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ORIGINAL RESEARCH

Utility of Metagenomic Next-Generation Sequencing for Diagnosis of Infectious Diseases in Critically Ill Immunocompromised Pediatric Patients

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Purpose: Infections cause high rates of illness and death in children worldwide. However, studies on the clinical value of metagenomic next-generation sequencing (mNGS) for immunocompromised children are still limited.

Patients and Methods: From June 2021 to December 2023, 119 samples were collected at Pediatric Intensive Care Unit (PICU) of a single-center pediatric hospital and classified into two groups based on their immune states. We compared the diagnostic performance of mNGS and conventional microbiological test (CMT) for pathogen identification, and assessed the clinical impacts of mNGS.

Results: Among the 119 samples, 48 (40.34%) belonged to the immunocompromised children. mNGS had a higher positivity rate than CMT (76.47% vs 55.46%, $P = 0.0006$). The positive percent agreement (PPA) of mNGS for immunocompromised children was higher compared to immunocompetent children (95.24% vs 77.78%). The most common pathogens for immunocompromised patients were gram-negative bacteria and herpesvirus. However, immunocompetent children showed a higher detection rate for gram-positive bacteria and respiratory viruses. Furthermore, the proportions of the positive impact of mNGS results were significantly higher in immunocompromised patients compared to immunocompetent patients for both diagnosis (91.67% vs 57.75%) and treatment (95.83% vs 64.79%) ($P < 0.0001$). Immunocompromised state, length of hospital stays, times stay in ICU, Pediatric Risk of Mortality (PRISM) score, neutrophil percentage (NEUT%) and the ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) were considered independent factors for poor prognosis in critically ill pediatric patients.

Conclusion: In patients from PICU, mNGS had a greater clinical significance in immunocompromised children compared to immunocompetent children. mNGS technology is an important auxiliary method for achieving accurate diagnosis and treatment of critically ill pediatric patients.

Keywords: infectious diseases, immunocompromised, pediatrics, mNGS, clinical impact

Introduction

In children, infectious diseases are the leading cause of morbidity and mortality worldwide.[1] Many survivors have persistent physical, cognitive, emotional, and psychological sequelae.[2] Therefore, improving diagnosis and management are critical to optimizing outcome for infected children. Early intervention in infectious diseases can reduce the risk of complications with underlying diseases and reduce the probability of death due to misdiagnosis.[3] Clinical diagnosis of infection has greatly benefited from conventional microbiological test (CMT), such as culture, smear, polymerase chain reaction (PCR), and serological tests. However, these methods have several limitations. For example, they can be time-consuming, have a narrow spectrum, low sensitivity, and are greatly affected by the use of antibiotics.[4,5] As a result, 15%

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to 60% of cases remain undiagnosed.[6,7] Therefore, timely and accurate identification of the causative pathogens is crucial for the diagnosis and treatment of infectious diseases.

Metagenomic next-generation sequencing (mNGS) can provide a comprehensive analysis of all nucleic acids in a clinical sample, making it a promising technology for microbial identification technology. mNGS is increasingly applied in clinical settings to identification of rare, emerging, and common microorganisms.[8–10] It is particularly useful in diagnosing infectious diseases, especially in the treatment of critically ill patients.[11,12]

Studies focused on the performance of mNGS in pediatric populations are still limited. Moreover, studies have shown that mNGS has important guiding significance for immunocompromised adults,[13–15] but its clinical value for immunocompromised children is still worth exploring. Therefore, this study aimed to assess the diagnostic ability and clinical impacts of mNGS in immunocompromised children compared to immunocompetent children from pediatric intensive care unit (PICU).

Material and Methods

Study Design

We retrospectively reviewed the records of patients with suspected infectious diseases and undertook mNGS in PICU of Children's Hospital, Zhejiang University School of Medicine during June 2021 and December 2023. Patients without conventional microbiological test results and was still in hospital for treatment was excluded. Patients were defined as immunocompromised when they had one or more of the following risk factors: (1) hematologic malignancies; (2) solid tumor with either neutropenia or chemotherapy; (3) known HIV infection with severe suppression; (4) chronic use of steroid or biologic drug for autoimmune diseases; (5) immunosuppressive therapy due to hematologic cancer or solid organ transplantation; (6) any immunocompromised state including congenital/genetic immunocompromise and asplenia.[16]

Collected samples included bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), blood, stool (OTH), peritoneal (PD), pleural (PE), pus, sputum, and swab. The conventional microbiological tests (CMT) were performed based on the clinician's judgment, including bacteria and fungi culture, bacterial smears, 1.3-β-D-glucan (BDG) test, T-spot, PCR of respiratory virus (including influenza A virus, influenza B virus, parainfluenza virus, adenovirus, respiratory syncytial virus, human metapneumovirus, rhinovirus, coronavirus, and bocavirus), and antibody or nucleic acid amplification tests for *Mycoplasma pneumoniae*.

Nucleic Acid Extraction, Library Preparation, and Sequencing

For nucleic acid extraction, the blood samples were centrifuged at 1900 × g and 4°C for 10 min to get plasma for subsequent processing. The sputum required liquefaction treatment. Plasma cell-free DNA (cfDNA) was extracted using PathoXtract® cell-free Nucleic Acid Kit (WYXM03010S, WillingMed Corp, Beijing, China). DNA from other sample types was extracted using PathoXtract® Basic Pathogen Nucleic Acid Kit (WYXM03211S, WillingMed Corp, Beijing, China) according to the manufacturer's protocol. RNA was extracted using PathoXtract® Virus DNA/RNA Isolation Kit (WYXM03009S, WillingMed Corp, Beijing, China). Extracted RNA was first reverse transcribed using SuperScript® Double-Stranded cDNA Synthesis Kit (11917020, Invitrogen, United States).

For cfDNA libraries construction, the KAPA DNA HyperPrep Kit (KK8504, KAPA, Kapa Biosystems, Wilmington, MA, United States) was used according to the manufacturer's protocol. Genomic DNA libraries were constructed using the Illumina® DNA Prep, (M) Tagmentation (20018705, Illumina, San Diego, USA) according to the manufacturer's protocol. The quality of the libraries was evaluated on Agilent 2100 Bioanalyzer (Agilent Technologies). Only the libraries with high quality were used for sequencing on the NextSeq™ 550Dx sequencer (Illumina, San Diego, USA) using a 75-bp single-end method. No-template control (NTC) and was set for each sequencing run to control the effect of contaminating DNA.

Bioinformatics Analyses and Criteria for Reporting mNGS Detection

The FASTQ-format data obtained by sequencing was processed with Trimmomatic[17] to filter out low-quality sequences, contaminated adapters, duplicated reads and reads shorter than 36 bp. Then the sequences were compared with the human reference genome GRCh37 (hg19) using Bowtie2 to remove human sequences.[18] For taxonomic classification and identification of microbial reads, we utilized Kraken2 with non-redundant nucleotide sequences database of National Center for Biotechnology Information (NCBI).[19]

To interpret the results of mNGS, the following criteria were applied to report the positive pathogens. Reads per ten million (RPTM) was used to quantify pathogen abundance. Bacteria and fungi with RPTM $\geq$ 20,[8] viruses with RPTM $\geq$ 3, and special pathogens (including *Cryptococcus, Mycobacterium, Mycoplasma, Chlamydia, Legionella*, and parasites) with RPTM $\geq$ 1, was identified as positive.[20,21]

Clinical Impacts of mNGS Results for Patients' Diagnosis and Treatment Management

To evaluate the impacts of mNGS results on clinical diagnosis and treatment, the mNGS results were classified into four categories: definite, probable, possible, unlikely, and false-negative.[22,23] (1) Definite: the mNGS results were consistent with those of CMT performed within 7 days of the NGS test; (2) Probable: microorganisms detected by NGS were probably the cause of infection; (3) Possible: mNGS-detected microorganisms have the potential to cause infection, but clinical experts have evaluated them as an uncommon cause based on the consideration of medical records; (4) Unlikely: the detection of microorganisms by mNGS was not identified as the possible cause of infection based on other clinical results or was inconsistent with the results of CMT.

The results of mNGS and CMTs was evaluated against the final clinical diagnoses by two experienced clinicians based on multiple clinical factors, including the clinical manifestations, laboratory examinations, and therapeutic outcomes. The pathogens classified as definite, probable, and possible were identified as the causes of the patient's disease. While the unlikely pathogens were classified as colonization.

The impact of mNGS on diagnosis and treatment was classified into three levels: positive, no effect, and negative.[22] For diagnosis, a positive effect indicated that mNGS helped with timing, co-infection diagnosis or possible etiology confirmation. When mNGS results were negative or the detected pathogen was classified as unlikely and with no clinical impact, or the detection time of pathogens consistent with CMT was later than that of CMT, mNGS was judged to have no effect. The negative effect indicates that the results of mNGS had resulted in additional ineffective treatment (Supplementary Table 1). For treatment, the positive effect indicates that mNGS helped adjust the antibiotics (including upgrade and downgrade of antibiotics or add and reduce some antibiotics) or confirmed empirical treatment. No effect represents there were no adjustment in treatment despite a positive or negative mNGS result, or the patient was discharged or dead when the mNGS report become available. A negative effect means unnecessary treatment or the addition of antibiotics based on the mNGS result.

Statistical Analysis

Statistical analyses were performed using Prism 9 (GraphPad, La Jolla, CA). The Wilcoxon-Mann–Whitney test was used for comparison between groups, and chi-square test was used for categorical variables. *P*-values with less than 0.05 were considered statistical significance. The correlation between the characteristic of the patients and immune status was evaluated by Spearman using R language. Multivariate analysis was performed by multiple logistic regression using R glmnet package.

Results

Patient Baseline Characteristics and Samples

A total of 119 samples were included in this study, with 48 (40.34%) of them being immunocompromised (Figure 1). The average age of immunocompromised children was higher than that of immunocompetent children (88.27 vs 68.72 months, $P = 0.0407$). Of the 48 immunocompromised children, mostly had leukemia (25/48, 52.08%) and solid tumors (10/48, 20.83%). Fever (84.87%), cough (29.41%), and convulsion (16.81%) were the most common symptoms for

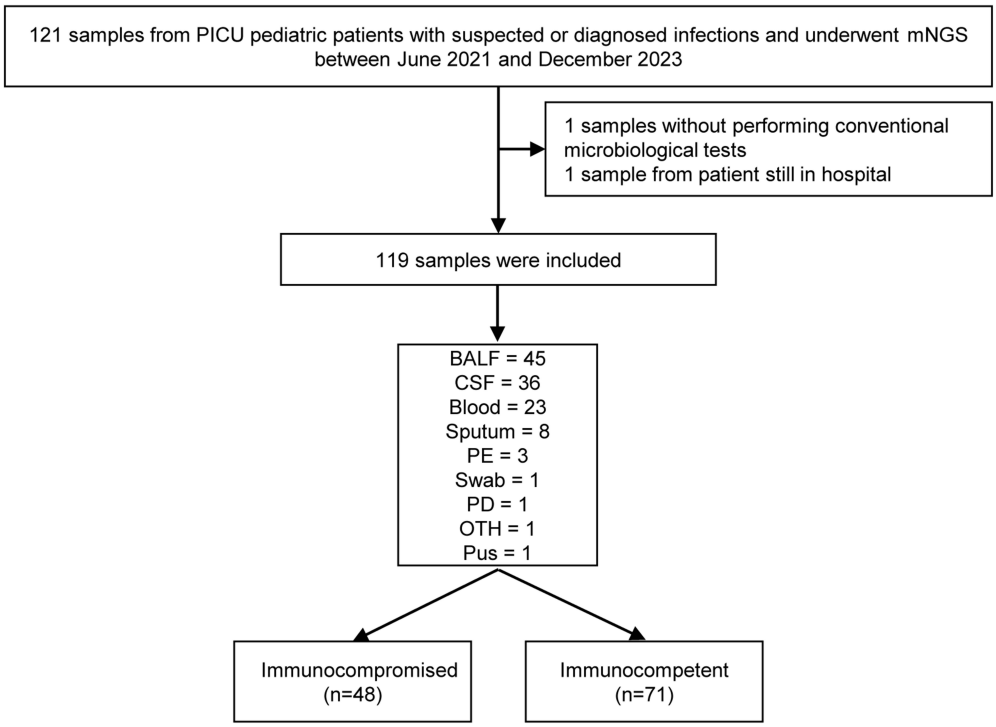

Figure 1 Flowchart of this study.

infection, and the proportion of convulsions was higher in immunocompetent children compared to immunocompromised children (12.68% vs 2.08%, $P = 0.0004$) (Table 1).

The immunocompromised children had a higher proportion of multiple sites co-infections compared to immunocompetent patients (Figure 2A). Respiratory tract infections were the most common, followed by bloodstream infections and central nervous system infections. The proportion of bloodstream infections in immunocompromised patients was

Table 1 Baseline and Clinical Characteristics of Immunocompromised and Immunocompetent Patients

Characteristics[a]	Immunocompromised (n=48)	Immunocompetent (n=71)	*P*-value
Age (Month)	88.27 ± 56.68	68.72 ± 46.02	0.0407
Gender (boy)	28 (58.33%)	40 (56.34%)	0.8292
Underlying disease			
Leukemia	25	1	<0.0001
Epilepsy	0	5	0.0603
Solid tumors	10	1	0.0003
Congenital heart disease	0	5	0.0603
After surgery	0	5	0.0603
Delayed brain development	0	3	0.1492

(*Continued*)

Table 1 (Continued).

Characteristics[a]	Immunocompromised (n=48)	Immunocompetent (n=71)	*P*-value
Symptoms			
Fever	37	64	0.0511
Cough	13	22	0.6467
Convulsion	1	19	0.0004
Disorders of consciousness	1	9	0.041
Shortness of breath	2	1	0.3464
Vomiting	1	2	0.8023
Drowning in water	0	5	0.0603
Lethargy	0	2	0.2409
Muscle weakness	0	2	0.2409
Abdominal pain	5	1	0.0276
Abdominal distention	1	2	0.8023
LOHS (days)	44.04 ± 35.46	27.25 ± 64.23	0.1021
ICU (days)	21.42 ± 21.91	12.51 ± 22.24	0.0331
Mortality	16	5	<0.0001

Abbreviations: [a]LOHS, length of hospital stays; ICU, length of ICU stays.

significantly higher than that in immunocompetent patients. Additionally, the percentage of central nervous system (CNS) infections was significantly lower in immunocompromised patients compared to immunocompetent patients (Figure 2B).

Comparison of the Diagnostic Performance of mNGS and CMT

The samples used for mNGS included BALF (n=45), CSF (n=36), blood (n=23), sputum (n=8), PE (n=3), and one sample each of swab, PD, OTH, and pus. Except for CSF, the positive rate of the other specimens was higher than 80% (Figure 3A). Compared to CMT, the positivity rate of mNGS was higher (76.47% vs 55.46%, $P = 0.0006$). Among immunocompromised patients, the pathogen detection rate of mNGS was significantly higher than that of CMT (93.75% vs 43.75%, $P < 0.0001$). However, there was no significant difference between the two groups in immunocompetent children (64.79% vs 63.38%, $P = 0.8611$) (Figure 3B). When the CMT results were used as the reference for correctness, the positive percent agreement (PPA) of mNGS in immunocompromised patients was higher compared to immunocompetent children (95.24% vs 77.78%) (Figure 3C).

For pathogen detection in immunocompromised patients, mNGS and CMT were both positive in 41.47% (20/48) cases and both negative in 4.17% (2/48) cases. Among the 20 immunocompromised patients who were positive by both methods, the percentages of complete match, partial match and mismatch were 10%, 65% and 25%, respectively. Among immunocompetent patients, 49.30% (35/71) cases were both positive and 21.13% (15/71) cases were both negative. The percentages of complete match, partial match and mismatch of both positive cases were 17.14%, 57.14% and 25.71%, respectively (Figure 3D).

In immunocompromised children, the detection rate of gram-negative bacteria was higher than that of gram-positive bacteria (56.25% vs 31.25%). The most common gram-negative bacteria were *Elizabethkingia, Pseudomonas, Acinetobacter baumannii*, and *Legionella pneumophila*. The most common gram-positive bacteria were *Streptococcus,*

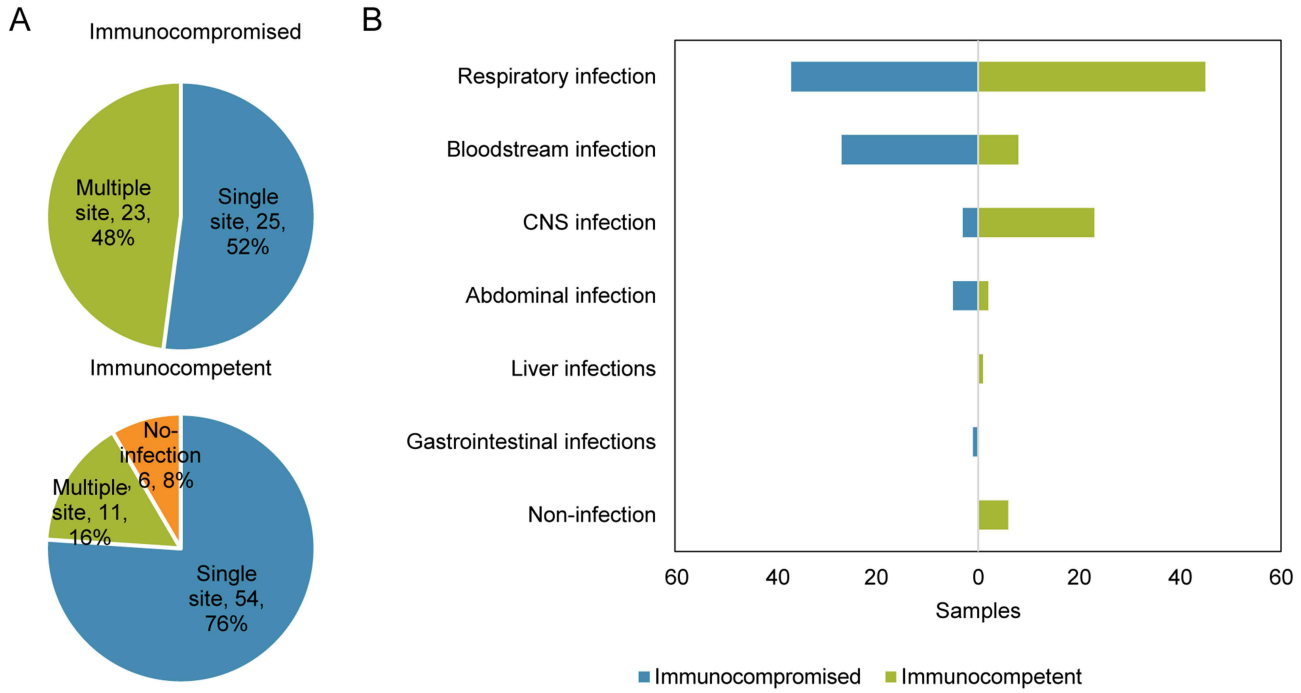

Figure 2 The infection site and types for the immunocompromised and immunocompetent patients. (**A**) Percentage of distribution of infection sites. (**B**) The distribution of infection sites for immunocompromised and immunocompetent patients. CNS: Central Nervous System.

Mycolicibacterium fluoranthenivorans, Staphylococcus, and *Enterococcus*. In the immunocompetent group, the detection rate of gram-positive bacteria was higher than gram-negative bacteria (80.28% vs 30.99%). *Streptococcus* and *Staphylococcus* were the most common gram-positive bacteria. Besides, special pathogen *Mycoplasma pneumoniae* was with a higher detection rate in immunocompetent children than in immunocompromised children (19.72% vs 12.50%, $P = 0.2861$). *Candida albicans* was the most common fungal infections, and the proportion of fungal infection in immunocompromised patients was higher than that in immunocompetent patients. *Pneumocystis jirovecii* (n=4) was identified by mNGS only in immunocompromised patients. Herpesvirus were detected more frequently in immunocompromised patients. Whereas rhinovirus and influenza virus showed a higher detection rate in immunocompetent patients (Figure 4) (Supplementary Table 2).

The Clinical Impact of mNGS on Diagnosis and Treatment of Infection

Among all the children, mNGS has a positive impact on the diagnosis and treatment of infection in 71.43% (85/119) and 77.31% (92/119) cases, respectively. No negative effects were observed. Then we compared the clinical impact of mNGS between the immunocompromised and immunocompetent children. The proportions of the positive impact of mNGS results were significantly higher in immunocompromised patients than in immunocompetent patients for both diagnosis (91.67% vs 57.75%) and treatment (95.83% vs 64.79%) ($P < 0.0001$) (Figure 5A and B). We also analyzed the clinical impact of mNGS on different kinds of infection type. Notably, in immunocompromised patients, mNGS has the highest diagnostic value for mixed infections, followed by single viral infection. In immunocompetent patients, mNGS also had the highest diagnostic value for mixed infection, followed by single bacterial infection (Figure 5C). Out of the 92 cases with positive treatment effects, 49 cases had their antibiotics usage adjusted. In the remaining 43 cases, although no antibiotic adjustment was made, positive mNGS results helped confirm that the pathogen had been covered by the agent. Of the 49 cases with adjusted antibiotics, 31 were immunocompromised patients, and the other 18 were immunocompetent patients. Immunocompromised children mainly received additional antifungal and antiviral agents, while the immunocompetent children had a higher proportion of antibiotic escalation, antibiotic de-escalation, and addition of antifungal agents (Figure 5D).

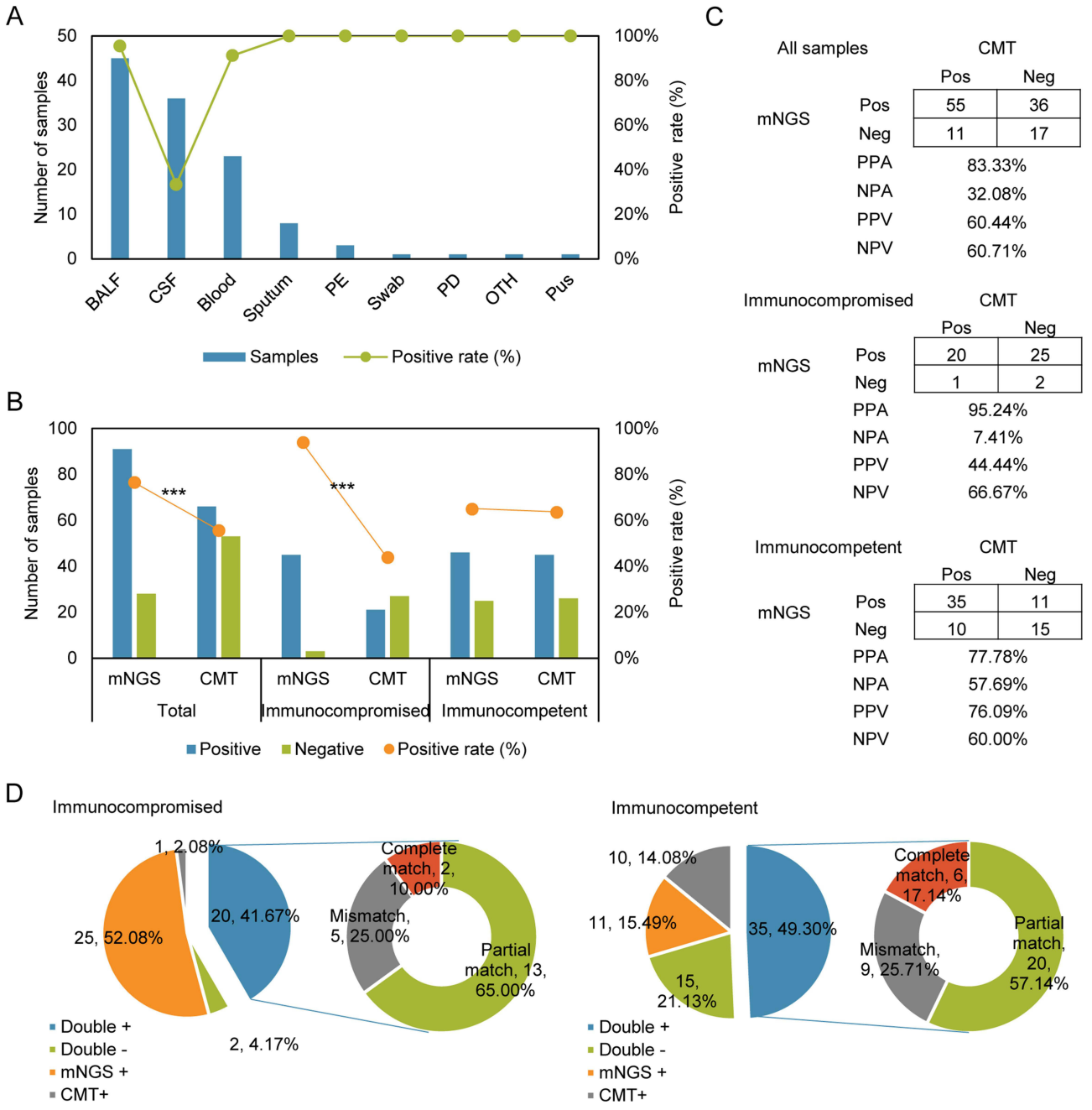

Figure 3 Diagnostic performance and consistence of mNGS and CMT for infection. (**A**) Positive rate of mNGS for different types of samples. (**B**) Positive rates of mNGS and CMT for immunocompromised and immunocompetent patients. (**C**) Positive and negative agreement percentages for mNGS and CMT in the analysis. PPA, positive percent agreement; NPA, negative percent agreement; PPV, positive predictive value; NPV, negative predictive value. (**D**) Concordance analysis between mNGS and CMT method for pathogen detection. The double-positive samples were divided into complete matched, partial matched (at least one pathogen detected by the two methods overlapped), and completely mismatched. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Analysis of Factors Affecting the Prognosis of Critically Ill Pediatric Patients

In this study, 21 out of 119 patients died, resulting in a mortality rate of 17.65%. The mortality rate was significantly higher in immunocompromised patients compared to immunocompetent patients (Table 1). We analyzed the correlation between patient outcomes and clinical indicators, infection symptoms, infection type and immune status. The study found a significant positive correlation between poor prognosis and immunocompromised status, length of hospital stays (LOHS), length of ICU stays (ICU), bloodstream infection, pediatric sequential organ failure assessment (pSOFA), pediatric risk of mortality score (PRISM), neutrophil percentage (NEUT%), total bilirubin (TBIL) and interleukin-6 (IL-6). Conversely, there was

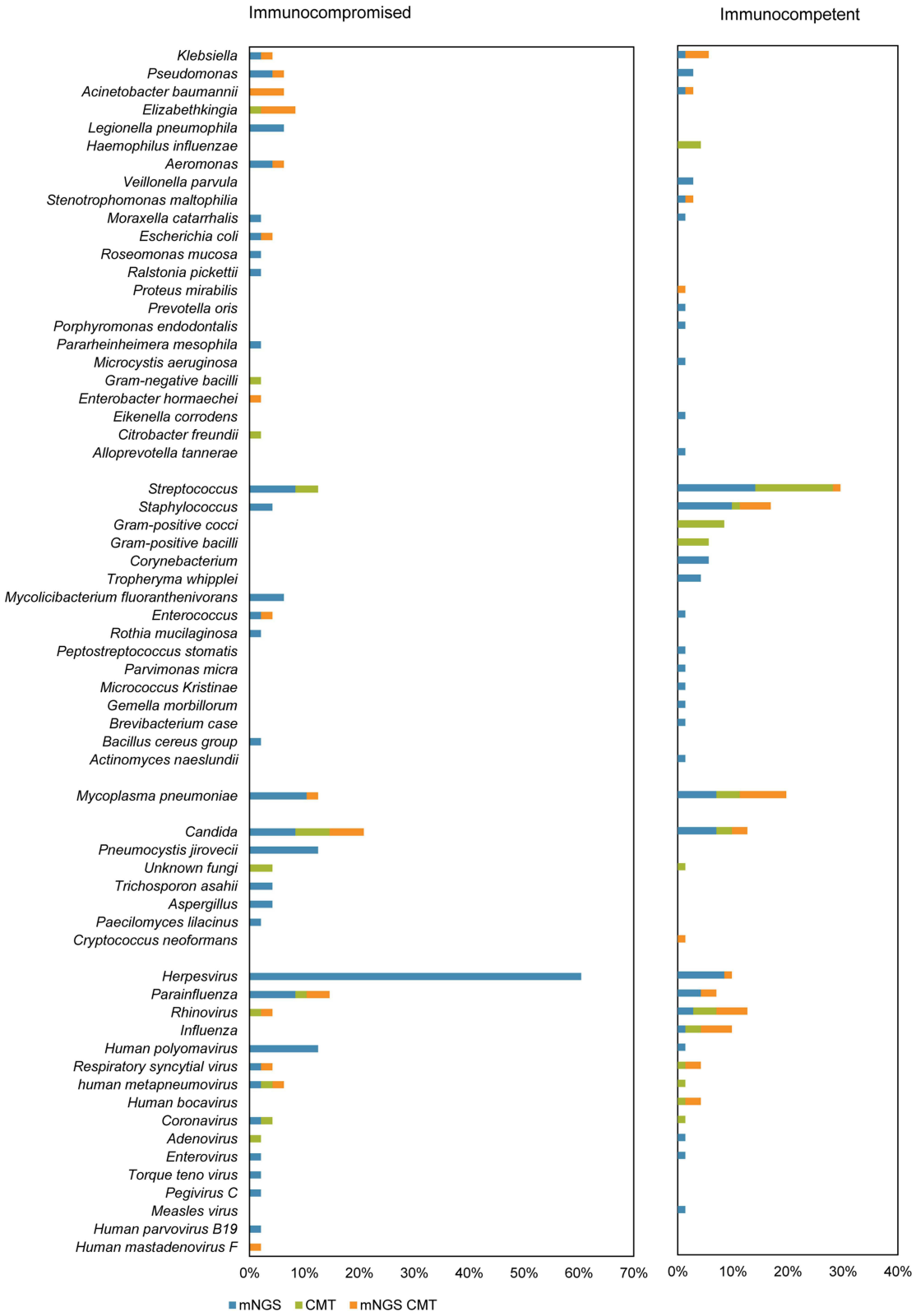

Figure 4 Pathogen profile identified by mNGS and CMT for immunocompromised and immunocompetent patients. The mNGS results only show pathogens that are judged to be possible causes, including the definite, probable and possible pathogen. The X-axis indicates the proportion of the pathogen detected in all immunocompromised or immunocompetent patients.

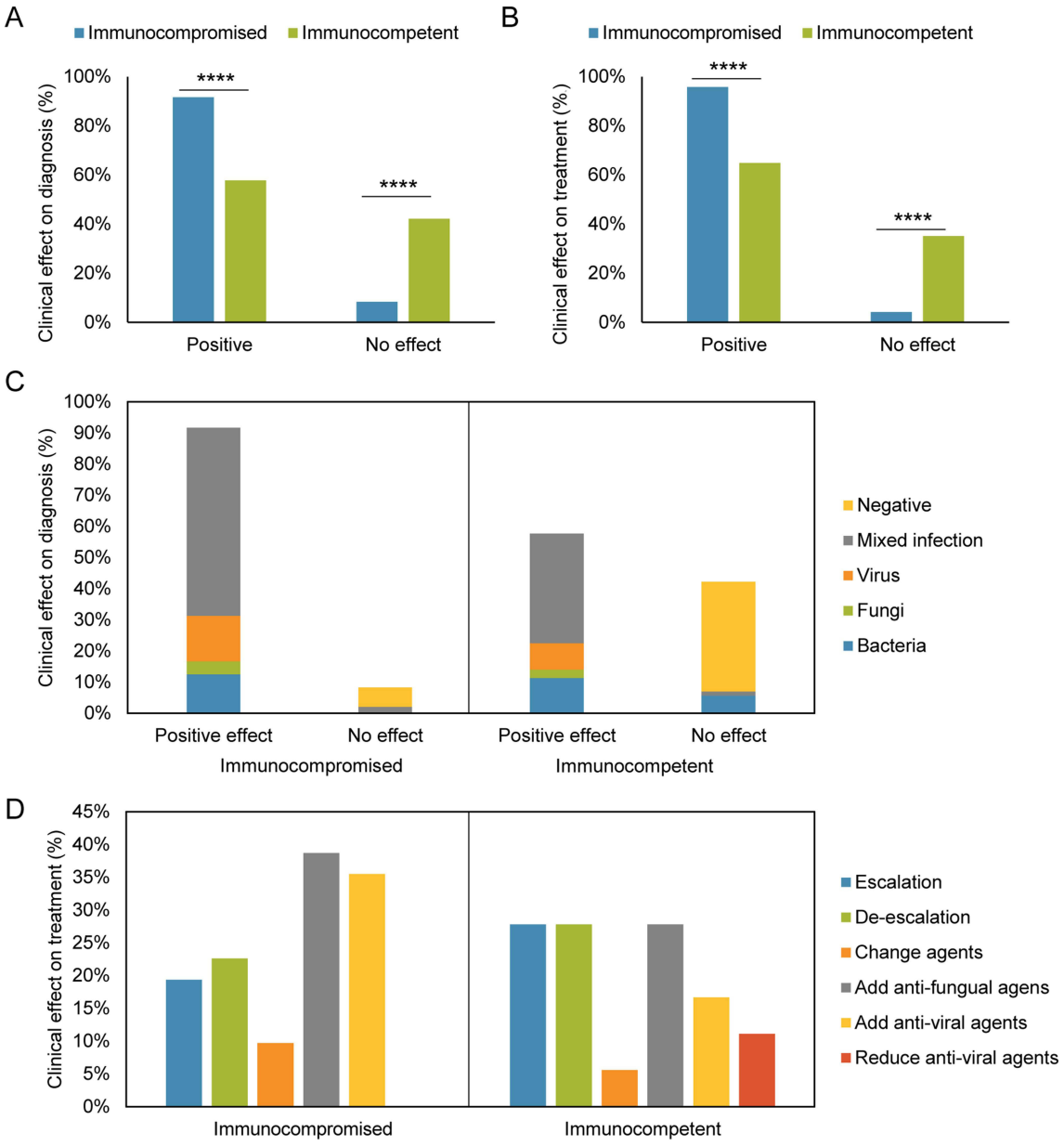

Figure 5 Comparison of clinical impact of mNGS on infection diagnosis and treatment between immunocompromised and immunocompetent patients. (**A**) Clinical effects of mNGS on the diagnose of infection. (**B**) Clinical impact of mNGS on the treatment of infection. ****$P < 0.0001$. (**C**) Clinical effects of mNGS on the diagnose of different types of infection. (**D**) Clinical impact of mNGS on the antibiotic use.

a negative correlation between poor prognosis and lymphocyte count (LY), lymphocyte percentage (LY%), blood platelet (PLT), hemoglobin (HBG) and the ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) (Supplementary Figure 1).

Next, we conducted a multivariate analysis using multiple logistic regression with the significantly correlated indices mentioned above. The results showed that immunocompromised state (OR = 5.62), LOHS (OR = 0.89), ICU (OR = 1.18), PRISM (OR = 1.37), NEUT% (OR = 1.06) and PaO2/FiO2 (OR = 0.9926) were considered independent factors for death (Table 2).

Table 2 Risk Factors for Poor Prognosis

Index[a]	OR[b]	95% CI	*P*-value
Immunocompromised	5.62	1.01–31.18	0.048
LOHS	0.89	0.8–0.98	0.021
ICU	1.18	1.04–1.33	0.009
PRISM	1.37	1.15–1.62	< 0.001
NEUP	1.06	1.01–1.1	0.012
TBIL	1.02	0.99–1.05	0.188
PaO2/FiO2	0.9926	0.9856–0.9996	0.038

Abbreviations: [a]LOHS, length of hospital stays; ICU, length of ICU stays; PRISM, pediatric risk of mortality score; NEUT%, neutrophil percentage; PaO2/FiO2, the ratio of arterial oxygen partial pressure to fractional inspired oxygen. [b]OR, odds ratio.

Discussion

mNGS has been utilized in clinical practice due to its advantages in pathogen identification. Immunocompromised hosts are particularly vulnerable to infection, often presenting with atypical symptoms or prolonged illness, even with more common infections. Diagnosis can be difficult due to low pathogen burden, a lack of standardized tests for uncommon organisms, and the unreliability of immunological tests such as serology in patients with compromised immunity or who are receiving antibody replacement therapy. In this retrospective study, we compared the diagnostic performance of mNGS with CMT, and evaluated the clinical impact of mNGS for critically ill pediatric patients. Our findings demonstrate that mNGS is effective for pathogen detection and treatment, particularly in immunocompromised children.

mNGS was found to be highly valuable in diagnosing critically ill pediatric patients. In immunocompromised patients, the positive rate of mNGS was approximately 50% higher than that of CMT, while no significant differences were observed in immunocompetent patients (Figure 3B). Additionally, compared to CMT results, mNGS demonstrated a PPA of 95.24% and 77.78% were observed for immunocompromised and immunocompetent patients, respectively (Figure 3C). Moreover, the study found that mNGS had a positive effect of 91.67% and 57.75% for diagnosing infections in critically ill immunocompromised and immunocompetent pediatric patients, respectively (Figure 5A). These results suggest that the diagnostic value of mNGS is higher in critically ill immunocompromised pediatric patients compared to immunocompetent patients. Similar results were found in studies conducted on adult critically ill patients,[13] and pediatric infectious diseases.[11] The reason for this phenomenon could be the proportion of multiple site infections in immunosuppressed children is higher than that in immunocompetent children, and the proportion of mixed infections with multiple pathogens is even higher,[15,24] and mNGS has a huge advantage in identifying mixed infections.[25,26] Another possible reasons for the lower impact of mNGS on immunocompetent patients in this study were investigated. We found that, in cases where mNGS results were negative but CMT results were positive for immunocompetent patients, the samples used for mNGS were mainly CSF. And more than 60% of the patients had performed CSF culture, but the culture results were all negative. In contrast, CMT detected respiratory viruses and *Mycoplasma pneumoniae* in blood or respiratory samples using PCR and *Mycoplasma pneumoniae* antibody detection methods. Children with respiratory infections can experience neurological complications, most of which are non-encephalitic encephalopathy.[27] The negative result of CSF mNGS is helpful to identify and distinguish whether the children have CNS infections.

Overall, the application of mNGS has a positive effect on the treatment of 95.83% of immunocompromised children, and on 64.79% of immunocompetent patients (Figure 5B). This suggests that mNGS provided a greater proportion of clinically beneficial anti-infective adjustments for the immunocompromised group compared to the immunocompetent group. A similar result was also found in a previous study conducted on adults.[13] mNGS plays a crucial role in guiding clinical medication adjustment (53.26%, 49/92) and assisting in the determination of the rationality of used drugs (46.74%, 43/92). Previous studies paid more attention to the guiding role of mNGS in clinical medication.[28,29] This

evaluation standard may have underestimated the indirect guiding significance of mNGS. Although clinicians did not adjust the use of antibiotics, positive mNGS results could still provide a basis for antibiotic treatment. A more comprehensive evaluation can better reflect the clinical impact of mNGS.[11,22] For immunocompromised children, mNGS has been used to guide the addition of antiviral and antifungal agents. For immunocompetent children, mNGS is mainly used to guide the escalation and de-escalation of antibiotics and the addition of antifungal agents (Figure 5D). This finding suggests that fungal and viral infections should be considered in the diagnosis and treatment of infections in immunocompromised patients.

Infectious diseases are the leading cause of morbidity and mortality worldwide in children. Immunocompromised pediatrics patients had worse clinical outcomes than immunocompetent patients (Table 1), and the same results were found in adult patients.[15] Identifying the independent risk factors that affect patient prognosis may help to achieve early warning of the disease. Currently, research on the independent risk factors of the prognosis of infectious diseases in children mainly focuses on the infection of specific pathogens. Yang et al identified IL-6 > 100 ng/L as an independent risk factor for the prognosis of children with *Chlamydia psittaci* pneumonia infection.[30] For adults, longer ICU stay, higher acute physiology and chronic health evaluation II (APACHE II) score and SOFA score were risk factors for the death of severe pneumonia patients with acute respiratory distress syndrome (ARDS), and the use of mNGS for clinical pathogen detection was found to be a protective factor.[31] Independent risk factors for 28-day mortality for ICU patients undergoing mechanical ventilation include not performing mNGS, having a high APACHE II score, and hypertension.[32] In our study, immunocompromised state, LOHS, ICU time, PRISM score, NEUT% and PaO2/FiO2 were considered as independent factors for death (Table 2). These studies demonstrate that there are variations in independent risk factors affecting the prognosis of different populations. However, important scoring criteria that represent the severity of the disease and appropriate etiological detection tools can achieve early warning of the disease.

This study has several limitations. It is a retrospective study, single-center review of mNGS with a relatively small sample size. The patients underwent mNGS analysis and CMT based on clinicians' decisions and the patient's preference, which may have led to selection bias. Furthermore, a large cohort study and multicenter studies are recommended to assess the value of mNGS in different pediatric populations.

Conclusion

This study compared the diagnostic performance of mNGS with CMT using multiple types of samples and patients with multiple infectious diseases, and evaluated the clinical impact of mNGS for critically ill pediatric patients. Results suggest that mNGS holds great potential for diagnosing and treating in critically ill pediatric patients with suspected infections, particularly in immunocompromised children. Immunocompromised state, LOHS, ICU time, PRISM score, NEUT% and PaO2/FiO2 were considered as independent factors for poor prognosis of PICU patients.

Ethics Statement

This study was conducted after an agreement from the Ethics Committee of Children's Hospital of Zhejiang University School of Medicine (2022-IRB-262). Due to the retrospective nature of the study, the Ethics Committee waived the requirement for patient consents. The patients were anonymized, and their information was nonidentifiable. In general, all data in this study were obtained in accordance with the Helsinki declaration.

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Disclosure

The authors declare no conflicts of interest in this work.

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