Pediatric sepsis cases diagnosed with group B streptococcal meningitis using next-generation sequencing: a report of two cases

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Abstract

Background: Group B Streptococcus (GBS) is an important cause of invasive infection in neonates and infants. Cerebrospinal fluid (CSF) findings and culture may not show evidence of infection early in GBS meningitis. Next-generation sequencing (NGS) has the potential to detect microbial genetic material in patients with infectious diseases. We report two cases of infantile sepsis of GBS meningitis with negative results for CSF culture tests, but positive results for NGS analysis.

Case presentation: Patient 1 was a 22-day-old male infant diagnosed with sepsis and meningitis. His CSF findings showed pleocytosis, decreased glucose, and increased protein levels. However, CSF and blood culture results at admission were negative. He received a total of 3 weeks of treatment with ampicillin and cefotaxime, and showed clinical improvement. GBS was detected through NGS analysis of CSF collected at admission. Patient 2 was a 51-day-old male infant with sepsis. CSF findings on admission were normal, and blood and CSF cultures were also negative. Intravenous ampicillin and cefotaxime treatment were initiated. Treatment was de-escalated to ampicillin alone because Enterococcus faecalis was cultured from urine. He was discharged after a total of 1 week of antibiotic treatment. Six days after discharge, he was re-hospitalized for sepsis. Blood and CSF cultures were negative, and E. faecalis was again cultured from urine. He received a total of 3 weeks of ampicillin treatment for enterococcal-induced nephritis and did not relapse thereafter. NGS pathogen searches were retrospectively performed on both blood and CSF collected at the first and second admission. GBS was detected in the CSF collected at the first admission, but no significant pathogen was detected in the other samples. Inadequate treatment for GBS meningitis at the first admission may have caused the recurrence of the disease.

Conclusion: Infantile sepsis may present bacterial meningitis that is not diagnosed by either culture testing or CSF findings. NGS analysis for CSF may be useful for confirming the diagnosis of bacterial meningitis.

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Background

Sepsis is one of the leading causes of death among children, and the percentage of all global deaths related to sepsis is highest in children under 1 year of age [1]. Despite its severity, pathogens are not identified in one-third of children with sepsis [2]. Group B streptococcus (Streptococcus agalactiae; GBS) is a leading cause of sepsis and bacterial meningitis in infants under 3 months of age. The estimated incidence of GBS infection in infants is approximately 0.5 per 1000 live births [3, 4]. GBS infection that occurs 7–89 days after birth is defined as a late-onset GBS infection. Sixty-five percent of late-onset GBS infection presents as unfocused bacteremia and 20–43% presents as meningitis [3, 5]. Late-onset GBS meningitis is associated with permanent neurologic sequelae in 50% of survivors [6] thus, accurate diagnoses and treatment are required.

Next-generation sequencing (NGS) technology makes it possible to analyze large amounts of nucleic acid sequence data contained in samples in a single assay. Therefore, untargeted metagenomic NGS of clinical samples has been applied for the comprehensive diagnosis of infections, including viruses, bacteria, fungi, and parasites [7]. We previously detected pathogens from blood samples and cerebrospinal fluid (CSF) from patients with sepsis and encephalitis [8, 9]. Here, we report two infants with sepsis that had GBS meningitis revealed through NGS.

Case presentation

We describe two pediatric cases of late-onset GBS meningitis with sepsis. They were born by vaginal delivery after uneventful pregnancies. Prenatal GBS screening through a vaginal swab was negative in both cases. Sepsis is defined as a systemic inflammatory response syndrome with an infectious disease. Systemic inflammatory response syndrome refers to four parameters: body temperature, tachycardia, hyperventilation, and white blood cell count. Specifically, it is diagnosed when body temperature is > 38.5 °C or < 36.0 °C, or leukocytosis or leukocytopenia is required, and a total of two or more parameters exist [10]. Tachycardia is defined as a heart rate > 180 beats per minute, and hyperventilation is defined as a respiratory rate > 40 per breathes minute (from 1 week to 1 month old) and > 34 breathes per minute (from 1 month to 1 year old).

Case 1

A 22-day-old male infant was seen in the emergency room of TOYOTA Memorial Hospital with a 7-h fever history to 38.6 °C and vomiting. He had a bulging anterior fontanelle and peripheral cyanosis. Initial blood examination showed a white blood cell count of 13.1 × 10^3/μL and a C-reactive protein (CRP) level of 12.0 mg/dL. A lumbar puncture was performed, and examination of the CSF showed pleocytosis (48,896 cells with 91% polymorphonuclear cells), a decreased glucose level of 25 mg/dL, and an increased protein level of 326 mg/dL. He also underwent a sepsis evaluation, including blood and CSF cultures. Leukocytes and bacteria were not seen in Gram staining. He was empirically administered ampicillin (280 mg/kg/day) and cefotaxime (280 mg/kg/day) for bacterial meningitis according to the ESCMID guidelines [11], and intravenous immunoglobulin therapy (1000 mg/kg) was administered on day 2 of admission. The fever broke on day 4 of admission. No conclusive cultures from either blood or CSF were found to guide treatment and de-escalation was not performed. He received a total of 3 weeks of antimicrobial treatment and showed clinical improvement. Magnetic resonance imaging of the brain was performed prior to discharge, and subdural edema and cerebral infarction were noted, but there were no clinical signs. He recovered without any noticeable sequelae after discharge. Later exploratory NGS identified GBS from his CSF collected at admission (Table 1, Fig. 1). The PCR result for GBS was negative using the CSF that showed positive results for NGS (data not shown).

Case 2

A 51-day-old male infant presented to the emergency room of Nagoya Memorial Hospital with a 4-h fever and irritability. Upon examination, body temperature was 38.9 °C, pulse rate was 214 beats per minute, respiratory rate was 48 breathes per minute, and SpO2 measured by a pulse oximeter was 99% in room air. Initial blood examination showed leukocytosis (27.4 × 10^3/μL) and a

### Table 1  Next-generation sequencing data of clinical samples

| Case No. | Admission | Sample   | Total Sequencing Read (reads) | All microorganism Derived Read (reads) | Streptococcus agalactiae Number of Reads (reads) | Mapping coverage (%) |
|----------|-----------|----------|-------------------------------|----------------------------------------|-----------------------------------------------|----------------------|
| 1        |           | CSF      | 8,797,854                     | 3754                                   | 1078                                          | 6.5                  |
| 2        | 1st       | CSF      | 181,132                       | 358                                    | 135                                           | 0.79                 |
| 2        | 1st       | Serum    | 155,378                       | 494                                    | 0                                             | 0                    |
| 2        | 2nd       | CSF      | 6,515,464                     | 3348                                   | 0                                             | 0                    |
| 2        | 2nd       | Serum    | 10,209,784                    | 638                                    | 0                                             | 0                    |

CSF cerebrospinal fluid
CRP elevation of 2.13 mg/dL. He also had peripheral cyanosis and was diagnosed with severe sepsis. He underwent a sepsis evaluation, including blood, CSF, and urine cultures. There was no leukocytosis in either the CSF or urine. He was initially administered intravenous ampicillin (300 mg/kg/day) and cefotaxime (300 mg/kg/day). On day 2 of admission, his vital signs normalized. Blood cultures and CSF cultures were negative, while Enterococcus faecalis (10^3 CFU/mL) was detected in urine cultures on day 4 of admission. He received ampicillin (200 mg/kg/day) for 1 week before discharge for enterococcal urinary tract infection. He was discharged after confirmation of a continued normal temperature, no abnormalities on physical examination, and improved blood test results. Six days after discharge, he returned to the hospital with a fever of 39.0 °C, tachycardia, and peripheral circulatory insufficiency. He underwent a sepsis evaluation with lumbar puncture and was empirically administered ampicillin (300 mg/kg/day) and cefotaxime (300 mg/kg/day) for sepsis. The CSF findings were within normal limits. No bacteria were cultured from the blood or CSF. A poorly enhanced area in the kidney was found using contrast-enhanced computed tomography (CT) on the day after admission. In addition, 10^3 CFU/mL E. faecalis was detected in urine culture, and ampicillin (200 mg/kg/day) was administered for enterococcal bacterial nephritis for 3 weeks. Voiding cystourethrogram revealed Grade 4 reflux findings on the right side strongly indicating bacterial nephritis. There was no recurrence of the disease. NGS analysis was performed retrospectively on serum and CSF collected at the first and second admission to detect pathogens. GBS was detected in the CSF collected at the first admission, but no significant pathogen could be detected in the other samples (Table 1, Fig. 1). The PCR result for GBS was negative using the CSF that showed positive results for NGS (data not shown).

**Sample collection and preparation of sequencing**

Serum and CSF were collected under aseptic conditions. DNA was extracted from 140 μL serum and CSF, using the QIAamp UCP Pathogen Mini Kit (Qiagen, Hilden, Germany). DNA sequencing libraries were prepared using a Nextera XT library Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions with slight modification [8]. An Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and a QX200® Droplet Digital PCR System (Bio-Rad, Richmond, CA, USA) were used for the quantification of NGS libraries. Sequencing was performed using the Illumina HiSeqX.

**Data analysis**

Sequence data were processed using the metagenomic analysis pipeline PATHDET version 1.0 [12] to detect pathogen-derived sequences. PATHDET reported the sequence-derived pathogen based on previously established threshold criteria [8].

![Relative abundance of microorganisms in two infantile sepsis cases. At first admission, Streptococcus (the genus level of taxonomic hierarchy) accounted for most of the detected microorganisms in both cases. Furthermore, Streptococcus agalactiae (arrowhead) was the most abundant among the Streptococcus](image-url)
NGS analysis that uses only small sample volumes is advantageous because sample volumes are often limited. There was a discrepancy in the results between NGS and PCR in the present cases; the NGS results for GBS were positive and the PCR results were negative. A previous report described the diagnosis of neurological infections using NGS with CSF samples; however, this report did not compare with the results using PCR methods [20]. The size of the amplified DNA was similar in this study; however, the efficacy of amplification may be influenced by unknown factors. Additionally, the region amplified by PCR was not covered by NGS. The difference in sensitivity may be due to differences in the amplification efficiency at different locations of the genome.

In Case 2, the NGS result for GBS was positive in CSF; however, the result was negative in blood. A previous report stated that blood cultures are positive in at least half of patients with bacterial meningitis [21, 22]. Other studies have reported that a substantial proportion (33 to 53%) of neonates with culture-proven meningitis have negative blood cultures [23]. The amounts of bacteria may be skewed in different types of clinical samples. Another possibility is that the efficiency for growth is different between CSF and blood samples. With regard to NGS, the huge amount of host-derived nucleic acids prevents detection of microorganism-derived sequences. In most cases using plasma samples, less than 1% of extracted DNA originates from microorganisms during blood-stream infection [8]. Moreover, NGS analysis using the blood of septic patients requires further efforts to remove human cell-free DNA because this may increase when systemic inflammation is progressing due to sepsis [24]. This study had a few limitations. First, we only experienced two cases. The number of cases assessed using NGS should be increased. Second, the diagnostic tests could not be repeated to confirm negative results because of the small number of CSF samples.

In conclusion, we report two cases of GBS meningitis that were revealed retrospectively by NGS analysis. We also showed that GBS meningitis was lurking in pediatric sepsis negative for CSF culture testing with normal CSF findings. NGS may be useful as a diagnostic tool in the specific clinical settings presented in the present report.

### Abbreviations

GBS: Group B streptococcus; NGS: Next-generation sequencing; CSF: Cerebrospinal fluid; CT: Computed tomography

### Acknowledgements

Not applicable.

### Authors’ contributions

KH, TOg, and YI designed the study, MS, MM, YK and SH collected clinical samples and data. KH, MY, TOk, and YT performed the experiments. KH and TS conducted the bioinformatics analyses. KH, MS, NT, SH, and JK were responsible for data interpretation. KH, TOg, and YI drafted the manuscript. All authors read and approved the final manuscript.
Funding
This work was supported by a Grant-in-Aid for Young Scientists 20K17464 and the Kawano Masanori Memorial Public Interest Incorporated Foundation for Promotion of Pediatrics, respectively. The funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request. Sequencing reads generated in this study were deposited at the DDBJ Sequence Read Archive under the following accession number: DRX 241918–241922.

Declarations

Ethics approval and consent to participate
This case series was approved by the Institutional Review Board of Nagoya University Hospital (no. 9069).

Consent for publication
Written informed consent was obtained from the guardians of both patients.

Competing interests
The authors declare no competing interests.

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Received: 11 January 2021 Accepted: 24 May 2021

Published online: 05 June 2021

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