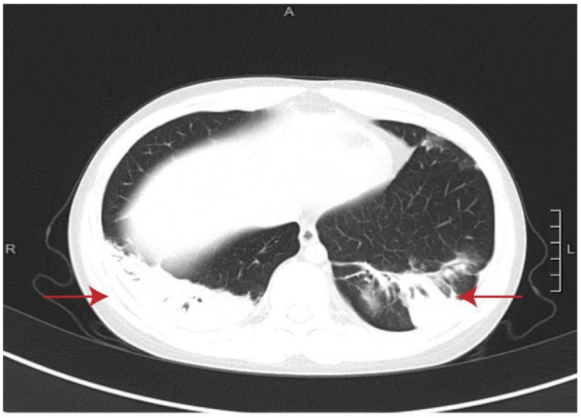

FIGURE 1
Chest CT in week 2 of the disease. The red arrows indicate wedge-shaped consolidations with pleural-based, hilar-pointing configurations.

TABLE 1 The basic information of the pediatric patient with SMPP.

Basic patient presentation	Case
Age (year)	9 years old
Gender	Boy
Embolism position	Bilateral pulmonary arteries
Fiberoptic bronchoscopy	Yes (hospital day 2 and 7)
Duration of hospitalization	31 days
Days of fever	28 days (intermittent)
Days of thrombus from onset	Hospital day 5
Days of thrombus disappear	3 months after leaving the hospital
Antibiotics before admission	(1) Azithromycin for 3 days and erythromycin for 6 days; (2) minocycline for 10 days and meropenem for 13 days
Antibiotics after admission	Linezolid and moxifloxacin
Anti-inflammatory therapy	Methylprednisolone
Anticoagulant therapy	Low-molecular-weight heparin and rivaroxaban

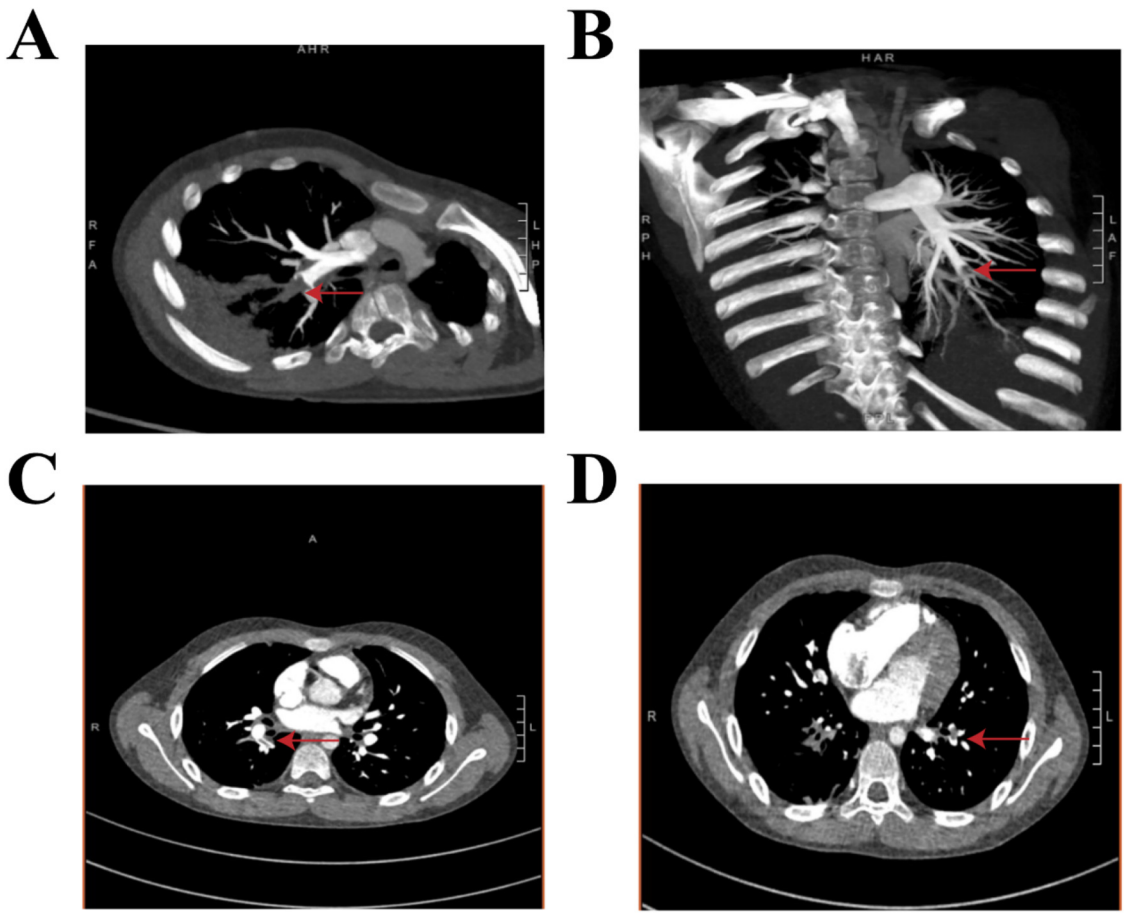

FIGURE 2
Contrast-enhanced chest CT obtained during the fourth week of illness demonstrates multiple pulmonary emboli in the bilateral lower lobe arteries. **(A,C)** The right lower lobe pulmonary artery shows filling defects involving the proximal basal segmental arteries (the red arrow) and their terminal branches. **(B,D)** The left lower lobe pulmonary artery shows occlusions at the origins of the basal segmental arteries (the red arrow), with distal propagation into branch vessels.

afebrile with no cough or wheezing. Contrast-enhanced chest CT showed no evidence of thromboembolism in the bilateral pulmonary arteries or veins, but revealed residual sequelae of right lower lobe atelectasis with minimal necrosis (Figure 3).

3 Discussion

In 2023, the post-COVID-19 era began, coinciding with a significant surge in MP infections, which reached epidemic levels globally (7). Furthermore, there has been a significant surge in reports of RMPP or severe MPP (SMPP) worldwide, with a particularly notable increase observed in Asia (8, 9). According to the *Guidelines for the Management of Community-acquired Pneumonia in Children* (2024 revision), RMPP is defined by the following criteria: (1) failure to respond to standard macrolide therapy for at least 1 week, (2) persistent fever, (3) progressive worsening of clinical symptoms, (4) deterioration of pulmonary imaging findings, and (5) presence of extrapulmonary complications involving multiple organ systems (10). RMPP mostly affects school-aged children (11). This child met the diagnostic criteria for both SMPP and RMPP, with the multifactorial pathogenesis attributable to a drug-resistant MP infection, hyperinflammatory response, and concurrent mixed infections. A delayed diagnosis likely contributed to disease progression, further complicating the clinical course. Moreover, the patient had residual sequelae of right lower lobe atelectasis. Early recognition and aggressive management of SMPP and RMPP are critical to minimizing the development of long-term sequelae (12). The optimal therapeutic window lies within 5–10 days post-fever onset (13). If the fever persists beyond 14 days of disease progression with no clinical improvement, patients are at high risk of irreversible complications (10). Given the clinical heterogeneity of MPP, individualized therapeutic regimens should be formulated based on disease subtypes (14, 15). Especially for SMPP cases, targeted multimodal interventions (e.g., combined anti-infective therapy, glucocorticoids, bronchoscopy, and anticoagulation) should be prioritized (16). It is also important to note that management must address both co-infections and precisely identify/control excessive

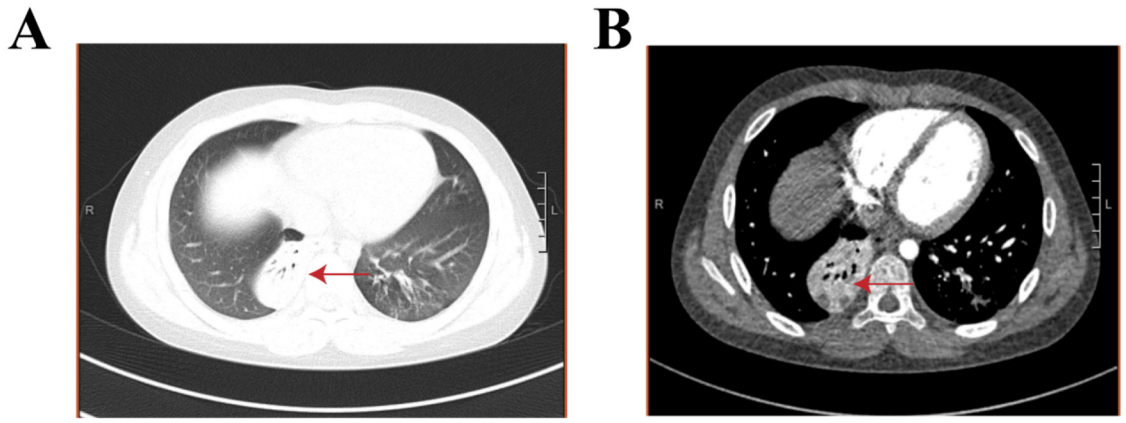

FIGURE 3
Chest contrast-enhanced CT with a three-dimensional reconstruction in month 4.5 of the disease. (A, B) The red arrows indicate right lower lobe pulmonary atelectasis with small areas of necrosis..

inflammatory responses, including cytokine storms. Failure to promptly control hyperinflammation may significantly increase the risk of secondary infections and long-term sequelae (17).

MP infections can give rise to both pulmonary and extrapulmonary complications, such as necrotizing pneumonia (NP), plastic bronchitis (PB), thromboembolism, myocarditis, hemolytic anemia, Stevens–Johnson syndrome, and erythema multiforme (18–22). Significantly, there has been a rising number of reported cases of MPP-associated thromboembolism (23, 24). The potential mechanisms underlying MP-associated thromboembolism include the following (25). First, MP can directly invade and damage pulmonary vascular endothelial cells, leading to endothelial activation and exposure of tissue factor, thus initiating the extrinsic coagulation cascade and promoting localized thrombus formation. Second, the MP infection triggers a robust inflammatory response characterized by elevated cytokines, thus inducing vascular wall inflammation and thrombotic vascular occlusion through endothelial dysfunction. Finally, through molecular mimicry, MP triggers autoimmune responses characterized by autoantibody production (antiphospholipid antibodies and antiprothrombin antibodies) and complement system activation, thereby elevating coagulation factor levels and establishing a systemic hypercoagulable state that predisposes the patient to thrombus formation. Thromboembolic events associated with MP infection can occur in any anatomical site, including PE, deep vein thrombosis (DVT) of the lower extremities, intracardiac thrombosis, aortic thrombosis, cerebral infarction, cerebral venous sinus thrombosis (CVST), pulmonary venous thrombosis, renal vein thrombosis, and splenic infarction (26–29). PE is the most common manifestation and serves as a major contributor to pulmonary necrosis, residual atelectasis, and organizing pneumonia (6). In this pediatric case, the patient developed a pulmonary embolism, pulmonary infarction, and residual right lower lobe atelectasis with focal necrosis during the disease course. The trigger for these complications may be a prolonged and excessive inflammatory response. The pathogenesis of this dysregulated hyperinflammation likely stems from multiple factors: delayed initiation and inadequate dosing of corticosteroid therapy, macrolide antibiotic resistance, and concurrent bacterial co-infection. Therefore, the early recognition of and prophylactic intervention for a PE hold significant clinical relevance, particularly in mitigating life-threatening complications and improving long-term outcomes.

Chest pain represents the most frequently reported symptom of a PE in children with an MP infection (6, 30). While dyspnea and hemoptysis may also occur, these manifestations are often overshadowed by the overlapping symptoms of MPP, complicating their identification. Notably, one-seventh to one-third of pediatric PE cases present asymptomatically (20, 31). Beyond clinical evaluation, D-dimer levels provide a valuable diagnostic clue for a PE in MP infections (32). Despite its high sensitivity for detecting a PE, the D-dimer level lacks specificity, as it can also be markedly elevated in patients with SMPP without thrombotic events (3, 33, 34). In addition, the most common diagnostic method for MPP combined with a PE is chest contrast-enhanced CT (35). The direct imaging signs of a PE on CT include filling defects or complete obstruction within the vascular lumen (36). Other associated findings may involve a localized reduction in pulmonary vascular markings, patchy lung shadows, and dilated bronchial arteries (37). A PE can often lead to secondary pulmonary infarction, with its classic imaging manifestation being a wedge-shaped consolidation beneath the pleura, with the apex pointing toward the hilum (38). However, some patients may also exhibit a reversed halo sign on imaging (39). These signs are critical for guiding the early diagnosis of a pulmonary embolism. Throughout the clinical course, this child did not exhibit typical symptoms of a PE. However, his D-dimer levels exceeded 5 mg/L by week 4 of illness, and contrast-enhanced chest CT at week 5 confirmed a PE with pulmonary infarction. Notably, wedge-shaped consolidations with pleural-based, hilar-pointing configurations were already evident in the bilateral lower lobes on CT imaging in approximately week 2 of the disease. For such cases, a PE should be suspected early

despite atypical presentations to mitigate thromboembolic complications and long-term sequelae. For SMPP complicated by a PE, anticoagulation is the cornerstone of therapy. Systemic thrombolysis should be considered if hemodynamic instability develops. Unfractionated heparin or LMWH is mostly used as an initial anticoagulation therapy. Moreover, for sequential anticoagulation, LMWH, warfarin, or rivaroxaban are generally selected (39). Anticoagulation therapy is typically continued for 3–6 months (40). In this case, the child received LMWH for 15 days during hospitalization, followed by oral rivaroxaban for 3 months, resulting in the resolution of the PE and favorable clinical outcomes. A phase 3 clinical trial evaluating rivaroxaban for pediatric acute venous thromboembolism reported that, compared to standard anticoagulants (LMWH or vitamin K antagonists), rivaroxaban reduced the risk of thrombotic recurrence without increasing bleeding risk (41). To prevent an MP-associated PE, the following strategies are recommended (10). First, early recognition and timely treatment of the MP infection to reduce progression to SMPP is recommended, thereby minimizing thrombotic complications. In addition, measures should be taken to minimize the risk of thrombosis, including ensuring adequate fluid intake to prevent dehydration caused by persistent high fever and avoiding prolonged immobility, among other precautions. Finally, it is necessary to monitor D-dimer levels in children with SMPP who have prolonged fever, extensive pulmonary consolidation, or elevated inflammatory markers. Clinicians should initiate prophylactic anticoagulation if the patient's D-dimer level is elevated and there is no bleeding risk. Continuous assessment of bleeding risk during anticoagulation therapy is also important.

CT technology can only detect differences in tissue density, whereas magnetic resonance imaging (MRI) technology offers superior tissue contrast, multiplanar imaging capabilities, sensitivity to blood flow, and absence of ionizing radiation. These features make MRI particularly suitable for detecting and diagnosing soft tissue lesions in the chest, especially for radiation-sensitive populations (e.g., children). Chest soft tissues include the heart, mediastinum, pleura, and chest wall. However, MRI has limited utility in evaluating pulmonary diseases due to motion artifacts caused by physiological respiratory movements, low signal intensity from air-filled lungs, and magnetic field inhomogeneities at air/soft tissue interfaces. Currently, some noble gas MRI contrast agents, such as hyperpolarized (HP) butane gas (42), HP diethyl ether (DE) gas (43), parahydrogen-hyperpolarized propane-d_6 gas (44), are revolutionizing functional pulmonary MRI. These agents enable high-resolution lung imaging and are compatible with virtually any MRI system, including emerging portable bedside low-field MRI systems.

4 Conclusion

This case highlights the critical risk of a PE in pediatric RMPP, even without classic thrombotic symptoms. Early recognition of subtle clinical clues, such as persistent fever unresponsive to conventional therapy and elevated inflammatory and coagulation markers (e.g., D-dimer), combined with contrasted chest CT, is essential for timely diagnosis. The successful outcome in this patient was achieved through a multidisciplinary approach integrating targeted anti-infective therapy, adjusted anticoagulation (escalated low-molecular-weight heparin followed by rivaroxaban), and immunomodulation. The complete resolution of the PE on follow-up imaging supports the efficacy and safety of a 3-month oral anticoagulation regimen in children. However, the residual pulmonary necrosis observed in this case emphasizes the importance of long-term monitoring for chronic sequelae. Proactive coagulation screening and early imaging in refractory cases are warranted. Further studies are essential to optimize pediatric-specific anticoagulation protocols and risk stratification.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Dalian Women and Children Medicine Group Medical Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

JZ: Data curation, Investigation, Resources, Writing – original draft. ZhZ: Data curation, Formal Analysis, Investigation, Visualization, Writing – original draft. ZiZ: Data curation, Formal Analysis, Writing – original draft. LC: Methodology, Writing – original draft. YS: Writing – review & editing, Supervision.

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Conflict of interest

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Immunological dysfunction of children with severe parapneumonic effusion

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Purpose: Despite the worldwide decrease in the incidence of serious pneumococcal infections following the introduction of the 13-valent pneumococcal conjugate vaccines (PCV13), invasive infections still occur. This study aimed to investigate the immunological function of children with severe parapneumonic effusion (PPE) both during their hospitalization and after full recovery.

Methods: This was a prospective, single-center study. Children with PPE were admitted to our clinic between 1 January 2011 and 30 June 2023, and participated in the study. Due to the severity of the effusion, all PPE cases required thoracic drainage and some children also underwent fibrinolysis and/or video-assisted thoracoscopic surgery. Demographic and clinical data and laboratory results were collected at admission. Extended immunological testing was performed at the time of clinical admission and again 6–8 weeks after discharge.

Results: A total of 66 episodes of PPE were identified. During hospitalization, one patient was diagnosed with human immunodeficiency virus infection and another with immunoglobulin A deficiency. Extended immunological evaluation was performed during follow-up in 49 patients. Within this cohort, seven patients were diagnosed with mannose-binding lectin deficiency and three with specific antibody deficiency. In total, immune dysfunction was confirmed in 12 patients. When comparing the immunocompromised and non-immunocompromised groups, the duration of hospitalization was longer in the immunocompromised group, with no other differences observed.

Conclusion: Although the incidence of severe PPE has declined since the introduction of PCV13, immunological evaluation remains essential for identifying underlying immunodeficiencies. Despite vaccination, screening patients with PPE for immune dysfunction is crucial. Early diagnosis and timely treatment can help prevent organ damage and reduce long-term morbidity.

KEYWORDS

parapneumonic effusion, community-acquired pneumonia, pneumococcal conjugate vaccine, immunodeficiency, children

Introduction

Pleural effusions can stem from a variety of infectious and non-infectious disorders. Infection is the most common cause (50%–70%), with parapneumonic effusion (PPE) being a prominent example. Less frequent causes include malignancy and congestive heart failure (1).

Despite the widespread use of pneumococcal conjugate vaccines since the early 2010s, *Streptococcus pneumoniae* remains the leading cause of PPE worldwide (2). Asymptomatic nasopharyngeal carriage of this pathogen occurs in up to 60% of healthy young children (3), with even higher rates observed in low- and lower-middle-income countries (4). *S. pneumoniae* can cause a spectrum of illnesses, ranging from mild infections to severe diseases, such as pneumonia, PPE, septicemia, and meningitis, all associated with significant morbidity and mortality (5). PPE remains a severe complication of community-acquired pneumonia, with a considerable number of affected children requiring admission to the intensive care unit (2).

The introduction of the pneumococcal conjugate vaccine (PCV) between 2008 and 2010 significantly reduced both the incidence of pneumonia and its complications, including the PPE incidence rate (1). In the United States, the incidence of PPE peaked at 4.2 cases per 100,000 children annually prior to the PCV era. Following vaccine implementation, this number decreased to approximately two hospital admissions per 100,000 children per year between 2008 and 2014 (6). A similar trend was observed in the United Kingdom. Saxena et al. reported that, before the introduction of PCV13 in 2010, the annual hospitalization rate for PPE was 3.9 per 100,000 children. By 2013, this rate had declined to 1.9 per 100,000 children (7).

However, data also indicate that while the incidence of pneumococcal PPE has declined, there has been a corresponding increase in infections caused by *Streptococcus pyogenes* (8).

In patients with inborn errors of immunity (IEI), severe bacterial infections commonly manifest around 4 months of age, coinciding with the waning of maternally acquired immunoglobulins. IEIs represent a genetically and clinically heterogeneous group of disorders, currently comprising at least 500 genetically defined single-gene defects (9). The hallmark of immunodeficiency (ID) is increased susceptibility to infection; however, the affected organs and pathogens vary depending on the type of immune defect. In immunocompromised patients, invasive pneumococcal disease (IPD) can lead to life-threatening complications. A meta-analysis by van Aalst et al. found that the incidence of IPD is approximately 6–30 times higher in patients with different types of ID compared to healthy controls (10). Despite this, limited data are available regarding the immune function of children with PPE. The primary aim of this study was to evaluate the immunological function and immune response to infection in children with PPE. Additionally, we aimed to estimate the prevalence of different types of immune deficiencies within this patient population.

Abbreviations

ANC, absolute neutrophil count; CAP, community-acquired pneumonia; CD, cluster of differentiation; CVID, common variable immunodeficiency disorder; CRP, C-reactive protein; HIV, human immunodeficiency virus; ID, immunodeficiency; IEI, inborn errors of immunity; Ig, immunoglobulin; IKBA, NF-kappa-B alpha inhibitor; IPD, invasive pneumococcal disease; IRAK-4, IL-1 receptor-associated kinase type 4; NEMO, NF-kappa-B essential modulator; MyD88, myeloid differentiation primary response 88; PCV, pneumococcal conjugate vaccine; PPE, parapneumonic effusion; SAD, specific antibody deficiency; VATS, video-assisted thoracoscopic surgery; WBC, white blood cell.

Materials and methods

Patients and methods

This was a single-center, prospective study conducted at the Department of Pediatrics, Medical School, University of Pécs. A total of 66 children (41 girls and 25 boys) were enrolled in the analysis between 1 January 2011 and 30 June 2023. Exclusion criteria included pleural fluid due to malignancy or trauma (hemothorax), iatrogenic causes of immunodeficiency (such as medications, nutritional status, or anatomic abnormalities), a known family history of immunodeficiency or autoimmune disease, and a history of previous bacterial infections.

The average age of the patients at admission was 4.8 ± 3.7 years (average ± SD). Before hospitalization, 60 patients (90.9%) had received prior vaccination with either PCV7 or PCV13, and 49 patients (74.2%) had received oral antibiotic treatment.

Upon hospitalization, all patients were treated empirically with parenteral antibiotic therapy with ceftriaxone (80 mg/kg/day), with or without clindamycin (40 mg/kg/day); this treatment was later adjusted based on microbiological results or the clinical response. Additionally, a thoracic drain was performed in all cases using an 8–12-Fr pigtail catheter. Indications for thoracic drainage included pleural fluid wider than 2 cm at the level of the hilus, the presence of septation or loculation in the pleural space (as detected by thoracic ultrasound), or severe respiratory distress in the presence of any pleural fluid. The thoracic drainage was initiated on the second (median) day of hospitalization [interquartile range (IQR): 1–3.5 days] and lasted for 6 (4–11.5) days. In cases of inadequate drainage or the appearance of loculation, intrapleural fibrinolytic therapy with tissue plasminogen activator (Alteplase, Genentech, USA) and/or thoracic surgery—either video-assisted thoracoscopic surgery (VATS) or decortication—was performed. Fibrinolytic treatment was administered in 56.1% (37/66) of cases. Due to insufficient conservative therapy, 18 patients (27.3%) required surgical intervention (decortication and/or VATS).

Extensive microbiological analysis of the pleural effusion was performed, utilizing standard culture, urinary antigen testing, and polymerase chain reaction (PCR) techniques. Simultaneously, blood culture was also obtained. At the beginning of hospitalization, an initial immunological screening test and routine blood tests, including complete blood count, C-reactive protein (CRP), procalcitonin, and blood culture, were performed. This included measuring serum immunoglobulin levels (IgA, IgE, IgG, IgM) and multiparameter flow cytometry analysis of lymphocyte subsets. Extended immunological evaluation was conducted 6–8 weeks after hospital discharge at the Allergy and Immunology Outpatient Care Unit of our clinic. At the outpatient unit, patients were clinically assessed, and peripheral venous blood samples were collected from all participants. Routine laboratory tests included complete blood count, CRP, serum immunoglobulin levels (IgA, IgE, IgG, IgM), and complement components (C3, C4). We also performed functional assessments of the classical, alternative, and lectin complement pathways. Additionally, peripheral blood

lymphocytes were analyzed using multiparameter flow cytometry (Beckton Dickson and Company Biosciences, San Jose, USA). The following cell types were evaluated: CD56+ natural killer cells, CD3 + CD56+ natural killer T cells, CD3 + CD8+ cytotoxic and CD3 + CD4+ helper T lymphocytes, CD19 + CD5+ B1 and CD19 + CD5− B2 B lymphocytes, CD4 + CD25 high+ regulatory T and CD3 + CD25 medium+ activated T cells, CD3 + HLADR+ activated T cells, CD8 + HLADR+ activated T cytotoxic cell, CD3 + CD45RA+ naïve and CD3 + CD45RO+ memory T cells, CD19 + IgD + CD27− naïve B cells, and CD19 + IgD + CD27+ non-switched B and CD19 + CD27 + IgD− switched B cells. Specific antibody titers (against *S. pneumoniae*, tetanus, diphtheria, and *Haemophilus influenzae* type b) were also measured.

The classical complement pathway was assessed using a hemolytic complement assay with sheep red blood cells. The alternative and lectin complement pathways were evaluated by ELISA. The results for the lectin complement pathways were expressed as a percentage of a standard control, with values below 30% considered abnormal. Abdominal ultrasound was performed to exclude asplenia. Immunodeficiencies were defined according to the criteria of the International Union of Immunological Societies(11). Based on the results of the immunological investigations, the patients were categorized into two groups: those with and those without impaired immune function.

The study was approved by the Local Ethics Committee (Medical School, University of Pécs). Informed consent was obtained from the parents or legal guardians of all participants.

Data analysis and statistics

SPSS for Windows 28.0 statistical software was used. Data distributions were tested for normality using the Kolmogorov–Smirnov test. Given that age, time and length of the drainage, lymphocyte count, the pleural fluid lactate dehydrogenase concentration, levels of the classical and alternative complement pathways, C3 and C4 levels, and pneumococcal antibody titer did not exhibit normal distribution, their median and IQR values are provided. For assessing statistically significant differences, Student's *t*-test (for normally distributed data), the Mann–Whitney test (for non-normally distributed data), and the χ^2 test or Fisher's exact test (for dichotomous variables) were used. A *p*-value of <0.05 was considered to be statistically significant.

Results

Clinical data

In this prospective study, *S. pneumoniae* was identified as the main etiological agent in 48 of 66 cases (72.7%). Only four patients (6.0%) required mechanical ventilation, while the remaining 60 children (90.9%) received supplemental oxygen therapy. The median duration of hospitalization was 16 days [interquartile range (IQR): 12–23.2], and no deaths occurred in the study population.

Seventeen patients were unavailable for follow-up and were therefore excluded from further analysis. Ultimately, 49 children were included in the extended immunological evaluation. There were no significant differences in age, sex, duration of drainage, length of oxygen therapy, or total hospital stay between those who participated in follow-up examinations and those who did not.

Immunological data

Impaired immune function was identified in 12 of 49 patients (24.5%). Of these, seven patients were diagnosed with mannose-binding lectin (MBL) deficiency, three with specific antibody deficiency (SAD), one with IgA deficiency, and one with human immunodeficiency virus (HIV) infection. Demographic characteristics of patients with and without immunodeficiency are summarized in Table 1. No significant differences were observed between the two groups regarding age, gender, the start of drainage, or the frequency of use of fibrinolytic and VATS/decortication procedures. However, the duration of hospitalization was significantly longer in immunocompromised patients than in those without immune dysfunction [19.0 (16.5–22.5) vs. 14.0 (12–20) days; $p = 0.006$].

Table 2 presents the pleural fluid characteristics and basic immunological findings of patients at ICU admission. Biochemical parameters of pleural fluid did not differ

TABLE 1 Demographic data and clinical characteristics of the participants.

Variables	Patients with immune dysfunction	Patients without immune dysfunction	*p*
n	12 (24.5%)	37 (75.5%)	
Sex (male/female)	3/9	16/21	0.22
Age (years)	3.6 (3.1–5.0)	4.1 (3.1–4.9)	0.56
Prehospital antibiotic therapy	8 (66.7%)	29 (78.4%)	0.32
Start of drainage (days)	1.5 (1.0–3.0)	2.0 (1.0–3.5)	0.56
Length of drainage (days)	6.5 (5.0–15.5)	6.0 (4.0–9.5)	0.56
Length of hospitalization (days)	19.0 (16.5–22.5)	14.0 (12.0–20.0)	0.006
Fibrinolysis	6 (50%)	20 (54.1%)	0.81
Video-assisted thoracoscopy	4 (33.3%)	9 (24.3%)	0.39

Variables with not normal distribution are presented with median (IQR). For dichotomous variables, the χ^2 test/Fisher's exact test was used, and the Kruskal–Wallis test was used for non-normally distributed data.

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TABLE 2 Common laboratory and basic immunological values at hospitalization of the participants.

Variables	Patients with immune dysfuntion	Patients without immune dysfunction	*p*
n	12 (24.5%)	37 (75.5%)	
C-reactive protein (mg/L)	261.6 ± 109.6	233.6 ± 100.7	0.21
Pleural fluid pH	6.98 ± 0.29	7.15 ± 0.25	0.055
Pleural fluid protein (g/L)	42.7 ± 4.7	40.7 ± 7.1	0.21
Pleural fluid LDH (U/L)	5,050 (1,220–19,300)	4,650 (910–10,970)	0.45
Immunoglobulin A (g/L)	2.36 ± 1.16	1.93 ± 0.88	0.11
Immunoglobulin M (g/L)	1.58 ± 0.54	1.29 ± 0.45	0.053
Immunoglobulin G (g/L)	14.4 (8.3–19.7)	9.6 (7.2–13.1)	0.33
White blood cells (/µl)	15 980 ± 6,550	19 720 ± 8,400	0.083
Absolute neutrophil count (/µl)	12 670 ± 5,660	15 870 ± 8,400	0.11
lymphocytes (/µl)	2,810 (1,560–3,200)	2,040 (1,310–3,320)	0.93
CD4+ T cells (/µl)	1,170 ± 630	1,210 ± 470	0.42
CD8+ T cells (/µl)	930 ± 400	800 ± 360	0.21
Active Th (%)	9.5 (4.8–24.8)	9.0 (6.7–13.1)	0.96
CD3/CD25+ T cells (%)	2.8 (1.2–5.0)	0.8 (0.7–1.8)	0.006
HLADR CD3+ T cells (%)	7.9 ± 8.5	5.9 ± 4.7	0.26
HLADR CD8+ T cells (%)	4.0 (1.5–11.4)	2.8 (1.8–5.2)	0.77
CD3/CD45RA+ T cells (/µl)	59.5 ± 12.6	56.2 ± 11.3	0.29
CD3/CD45RO T cells (/µl)	19.0 (12.9–34.5)	17.4 (14.5–22.2)	0.73
CD19+ lymphocytes (/µl)	373 (200–665)	400 (300–545)	0.48
Natural killer CD56+ cells (%)	3.9 (2.2–5.1)	4.2 (2.9–7.5)	0.48
Natural killer T cells (%)	2.5 (0.9–4.9)	1.4 (0.8–2.5)	0.29

Variables with normal distribution are presented as mean ± SD, and those with non-normal distribution are presented with median (IQR).

significantly between groups. Both groups exhibited elevated white blood cell and absolute neutrophil count levels without significant difference. Neutropenia was not observed in any patient. One 5-year-old patient, diagnosed with IgA deficiency, had an IgA level below 0.05 g/L. In the immunocompromised group, both IgM and IgG levels showed a non-significant increase. While median lymphocyte counts and subpopulations remained within the normal range, the percentage of $CD3^+CD25^+$ activated T cells was significantly higher in patients with immune dysfunction [2.8 (1.2–5.0) vs. 0.8 (0.7–1.8)%; $p = 0.006$]. Severe $CD4^+$ lymphopenia (146/µl) was detected in a patient with confirmed HIV infection by serology.

Table 3 details the extended immunological findings from outpatient follow-up. The total $CD19^+$ naïve B lymphocyte counts and the age-matched Ig levels were within the normal range and did not differ significantly between groups. However, three patients exhibited low pneumococcal antibody concentrations (19.6, 26.3, and 37.2 mg/L; normal range: >50 mg/L) despite confirmed severe infection and vaccination. Since revaccination did not increase antibody titers, they were diagnosed with specific antibody deficiency. All three patients began immunoglobulin replacement therapy.

Antibody titers for tetanus, diphtheria, and *Haemophilus* were within the normal range in both groups. T-cell numbers, subsets, and activation markers were also within the normal range, with no significant difference observed between groups.

The classical and alternative complement pathways were within normal ranges; however, seven children exhibited reduced lectin pathway activity and were diagnosed with MBL deficiency.

No cases of asplenia were detected by abdominal ultrasound examination.

Discussion

To our knowledge, the study represents the first investigation into extended immune function within a homogeneous cohort of children presenting with severe PPE and requiring intensive care treatment. Our screening protocol revealed that approximately one in four children with severe PPE exhibited some degree of immune dysfunction.

Severe infections can occur in vulnerable patients who are immunocompromised by immunosuppressive treatment, HIV infection, or chronic inflammatory diseases (12). For cases where no identifiable risk factor is apparent at presentation, comprehensive immunological exploration is crucial for detecting potential primary or acquired immunodeficiencies (13). While severe bacterial infection may be the initial presenting symptom of IEI, immunological screening is not yet standard practice in many clinical settings (14).

A retrospective study by Strasser et al. identified significantly impaired immune function in 6% of pediatric patients with severe bacterial infection requiring intensive care (15). Furthermore, an additional 11% of subjects exhibited mild immunological abnormalities, which were potentially attributable to delayed immune system maturation. This suggests that approximately 17% of their heterogeneous patient population—which included children with sepsis, meningitis, and PPE—demonstrated varying degrees of immune dysfunction. Our prospective study, focusing exclusively on a homogeneous PPE cohort, found a similar prevalence (15). Previous studies indicate that 1%–10% of children presenting with invasive pneumococcal disease possess an underlying IEI (16–18).

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TABLE 3 Detailed immunological characteristics of patients at the outpatient unit.

Variables	Patients with immune dysfunction	Patients without immune dysfunction	*p*
n	12 (24.5%)	37 (75.5%)	
Immunoglobulin A (g/L)	1.15 (0.62–1.78)	1.04 (0.7–1.45)	0.33
Immunoglobulin M (g/L)	1.16 (0.9–1.8)	1.1 (0.8–1.42)	0.42
Immunoglobulin G (g/L)	9.9 ± 3.7	9.8 ± 2.3	0.46
Pneumococcal antibody level (mg/L)	62.7 (26–313)	107 (70.2–323.4)	0.28
CD3+ lymphocytes (/μl)	2,900 ± 1,490	2,730 ± 1,090	0.35
CD4+ T cells (/μl)	1,470 ± 820	1,330 ± 560	0.30
CD8+ T cells (/μl)	1,030 (578–1,478)	1,060 (817–1,445)	0.89
Active Th (%)	16.8 ± 8.8	13.7 ± 8.5	0.16
CD3/CD25+ T cells (%)	1.9 (0.75–3.5)	1.6 (0.9–3.2)	0.59
HLADR CD3+ T cells (%)	4.4 (2.9–8.2)	5.6 (4.0–9.2)	0.38
HLADR CD8+ T cells (%)	4.4 (2.5–6.3)	4.1 (2.4–7.2)	0.59
CD3/CD45RA+ T cells (/μl)	48.2 ± 16.3	46.9 ± 8.8	0.41
CD3/CD45RO T cells (/μl)	26.0 ± 11.5	28.3 ± 8.3	0.24
CD19+ lymphocytes (/μl)	284 (164–518)	332 (217–403)	0.52
Natural killer CD56+ cells (%)	12.6 ± 8.8	10.0 ± 5.5	0.20
Natural killer T cells (%)	1.3 (0.6–2.5)	1.9 (1.1–4.8)	0.33
Complement 3 (g/L)	1.19 ± 0.26	1.2 ± 0.24	0.40
Complement 4 (g/L)	0.24 (0.18–0.35)	0.22 (0.18–0.32)	0.46
Classical complement pathway (%)	61.2 ± 21.6	55.5 ± 17.7	0.19
Alternative complement pathway (%)	80.5 (71–92)	82 (59–92)	0.45
Lectin pathway (%)	44.4 ± 49.0	78.8 ± 41.6	0.013

Results of detailed immunological analysis among patients with or without immunodeficiency after 6–8 weeks of hospitalization. Variables with normal distribution are presented as mean ± SD, and those with non-normal distribution are presented with median (IQR).

Pulmonary disease is common among patients with IEI (19). While individual disorders are rare, collectively, they represent a significant cause of morbidity and mortality (20).

S. pneumoniae is the primary pathogen responsible for PPE. The severity and localization of pneumococcal infection vary from asymptomatic colonization to invasive disease. The pathomechanism underlying the progression from asymptomatic colonization to invasive pneumococcal infection remains poorly understood. Factors such as strain-specific virulence and the presence of underlying chronic disorders or immunodeficiencies may contribute to this progression (21).

The immune defense against pneumococcal infection involves complex pathomechanisms. The innate immune system functions as the first line of defense. Peptidoglycans found in Gram-positive bacteria primarily activate the complement system via the alternative pathway. Additionally, bacteria expressing mannose residues on their surface can bind MBL, thereby facilitating opsonization and activating the complement system via the lectin pathway (22). MBL, a soluble pattern recognition molecule, contributes to the elimination of a broad spectrum of pathogenic microorganisms by activating the lectin complement pathway and promoting opsonophagocytosis (22).

The synthesis of MBL is influenced by polymorphisms in the *MBL2* gene. However, serum MBL levels are not solely determined by genotype, as other genetic and environmental factors also affect their expression. Therefore, measuring serum MBL levels is considered a more reliable method for diagnosing MBL deficiency than relying solely on genotyping (23). Clinical studies have demonstrated that MBL deficiency predisposes individuals to severe respiratory tract infections (24).

Nasopharyngeal carriage of *S. pneumoniae* is approximately twice as prevalent in MBL-deficient children compared to healthy controls (25). Furthermore, MBL deficiency plays a more prominent role in infection susceptibility in individuals with predisposing risk factors, such as chronic lung disease and cystic fibrosis, and in infants with immature adaptive immune systems (24). The absence of these above-mentioned risk factors in our patients underscores the importance of comprehensive immunological screening.

Several studies have reported an increased risk of invasive pneumococcal disease in MBL-deficient children and adults (26–28). In our study, MBL deficiency was the most common immunodeficiency identified. Although no serious infections occurred during the follow-up period, upper respiratory tract infections were more frequent in these patients than in healthy controls. Currently, no specific therapy exists for this complement deficiency; however, immunization of family members and household contacts is recommended to reduce the risk of infections (29).

The cellular immune response also plays a critical role in protection against pneumococcal infection (30). The Th17 response has been shown to be a key component of pneumococcal immunity in both mouse models and immunodeficiency conditions, such as hyper-IgE syndrome (31, 32). Serum immunoglobulin E levels in our patients were below 2,000 IU/ml, thereby effectively ruling out hyper-IgE syndrome. The severe $CD4^+$ lymphopenia in our HIV-infected patient further highlights the importance of screening for secondary immunodeficiencies. The significantly elevated $CD3^+CD25^+$ T-cell count within the immunocompromised group is likely indicative of immunocompensatory mechanisms.

Recurrent sinopulmonary infections represent a typical symptom of humoral immune deficiency (33), which is the most common form of IEI. This condition can result from defects in B-cell development, abnormal B-cell activation, or impaired antibody synthesis. Screening for humoral IEI typically involves flow cytometric analysis of B-cell subsets, quantitative measurement of serum immunoglobulin levels, and evaluation of specific antibody responses to infection and/or vaccination (34, 35).

Within our cohort, one patient was diagnosed with selective IgA deficiency (sIgAD). Most individuals with sIgAD are asymptomatic or experience mild sinopulmonary and gastrointestinal infections (36). However, Gaschignard et al. reported a notably higher prevalence of IgA deficiency (5%–7%) in children with severe bacterial infection compared to the general population (<1%) (16). It is important to emphasize that during immunological follow-up, 20%–30% of patients with IgA deficiency may develop autoimmune disorders, such as celiac disease or thyroiditis (37).

Three patients were diagnosed with SAD, which is characterized by an isolated, impaired antibody response to polysaccharide antigens despite normal levels of serum IgG, IgG subclass, IgA, and IgM, as well as normal T-cell subpopulations (38). Several monogenic disorders, including common variable immunodeficiency disorders, ataxia telangiectasia, Wiskott–Aldrich syndrome, and nuclear factor-kappa-B essential modulator deficiency, are associated with SAD. Identification of these patients is critical for appropriate management and prevention of complications. Early antibiotic treatment and regular immunoglobulin replacement therapy can prevent long-term complications such as mastoiditis and bronchiectasis (39). In our cohort, we observed no infections after initiating immunoglobulin substitution therapy. In addition to infectious complications, non-infectious manifestations such as autoimmunity and hematopoietic malignancies have also been reported in patients with SAD.

Rare primary immunodeficiency syndromes may also predispose patients to severe pneumococcal infections. Autosomal recessive disorders, such as NF-kappa-B essential modulator (NEMO) or NF-kappa-B alpha inhibitor (IKBA) deficiency, along with IL-1 receptor-associated kinase type 4 (IRAK-4) and myeloid differentiation primary response 88 (MyD88) deficiency, disrupt Toll-like receptor and interleukin-1 receptor signaling pathways, leading to increased susceptibility to severe infections (40).

In our study, it was not possible to investigate interleukin 6 production by white blood cells; therefore, the presence of MyD88 deficiency and disruptions in Toll-like receptor signaling were not assessed. Additionally, genetic testing was not performed on our patients. It is worth noting that, however, genetic testing is not routinely performed in clinical practice (41).

Primary immunodeficiency diseases and acquired immunodeficiencies can also predispose to severe infection. HIV infection is the most common acquired immunodeficiency. The prevalence of pleural effusion among patients hospitalized with AIDS ranges from 2% to 20%, often caused by bacterial infection, tuberculous pleuritis, or other malignancies (42). *Staphylococcus aureus* was more common in the HIV-positive group, along with anaerobic and opportunistic infections. In our study, the patient's severe infection, dystrophic appearance, and severe stomatitis raised suspicion of HIV infection, which was subsequently confirmed by serological testing.

Conclusion

Pulmonary disease is a common manifestation of many IEIs and continues to be a leading cause of morbidity and mortality. In our study, varying degrees of immune impairment were identified in approximately one-quarter of children presenting with severe PPE. We advocate for detailed immunological screening in children with severe PPE. Early diagnosis and timely initiation of appropriate treatment are crucial for improving the prognosis of patients with primary immunodeficiencies.

Study limitations

Our study has several limitations. First, it single-hospital setting may limit the generalizability of our findings. Second, not all children with PPE were tested for detailed immune function; therefore, the true prevalence of immunodeficiencies in this population may differ from that reported here. However, basic physiological and laboratory parameters did not significantly differ between the tested and untested groups. Additionally, pneumococcal serotyping of patients has only been feasible in recent years, so serotype data are not available for all cases. Among the serotyped isolates, serotype 3 was the predominant strain (unpublished data).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Ethics statement

The studies involving humans were approved by the Local Ethics Committee, Medical School, University of Pécs. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

BR: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing. DS: Methodology, Validation, Writing – review & editing. TB: Methodology, Supervision, Validation, Writing – review & editing. GK: Data

curation, Investigation, Project administration, Writing – original draft. BM: Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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The protective effect of biologic and targeted-synthetic therapies on developing multisystem inflammatory syndrome in children

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Background: Multisystem Inflammatory Syndrome in Children (MIS-C) is a severe, life threatening, complication that arises weeks after acute Coronavirus disease 2019 (COVID-19) infection, often presenting with fever and diverse systemic symptoms. Limited data exists on the effectiveness of biologic and targeted-synthetic therapies in preventing MIS-C development. Therefore, our aim was to investigate whether biologic and targeted-synthetic therapies can prevent the occurrence of MIS-C.
Methods: We assessed the Clalit Health Services database, the largest health care organization in Israel, data from 793,909 children aged 0–18 years who tested positive for COVID-19 were analyzed. The diagnosis of MIS-C was adjudicated using the case definition used by the Centers for Disease Control and Prevention (CDC) or by the World Health Organization (WHO). Patients receiving biologic and targeted-synthetic therapies were compared to a control group.
Results: Among 793,909 cases, 573 children received biologic and targeted-synthetic therapies, and 143 cases of MIS-C were identified. Notably, none of the individuals treated with biologic and targeted-synthetic therapies developed MIS-C.
Conclusion: Our study highlights our hypothesis on the efficacy of biological treatments in preventing MIS-C. Although statistical significance was not achieved due to the absence of MIS-C cases in patients receiving biologic and targeted-synthetic therapies, our study shows a possible association between biological therapies and reduced risk of MIS-C following COVID-19 infection in children. Further research, including prospective studies with larger cohorts, is warranted to confirm these findings and elucidate underlying mechanisms.

KEYWORDS

MIS-C, COVID-19, biological treatments, pediatric, targeted-synthetic therapies

Background

Multisystem Inflammatory Syndrome in Children (MIS-C) is a systemic inflammatory syndrome that occurs 2–6 weeks after acute Coronavirus disease 2019 (COVID-19) infection (1). Clinical presentation typically includes fever and often gastrointestinal or cardiac symptoms but can involve other body systems as well (2). Additionally, many

children present with symptoms resembling Kawasaki-like disease such as conjunctival injection, cervical lymphadenopathy, and appropriate skin involvement (3). Most patients with MIS-C require hospitalization for aggressive management due to significant cardiac involvement, which may lead to cardiogenic shock, and approximately 2% of them succumb to this condition (4, 5).

Two diagnostic criteria for MIS-C are accepted worldwide: the criteria from the World Health Organization (WHO) and those from the Centers for Disease Control and Prevention (CDC) (6, 7). According to the CDC, patients up to the age of 21 are included in the criteria, whereas the WHO includes patients only up to the age of 19.

The pathogenesis of MIS-C involves dysregulation of various proinflammatory cytokines including IL-1, IL-6, IL-18, TNF-α, and IFN-γ, leading to cytokine storm and widespread systemic inflammation (8).

The first line of treatment for MIS-C includes steroids and intravenous immunoglobulin (IVIG) along with anti-thrombotic and anticoagulant therapies (9). Children who do not respond to these treatments receive different biological therapies such as anti-TNF or anti-IL1 and anti- IL6 (10).

To date, there is limited data on the proportion of children receiving biologic and targeted-synthetic therapies who had developed MIS-C. In a cohort of 55 children with rheumatic diseases who had COVID-19, 31 of them were treated with biologic and targeted-synthetic therapies, there were no cases of MIS-C (11). Conversely, in a study with a cohort of 26 rheumatologic patients treated with biological therapies who had COVID-19 infection, 5 of them (19.2%) developed MIS-C (12). In another study with a cohort of 113 children who had COVID-19 and received biological treatments, the MIS-C rate was 4.4% (13).

In this "big data" study we assessed the Clalit medical database in a cohort study to compare the percentage of children and adolescents up to the age of 18 who were treated with biologic and targeted-synthetic therapies and developed MIS-C after contracting COVID-19, vs. the percentage of individuals in the same age group who did not receive biological therapy, contracted COVID-19, and developed MIS-C in order to evaluate whether biologic and targeted-synthetic therapies may have a protective effect from the devastating MIS-C.

Methods

The study is a retrospective cohort study based on the Clalit health service database, represent the major Israeli health organization and one of the largest health service organizations in world, delivering health care services to about 5,000,000 insured subjects, highly computerized and continuously updated.

This study included children and adolescents aged 0–18 who contracted COVID-19 between March 2020 and February 2023, examining whether these patients developed MIS-C according to the CDC or WHO diagnostic criteria. The research group comprised patients who had received biologic and targeted-synthetic therapies within 31 days before their positive COVID-19 test date, compared to the control group who had not received such treatments. This timeframe was chosen because all biologic and targeted-synthetic therapies included in the study are typically administered on a monthly or more frequent schedule in pediatric clinical practice. Positive COVID-19 cases were determined based on either a positive RT-PCR test or a positive COVID-19 antigen test.

Our primary outcome was the percentage of COVID-19 patients who developed MIS-C in both groups. Secondary outcomes included demographic data (gender, age, origin), data regarding underlying diseases and biological treatment, data regarding COVID-19 infection, and data regarding MIS-C.

The biologic and targeted-synthetic therapies included in this study were the biological agents in use among children and adolescents including Adalimumab, Etanercept, Golimumab, Infliximab, Tocilizumab, Canakinumab, Anakinra, Tofacitinib, Baricitinib, Ustekinumab, Abatacept, Rituximab, Upadacitinib, Secukinumab.

Statistical analysis

Categorical variables were compared using Fisher's exact test or chi-square, as appropriate, while continuous variables were compared using the two-sample Wilcoxon test. The analysis was performed using R software (version 4.3.1) from the R Foundation for Statistical Computing.

Results

Basic characteristics of the study population

This study examined data from 793,909 cases aged 0–18 who tested positive for COVID-19, of whom 143 (0.02%) developed MIS-C. The median age at the time of COVID-19 infection was slightly lower in the MIS-C group compared to those without MIS-C [7.93 years (IQR 4.85–10.60) vs. 9.27 years (IQR 5.52–13.04), $p = 0.2$]. Biologic and targeted-synthetic therapies were administered to only 0.1% of the total cohort ($n = 573$), all within the non-MIS-C group (Table 1). The process of patient selection and inclusion is pictorially shown in Figure 1.

Patients treated with biologic and targeted-synthetic therapies

Regarding the 573 patients treated with biologic and targeted-synthetic therapies; the average duration between biologic and targeted-synthetic therapies administration and the positive COVID-19 test was 16 ± 9.2 days. The most prevalent biological drug used was Adalimumab, in 236

Abbreviations

MIS-C, multisystem inflammatory syndrome in children; WHO, world health organization; CDC, centers for disease control and prevention; COVID-19, coronavirus disease 2019; IVIG, intravenous immunoglobulin; IBD, inflammatory bowel disease; JIA, Juvenile idiopathic arthritis.

TABLE 1 Comparison between patients without MIS-C and patients with MIS-C.

Characteristic	Patients without MIS-C (n = 793,766)	Patients with MIS-C (n = 143)	P-value
Patients not receiving biologic and targeted-synthetic therapies	793,192 (99.9%)	143 (100%)	
Patients receiving biologic and targeted-synthetic therapies	573 (0.1%)	0 (0%)	
Age at covid infection, median (IQR)	9.27 (5.52, 13.04)	7.93 (4.85,10.60)	0.2
Gender (Male), n (%)	3,97,626 (50%)	82 (57%)	0.10

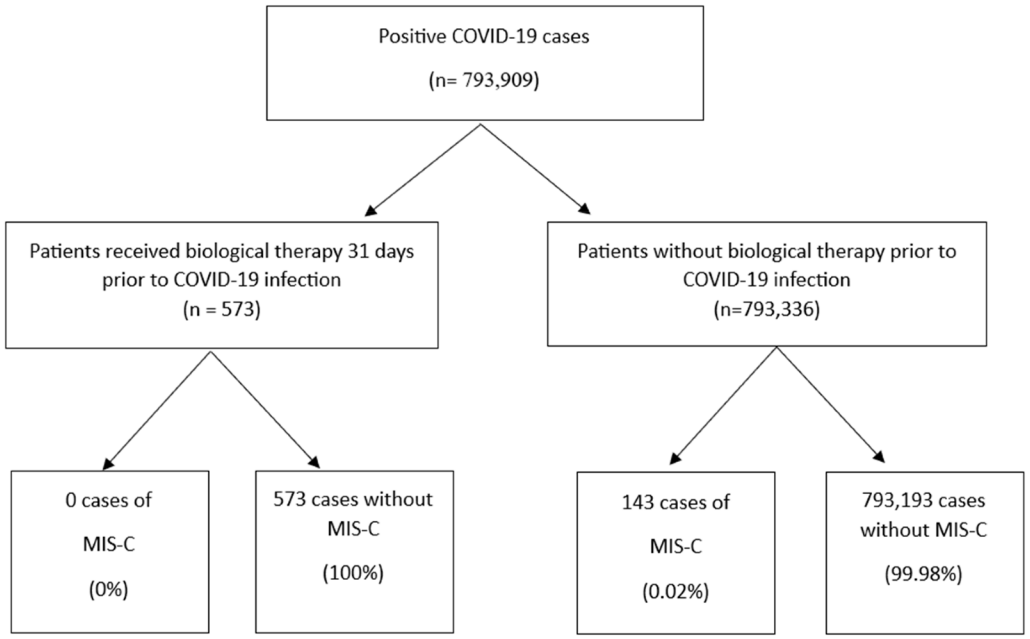

FIGURE 1
The process of patient selection and inclusion.

(41.2%) patients, followed by Infliximab in 174 (30.4%) patients; both are anti-TNF α drugs. Etanercept, another anti-TNF α drug, was used in 35 (6.1%) of the patients, making anti-TNF α drugs the most used drugs in our cohort. Table 2 presents the prevalence of the biologic and targeted-synthetic therapies used for treatment in this cohort. Additionally, regarding the medical conditions that required treatment with biological or targeted-synthetic therapies, some patients had more than one diagnosis. Unfortunately, most of the patients had no obvious diagnosis warranting treatment with biological therapies according to the medical records. Among the known diagnoses, Inflammatory Bowel Disease (IBD) was the most prevalent, affecting 189 (15%) of the patients, followed by Juvenile Idiopathic Arthritis (JIA), which affected 93 (7.2%) of the patients. Table 3 presents all the medical conditions and their prevalences.

MIS-C cases

MIS-C was identified in 143 children of the whole cohort (18 cases per 100,000 participants) between 28 and 42 days after their positive COVID-19 test. The average duration from the positive COVID-19 test to MIS-C diagnosis was 40.8 ± 3.2 days. The patients diagnosed with MIS-C had a median age of 7.93 (IQR: 4.85,10.60), with 57% being males (Table 1). Remarkably, none of the participants who received biologic and targeted-synthetic therapies were diagnosed with MIS-C.

Among 793,335 patients who were not treated with biologic and targeted-synthetic therapies, 143 MIS-C cases were detected (risk = 0.00018). In contrast, no MIS-C cases were recorded among the 573 individuals who received biologic and targeted-synthetic therapies. The calculated relative risk (RR) was 0, indicating no observed association between biological treatment status and the occurrence of MIS-C. The uncorrected odds ratio was also 0 [95% CI: (0, 0.38)], and Fisher's exact test returned a non-significant p-value ($p = 1$). These counterintuitive results reflect the extremely low frequency of MIS-C cases overall and the conservative nature of the Fisher test when applied to highly unbalanced tables with small expected counts. Despite the non-significant p-value from Fisher's test, and the 0 RR and OR, the absence of events in the biologic treatment group suggests a clinically relevant reduction in risk.

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TABLE 2 Biologic and targeted-synthetic therapies.

Biological Drug	Number of patients (%)
Adalimumab	236 (41.2%)
Infliximab	174 (30.4%)
Canakinumab	62 (10.8%)
Etanercept	35 (6.1%)
Rituximab	21 (3.7%)
Ustekinumab	17 (3%)
Tocilizumab	13 (2.3%)
Tofacitinib	6 (1%)
Abatacept	4 (0.7%)
Anakinra	3 (0.5%)
Golimumab	2 (0.3%)

TABLE 3 Medical condition treated with biologic and targeted-synthetic therapies.

Medical condition	*N* = 1,283[a]
Unknown	877 (68.3%)
IBD	189 (15%)
JIA	93 (7.2%)
Psoriasis	42 (3.3%)
FMF	34 (2.7%)
Hidradenitis Suppurativa	26 (2.0%)
Optic Neuritis	7 (0.5%)
Behçet's disease	6 (0.5%)
Rheumatic/Autoimmune	5 (0.4%)
CRMO	1 (<0.1%)
SLE	1 (<0.1%)
Synovitis And Tenosynovitis	1 (<0.1%)
Takayasu arteritis	1 (<0.1%)

CRMO, chronic recurrent multifocal osteomyelitis; FMF, familial mediterranean fever; IBD, inflammatory bowel disease; JIA, Juvenile idiopathic arthritis; SLE, systemic lupus erythematous.

[a]The number of diagnoses exceeds the number of patients as some individuals had multiple medical conditions.

Discussion

MIS-C presents a significant challenge in the context of the COVID-19 pandemic due to its potentially severe clinical manifestations, including significant cardiac involvement and a mortality rate of approximately 2% (4). Understanding the factors that may influence the development of MIS-C is crucial for guiding clinical management and improving patient outcomes.

Biological therapies have emerged as a potential treatment option for MIS-C, targeting the dysregulated proinflammatory cytokines implicated in its pathogenesis (5). However, the relationship between biological therapy and the risk of developing MIS-C in the context of COVID-19 infection remains unclear. This study aimed to address this gap by comparing the incidence of MIS-C among individuals treated with biologic and targeted-synthetic therapies vs. those who did not receive such treatments.

In this "big data" study, none of the children who received biologic and targeted-synthetic therapies were diagnosed with MIS-C, indicating a potential protective effect. Although Fisher's exact test and logistic regression analysis were not applicable due to the absence of MIS-C cases among patients treated with biologic and targeted-synthetic therapies, the findings of this study suggest that biologic and targeted-synthetic therapies may indeed play a role in mitigating the risk of MIS-C following COVID-19 infection in children despite the inability of statistical tests to demonstrate this mathematically due to the lack of observed cases. Given that MIS-C is rare, affecting only 0.02% of patients not receiving biologic and targeted-synthetic therapies, statistical proof of this protective effect remains challenging. Nonetheless, the lack of MIS-C cases in the biological therapy group supports this as a reasonable assumption.

Our results align with a previous study involving a cohort of 31 patients previously treated with biological therapies, which reported zero cases of MIS-C in this group (11). However, they contradict two other studies involving cohorts of 26 (12) and 113 (13) patients, which revealed a percentage of 4.4% to 19.2% of MIS-C development in patients treated with biological drugs. Based on a large number of patients treated with biological drugs from multiple medical centers across the country, our study is less susceptible to selection biases that often affect single-center studies. In addition, it contributes significantly to the existing data and may influence the understanding of MIS-C occurrence in patients treated with biological drugs, potentially tilting the scale towards a zero percentage.

In addition, these findings contribute to the growing body of literature on the management of MIS-C and underscore the importance of considering biologic and targeted-synthetic therapies as a potential therapeutic option in this population (14).

The potential protective effect of biological therapies in preventing MIS-C may be linked to their ability to modulate the immune response, particularly through the regulation of cytokine storms and inflammation. Biological therapies, such as TNF inhibitors, IL-6 inhibitors, and other targeted agents, are known to interfere with the dysregulated immune signaling that often occurs in severe COVID-19 and related complications.

Understanding the immune responses in inflammatory conditions is crucial for elucidating the pathogenesis of MIS-C. For instance, disturbances in the interaction between gut-resident macrophages and the gut microbiota can lead to IBD (15). The dysregulated immune responses observed in IBD may share similarities with those in MIS-C, suggesting that alterations in macrophage function could contribute to the development of MIS-C. Additionally, oxidative stress can lead to the depletion of tetrahydrobiopterin (BH4), a critical cofactor in nitric oxide synthesis, impairing endothelial function (16). Given that endothelial dysfunction is a hallmark of MIS-C, these insights highlight the importance of redox balance in the pathogenesis of MIS-C. Furthermore, the plasticity of immune cells, particularly macrophages, allows for a dynamic response to inflammatory stimuli (17). This adaptability may influence the severity and progression of inflammatory conditions, including MIS-C, underscoring the need for targeted therapeutic strategies that modulate immune cell function.

In the case of MIS-C, a hyper-inflammatory response, characterized by an overproduction of pro-inflammatory cytokines, plays a crucial role in the development of the

syndrome. By targeting these inflammatory pathways, biologic and targeted-synthetic therapies may help reduce excessive immune activation, thereby potentially preventing the onset of MIS-C. However, further research is needed to fully elucidate the exact mechanisms through which these therapies influence the progression of MIS-C and confirm their therapeutic potential in this context.

Several limitations should be acknowledged. One of the major limitations of this study is the low number of MIS-C events, due to the rarity of the disease, which may affect the statistical power and reliability of the findings. An additional significant limitation is the lack of data on the clinical reasons for initiating biologic and targeted-synthetic therapies in most of the children treated. This absence of indication data limits our ability to fully interpret the results and increases the potential for residual confounding. Furthermore, the retrospective design and the data structure of this large-scale, real-world dataset prevented a more refined analysis of pharmacokinetics and the specific dosages of biologic and targeted-synthetic therapies used, and whether other anti-inflammatory or immunomodulatory treatments were administered simultaneously, which may influence their effectiveness in preventing MIS-C. Finally, the study population was limited to children aged 0–18, and individuals with underlying comorbidities were excluded, which limits the generalizability of the findings.

Conclusion

The results of this study suggest a possible association between biological and targeted-synthetic therapies and reduced risk of MISC following COVID-19 infection in children. However, due to the observational nature of the study, further research is needed to confirm these observations and establish causality. Prospective studies with larger sample sizes and longer follow-up periods are essential to provide more definitive evidence and better inform clinical practice regarding the optimal management of MIS-C in pediatric patients with COVID-19.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Helsinki Institutional Review Bord in Carmel Medical Center, Israel. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

LK: Writing – review & editing, Investigation, Writing – original draft, Visualization, Validation, Data curation. AM-B: Investigation, Writing – review & editing, Conceptualization. SS: Investigation, Writing – review & editing. HC: Validation, Formal analysis, Methodology, Data curation, Investigation, Writing – review & editing. DH: Investigation, Validation, Writing – review & editing, Formal analysis, Methodology, Data curation. MH: Validation, Conceptualization, Visualization, Investigation, Data curation, Project administration, Writing – review & editing, Formal analysis.

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Development and validation of a CD4+/CD8+ ratio-based nomogram to predict plastic bronchitis in pediatric *Mycoplasma pneumoniae* pneumonia

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Introduction: Plastic bronchitis (PB) is a rare but severe complication of *Mycoplasma pneumoniae* pneumonia (MPP) in children. Early identification of PB is critical for timely intervention. This study aimed to develop and validate a nomogram incorporating the CD4+/CD8 + ratio to predict PB risk in children with MPP who underwent bronchoscopy.

Methods: This single-center, retrospective cohort study was conducted at Fujian Children's Hospital, China, from January 2023 to December 2024. A total of 281 children hospitalized with MPP, including 39 patients who developed PB, were included. Key predictors for nomogram were selected out of 19 variables using least absolute shrinkage and selection operator regression, followed by multivariable logistic regression. Model performance was evaluated through discrimination, calibration, and decision curve analysis (DCA). Internal validation was performed using the Bootstrap method with 1,000 resamples.

Results: The nomogram incorporated four independent predictors—fever duration, atelectasis, elevated D-dimer, and reduced CD4+/CD8 + ratio. It demonstrated strong discrimination (AUC = 0.83, 95% CI 0.77–0.90) and calibration (Hosmer-Lemeshow $P = 0.303$), with superior net benefit across risk thresholds of 0.1–0.7 by decision curve analysis, supporting its clinical utility for early risk stratification. Bootstrap validation confirmed robust performance with minimal overfitting.

Discussion: CD4+/CD8 + ratio based nomogram provides a practical tool for predicting PB risk in children with MPP, which may facilitate early bronchoscopy leading to improved patient outcomes.

KEYWORDS

plastic bronchitis, *Mycoplasma pneumoniae*, pneumonia, nomogram, CD4+/CD8+ ratio, children, LASSO regression, bootstrap validation

1 Introduction

Mycoplasma pneumoniae Pneumonia (MPP) is the leading cause of community-acquired pneumonia in children, accounting for 10%–40% of cases globally (1). Plastic bronchitis (PB) is a rare but critical complication of MPP that involves the formation of bronchial casts that obstruct airways. PB can lead to respiratory failure if not addressed promptly (2, 3).

Recent studies have reported an increasing incidence of PB in MPP cases, particularly among school-aged children, associated with prolonged fever, heightened inflammatory responses, and immune dysregulation (4, 5). The early identification of PB in MPP remains challenging due to its nonspecific clinical presentation and overlap with other respiratory conditions. Although bronchoscopy is the gold standard for diagnosis, its invasive nature limits routine use, highlighting the need for predictive tools based on accessible clinical and laboratory markers (6).

A number of predictive models have been proposed previously that included clinical and laboratory variables, such as fever duration, lactate dehydrogenase (LDH), D-dimer, and atelectasis, as as potential predictors of PB in RMPP (7). However, despite evidence of immune-mediated mechanisms in MPP severity, existing models overlook immune markers, such as the CD4 +/CD8 + ratio (8). Immune dysregulation, particularly imbalances in T-cell subsets like the CD4+/CD8 + ratio, has been implicated in chronic inflammation and airway remodeling, which may predispose patients to the formation of bronchial casts in PB.Moreover, existing models lack validation in broader MPP cohorts and consensus on optimal predictors, limiting their generalizability. This study aimed to develop and validate a nomogram incorporating the CD4+/CD8 + ratio, alongside established clinical and laboratory variables, to predict PB risk in pediatric patients children with MPP.This model is specifically designed to identify high-risk individuals among MPP patients considered for bronchoscopy.

2 Methods

2.1 Study design and settings

This retrospective study was conducted at Fujian Children's Hospital, Fuzhou, China, from January 2023 to December 2024, to develop and validate a nomogram incorporating the CD4 +/CD8 + ratio for predicting plastic bronchitis (PB) in children with MPP.

The study was approved by the Ethics Committee of Fujian Children's Hospital (Approval No. 2025ETKLRK04004), and informed consent requirement was waived due to the retrospective design, in accordance with the Declaration of Helsinki.

2.2 Inclusion and exclusion criteria

All patients with the diagnosis of MPP as per the Diagnostic and Treatment Guidelines for Child Pneumonia Caused by *Mycoplasma pneumoniae* (2023 Edition) (9) were included in the study if they fulfilled the bronchoscopy indications according to the Chinese Pediatric Flexible Bronchoscope Technique Guidelines (2018 Edition) (10).

A patient was diagnosed with MPP on the basis of (a) primary clinical manifestations of fever and cough, potentially accompanied by headache, nasal discharge, sore throat, or otalgia; (b) chest x-ray or CT showing thickened peribronchovascular textures, bronchial wall thickening, and possible ground-glass opacities, tree-in-bud sign, thickened interlobular septa, or reticular shadows; (c) single serum MP antibody titer ≥1:160 [Particle Agglutination (PA) method] or a four-fold increase in paired sera during the disease course; (d) positive MP-DNA or RNA detection. Diagnosis required clinical and imaging findings combined with either criterion (c) or (d).

Bronchoscopy indications included (a) pulmonary imaging anomalies, such as atelectasis or pleural changes, needing differential assessment; (b) determination and management of pathogens causing lung infections, with bronchoscopy conducted during hospitalization.

The exclusion criteria were as follows: (1) past or current tuberculosis, signs of tuberculous infection, repeated respiratory tract infections, persistent pulmonary conditions, asthma, primary or acquired immunodeficiency, hepatic or renal disorders, or cardiovascular conditions and (2) co-infection with other pathogens confirmed by laboratory testing.

2.3 Data collection

Data, including demographic information (age and sex), clinical features (fever duration,defined as the number of days from fever onset to hospital admission, wet rales, and wheezing), laboratory parameters (white blood cell count [WBC]; neutrophil percentage [N%]; erythrocyte sedimentation rate [ESR]; procalcitonin [PCT], C-reactive protein [CRP], lactate dehydrogenase [LDH], alanine aminotransferase [ALT], aspartate aminotransferase [AST], ferritin, fibrinogen, D-dimer, and interleukin-6 [IL-6] levels; and CD4+/CD8 + ratio, and imaging findings (atelectasis) were extracted from electronic medical records by two independent researchers to ensure accuracy. All data were collected at admission,prior to bronchoscopy, to ensure consistency in timing and clinical context for predictive modeling.

2.4 Pathogen detection via tNGS

Mycoplasma pneumoniae (MP) as the primary pathogen and exclusion of co-infections was based on targeted next-generation sequencing (tNGS) of bronchoalveolar lavage fluid (BALF) samples. The tNGS panel enabled detection of 153 pathogens, including bacteria, viruses, fungi, and atypical pathogens such as MP, *Chlamydia pneumoniae*, and *Legionella pneumophila*, as previously validated for comprehensive pathogen identification (11). The process involved nucleic acid extraction, library construction, high-throughput sequencing, and data analysis,

conducted by Fuzhou Kingmed Diagnostics Company. BALF-tNGS results served as the diagnostic standard for MP infection, ensuring specificity in identifying PB cases associated with MP.

2.5 Statistical analysis

All statistical analyses were performed using R software (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria) and MSTATA software (https://www.mstata.com/). The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data (e.g., WBC, N%, ESR etc.) or median [interquartile range (IQR)] for non-normally distributed data (e.g., fever duration, D-dimer etc.) ategorical variables were presented as frequencies and percentages. Differences between the PB and non-PB groups were assessed using the *t*-test for normally distributed continuous variables, the Mann–Whitney *U*-test for non-normally distributed continuous variables, and the chi-square test for categorical variables, with a significance threshold of $P < 0.05$.

Variable selection was conducted using least absolute shrinkage and selection operator (LASSO) regression to identify key predictors of PB from candidate variables. LASSO regression was performed with a penalty parameter (λ) selected via tenfold cross-validation, minimizing the binomial deviance. Variables with non-zero coefficients at the optimal λ (0.054) were selected and incorporated into a multivariable logistic regression model to construct a nomogram for predicting PB risk. Model performance was evaluated as follows: (1) discrimination was assessed by the area under the receiver operating characteristic (ROC) curve (AUC); (2) calibration was evaluated using a calibration curve and the Hosmer–Lemeshow test; (3) clinical utility was assessed via decision curve analysis (DCA); and (4) internal validation was performed using the bootstrap method with 1,000 resamples, adjusting for optimism in the C-index to quantify overfitting and ensure model robustness. All statistical tests were two-sided, and *P*-values of < 0.05 were considered statistically significant.

3 Results

Total 848 patients were diagnosed with Mycoplasma pneumoniae pneumonia (MPP) during the study period at Fujian Children's Hospital. However, 567 patients were excluded due to incomplete laboratory data (e.g., missing CD4+/CD8 + ratio, $n = 190$), refusal to undergo bronchoscopy ($n = 121$), or not meeting inclusion criteria ($n = 256$). The 256 excluded patients included 2 with past tuberculosis infection, 32 with recurrent respiratory infections or chronic lung diseases (e.g., bronchiectasis), 183 with co-infections confirmed by targeted next-generation sequencing (tNGS), 28 with bronchial asthma on inhaled corticosteroid treatment, 9 with immune diseases, and 28 with liver, kidney, or cardiovascular diseases (e.g., patent ductus arteriosus), with some having overlapping conditions. Hence, total 281 pediatric patients with MPP were included. Out of these 281 patients 39 (13.88%) patients had confirmed plastic bronchitis (PB) on bronchoscopy (Figure 1).

3.1 Comparison of clinical characteristics and laboratory findings

The baseline characteristics and laboratory findings of the study population are summarized in Table 1. No significant differences were observed between the non-PB and PB groups in terms of age ($P = 0.787$), sex distribution ($P = 0.242$), the prevalence of wheezing ($P = 0.167$), or most of the laboratory parameters including WBC, N%, PCT, ESR, CRP, ALT, AST, fibrinogen) (Table 1). However, the PB group exhibited a significantly longer fever duration ($P < 0.001$), higher incidence of wet rales ($P = 0.035$), and atelectasis ($P < 0.001$) compared to the non-PB group. Laboratory findings revealed that the PB group had significantly higher levels of lactate dehydrogenase (LDH), D-dimer, and interleukin-6 (IL-6), alongside lower CD4+/CD8 + ratio and ferritin levels (all $P < 0.05$). These findings indicate that the PB group presents a distinct clinical and immunological profile compared to the non-PB group, suggesting specific pathophysiological pathways involved in PB development.

3.2 Variable selection using LASSO regression

Nineteen variables, including sex, age, fever duration, presence of wet rales, wheezing, atelectasis, white blood cell (WBC) count, neutrophil percentage (N%), procalcitonin (PCT) level, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, lactate dehydrogenase (LDH) level, alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, ferritin level, fibrinogen level, D-dimer level, CD4+/CD8 + ratio, and interleukin-6 (IL-6) level, were included in the LASSO regression to identify key predictors of PB in children with MPP (Figure 2A). As penalties increased, LASSO compressed the coefficients of most variables to zero, selecting those with non-zero coefficients (Figure 2A). The optimal penalty parameter (λ) was determined using tenfold cross-validation by minimizing the binomial deviance, resulting in the selection of four predictors: fever duration, presence of atelectasis, D-dimer levels, and CD4+/CD8 + ratio (Figure 2B). These selected variables represent the most influential factors in predicting PB risk, providing a robust foundation for the subsequent nomogram construction.To evaluate collinearity, variance inflation factors (VIFs) for the four predictors were calculated, all of which were less than 5, confirming the absence of multicollinearity.

3.3 Multivariable logistic regression analysis

The four predictors identified through LASSO regression—fever duration, presence of atelectasis, elevated D-dimer levels, and reduced CD4+/CD8 + ratio—were analyzed using

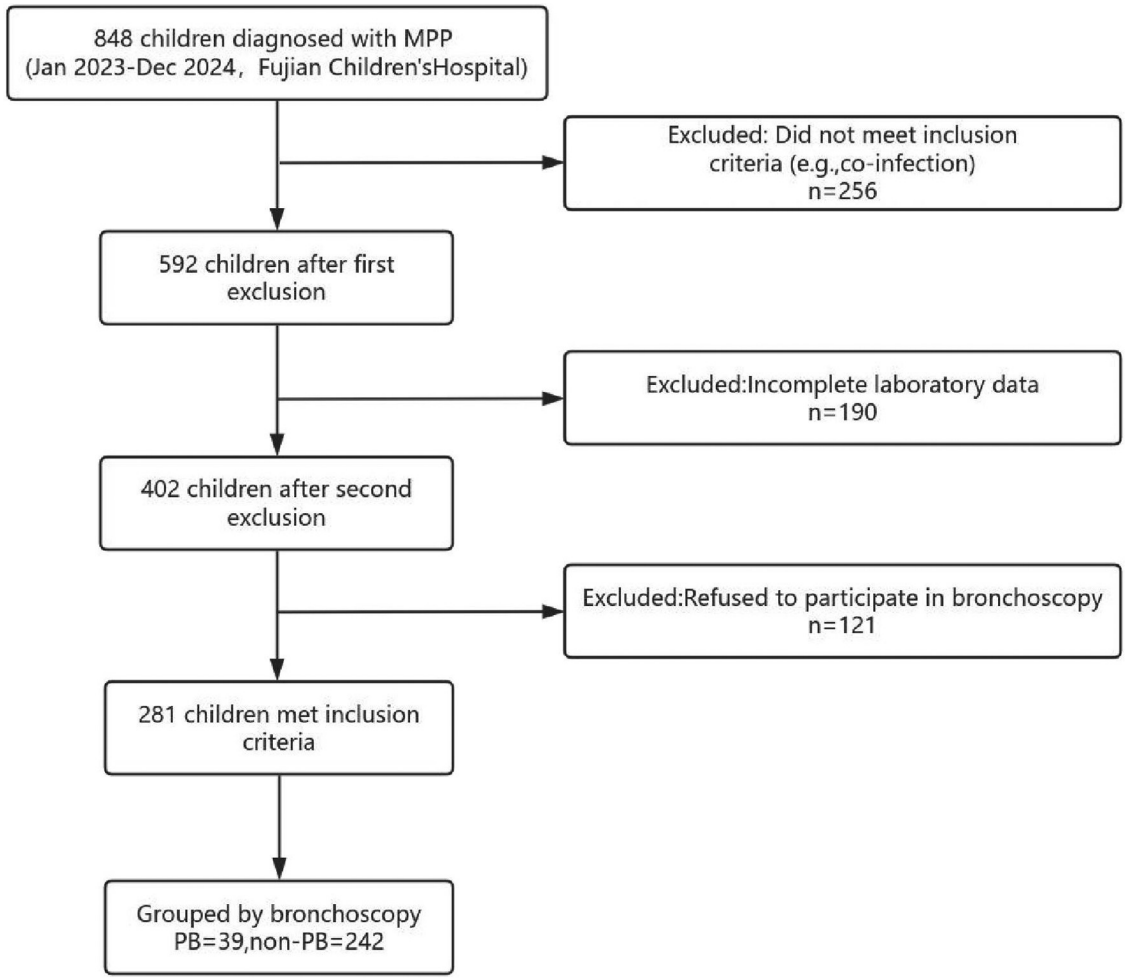

FIGURE 1
Flowchart of the participant inclusion process.

multivariable logistic regression to determine their independent associations with PB in children with MPP. The Results are summarized in Table 2. The presence of atelectasis exhibited the strongest association with PB ($\beta = 1.81$, OR = 6.11, 95% CI 2.56–14.63, $P < 0.001$), followed by a reduced CD4+/CD8 + ratio ($\beta = -1.78$, OR = 0.17, 95% CI 0.06–0.50, $P = 0.001$). Fever duration ($\beta = 0.17$, OR = 1.19 per day, 95% CI 1.05–1.35, $P = 0.007$) and elevated D-dimer levels ($\beta = 0.38$, OR = 1.46 per mg/L, 95% CI 1.04–2.05, $P = 0.030$) were also significant predictors of PB. These findings confirm the independent predictive roles of these variables in PB development, highlighting their significance as key indicators for early identification and intervention strategies.

3.4 Nomogram for PB risk prediction

The variables identified in the multivariable logistic regression analysis including fever duration, presence of atelectasis, elevated D-dimer levels, and reduced CD4+/CD8 + ratio were used to construct a nomogram (Figure 3). Total points, based on the sum of points assigned to each predictor in the nomogram, are associated with the risk of PB, ranging from 0.1 to 0.9. This nomogram provides a visual and intuitive tool for clinicians to estimate individual patient risk of PB based on their specific clinical and laboratory profiles.

3.5 Nomogram performance evaluation

The performance of the nomogram was evaluated for discrimination, calibration, and clinical utility (Figure 4), and it demonstrated good discriminatory performance with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.83 (95% CI 0.77–0.90) (Figure 4A), good calibration with a Hosmer–Lemeshow test p-value of 0.303 (Figure 4B), and superior clinical utility compared to default strategies across risk thresholds of 0.1–0.7 (Figure 4C), demonstrating its potential to significantly improve early risk stratification and guide clinical decision-making.

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TABLE 1 Comparison of baseline characteristics and laboratory parameters between the Non-plastic bronchitis (Non-PB) and plastic bronchitis (PB) groups.

Variables	Non-PB (*n* = 242)	PB (*n* = 39)	Statistic	*P*-value
Sex, *n* (%)			$\chi^2 = 1.37$	0.242
Male	106 (43.80)	21 (53.85)		
Female	136 (56.20)	18 (46.15)		
Wet rales, *n* (%)			$\chi^2 = 4.45$	0.035
No	70 (28.93)	5 (12.82)		
Yes	172 (71.07)	34 (87.18)		
Wheezing, *n* (%)			$\chi^2 = 1.91$	0.167
No	202 (83.47)	29 (74.36)		
Yes	40 (16.53)	10 (25.64)		
Atelectasis, *n* (%)			$\chi^2 = 30.74$	<.001
No	221 (91.32)	23 (58.97)		
Yes	21 (8.68)	16 (41.03)		
WBC ($\times 10^9$/L), Mean ± SD	8.27 ± 3.36	7.15 ± 3.93	t = 1.88	0.061
N%, Mean ± SD	60.76 ± 14.57	60.81 ± 22.37	t = −0.01	0.990
ESR (mm/h), Mean ± SD	51.24 ± 22.39	48.28 ± 23.55	t = 0.76	0.447
Age, M (Q_1, Q_3)	6.00 (5.00, 8.00)	6.00 (5.00, 8.00)	Z = −0.27	0.787
Fever duration, M (Q_1, Q_3)	6.69 (4.00, 8.65)	8.10 (7.00, 10.00)	Z = −3.78	<.001
PCT (ng/ml), M (Q_1, Q_3)	0.38 (0.28, 0.50)	0.40 (0.31, 0.59)	Z = −0.92	0.355
CRP (mg/L), M (Q_1, Q_3)	21.74 (16.27, 30.38)	22.39 (17.63, 31.47)	Z = −0.45	0.651
LDH (U/L), M (Q_1, Q_3)	323.30 (241.25, 481.00)	498.00 (345.10, 603.55)	Z = −3.50	<.001
ALT (U/L), M (Q_1, Q_3)	15.63 (11.48, 22.40)	14.95 (10.67, 22.63)	Z = −0.63	0.532
AST (U/L), M (Q_1, Q_3)	31.61 (24.18, 41.25)	32.56 (25.71, 43.40)	Z = −0.74	0.457
Ferritin (ng/ml), M (Q_1, Q_3)	251.21 (191.82, 350.78)	222.27 (177.10, 281.02)	Z = −2.33	0.020
Fibrinogen (g/L), M (Q_1, Q_3)	3.82 (3.23, 4.38)	4.10 (3.33, 4.53)	Z = −0.69	0.491
D-dimer (mg/L), M (Q_1, Q_3)	0.68 (0.47, 0.93)	1.15 (0.67, 1.88)	Z = −3.76	<.001
CD4+/CD8+ Ratio, M (Q_1, Q_3)	1.50 (1.23, 1.83)	1.11 (0.88, 1.41)	Z = −4.98	<.001
IL-6 (pg/ml), M (Q_1, Q_3)	15.01 (4.76, 28.25)	30.40 (20.00, 51.05)	Z = -4.21	<.001

t: *t*-test, Z: Mann–Whitney test, χ^2: Chi-square test.

SD, standard deviation, M, Median, Q_1, 1st Quartile, Q_3, 3st Quartile; WBC, white blood cell; N%, neutrophil percentage; PCT, procalcitonin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IL-6, interleukin-6.

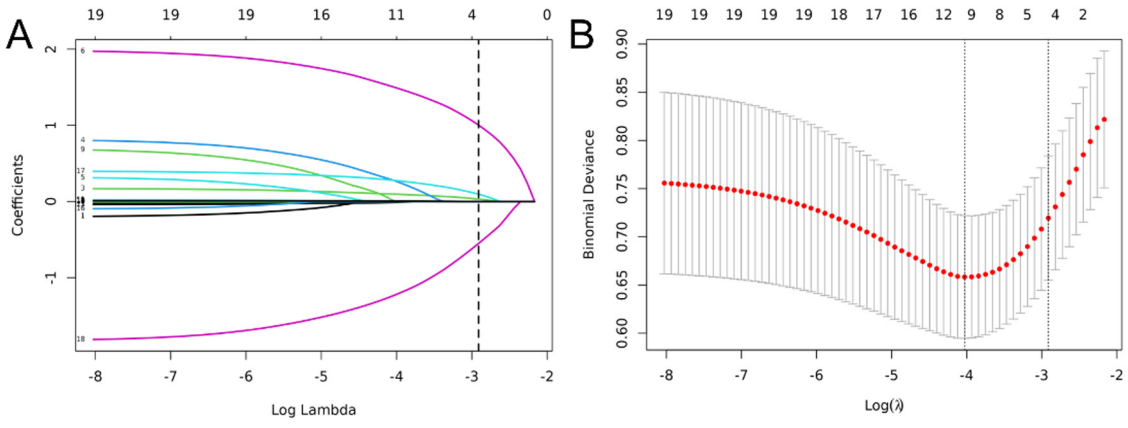

FIGURE 2
Variable selection using least absolute shrinkage and selection operator (LASSO) logistic regression. **(A)** LASSO coefficient profile of the 19 variables. With increasing penalties, coefficients of more variables are compressed to zero, ultimately selecting 4 variables with non-zero coefficients. **(B)** The optimal penalty coefficient lambda was selected using a tenfold cross-validation and minimization criterion. The binomial deviance curve was plotted vs. log(lambda), with dotted vertical lines drawn based on 1 standard error criterion. Four variables with non-zero coefficients were selected at the optimal lambda (λ = 0.054).

TABLE 2 Multivariable logistic regression for PB predictors in MPP children.

Variables	β	S.E	Z	P	OR (95%CI)
Atelectasis	1.81	0.44	4.07	<.001	6.11 (2.56–14.63)
Fever duration	0.17	0.06	2.71	0.007	1.19 (1.05–1.35)
D-dimer（mg/L）	0.38	0.17	2.17	0.030	1.46 (1.04–2.05)
CD4+/CD8+ Ratio	−1.78	0.55	−3.22	0.001	0.17 (0.06–0.50)

OR, odds ratio; CI, confidence interval.

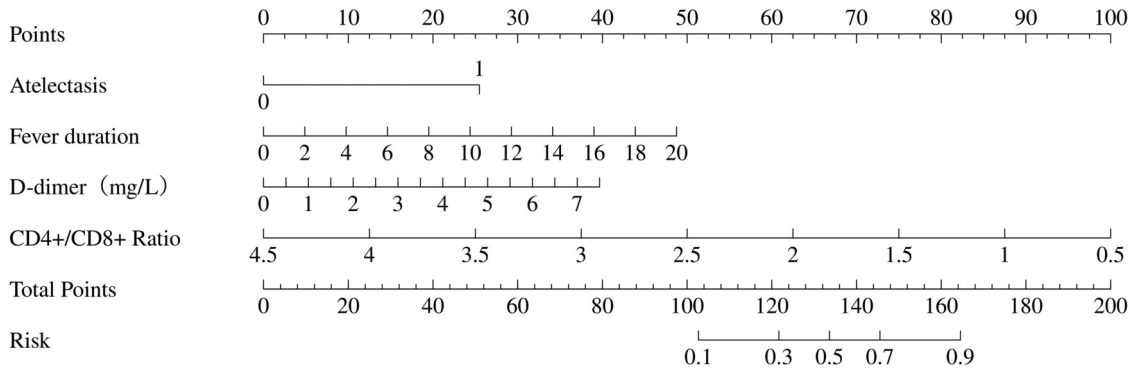

FIGURE 3
Nomogram to predict plastic bronchitis (PB) risk in children with *Mycoplasma pneumoniae* pneumonia (MPP). The nomogram is based on four independent predictors identified from multivariable logistic regression: fever duration, atelectasis, D-dimer, and CD4+/CD8 + ratio. To use the nomogram, mark the value of each predictor on its corresponding axis, draw a vertical line to the top "Points" axis to obtain the points, sum the points from all predictors, locate the total on the "Total Points" scale, and project it vertically to the "Risk" axis to determine the PB risk (0.1–0.9).

3.6 Internal validation

Internal validation of the nomogram was performed using the Bootstrap method with 1,000 resamples to assess the stability of the model's discriminatory performance. As shown in Figure 5, the C-index after Bootstrap validation was 0.834 (95% CI 0.767–0.899), consistent with the original AUC of 0.83, indicating good discriminatory ability with minimal overfitting, thus confirming the model's robustness and generalizability to similar patient populations.

4 Discussion

This study reports a plastic bronchitis (PB) incidence of 13.88% among 281 Mycoplasma pneumoniae pneumonia (MPP) patients, consistent with the 14.2% reported by Zhong et al. in a similar cohort requiring bronchoscopy (8). These findings highlight PB as a significant complication in severe MPP, necessitating early identification to guide timely bronchoscopic intervention and prevent outcomes like respiratory failure. Our nomogram, incorporating fever duration, atelectasis, D-dimer, and CD4 +/CD8 + ratio, achieves robust discrimination (AUC = 0.83) and clinical utility (decision curve analysis, thresholds 0.1–0.7). By quantifying PB risk at admission, it enables clinicians to prioritize high-risk patients for bronchoscopy, potentially reducing complications such as airway obstruction or atelectasis progression. Unlike invasive diagnostic approaches, this nomogram leverages routine clinical and immunological markers, enhancing feasibility in pediatric settings. This tool addresses diagnostic delays in PB, reinforcing the study's rationale for advancing early detection and management in pediatric MPP.

Multivariate analysis identified four key predictors for the nomogram: fever duration, the presence of atelectasis, elevated D-dimer levels, and reduced CD4+/CD8 + ratio. The presence of atelectasis was the strongest predictor, reflecting its mechanistic link to cast-induced airway obstruction, a feature well-documented in severe respiratory infections (12). Atelectasis likely exacerbates airway obstruction by promoting mucus stasis and cast formation, creating a vicious cycle that worsens respiratory distress in patients with PB. Prolonged fever may reflect an ongoing inflammatory cascade, potentially driven by *Mycoplasma pneumoniae*'s ability to evade host immune responses, leading to sustained cytokine release and tissue damage that predisposes to the development of PB. Elevated D-dimer levels suggest a hypercoagulable state, aligning with coagulation abnormalities in severe MPP (13). This finding may indicate microvascular thrombosis or endothelial dysfunction in the airways, contributing to cast formation through fibrin deposition, a process often exacerbated by the inflammatory milieu in MPP (14). The reduced CD4+/CD8 + ratio, a novel predictor in this context, points to immune dysregulation, potentially driven by excessive cytotoxic T-cell activity, a hallmark of the immune response in MPP (15, 16). Jia et al.

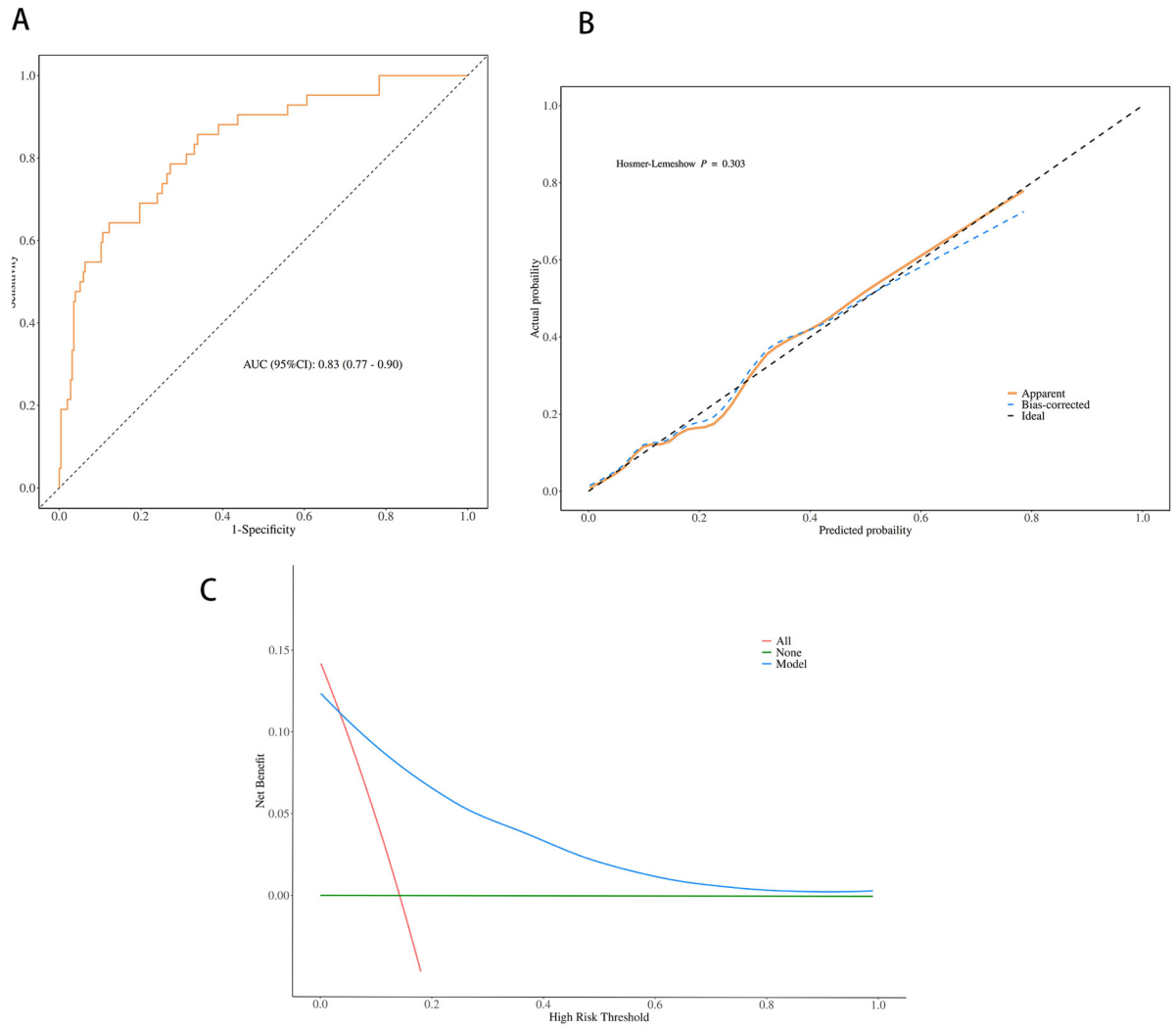

FIGURE 4
Performance evaluation of the nomogram for predicting plastic bronchitis (PB) risk in children with *Mycoplasma pneumoniae* pneumonia (MPP). **(A)** Receiver operating characteristic (ROC) curve; area under the curve (AUC) = 0.83 (95% CI 0.77–0.90). **(B)** Calibration curve; the Hosmer-Lemeshow test yielded a *p*-value of 0.303 ($P > 0.05$). **(C)** Decision curve analysis (DCA); the nomogram shows higher net benefit than "treat all" and "treat none" strategies across risk thresholds of 0.1 to 0.7.

demonstrate that severe MPP (S-MPP) is characterized by increased Th1 cell frequencies and CD8+ T cells with an exhausted phenotype marked by elevated PD-1 expression (17). In our PB cohort, this decreased ratio may reflect an increased CD8+ T-cell proportion, impairing pathogen clearance and sustaining Th1-driven inflammation. Elevated IL-6 levels in our PB group support this inflammatory response but are not an independent predictor (18). Combined with increased D-dimer levels, consistent with S-MPP findings (17), this suggests that Th1-mediated inflammation promotes fibrin deposition and airway obstruction, critical for bronchial cast formation in PB. Thus, the reduced CD4+/CD8 + ratio serves as a key immunological marker linking persistent inflammation to PB's coagulative pathology.

The univariate analysis revealed elevated lactate dehydrogenase (LDH) and interleukin-6 (IL-6) levels in the PB group. These findings are consistent with the known roles of LDH as a marker of tissue injury and cell death, reflecting the extent of lung damage and inflammation in PB patients. Similarly, IL-6, a key pro-inflammatory cytokine, plays a central role in driving systemic inflammation and immune responses, contributing to the formation of bronchial casts and airway obstruction in PB. While these indicators were not identified as independent predictors in our multivariate model, likely due to their overlap with other significant factors, their elevation underscores the critical involvement of both direct tissue damage and systemic inflammatory processes in PB pathogenesis (19). Li et al. emphasized the role of C-reactive protein (CRP) and drug resistance in PB, highlighting heightened inflammation and treatment challenges posed by resistant strains (20). The lower white blood cell (WBC) count in PB cases ($P = 0.061$) mirrored variability seen in severe MPP, but did not reach statistical

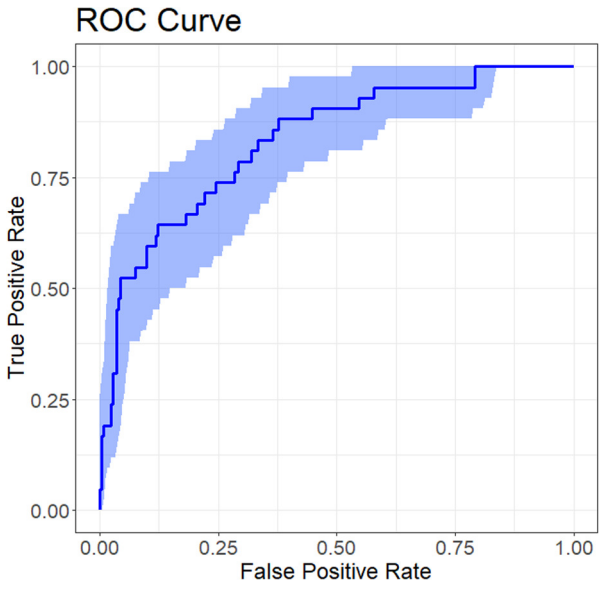

FIGURE 5
ROC curve for internal validation of the nomogram predicting plastic bronchitis (PB) risk in children with *Mycoplasma pneumoniae* pneumonia (MPP). The ROC curve illustrates the nomogram's discriminatory performance after internal validation using the Bootstrap method (1,000 resamples).

significance, suggesting no significant difference between the groups in this study (21). Our nomogram demonstrated strong performance and good calibration, with minimal overfitting confirmed by internal validation. The decision curve analysis indicated superior net benefit across a 0.1–0.7 risk threshold compared to uniform intervention strategies. Compared to Zhang et al.'s RMPP nomogram and Li et al.'s decision tree, this model enhances practicality by utilizing fewer variables (14, 22).

Clinically, the nomogram facilitates early PB detection using routine admission data, guiding targeted bronchoscopy, which is critical as PB prevalence increases among school-aged children with robust immune responses (23). Zhong et al. reported prolonged hospital stays in PB cases (12 vs. 7 days), a burden this tool could reduce through timely intervention (8). The role of the CD4+/CD8 + ratio suggests potential for immunomodulatory therapies, such as corticosteroids, though their efficacy needs further study (24). Zhang et al. linked PB to sequelae like bronchiolitis obliterans, underscoring the need for early intervention to improve long-term outcomes (22). By leveraging accessible markers, the nomogram supports resource-efficient management across diverse healthcare settings, aligning with actionable infectious disease strategies.

Our study possesses several key strengths. Firstly, it introduces the CD4+/CD8 + ratio as a novel immunological marker for predicting PB risk in MPP, addressing a critical gap in existing predictive models that often overlook immune dysregulation. Secondly, we employed robust statistical methodologies, including LASSO regression for rigorous variable selection, which minimizes overfitting and enhances model parsimony. Furthermore, the comprehensive evaluation of model performance through discrimination (AUC), calibration (Hosmer-Lemeshow test), and clinical utility (Decision Curve Analysis) provides a thorough assessment of the nomogram's predictive capabilities. The internal validation using Bootstrap resampling further confirms the model's robustness and generalizability within similar patient populations. These methodological strengths contribute to the reliability and clinical applicability of our nomogram, offering a practical tool for early risk stratification in pediatric MPP patients requiring bronchoscopy.

This study had some limitations. Firstly, the retrospective single-center design may introduce bronchoscopy-related selection bias. Secondly, the modest PB cohort of 39 cases (13.88%) may limit the model's generalizability and statistical power. Thirdly, the absence of longitudinal data to clarify the mechanistic role of the CD4+/CD8 + ratio requires cautious interpretation. The small PB cohort may also affect the model's stability, particularly for rare outcomes like PB, necessitating validation in larger cohorts. Future research prioritizing multicenter validation, longitudinal immune profiling to investigate T-cell dynamics, such as flow cytometry to assess T-cell subsets, and assess resistance patterns, such as A2063G mutation, using genomic sequencing to refine PB pathogenesis models is recommended.

In conclusion, this study identified fever duration, presence of atelectasis, elevated D-dimer levels, and reduced CD4+/CD8 + ratio as predictors of PB in MPP, developing a nomogram that enhances early risk stratification. This tool provides a practical approach to guide bronchoscopy and optimize outcomes in pediatric MPP, requiring further validation to advance infectious disease management.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Fujian Children's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because The study was approved by the Ethics Committee of Fujian Children's Hospital (Approval No. 2025ETKLRK04004), and written informed consent was waived because this study is a retrospective analysis of data collected from electronic medical records, with privacy protection measures applied, in accordance with the Declaration of Helsinki.

Author contributions

DL: Data curation, Visualization, Writing – original draft, Writing – review & editing. CL: Conceptualization, Methodology, Validation, Writing – original draft. XD: Conceptualization, Data curation, Resources, Visualization, Writing – original draft. JW: Methodology, Validation, Writing – original draft. XH: Data curation, Investigation, Supervision, Writing – original draft. HJ: Data curation, Software, Writing – original draft. QT: Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

XD was employed by Fuzhou KingMed for Clinical Laboratory Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BCG vaccination: historical role, modern applications, and future perspectives in tuberculosis and beyond

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Tuberculosis (TB) remains a fatal disease primarily transmitted through airborne droplets, with children who are the most susceptible, particularly in the areas with poor tuberculosis control. The BCG vaccine, developed by Albert Calmette and Camille Guérin, has a history spanning a century. This vaccine has been implemented in numerous countries, significantly reducing child mortality in regions heavily affected by TB. In this review, we aim to revisit the vaccine's development and rollout, while also highlighting its current attributes and the successful application in the Russian Federation, where 90% of newborns receive the anti-tuberculosis vaccination. Due to that practice, only a few isolated cases of young children with generalized tuberculosis (about five to seven annually) are observed in Russia. Research on the BCG vaccine is ongoing, revealing significant genetic alterations in BCG strains that have evolved from the original variant. These genetic differences may contribute to variations in vaccine efficacy, making screening important to predict effectiveness. The BCG vaccine can initiate a localized mucosal immune response, offering, besides the anti-TB effect, some protection against infections involving mucous membranes, including salmonellosis, HIV, and acute viral respiratory infections. It is essential to investigate the role of BCG in various applications; however, this exploration should not detract from its main protective benefits against tuberculosis (TB). Future studies may provide evidence of the vaccine's safety and efficacy to support its use beyond TB prevention. While BCG vaccination does not lower the risk of infection with *Mycobacterium tuberculosis*, it does prevent the progression to the most severe clinical manifestations (such as miliary TB and tuberculous meningitis) caused by hematogenous spread of *M.tuberculosis*. The challenge of protecting HIV-infected children from TB remains urgent, especially in regions burdened with drug-resistant TB, highlighting the need for robust protective measures.

KEYWORDS

BCG vaccine, immune response, COVID-19, laboratory diagnostics, tuberculosis, preventive therapy

1 Introduction

Tuberculosis (TB) remains one of the leading infectious diseases responsible for a significant number of deaths worldwide. The World Health Organization (WHO) reported that in 2023, approximately 10.8 million new TB cases were identified, marking a 3.5% rise from the 10.3 million cases recorded in 2021. Children are among the most at-risk populations, particularly in regions or countries with a high TB prevalence. In 2018, the inability to provide timely and accurate diagnoses for over 600,000 children resulted in the tragic deaths of 200,000 children of various ages. By 2020, more than 226,000 children under the age of 15 had succumbed to the disease, and recent statistics indicate that around 25,000 children contracted tuberculosis from patients with multidrug-resistant tuberculosis (1).

Vaccination efforts have achieved significant progress, leading to a dramatic decline in the incidence of many diseases such as measles, diphtheria, tetanus, rubella, epidemic parotitis, and hepatitis B, all of which are now limited to only a few isolated cases. Polio has been eradicated in most regions, e.g., Russia maintaining its polio-free status since 2002 as part of the European Region. The USA became the first region in the world to be declared free of endemic measles on September 26, 2016. Furthermore, smallpox was declared globally eradicated in 1980.

Immunoprevention of infectious diseases encompasses a range of individual and mass strategies aimed at preventing disease onset, controlling pathogen spread, reducing infection severity, and eradicating particularly hazardous infectious diseases. At present, the implementation of early diagnostic and preventive measures for tuberculosis infection is of particular importance, as it directly correlates with the prevention of active tuberculosis transmission. It is well established that a single individual with active tuberculosis can infect up to 30–50 people per day, especially through close contact. Additionally, it focuses on enhancing the immune response to specific pathogens (2). Under special circumstances, individual immunization may also serve a therapeutic role. Mass immunization is employed during epidemics when there is a risk of widespread infectious diseases. The term "*vaccine*" is derived from the Latin word vacca, meaning cow. A vaccine is a medical or veterinary preparation designed to create active immunity to infectious diseases. Vaccines are developed using attenuated or inactivated microorganisms, byproducts of their biological activity, or antigens produced through genetic engineering or chemical methods (3). Live vaccines are created using attenuated strains of microorganisms that retain consistent avirulence (non-pathogenic properties). Once administered, these strains replicate within the host cells, leading to a controlled vaccine-induced infection. Examples of live vaccines include those for rubella, measles, polio, tuberculosis, and mumps. Because antigens of live vaccines are produced within host cells by the persisting pathogens, they are processed and presented mostly via intracellular (although also via extracellular, phagocytosis-associated) routes, in the context of both MHC Class I and Class II proteins. Hence, this type of vaccine is able to induce active cellular, as well as humoral adaptive immune responses, making it the most effective. The history of immunoprophylaxis began with a milestone achieved by English physician Edward Jenner. In 1796, he vaccinated the eight-year-old son of his gardener using live cowpox virus. Jenner proposed that material derived from cowpox lesions could be used for immunization, and individuals who received this inoculation were protected from smallpox. However, there is historical evidence that Jenner's experiment was preceded by analogous successful procedure performed on three children in 1791 by a German schoolteacher, Peter Plett, who has reported his experience at the University of Kiel, but the medical professors severely criticized «*the amateur without M.D. Degree*», so first publication of Plett's results was postponed until 1802. In 1880, Louis Pasteur developed a vaccine against anthrax, followed later by vaccines against cholera and rabies. Then, in 1921, Albert Calmette and Camille Guérin announced the development of a vaccine against tuberculosis (4). Vaccines can be derived from pathogens and their byproducts. They are classified into two main types: Live vaccines, which contain attenuated pathogens, and non-live (inactivated) vaccines, which do not contain any live microorganisms (1).

2 History of the BCG vaccine

The BCG vaccine (Bacillus Calmette–Guérin, BCG) is prepared from a strain of attenuated *Mycobacterium bovis* BCG. This strain is produced in an artificial environment and has low virulence in humans. The vaccine was developed due to the collaborative efforts of two French scientists: navy physician and bacteriologist Léon Charles Albert Calmette, and veterinarian and immunologist Jean-Marie Camille Guérin. Being a student, Camille Guérin began working as an assistant to the renowned pathologist Edmond Nocard (4). In 1902, Nocard isolated a culture of *M. bovis* from a cow suffering from tuberculosis. He authored a monograph: *La Tuberculose Bovine: ses Dangers, ses Rapports avec la Tuberculose Humaine*. In 1912, Nocard and Norwegian veterinarian Christian Feyer Andvord (who coined the idea to use ox bile to weaken pathogens), after 96 serial inoculations, succeeded in obtaining an attenuated culture of *M. bovis* using a nutrient medium composed of bile, potato, and glycerol (4) (Figure 1).

After considerable delay caused by The Great War, in 1919, Albert Calmette established a working group at the Pasteur Institute in Paris to develop a tuberculosis vaccine. By that same year, researchers conducted 230 serial passages, demonstrating changes in the morphological and cultural characteristics of M. bovis, as well as a reduction in its virulence in experimental models (5). The safety and effectiveness of the tuberculosis vaccine in veterinary medicine were confirmed at the experimental farm in Fécamp in 1921. That year, scientists

Abbreviations

CM, central memory; EM, effector memory; MDR, multiple drug-resistant; RF, Russian Federation; TB, tuberculosis; TRM, tissue-resident memory; TSCM, stem cell-like memory T cells.

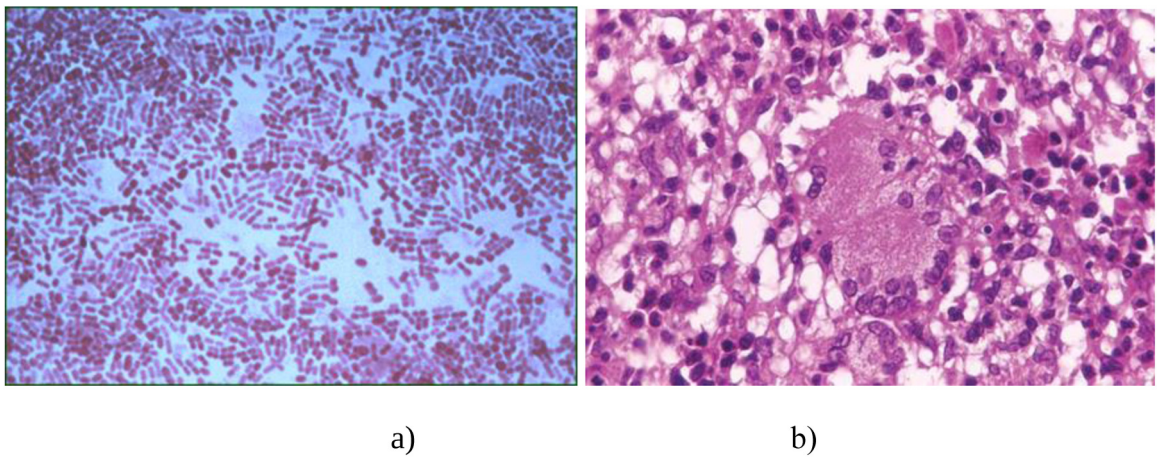

FIGURE 1
Diagnosis of BCG infection according to histological findings. **(a)** Gram staining of mycobacteria; **(b)** The microscopic structure of a tuberculosis granuloma.

announced that the BCG vaccine was ready for practical use, marking the beginning of mass vaccination efforts. On July 18, 1921, pediatricians Benjamin Weill-Hallé and Raymond Turpin initiated vaccinations for newborns at the Hôpital de la Charité in Paris (6). The first infant to receive the vaccine (initially designed for veterinary practice) was born to a mother who had died from tuberculosis just a few hours after childbirth. The child was administered the vaccine orally on the 3rd, 4th, and 7th days of life.

After six months of monitoring, it was determined that the child exhibited no signs of illness, despite being in close contact with the infected mother. From 1921–1924, tuberculosis vaccination was gradually extended to include other newborns at the Hôpital de la Charité. In 1921, Weill-Hallé introduced oral administration of BCG at the hospital (7, 8). This method was further developed by Boquet and Nègre, who continued the use of oral BCG emulsions. The effectiveness of this delivery pathway depends on the status of the gastrointestinal tract, with post-vaccination allergic reactions observed in 30% of cases (8).

Starting in 1924, BCG vaccination was implemented in French healthcare dispensaries. Between 1924 and 1925, the vaccination campaign expanded to Madagascar and Indochina. In 1925, Canada established the Tuberculosis and BCG Research Committee under the Medical Research Council, aimed at evaluating the vaccine's effectiveness in both humans and animals. That same year, F.A. Baudouin began clinical trials of the vaccine (9). By 1927, Calmette published a study detailing the vaccination outcomes of 21,200 newborns, providing compelling evidence of the BCG vaccine's efficacy. The same year, Swedish pediatrician Karl Nöslund also demonstrated, using large statistical data, that BCG vaccination significantly reduced infant mortality, which contributed to its public acceptance across Scandinavia. In 1928, the League of Nations officially recognized the vaccine. Also in 1927, Luis Sayé in Barcelona, Arvid Wallgren in Gothenburg, and Johannes Heimbeck in Oslo were the first to administer BCG intradermally using the multiple-injection technique (10) (Figure 2).

The method was modified and applied in France and further in the USA using a multiple injection apparatus developed by Konrad Birkhaug (1927) (11).

2.1 The Lübeck tragedy

Probably due to differences in the versions of the vaccine used at different times and in various places, and even more so due to the influence of subjective and random factors, as well as non-scientific circumstances influencing public opinion, the social acceptance of BCG in many parts of the world was greatly delayed or did not occur at all. In 1930, the Lübeck tragedy broke out (Figure 3). Four to six weeks after BCG vaccination, 72 out of 251 newborns died within a year from generalized tuberculosis (10, 12).

A total of 131 children developed clinical tuberculosis, which was ultimately treated successfully. As a result, the German government filed a lawsuit against the Pasteur Institute. An investigation was launched in late 1931, led by Professor Bruno Lange of the Robert Koch Institute in Berlin and Professor Ludwig Lange of the German Ministry of Health (10). After 20 months of thorough inquiry, the BCG vaccine was exonerated, while the Lübeck laboratory was found responsible for contaminating vaccine batches with virulent strains of *Mycobacterium tuberculosis*. Two medical professionals were convicted and sentenced to prison. Additionally, in August 1930, at the International Union Against Tuberculosis congress in Oslo, Calmette publicly defended the BCG vaccine, reaffirming its safety and efficacy. However, the tragedy in Lübeck delayed the acceptance of BCG by the German healthcare system. Moreover, during that period, the BCG vaccine was not adopted for use either in Great Britain or in the USA. Its introduction into medical practice in Britain was prevented by the position of the authoritative microbiologist M. Greenwood, who in 1928 sharply criticized the methodology of Calmette's statistical

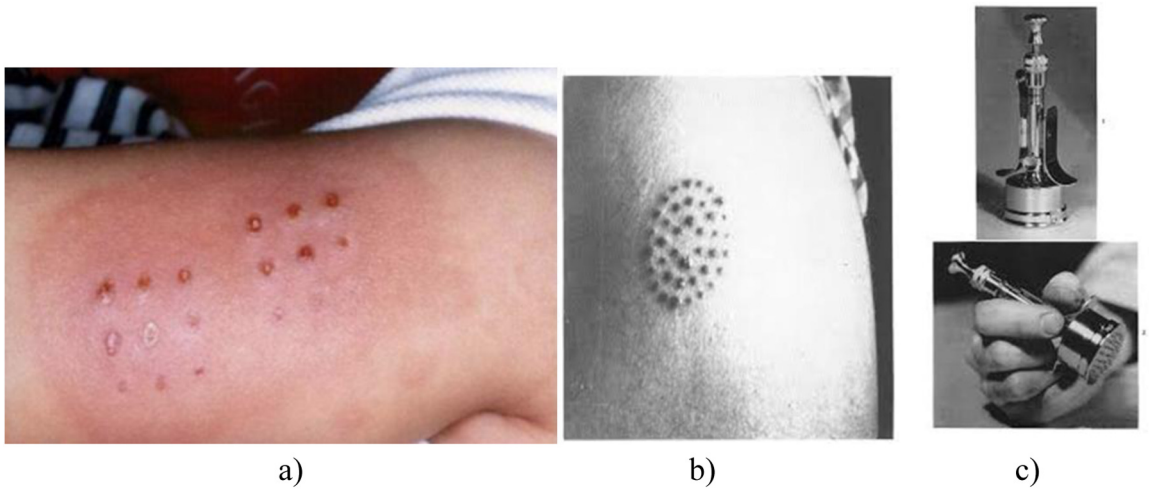

FIGURE 2
Multiple jabs during intradermal administration of BCG vaccine (10). (a)—multiple vaccination marks; (b)—the multiple injection technique; (c)—multiple injection vaccination machine).

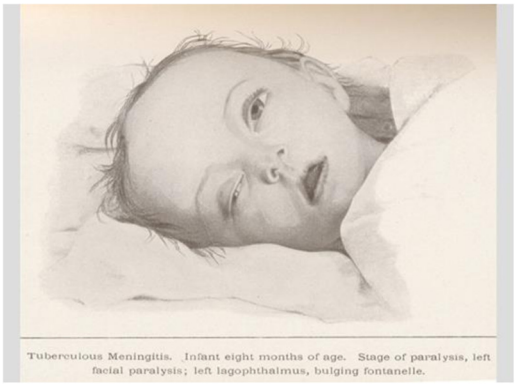

FIGURE 3
Child with generalized tuberculosis after BCG vaccination in the Lübeck tragedy (10).

calculations in the high-impact "British Medical Journal". In the USA, S.A. Petroff and co-authors at the largest phthisiology center, the Trudeau Sanatorium, analyzing in 1929 a sample sent by A. Calmette, found virulent *Mycobacterium tuberculosis* in it (11), which practically buried the prospects for the rapid introduction of the new product overseas.

Meanwhile, B. Weill-Hallé (1930) applied a subcutaneous method of BCG administration (Figure 4). Unfortunately, there were many complications, including cold abscesses that persisted for a long time (12, 13).

2.2 Percutaneous administration of BCG emulsion

Roy Rosenthal (1939) applied the method of multiple needle pricks of the skin in place of a drop of BCG emulsion and B. Weill-Hallé (1939) applied the scarification method (14). A drop of emulsion is scratched through the entire epidermis followed by a compress made of gauze soaked in vaccine. However, this method has not been widely used (13).

3 Use of BCG vaccine in the world and in Russia

Historically, the earliest recognition and pioneering use of BCG for mass vaccination occurred in its country of origin—France—as well as in several Scandinavian countries and the USSR (see below). However, prior to World War II, BCG vaccination was not mandatory in any of these countries. In 1946, the Danish Red Cross coordinated BCG vaccination programs in Poland, Austria, Hungary, and Yugoslavia (15). Norway mandated BCG vaccination for individuals with negative tuberculin skin tests in 1947, followed by France in 1950, which introduced compulsory vaccination. That same year, the Soviet Union also implemented mandatory BCG vaccination for all newborns. In 1974, the BCG vaccine was incorporated in the Expanded Programme on Immunization (EPI) by the United Nations International Children's Emergency Fund (UNICEF). In the United States, BCG vaccination was reserved for individuals at high risk of tuberculosis exposure. According to World Health Organization (WHO) guidelines, BCG vaccination remains a cornerstone of global TB prevention strategies. Currently, BCG vaccination is mandatory in 64 countries and recommended in an additional 118 countries and territories. Following World War II, the issue of mass BCG vaccination was reconsidered in both the United Kingdom and the United States. However, findings from cohort studies differed significantly between the two countries: high effectiveness was reported in the UK, while no significant benefit was observed in the US. This discrepancy is thought to be

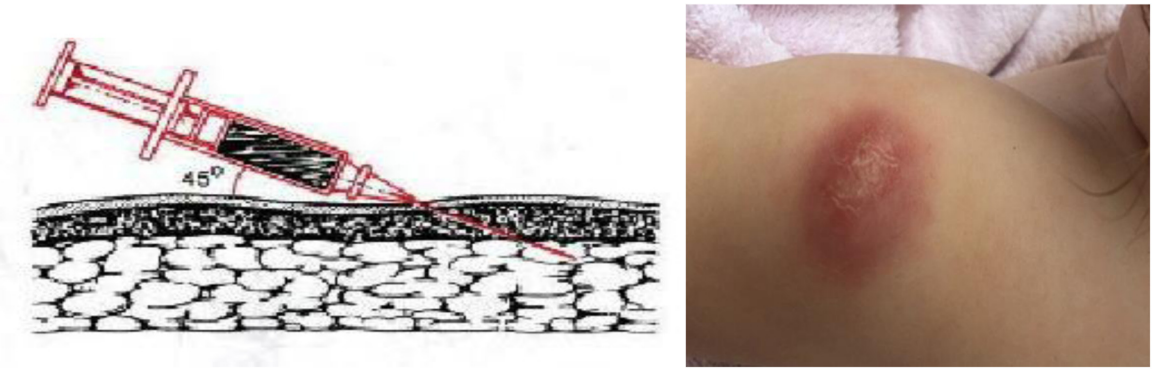

FIGURE 4
BCG vaccine subcutaneous injection technique and cold abscess formation.

attributable to methodological differences—such as the use of various BCG strains, stricter age matching in the British cohort, and limited control in the American studies due to populations residing in regions with frequent exposure to animals carrying *Mycobacteria* (12). Notably, neither the United States nor the Netherlands has ever implemented a universal BCG vaccination program. A meta-analysis published in 1995 demonstrated that BCG vaccination in neonates and infants reduces the risk of developing tuberculosis by an average of more than 50% (16). A robust and protective immune response following BCG vaccination has been observed across diverse populations, study designs, and TB manifestations.

In 1925, Albert Calmette, a disciple of Ilya Metchnikoff, provided a prototype BCG strain to another of Metchnikoff's students—Soviet Professor Leo A. Tarasevich in Moscow—where it was designated as BCG-1. The first BCG vaccinations of newborns in tuberculosis-endemic regions of the Soviet Union began in 1928 (5). A national policy mandating BCG vaccination for newborns in urban maternity hospitals was introduced in 1942 (Order of the People's Commissariat of Health No. 448, dated August 31, 1942). By 1953, BCG vaccination coverage had expanded to include newborns in rural areas, as well as primary vaccination and revaccination of all children of preschool age and schoolchildren not infected with *Mycobacterium tuberculosis* (in accordance with Decree of the USSR Council of Ministers No. 3989, dated October 25, 1948, and Orders of the USSR Ministry of Health No. 676 of November 12, 1948, and No. 384 of July 3, 1952). BCG vaccination has been continuously administered in the USSR and subsequently in the Russian Federation since 1953.

Until 1962, oral administration was the predominant method for newborns, with percutaneous administration used less frequently. Since 1962, the intradermal route has been adopted as the standard one due to its superior immunogenicity. In the Russian Federation, a single BCG revaccination is administered at age seven for children with a negative Mantoux test with 2 TE PPD-L. Re-vaccination at age 14 was discontinued in Russia in 2014. Trends in tuberculosis incidence among children and adolescents in the USSR following the introduction of intradermal BCG immunization (per 100,000 population) are illustrated in Figure 5.

Since 1961, a steady decline in tuberculosis incidence has been observed among both children and adolescents throughout the USSR. The implementation of BCG vaccination in Russia has contributed to several notable public health achievements, including:

- The elimination of fatal tuberculosis cases in children during periods of high TB incidence, particularly the eradication in young children with tuberculous meningitis and miliary tuberculosis (Figure 5);
- Stable TB prevalence rates, with no observed increase in complicated or disseminated forms; A predominance of lymph node involvement over systemic disease manifestations.

The epidemiological trends of tuberculous meningitis following the introduction of mass BCG vaccination in the USSR are depicted in Figure 6.

Currently, tuberculosis vaccination coverage among newborns in the Russian Federation reaches 90%–95% (2). National surveillance data from the past decade indicate that only five to seven cases of tuberculous meningitis are reported annually. In 2023, a total of ten confirmed cases were reported (17).

4 BCG vaccine strains

Since 2004, the following strains have accounted for 90% of BCG vaccinations worldwide (18, 19) (Figure 7):

- Pasteur 1,173 P2 (France);
- Danish 1,331 (Denmark);
- Glaxo 1,077 (Danish derivative)
- Tokyo 172-1 (Japan);
- BCG-1 (Russia).

According to the most recent update of the BCG World Atlas (2020), the most widely used BCG vaccine strains globally are Danish 1,331 (16.6%), Pasteur 1173P2 (9.2%), and Tokyo 172 (7.3%) (19). The present study aimed to analyze the complete

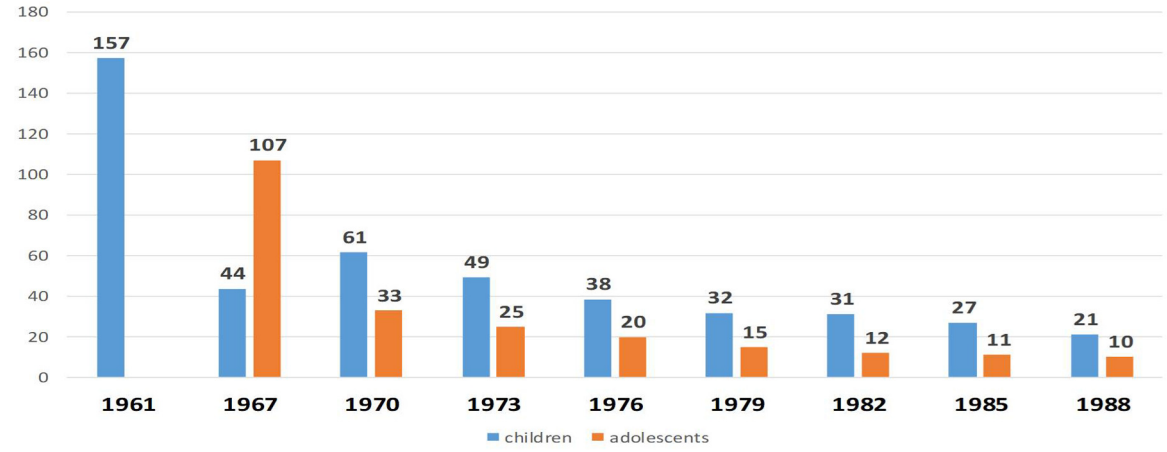

FIGURE 5
The dynamics of morbidity among children and adolescents with tuberculosis in the USSR after the introduction of intradermal immunization with BCG vaccine (cases per 100,000 population)[y, years; x, children (blue) and adolescents (orange)].

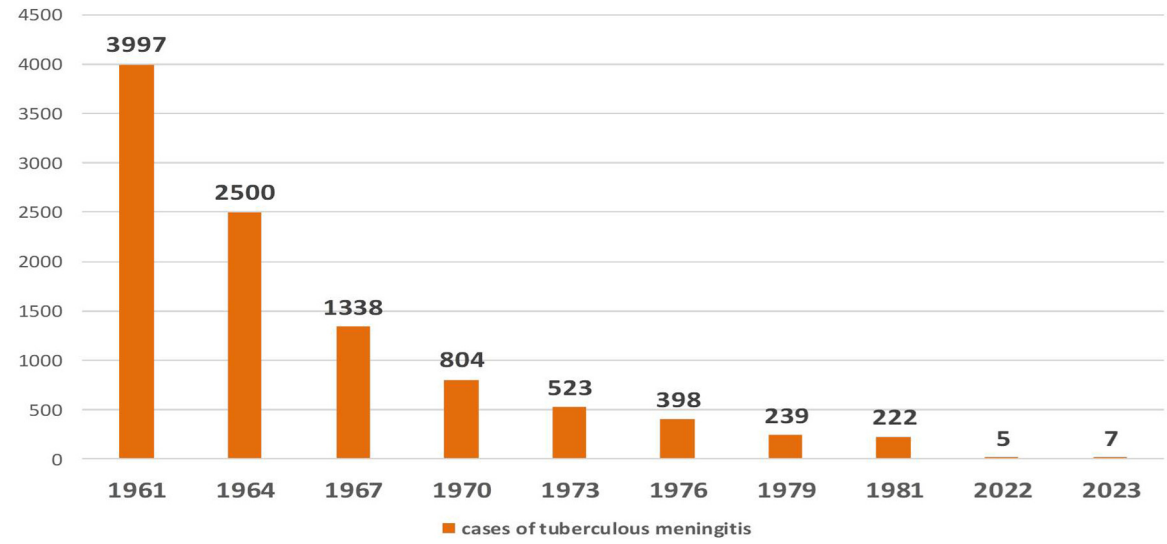

FIGURE 6
Dynamics of the letal cases of, tuberculous meningitis after the introduction of mass BCG vaccination in the USSR. (y, years; x, number of children with meningitis).

genome sequences of two of the most widely used BCG strains: the WHO-recommended reference strain (Danish 1,331) and the Pasteur 1173P2 strain used in Iran. A total of 4,060 genes were identified in Pasteur 1173P2 and 4,037 genes in Danish 1,331 using a comparative annotation framework. Among them, 4,006 coding sequences (CDSs) and 50 tRNA genes were found in Pasteur 1173P2, while Danish 1,331 contained 3,982 CDSs and 51 tRNA genes. Both strains contain three rRNA genes (5S, 16S, and 23S) and a single tmRNA gene (ssrA) (19).

When compared to the *Mycobacterium tuberculosis* H37Rv reference genome, both strains were found to contain 58 PPE genes and 31 PE family genes. Additionally, 58 PE_PGRS subfamily genes were identified in Pasteur 1173P2, compared to 59 in Danish 1,331. Notably, specific genomic deletions and insertions—referred to as regions of difference (RDs)—that distinguish BCG sub-strains were identified: RD14 and N-RD18 were present in Pasteur 1173P2 but absent in Danish 1,331.Conversely, a DU1-like region spanning 14,577 base pairs was found in the Danish 1,331 strain.

There is growing evidence that genetic drift among BCG strains has led to significant variability in their immunogenicity and protective efficacy (16). Identifying these genetic differences is essential for understanding clinical heterogeneity and informing future vaccine development.

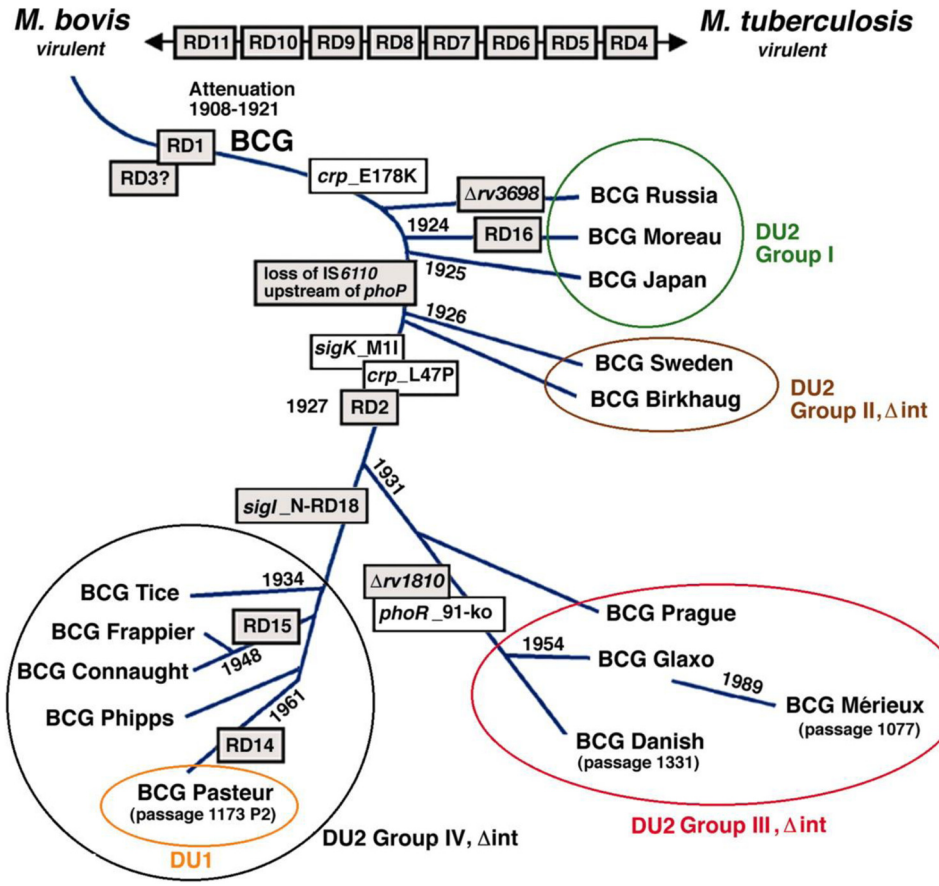

FIGURE 7
Phylogeny of BCG sub-strains with shwing genetic deletions in the different vaccine sub-strains over time (18).

4.1 Genetic evolution and virulence of BCG sub-strains

The phylogenetic lineage of modern BCG sub-strains can be traced back to the original *Mycobacterium bovis* BCG strain developed at the Pasteur Institute. These sub-strains have diverged over time, with some exhibiting abrupt genetic changes after 1927, including gene deletions and alterations in biochemical phenotypes.

Historical and molecular evidence supports the hypothesis that BCG strains produced at the Pasteur Institute in the late 1920s underwent attenuation of virulence (19). Today, BCG vaccine strains vary in their ability to induce immune responses. Multiple studies have demonstrated significant differences in both the magnitude and quality of neonatal immune responses elicited by various strains (20). In particular, BCG-Danish and BCG-Japan have been shown to induce higher frequencies of polyfunctional cytotoxic T lymphocytes and elevated production of Th1-type cytokines.

Despite its widespread use, the full protective potential of the BCG vaccine has not yet been fully realized (20). Although it confers strong protection against severe forms of tuberculosis in children—such as miliary TB and tuberculous meningitis (16)—its efficacy in preventing adult pulmonary TB, particularly in high-burden settings, remains limited.

Given that approximately one-quarter of the global population is infected with *Mycobacterium tuberculosis* (Mtb), there is an urgent need for novel vaccines designed for both pre-exposure (preventive) and post-exposure (therapeutic) use. Current clinical trials are evaluating endpoints such as prevention of infection, disease progression, and relapse. Most vaccine candidates target T cell–mediated immune responses, particularly $CD4^+$ and $CD8^+$ T lymphocytes, which are essential for controlling Mtb infection.

5 Next-generation TB vaccines

Despite widespread use, the full protective potential of the BCG vaccine has yet to be fully realized (20). While it provides strong protection against severe forms tuberculosis in Children, such as miliary tuberculosis and tuberculous meningitis (16), BCG is less effective in preventing adult pulmonary TB, particularly in endemic settings.

Given that approximately one-quarter of the global population is infected with *M. tuberculosis* (Mtb), there is a pressing need for new vaccines designed for both pre-exposure (preventive) and

post-exposure (therapeutic) applications. Clinical trial endpoints currently under investigation include prevention of infection, disease progression, and relapse. TB vaccine development primarily targets T-cell–mediated immune responses, particularly $CD4^+$ and $CD8^+$ T lymphocytes, which are key to containing Mtb infection.

Current vaccine strategies include:

1. Whole-Cell Vaccines
   - Recombinant BCG (rBCG) strains engineered to express foreign antigens (e.g., *ESAT-6* encoded in RD1 regions);
   - Incorporation of the Listeria monocytogenes hemolysin gene to enhance phagosomal escape;
   - Addition of immunodominant antigens such as Ag85A, although some constructs inadvertently confer antibiotic resistance (21, 22).
2. Subunit Vaccines
   - Fusion proteins of mycobacterial antigens combined with Th1-stimulating adjuvants (e.g., monophosphoryl lipid A).
   - Examples include Mtb72F, a fusion of two immunodominant antigens;
   - Other candidates target Ag85, ESAT-6, CFP-10, or heat shock proteins like DnaK.
3. DNA Vaccines
   - Plasmid-based constructs encoding Mtb antigens;
   - Capable of inducing cytotoxic T lymphocyte (CTL) responses and long-lasting immunity;
   - Some vaccines combine antigens of 6 kDa and 32 kDa with lipophilic adjuvants.

A promising candidate, H4:IC31, underwent Phase II clinical trials in South Africa in 2018. It was utilized a viral vector presenting the Ag85 antigen via the Modified Vaccinia Ankara (MVA85A) platform. However, its efficacy was inferior to that of BCG (23).

Two other genetically modified live vaccines, VPM1002 and MTBVAC, are currently in various phases of clinical development.

5.1 Russian contributions: GamTBvac and GamLTBvac

The Gamaleya National Research Center for Epidemiology and Microbiology (Russia) has developed two novel TB vaccines: the prophylactic GamTBvac and the therapeutic GamLTBvac. Currently in Phase III clinical trials, GamTBvac is designed as a booster vaccine containing the Ag85A and ESAT 6/CFP 10 antigens, formulated with a CpG oligodeoxynucleotide (ODN) adjuvant. It is considered the most advanced subunit TB vaccine currently under evaluation in the Russian Federation. Phase I/II trial data confirmed the vaccine's safety and immunogenicity, with durable T cell responses observed in 94%–98% of participants (24). Unlike BCG—which is primarily effective in children—GamTBvac targets adolescents and adults and does not contain live mycobacteria, making it suitable for use in immunocompromised individuals.

6 Major types of memory T cells and broad spectrum of BCG vaccine effects

Currently, several antigen-specific memory T cell subsets are recognized, each with distinct functional characteristics and phenotypic markers (24). Initially, circulating $CD4^+$ and $CD8^+$ memory T cells were classified based on their homing receptor expression—CD62l and CCR7—reflecting their capacity to migrate to secondary lymphoid organs (25). This classification defined two primary subsets: central memory T cells (TCM, $CD62l^+CCR7^+$) and effector memory T cells (TEM, $CD62l^-CCR7^-$).

TCM cells exhibit high proliferative and clonal expansion potential upon antigen re exposure and secrete substantial amounts of IL 2, although they lack immediate effector functions. In contrast, TEM cells are characterized by migration-associated molecule expression, limited proliferative capacity, and abundant effector cytokine production upon activation (26).

In addition to circulating subsets, tissue resident memory T cells (TRM) have been identified in non inflamed peripheral tissues, particularly at mucosal and epithelial barriers.

These cells exhibit minimal recirculation through blood or lymphatic systems but mount rapid effector responses upon local antigen re encounter, contributing to early mucosal defense prior to systemic immunity.

Phenotypically, TRM cells can be distinguished by surface markers CD69 and CD103; however, their biology and function in humans remain incompletely understood (27). Another distinct subset, stem cell like memory T cells (TSCM), exhibit a naïve like phenotype—expressing CD45RA, CCR7, CD62l, CD27, and CD28—while also expressing memory-associated markers such as CD95, CD122, and CXCR3 (28). TSCM cells are characterized by longevity, self-renewal capacity, and high proliferative potential. Upon antigenic restimulation, they can differentiate into other memory and effector subsets, thus contributing to durable immunological memory and sustained protective responses (29). Given the critical role of TSCM cells in durable immunity, vaccine strategies—including those targeting *Mycobacterium tuberculosis*—should aim to promote their development.

Early studies on BCG-specific memory T cell phenotypes demonstrated that primary BCG vaccination in neonates induces both central and effector memory $CD4^+$ T cell responses (30). IFN-γ and IL-2-expressing $CD4^+$ T cells were initially characterized as $CD45RA^-CCR7^-CD27^+$, consistent with effector memory phenotype. However, a substantial proportion of IFN-γ^+ $CD4^+$ and $CD8^+$ T cells exhibiting a $CD45RA^+CCR7^+CD27^+$ phenotype were also detected. At the time, these were wrong classified as central memory cells, since the TSCM subset had not yet been defined. Similar observations were made in murine *in vivo* models, where BCG vaccination led to accumulation of $CD4^+CD44^{hi}CD62L^{lo}$ effector cells in the lungs capable of producing IFN-γ. Simultaneously, a population of T cells with a naïve-like phenotype also emerged (31).

Notably, adoptive transfer experiments demonstrated that BCG-specific $CD44^{lo}CD62L^{hi}$ T cells—but not their

$CD44^{hi}CD62L^{lo}$ counterparts—were the key to protecting $Rag^{-/-}$ mice from experimental *M. tuberculosis* infection. This finding underscores the essential role of TSCM cells in protective immunity. More recently, BCG-specific TSCM cells have also been identified in humans (32). Mpande et al. demonstrated that $CD45RA^{+}CCR7^{+}CD27^{+}$ $CD4^{+}$ TSCM cells secreting IFN-γ, TNF-α, or IL-2 were abundant in the peripheral blood of QFT-positive, HIV-negative, TB-naive individuals. These cells also expressed CD95 and CXCR3, characteristic of the TSCM phenotype, and their frequency positively correlated with the proliferative potential of BCG-specific $CD4^{+}$ T cells measured 10 months post-vaccination. These findings suggest that BCG-induced differentiation and expansion of TSCM cells contribute to long-term immunological memory and robust recall responses against *Mycobacterium tuberculosis* (33).

Despite the emergence of TSCM, the predominant phenotype among BCG-reactive $CD4^{+}$ T cells remains the $CD45RA^{-}CCR7^{-}$ effector memory subset. Upon BCG re-vaccination, this population further expands in peripheral blood, while increases in central memory and effector populations are less pronounced, and levels of naïve-like $CD45RA^{+}CCR7^{+}$ cells remain largely unchanged (33). Experimental evidence also supports the critical role of BCG-induced TRM cells in protective immunity. For instance, intratracheal BCG vaccination in mice significantly enhanced the formation of both $CD4^{+}$ and $CD8^{+}$ TRM cells in the lungs, leading to superior protection compared to subcutaneous immunization (34, 35). Additionally, adoptive transfer of pulmonary TRM cells into naive mice conferred resistance to subsequent *M. tuberculosis* infection, highlighting the importance of mucosal vaccination routes (36).

Another critical correlate of vaccine-induced protection is the generation of "polyfunctional" memory T cells capable of simultaneously producing IFN-γ, TNF-α, and IL-2 in response to antigenic stimulation (37). The role of these polyfunctional cells in protection against TB was first demonstrated by Darrah et al. in both murine models and human studies (37). These cells were present in the lungs and spleens of vaccinated mice 2–8 months post-immunization but were undetectable at 14 months (38, 39). Nonetheless, epidemiological studies in humans have shown that BCG-induced protection can persist until school age (40), and some evidence even suggests protection may last 50–60 years (41).

BCG vaccination in neonates has consistently been shown to induce polyfunctional memory T cells across diverse populations and settings (42). Interestingly, the timing of vaccination—whether administered immediately after birth or 6–10 months later—did not significantly influence the magnitude of these responses (39, 42, 43). Studies by Kagina et al. and Smith et al. found that BCG-specific polyfunctional T cells peaked in circulation 6–10 weeks post-vaccination but remained detectable up to 12 months (39, 41).

In adults, approximately 50% of the BCG-specific memory T cell pool consists of polyfunctional cells (44). Boer et al. reported that their frequency peaks around 8 weeks post-vaccination, followed by a notable decline after one year (45). Nonetheless, the development of polyfunctional memory T cells appears to be a consistent feature of BCG vaccination in both children and adults, with a minimum induction period of approximately 10 weeks (46).

Finally, evidence supports the involvement of BCG in initiating mucosal immune responses critical for protection against infections with transmucosal entry points, including tuberculosis, HIV, salmonelloses, and acute respiratory infections (47).

The BCG is a classical adjuvant, because it contains the components able to enhance the immune responses not only against target tuberculosis germ and other *Mycobacteria* (for example, those causing leprosy and Buruli ulcer), but also towards many other pathogens. It is not occasional that Jules Freund included inactivated dried *M. tuberculosis* into composition of his famous complete Freund's adjuvant broadly used since 1942 in experimental immunology (48).The type of adjuvant effect inherent in the BCG vaccine, caused by early contact of the immune system with factors modifying interactions between immunocompetent cells regardless of their clonal affiliation, has in recent years come to be referred to as "trained immunity" (49).

This phenomenon is addressed to the complex of links of the adaptive immune response. It is mediated also by the influence on the initiation of innate immunity through epigenetic mechanisms. Trained immunity as a type of adjuvant effect has its own characteristics. This phenomenon is inherent in very early and prolonged effects on the developing immune system, which is ensured by the neonatal administration of the live the BCG vaccine, essentially creating a symbiosis of the host organism and the vaccine strain of *Mycobacteria*. Due to this, epigenetic changes occur in the regulation of genome expression in myeloid progenitor cells and, in particular, in lymphoid elements of innate pools and diffuse non-encapsulated mucosa-associated lymphoid tissue. It changes the course of their differentiation, leading to greater accessibility of the genes of NOD2-dependent reactions of innate immunity for bioregulators, and reprogramming of cells for more effective production of a number of cytokines and increased expression of several Toll-like receptors. The phenomenon affects the debut phase of anti-infective protection and promotes a more active interferon response from lymphocytes (50, 51).The spectrum of documented enhancing effects of BCG vaccination on various aspects of immune protection beyond phthisiology is quite broad. It reduced the viral load and increased resistance to yellow fever, changing the cytokine profile of vaccinated individuals accordingly (52). It also proved to be an effective means of increasing antimalarial resistance in children of sub-Saharan Africa as well as in experiments on mice (53). Of special interest is use of BCG vaccine for activation of anti-neoplastic immunity. In treatment of bladder cancer, the BCG vaccine has been used locally for over 45 years. After intravesical administration of the BCG vaccine, a local immunological reaction of bladder mucosa was detected with increase in the number and activity of local immunocompetent cells (54). The comparative epidemiological data from East Germany (where BCG vaccination was mandatory between 1953 and 1991 and recommended during 1951-1952 and in 1992-1998) and West Germany (where it never was mandatory, just voluntary recommended in 1955-1998) witness

for lower incidence of lymphomas and acute lymphoblastic leukemia in cohorts immunized by BCG compared to those non-immunized by this vaccine. After cancellation of mandatory BCG vaccination the incidence of lymphoid malignancies in Eastern lands of Germany tended to increase reaching the level of its Western part (55, 56).

7 COVID-19 and BCG vaccination

During the COVID-19 pandemic, the BCG vaccine attracted considerable attention due to accumulating evidence suggesting a correlation between BCG vaccination and reduced COVID-19 morbidity and mortality (57). Comparative analyses of COVID-19 outcomes in countries with and without universal BCG vaccination programs revealed that nations lacking such policies—such as Italy, the Netherlands, the USA, Belgium, Spain, and Sweden—experienced higher morbidity and mortality rates compared to countries with longstanding BCG vaccination practices, including former Soviet republics, Eastern European nations, South and East Asian countries, Japan, Finland, and several African states. Notably, Sweden, which ceased mandatory BCG vaccination in 1975 and did not reinstate it after 1986, reported an incidence rate approximately 4.5 times higher than that of neighboring Scandinavian countries (8,305 cases per million) and a significantly elevated mortality rate (576 deaths per million) (47, 57). Furthermore, some studies observed that countries where older generations had received BCG vaccination exhibited comparatively lower COVID-19 morbidity and mortality (58).

The highest COVID-19 mortality rates were recorded in countries that either never implemented universal BCG vaccination or did so only recently. For instance, Iran introduced universal BCG vaccination in 1984 and exhibited elevated mortality rates, supporting the notion that protection is most pronounced in previously vaccinated elderly cohorts. Countries without universal BCG programs or with discontinued policies—such as San Marino, Belgium, Andorra, Spain, Italy, Sweden, the USA, Saint Maarten, and the Netherlands—rank among those with the highest mortality rates (59).

Multiple epidemiological studies have documented a negative correlation between national BCG vaccination policies and COVID-19 incidence and mortality (60, 61). By the end of 2020, the lowest COVID-19 incidence and mortality rates were observed in countries maintaining mandatory triple-dose BCG vaccination until 2011 (e.g., Belarus, Kazakhstan, Uzbekistan) (62, 63). These findings contradict the null hypothesis of no association between BCG vaccination and COVID-19 outcomes, supporting a potential protective role for BCG (64).

Additionally, epidemiological research has linked BCG vaccination to reductions in respiratory infections, such as respiratory syncytial virus (RSV) and influenza, as well as sepsis in children, with some studies reporting nearly 50% reductions in neonatal mortality in high-risk settings. Timely neonatal BCG administration may significantly improve health outcomes in HIV-1–infected children. Enhanced production of tumor necrosis factor (TNF), interleukins (IL)-1β, IL-6, and interferon-γ has been observed in cells from BCG-vaccinated individuals in response to both mycobacterial and heterologous antigens (65, 66).

A double-blind, placebo-controlled phase III trial demonstrated that multi-dose BCG vaccination protects adults with type 1 diabetes against COVID-19 and other infections (67). From April 2021 to November 2022, BCG vaccines derived from the Tokyo strain conferred significant protection against COVID-19 ($p = 0.023$) and robust cross-protection against infectious diseases overall ($p < 0.0001$). Notably, mRNA-based COVID-19 vaccines alone did not demonstrate comparable protection against COVID-19 ($p = 0.43$), and their administration neither enhanced nor interfered with the BCG-dependent protective effect.

In a comparative study of young adults, 11.7% of BCG-vaccinated individuals tested positive for COVID-19 vs. 10.4% of unvaccinated individuals (68). Australian researchers emphasized the continued necessity of BCG vaccination as a safe, effective, and cost-efficient method for tuberculosis prevention, particularly in children, both during and after the COVID-19 pandemic (69).

The pathogenesis of COVID-19 involves hyperinflammation, epithelial barrier dysfunction, and excessive systemic production of inflammatory mediators, especially cytokines. BCG vaccination induces lymphocyte production of INF-γ, which modulates multiple interleukins and may mitigate the severity of COVID-19 by attenuating IL-12 and IL-18–dependent inflammatory responses. Moreover, evidence suggests antigenic cross-reactivity between mycobacterial pathogens and SARS-CoV-2 due to shared peptide sequences and epitope mimicry, implying that adaptive immune responses elicited by BCG vaccination may partially cross-protect against SARS-CoV-2 infection (70, 71).

It remains essential to further investigate the broader immunomodulatory effects of the BCG vaccine without undermining its well-established efficacy in tuberculosis prevention (72, 73). Future studies must rigorously evaluate the safety and effectiveness of BCG vaccination for indications beyond tuberculosis (74).

In the Russian Federation, tuberculosis prevention strategies include the use of both BCG and BCG-M vaccines (75). Both vaccines comply with WHO standards for live attenuated vaccines. While BCG vaccination does not prevent *Mycobacterium tuberculosis* infection *per se*, it effectively protects against severe clinical manifestations, such as miliary tuberculosis and tuberculous meningitis, which result from hematogenous dissemination. Newborns are vaccinated in maternity hospitals between days 3 and 7 of life, with revaccination performed at 6–7 years of age. The Russian Federation enforces strict quality control standards for both BCG and BCG-M vaccines (76).

8 HIV infection and BCG vaccination

Children who are exposed to HIV and come into contact with a patients, suffering from tuberculosis, face a significant risk of developing complicated tuberculosis (77). The likelihood of contracting tuberculosis and experiencing severe complications is considerably greater among children with HIV. The BCG vaccine

is both safe and effective for infants with HIV, as it prevents severe course of tuberculosis in susceptible children. Early and carefully timed immunization, aligned with traditional vaccination timing and criteria, results in sufficient, moderately strong anti-tuberculosis immunity, as evidenced by local post-vaccination reactions and tuberculin skin tests. The high safety profile of early, cautious BCG vaccination in children with perinatal HIV infection has been established. In contrast, the clinical progression of tuberculosis in unvaccinated children, particularly in younger age groups, tends to be severe and complicated, often leading to rapid deterioration (78).

However, it is important to note that vaccination against tuberculosis in children with HIV does not consistently yield strong immunologic or clinical responses. For example, the Mantoux test (2 TU) is positive in only about one-third of vaccinated HIV-infected individuals. Furthermore, analyses indicate that the incidence of disseminated tuberculosis does not differ significantly between vaccinated and unvaccinated children who are exposed to HIV (79). Children with HIV are at increased risk of disseminated complications, particularly generalized BCG infection, within three years following vaccination.

Currently, children infected with HIV receive vaccinations in accordance with the preventive vaccination schedule (80). Those born to mothers with HIV infection who have undergone three-stage chemoprophylaxis to prevent mother-to-child transmission of HIV are vaccinated against tuberculosis in the maternity hospital (with BCG-M vaccine). Children with confirmed HIV infection using molecular tests for HIV DNA are excluded from BCG vaccination. Vaccination is administered either in the maternity hospital or thereafter, provided there are no clinical or laboratory signs of immunodeficiency (80).

Administration of live vaccines is contraindicated in children with immunodeficiency. Immunization may induce a transient increase in HIV viral replication. Following BCG vaccination, infants with HIV exhibit elevated levels of CCR5+ CD4+ T cells —preferential targets for HIV infection—which can persist for up to eight weeks post-vaccination (43). The risk of HIV acquisition during breastfeeding is also heightened for infants born to mothers with HIV-positive, underscoring the complexity of vaccination decisions in this group.

BCG vaccination in children with HIV involves a delicate balance between benefits and risks, especially concerning vaccine safety, efficacy, and immune response. Infants with HIV are at significant risk for severe BCG-related complications, including disseminated disease, with incidence rates of 329–417 cases per 100,000 vaccinated infants reported in regions of high HIV prevalence (78). Disseminated BCG disease can result in systemic infections involving lungs, bones, or lymph nodes and may carry mortality rates as high as 75%. Additionally, BCG-associated Immune Reconstitution Inflammatory Syndrome (IRIS) frequently occurs after initiation of antiretroviral therapy (ART), presenting as inflammatory lymphadenitis or abscess formation at the vaccination site. Younger age and high baseline viral load are among factors increasing IRIS risk.

Children infected with HIV generally mount suboptimal immune responses to BCG vaccination, characterized by lower levels of protective CD4+ T cells and diminished interferon-gamma production. This inadequate immune response compromises protection against tuberculosis, particularly severe manifestations such as tuberculous meningitis. Consequently, BCG vaccination is not recommended for infants with confirmed HIV infection prior to ART initiation to reduce IRIS risk (68, 81).

Conversely, timely BCG vaccination may confer important non-specific protective effects in infants with HIV. Emerging data provide a foundation for optimizing the timing of BCG vaccination in this population (82). In regions with high tuberculosis incidence, BCG vaccination at birth is recommended when HIV status is unknown, as benefits outweigh risks. Some studies suggest delaying vaccination until HIV status confirmation (e.g., 8–14 weeks) to minimize risks, though this delay may postpone protection against tuberculosis.

BCG vaccination induces immune alterations in infants with HIV, notably increasing activated CCR5+ CD4+ T cells, which could theoretically enhance susceptibility to HIV infection during breastfeeding. However, available primate studies have not demonstrated significant increases in HIV transmission following BCG vaccination (65). These findings highlight the need to carefully balance the timing of BCG vaccination to minimize HIV transmission risk while maximizing vaccine benefits (81).

Several studies suggest that BCG vaccination reduces all-cause mortality in infants with HIV by providing protection against non-tuberculosis infections, such as respiratory viruses, through mechanisms of trained immunity. BCG induces epigenetic and metabolic reprogramming of innate immune cells—including monocytes and macrophages—enhancing their responsiveness to diverse pathogens. This immunomodulatory effect may underlie the observed reductions in mortality among vaccinated infants (83, 84).

9 BCG in preterm infants

Children who are exposed to HIV and infected with TB infection face a significant risk of developing complicated tuberculosis (77). The likelihood of contracting tuberculosis and experiencing severe complications is considerably greater among children infected with HIV. The BCG vaccine is both safe and effective for infants with HIV, as it helps prevent the onset of severe tuberculosis in susceptible children. Research indicates that early and carefully timed immunization, aligned with traditional vaccination timing and criteria, results in sufficient, moderately robust anti-tuberculosis immunity, as evidenced by local post-vaccination reactions and tuberculin skin tests. The high safety profile of early, cautious BCG vaccination in children with perinatal HIV infection has been established. In contrast, the clinical progression of tuberculosis in unvaccinated children, particularly in younger age groups, tends to be severe and complicated, often leading to rapid deterioration (78).

It is important to note that vaccination with the TB prevention vaccine in children with HIV does not demonstrate sufficient immunological and clinical efficacy. The Mantoux test with 2TU indicates a positive reaction in only about one-third of

vaccinated individuals. An analysis of additional data revealed that the occurrence of disseminated processes in vaccinated patients was not significantly different from that in unvaccinated children exposed to HIV (79). Research has indicated that children with HIV are at an increased risk of developing disseminated complications, particularly generalized BCG infections, within three years post-vaccination. Currently, children infected with HIV receive vaccinations in accordance with the preventive vaccination schedule (80). Those born to mothers with HIV-positive who have undergone three-stage chemoprophylaxis to prevent mother-to-child transmission of HIV are vaccinated against tuberculosis in the maternity hospital (BCG-M). Children who test positive for HIV using molecular methods are excluded from vaccination. BCG vaccination is administered either in the maternity hospital or after the mother and child are discharged, using the BCG-M vaccine, provided there are no clinical or laboratory indications of immunodeficiency (70).

The administration of live vaccines is contraindicated in individuals exhibiting signs of immunodeficiency. Immunization may lead to a temporary rise in HIV viral replication. Research indicates that infants exposed to HIV exhibit an increase in CCR5+ CD4+ T-cell levels following BCG vaccination, with this increase lasting for up to 8 weeks post-vaccination (43). Furthermore, BCG-vaccinated infants aged 8 weeks also show elevated levels of these cells that are preferential targets for HIV. Infants born to mothers with HIV-positive face a heightened risk of HIV infection during breastfeeding, underscoring the importance of vaccination for this vulnerable group. The implications of BCG vaccination in children -infected with HIV involve a complex balance of risks and benefits, particularly concerning vaccine safety, efficacy, and immunological responses. HIV-positive infants are at significant risk for serious complications from BCG, including disseminated disease, with reported incidence rates of 329–417 cases per 100,000 vaccinated infants in regions with high HIV prevalence (78). Such complications can lead to systemic infections affecting the lungs, bones, or lymph nodes, with severe cases carrying a mortality rate as high as 75%. Additionally, Immune Reconstitution Inflammatory Syndrome (IRIS) associated with BCG vaccination is frequently observed after the initiation of antiretroviral therapy (ART), manifesting as inflammatory lymphadenitis or abscesses at the vaccination site. Factors such as younger age and a high baseline viral load increase the risk of IRIS. Children with HIV generally demonstrate suboptimal immune responses to BCG, characterized by lower levels of protective CD4+ T cells and diminished interferon-gamma production. This inadequacy undermines their protection against TB, especially against severe forms such as TB meningitis. Consequently, BCG vaccination is not recommended for confirmed HIV-infected infants prior to starting ART to mitigate the risk of IRIS (68, 81).

At the same time, timely BCG vaccination may have important non-specific protective effects in infants infected with HIV-1. This study may provide a basis for developing optimal timing of BCG vaccination for infants infected with HIV-1 (82). If the HIV status of infants is unknown, BCG vaccination is recommended at birth in areas of high TB incidence, as the benefits outweigh the risks. Some studies suggest delaying BCG until HIV status is confirmed (e.g., at 8–14 weeks) to reduce risks, although this may postpone protection against TB. BCG vaccination increases the number of activated CCR5+ CD4+ T cells (HIV target cells) in HIV-exposed infants, potentially increasing susceptibility to HIV infection during breastfeeding. However, studies in primates have not shown a significant increase in transmission rates (65).

Simultaneously, BCG vaccination triggers immune alterations in infants exposed to HIV, notably increasing the proportion of activated CCR5+ CD4+ cells, which are targets for HIV. These findings provide valuable insights into the balance needed for the timing of BCG vaccine administration, aimed at minimizing the risk of HIV transmission to already infected infants while still providing the potential benefits of the vaccine (81). Several studies indicate that BCG may lower all-cause mortality among infants infected with HIV by offering protection against infections unrelated to tuberculosis (such as respiratory viruses) through trained immunity mechanisms. The BCG is known to induce epigenetic and metabolic changes in innate immune cells, such as monocytes and macrophages, thereby enhancing their response to various pathogens, including respiratory viruses. This could help explain the noted decrease in all-cause mortality among infants who received the vaccination (75, 76).

Using of the BCG vaccine in preterm infants is associated with some concerns due to their immunological immaturity and increased risk of infections. The BCG is generally safe in clinically stable preterm infants (those born at more than 30 weeks' gestation or weighing more than 1.5 kg), with no increased risk of systemic adverse events (e.g., disseminated BCG disease).

A meta-analysis of 10,568 preterm and low birth weight infants found no deaths or systemic reactions associated with BCG vaccination within 7 days after birth. Forty studies were included in the meta-analysis, involving preterm infants (born at 26–37 weeks of gestational age) and/or small-for-date infants (0.69–2.5 kg at birth). Based on the available data, early BCG vaccination of healthy preterm infants and/or children with low birth weights in order to improve vaccine efficacy is justified. Local reactions (e.g., lymphadenitis, ulceration) occurred with the same frequency (0%–4.2%) as reported in term infants (85).

Extremely preterm infants (<30 weeks or <1.5 kg) and those with immunodeficiency (e.g., SCID, HIV) face higher risks of disseminated BCG infection, which can be fatal (86). In China, severe adverse events (e.g., interstitial pneumonia, and sepsis) were rare (8 per million) but had a 100% mortality rate in preterm infants (87).

Preterm infants mount cell-mediated immune responses (e.g., tuberculin skin test conversion, lymphocyte proliferation) similar to term infants when vaccinated at 34–40 weeks' postconceptional age. No significant differences in scar formation or cytokine profiles were observed between early (34–35 weeks) and late (38–40 weeks) vaccination terms (88).

BCG may enhance trained immunity, reducing all-cause mortality and respiratory infections in preterm infants. It is recommended for stable preterm infants (>30 weeks, >1.5 kg) to improve coverage and leverage potential non-specific immune benefits. Delayed vaccination increases dropout rates in high-TB-

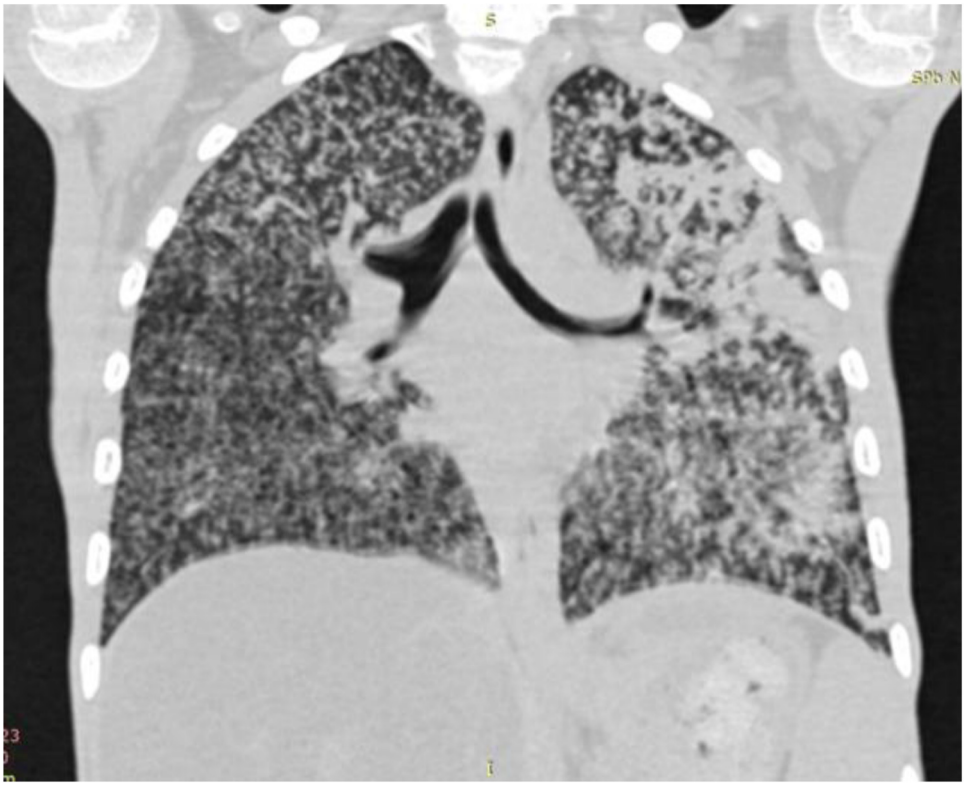

FIGURE 8
X-ray of a child with pulmonary myliary dissemination BCG infection.

burden settings. Vaccination is advised to be postponed only for extremely preterm infants or those with comorbidities, until they reach approximately 34 weeks' postconceptional age or achieve clinical stability.

WHO supports BCG vaccination for preterm infants in high-TB-burden regions but emphasizes the importance of personalized risk assessment (89).

10 BCG complications in primary immunodeficiencies

The development of complications after BCG vaccination may indicate the presence of primary immunodeficiency. It is particularly important to be aware of this fact if there is a family history of BCG complications, immunodeficiency, or unexplained deaths of children following vaccination.

Immunological screening of patients with suspected immunodeficiency is relevant, as it can help prevent the development of severe complications after vaccine prophylaxis and reduce morbidity and mortality associated with BCG vaccination (90, 91). The most severe complications associated with BCG vaccination in the context of primary immunodeficiency include: generalized BCG infection, BCG osteitis (osteomyelitis), and disseminated BCG lymphadenitis (92, 93).

Generalized BCG infection is the most severe complication resulting from dissemination of BCG *Mycobacteria* throughout the body. Fever, weight loss, hepatosplenomegaly, and involvement of lymph nodes, skin, and lungs are common clinical features of that entity. Lethality exceeds 50%. It is more common in severe combined immunodeficiency (SCID) and chronic granulomatous disease. Immunocompromised patients with generalized BCG infection may present with sole axillary lymphadenopathy in 64%, combined axillary, cervical or supraclavicular lymphadenopathy in 32%, hepatomegaly and splenomegaly in 50%, pneumonia in 36%, gastrointestinal symptoms in 10%, skin rash in 15%, meningitis in 5%, and osteomyelitis in 1% (90) (Figure 8).

BCG dissemination can occur in 65% with a fatality rate of 36%. Delaying BCG vaccination until 6 months of age significantly reduces the incidence of BCG-related complications in patients suffering from SCID. It is the importance of developing individualized vaccination schedules for high-risk groups (Figure 9). Early newborn screening and timely diagnosis of immunodeficiencies are essential to further reduce complication rates (94, 95).

Localized reactions such as cold abscess, ulcers, keloid scars may also occur (84).

BCG osteitis (osteomyelitis) occurs in bones and joints, developing several months or even years after vaccination. Most often metaphyses of long tubular bones, vertebrae, and sternum

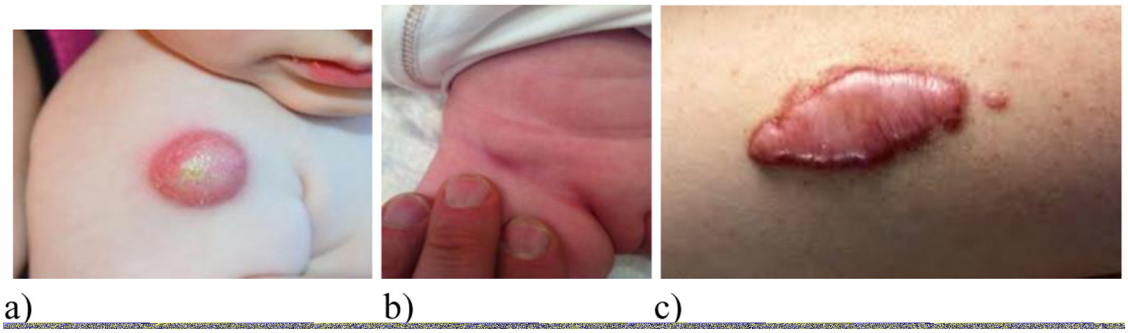

FIGURE 9
Variants of local manifestations of complicated course of BCG vaccination **(A)** cold abscess, **(B)** inguinal lymphadenitis after BCG vaccination in the buttock (Sweden); **(C)** keloid scar after vaccination).

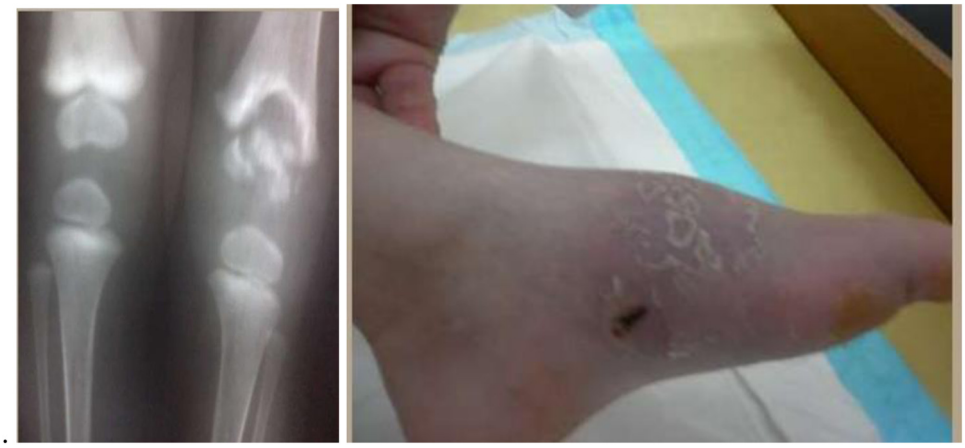

FIGURE 10
BCG ostiomyelitis in a 4-year-old child (radiographs and tibial fistula). BCG bovis DNA was obtained.

are affected. This complication is associated with defects in the IL-12/IFN-γ pathway leading to impaired immune response against BCG *Mycobacteria* (96, 97) (Figure 10).

Disseminated BCG lymphadenitis can occur not only in regional (axillary an/or cervical) lymph nodes, but also in their distant groups. It may be accompanied by suppuration, fistula formation. It often occurs in HIV infection and combined immunodeficiencies (90).

11 Latest advances in vaccine strategies and noninvasive imaging methods for tuberculosis diagnosis and monitoring

The development of new vaccines remains a strategically important and urgent task in the global fight against tuberculosis (TB). In 2023, the WHO Council for Accelerating the Development of a Tuberculosis Vaccine was established to expedite vaccine development by leveraging lessons learned from the COVID-19 pandemic (98, 99). Several vaccine candidates, including RUTI and DAR-901, are currently being evaluated as immunotherapies aimed at shortening treatment duration and preventing relapse, particularly in cases of drug-resistant TB. Novel platforms such as mRNA-based and viral vector vaccines show promising early results. For instance, mRNA vaccines like BNT164 (BioNTech) and viral vector vaccines such as ChAdOx1-85A are in early clinical trials designed to elicit strong T-cell immune responses (85, 99, 100).

A notable example is the mRNACV2 vaccine, an mRNA-based subunit vaccine formulated with lipid nanoparticles (LNP) and an adjuvant. In preclinical studies, intramuscular administration of mRNACV2 to female C57BL/6 mice—either as a standalone vaccine or as a booster following BCG vaccination—induced a high frequency of multifunctional, antigen-specific Th1 CD4+ T cells in blood and lungs. This immune activation was associated with rapid recruitment of both innate and adaptive immune cells to draining lymph nodes. Importantly, mRNACV2 vaccination conferred significant lung protection in mice infected

M. tuberculosis- by reducing bacterial load and inflammatory infiltration. When used as a booster, mRNACV2 enhanced immune responses and provided durable protection in BCG-vaccinated mice (100, 101). It is also known that circRNA vaccines represent a potential new direction in the vaccine era. Several circRNA vaccines have recently been synthesized and tested *in vitro* and *in vivo* (98).

In several publications, researchers have described the development of a lipid nanoparticle (LNP)–mRNA-based vaccine, referred to as mRNACV2, which encodes the *Mycobacterium tuberculosis* fusion protein CysVac2. Previously, this vaccine was classified as an adjuvanted subunit vaccine. The LNP–mRNA vaccine was administered intramuscularly to female C57BL/6 mice either as a standalone immunization or as a booster following BCG vaccination, to assess the immunogenicity and efficacy of the construct. Notably, mRNACV2 enhanced immune responses and provided long-term protection when used as a booster in BCG-vaccinated mice. The findings underscore the potential of the LNP–mRNA platform for tuberculosis control and support further research to facilitate its translation to human use (99).

Challenges in TB vaccine development and implementation include variable vaccine efficacy across different population groups and regions. The inconsistent protection offered by BCG highlights the need for new vaccines that confer effective immunity across all age groups and geographic areas. Funding shortages pose a significant barrier; currently, only 26% of the estimated $22 billion required annually for TB control programs is available, risking delays in vaccine rollout (88, 89).

In parallel with vaccine development, there is a critical need to advance non-invasive imaging technologies for TB diagnosis and treatment monitoring (101, 102). Molecular imaging using SPECT/CT targeting the translocator protein (TSPO) with radioligands such as [^125I]iodo-DPA-713 has shown high specificity for TB-associated inflammation in macrophages, with superior signal-to-noise ratios compared to conventional [^18F] FDG-PET. This enables real-time 3D visualization of TB lesions, overcoming limitations of sputum-based diagnostics, which cannot detect non-respiratory tract lesions (102). Furthermore, novel PET/CT radiopharmaceuticals like ^68Ga-labelled somatostatin analogues are being introduced to improve specificity over [^18F] FDG, which may be confounded by non-TB inflammation. PET/CT enables longitudinal assessment of treatment response and drug penetration into granulomas, critical parameters for evaluating novel therapies (103). However, these imaging modalities are limited by high costs and lack of pathogen-specificity, restricting their use in resource-limited settings.

Near-infrared (NIR) spectroscopy using semiconductor sensors represents a promising, low-cost diagnostic alternative. Diluted III-V semiconductors, such as nitrogen-doped GaAs, enhance NIR sensitivity to detect TB biomarkers in sputum or breath samples rapidly and in mobile formats, potentially addressing diagnostic gaps in low-resource environments (104). Additionally, fluorescence labeling with iron transport protein (IrtAB) enables detection of M. tuberculosis in saliva within 10 min, considerably faster than culture-based methods.

12 Conclusion

The BCG vaccine has a well-established history of development and use spanning more than a century. Its widespread administration has contributed significantly to reducing childhood mortality from tuberculosis worldwide (105, 106). In the Russian Federation, approximately 90% of newborns receive the BCG vaccine, and as a result, the incidence of disseminated tuberculosis in young children remains low.

Ongoing research has revealed that BCG strains have genetically diverged from the original strain, resulting in notable genomic differences. Although these variations have been associated with changes in scar formation, reactogenicity, and immune response profiles, their impact on overall vaccine efficacy remains inconclusive (105, 107,108,109,110,111).

The BCG vaccine has demonstrated the capacity to induce mucosal and systemic immune responses, offering partial protection not only against tuberculosis but also against other infections involving mucosal surfaces, such as salmonellosis, certain viral respiratory infections, and possibly HIV. Through mechanisms of trained immunity, early BCG vaccination may enhance resistance to malaria and lower the incidence of lymphoid malignancies. It is also used intravesically as an immunotherapy for bladder cancer. While it is important to explore the broader immunomodulatory potential of BCG, such efforts should not overshadow its established efficacy in preventing severe tuberculosis (93, 98).

Although BCG vaccination does not eliminate infection with *Mycobacterium tuberculosis*, it effectively prevents severe disease manifestations such as miliary tuberculosis and tuberculous meningitis, which are associated with hematogenous dissemination. To minimize the risk of serious complications, including generalized BCG infection, neonatal screening for primary immunodeficiencies should be considered before vaccine administration.

Some restrictions remain on the use of BCG in preterm infants due to concerns regarding their immature immune systems and increased susceptibility to infections. Nevertheless, the vaccine is generally considered safe for clinically stable preterm infants born after 30 weeks of gestation or weighing more than 1.5 kg.

A persistent challenge involves protecting children with HIV-infected and tuberculosis while minimizing the risk of vaccine-related adverse events in those with underlying immunodeficiencies. In addition, the development of new vaccines effective against drug-resistant *M. tuberculosis* strains, and suitable for use across different age groups and populations, remains a critical priority.

In summary, the BCG vaccine remains one of the most essential tools in global tuberculosis prevention. Beyond its role in TB control, it contributes to the modulation of immune reactivity through its adjuvant properties, making it a subject of continued scientific interest.

Author contributions

AS: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – original draft,

Writing – review & editing. IK: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. AR: Formal analysis, Methodology, Writing – original draft. ID: Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. AK: Data curation, Formal analysis, Writing – original draft. LPC: Data curation, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. DK: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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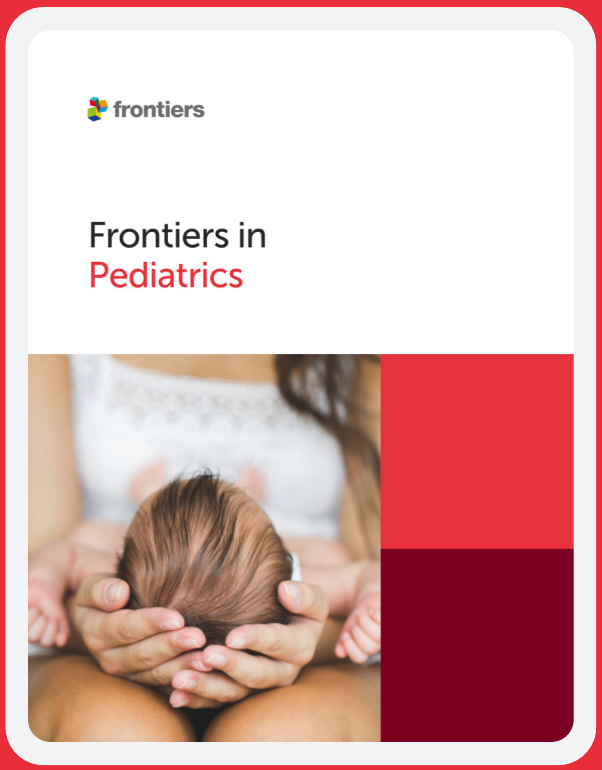

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