Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.49.007861

Usefulness of the Acute Phase Reactants (APR) Score: Early Detection of Delayed Onset of Sepsis in Extremely Low Birth Weight Infants by APR Score

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ARTICLE INFO

Received: March 31, 2023

Published: April 18, 2023

Citation: Toshihiko Nakamura, Haruo Goto, Jin Xiuri and Shingo Yamada. Usefulness of the Acute Phase Reactants (APR) Score: Early Detection of Delayed Onset of Sepsis in Extremely Low Birth Weight Infants by APR Score. Biomed J Sci & Tech Res 49(5)-2023. BJSTR. MS.ID.007861.

ABSTRACT

Background: The production of acute phase reactants (APRs) in the liver is stimulated by increased inflammatory cytokines. Therefore, inflammatory cytokines are more advantageous than APRs for early diagnosis of neonatal sepsis. There is a machine Latessier TM that can produce results after about 3 minutes with a small amount of sample of 10 μL each at the same time of C-reactive protein (CRP), α1 acid glycoprotein (AGP), and haptoglobin (Hp). This instrument will be used to search for an advantageous method for diagnosing late-onset sepsis in extremely low birth weight infants (ELBWI).

Methods: Twenty-nine ELBWI who were diagnosed with clinical late-onset sepsis (LOS) and underwent sepsis work-up to make a definitive diagnosis were retrospectively examined for changes in CRP, AGP, and Hp over time. Sixty-six ELBWI without sepsis were selected as control.

Results: At the time of sepsis work-up, 10 patients (34.5%) had no significant increase in CRP. However, AGP was already significantly elevated in 26 cases (89.7%). Furthermore, 26 cases (89.7%) had an increase in AGP prior to an increase in CRP (this phenomenon is called warning sign). Only one patient had neither CRP nor AGP elevated at sepsis work-up. This warning sign is associated with 89.7% sensitivity and 69.7% specificity in diagnosing sepsis in ELBWI.

Conclusion: By simultaneously measuring and tracking APRs in microsamples, if AGP rises prior to CRP elevation (warning sign), continue follow-up with caution. And, when clinical sepsis symptoms are identified, begin sepsis work-up and treatment immediately. Measurement of APRs in this manner can help in the early diagnosis of LOS. The usefulness of APR score was also confirmed in this respect.

Keywords: Acute Phase Reactant Score; α1-Acid Glycoprotein; C-Reactive Protein; Extremely Low Birth Weight Infant; Late-Onset Sepsis

Abbreviations: APRs: Acute Phase Reactants; CRP: C-Reactive Protein; AGP: α1 Acid Glycoprotein; Hp: Haptoglobin; ELBWI: Extremely Low Birth Weight Infants; NICU: Neonatal Intensive Care Unit; PICC: Peripherally Inserted Central Venous Catheter

Introduction

Neonatal infection has few specific symptoms. In particular, sepsis in extremely low birth weight infants (ELBWI) is a serious complication that affects the prognosis of these infants, and thus, early diagnosis and early treatment of this condition are essential [1-5]. We have focused on three substances, C-reactive protein (CRP), α1-acid glycoprotein (AGP), and haptoglobin (Hp), which are considered acute phase reactants (APRs) [6]. Thirty-five years have passed since we first proposed a system of APR scoring ranging from 0 to 3 points, with a score of 1 point for each of the three APRs if they exceed their reference values according to the number of days after birth, and a score of 0 if not [7]. Subsequently, we compared the APR score and cytokine profile simultaneously and confirmed that the cytokine profile was superior in early detection of infections in the early neonatal period. Simultaneously, we found that the APR score was superior to the cytokine profile in timing treatment initiation and termination of antibiotic administration [8]. Cytokine profiles cannot be measured at the bedside and are not suitable for real-time diagnostics in the clinical setting. Therefore, we now report on the discovery of a phenomenon that enables early diagnosis even with the same test that is based on the accumulation of daily APR score results.

Material and Methods

In total, 95 ELBWI with serial measurement of APR score, who were born at the Nagoya City University West Medical Center and the Japanese Red Cross Musashino Hospital and were immediately admitted to the neonatal intensive care unit (NICU) over the 11 years and 8 months from January 2011 to August 2022, were selected. In neonates who were diagnosed as having clinical sepsis and whose causative organism was identified as a result of immediate blood culture, onset beyond 72 hours after birth was defined as late-onset sepsis (LOS) [9,10], and these infants were included in this study. At our facility, an ELBWI whose mother suffered premature rupture of membranes or is suspected of having intrauterine infection undergoes various cultures including blood culture on admission, and then two antibiotics, ampicillin and gentamicin, are given as empiric therapy. If the APR score is within the range of 0 to 1 indicating non-infection, antibiotics administration is continued for 3 days until a negative blood culture is confirmed [7]. Bifidobacterium as a prebiotic is enterally administered with mother's milk from day 1.

To calculate the APR score, capillary blood is centrifuged, evaluated for jaundice, the separated serum portion is used for the measurement, and the remainder is discarded. Measurement is performed 2–3 times a day from 0 to 3 days of age, and at least 1 time per day thereafter, and as a principle, follow-up continues until confirmation of a decline in CRP, AGP, and Hp levels. Diagnosis of clinical sepsis is made according to the following factors: results of various culture tests including that of blood and blood tests (white blood cell count and its fraction, platelet count, blood sugar level, blood gas analysis, and APR score); presence of hyperthermia (hypothermia is predominant in very low birth weight infants); cardiovascular instability (e.g., low blood pressure, increased episodes of bradycardia); skin petechiae, red rash, sclerema, etc.; respiratory system instability (exacerbation of apnea attacks, increased inspiratory oxygen concentration required, increased number of respiratory conditions, etc.); digestive system abnormalities (feeding intolerance, increased distension, etc.); and instability of the nervous system (irritability, lethargy, hypotonia, etc.) [10].

For calculation of the APR score, the Latessier TM, a dedicated device manufactured by ARKRAY, Inc. (Kyoto, Japan), was used. Measurement of the three APRs requires 10 μL of serum for each APR. In our experience, centrifugation of a single 80-μL capillary tube for jaundice measurement is sufficient for ELBWI unless the blood is polycythemic with a hematocrit value of more than 60%. After first entering the birth weight and age of the baby, the Latessier TM recognizes the reference values of the three APRs at the time of measurement, calculates 1 point if the upper limit is exceeded, 0 points if not, and finally totals the score. Results are given on a scale of 0 to 3. A micropipette with an application sensor is attached using a dedicated tip that can directly aspirate serum from a centrifuged capillary tube. CRP is measured automatically using latex immunoturbidimetry, whereas AGP and Hp are measured using immunonephelometric. Measurement times are as short as 30, 60, and 60 seconds, respectively, and if the reagents for application are incubated in advance, it takes only about 3 minutes from input to calculation of the results. It is potentially possible to measure the values at the bedside and to measure the infant's APR in real time. Measurement sensitivity in the APR score calculation mode is 0.25 to 15.0 mg/dL for CRP, 20 to 300 mg/dL for AGP, and 20 to 300 mg/dL for Hp. Furthermore, the scoring of low birth weight infants including the ELBWI in this study was as follows: CRP ≥ 1.0 mg/dL: 1 point; AGP ≥ 30 mg/dL from 72 to 96 hours after birth: 1 point and ≥ 40 mg/dL after 96 hours after birth: 1 point; and Hp ≥ 20 mg/dL until 72 hours after birth: 1 point and ≥ 50 mg/dL after 72 hours after birth: 1 point. Infectious disease is diagnosed when the APR score is 3 points or 2 points, with CRP and AGP scoring 1 point each [6,7].

In Japan, APR score measurement is already covered by health insurance and is one of the infection control measures undertaken for newborns. Review of the present study by the involved hospitals' ethics committees was judged as unnecessary because this is a retrospective review, and the individuals in this study cannot be identified. When explaining the treatment plan for their infant to parents mainly at the time of admission, we indicate that the APR examination is necessary for systemic management and obtain their informed consent at that time. Comparisons between two proportions were performed with

Chi-square or Fisher's exact test. Stat Mate IVTM (Tokyo, Japan) statistical software was used for the statistical analysis. A P value of 0.05 was considered statistically significant.

Results

Patient Background

During the study period, 124 ELBWI were hospitalized in total and 95 ELBWI were selected. Birth weight ranged from 382 to 988 g, gestational age was 22 weeks 2 days to 31 weeks 5 days, and the male: female ratio was 50: 46. Of the 124 patients, 6 died (including those with congenital malformations), resulting in a survival rate of 95%. Twenty-nine patients (23.4%) with blood culture-positive LOS were included in this study. 66 patients without LOS ware included in control. Two cases of sepsis onset occurred after 50 days of age, but 26 cases (89.7%) were concentrated within 1 month after birth, between 4 and 31 days of age. Furthermore, 16 patients (62.1%) developed symptoms within 2 weeks after birth, and even within 1 month after birth, there was a tendency for sepsis to be more frequent in the first half of the month.

Pathogens Causing LOS

Staphylococcus epidermidis accounted for 14 cases, S. aureus (including methicillin-resistant S. aureus) for 13 cases, and Staphylococcus spp. accounting for the majority (93.1%). One patient each had group B hemolytic streptococci, Escherichia coli. And Pseudomonas aeruginosa and fungi were not identified in any of the patients.

Postnatal Age and CRP Level at Time of Blood Culture

The distribution of CRP levels at the time blood culture was performed after diagnosis of clinical sepsis is shown in Figure 1. There were 10 patients (34.5%) in whom the CRP was < 1 mg/dL and no significant increase was observed, and the APR score for CRP was 0 points. Thus, approximately one-third of the patients with blood culture-positive blood who were suspected of having clinical sepsis had no significant elevation of their CRP level.

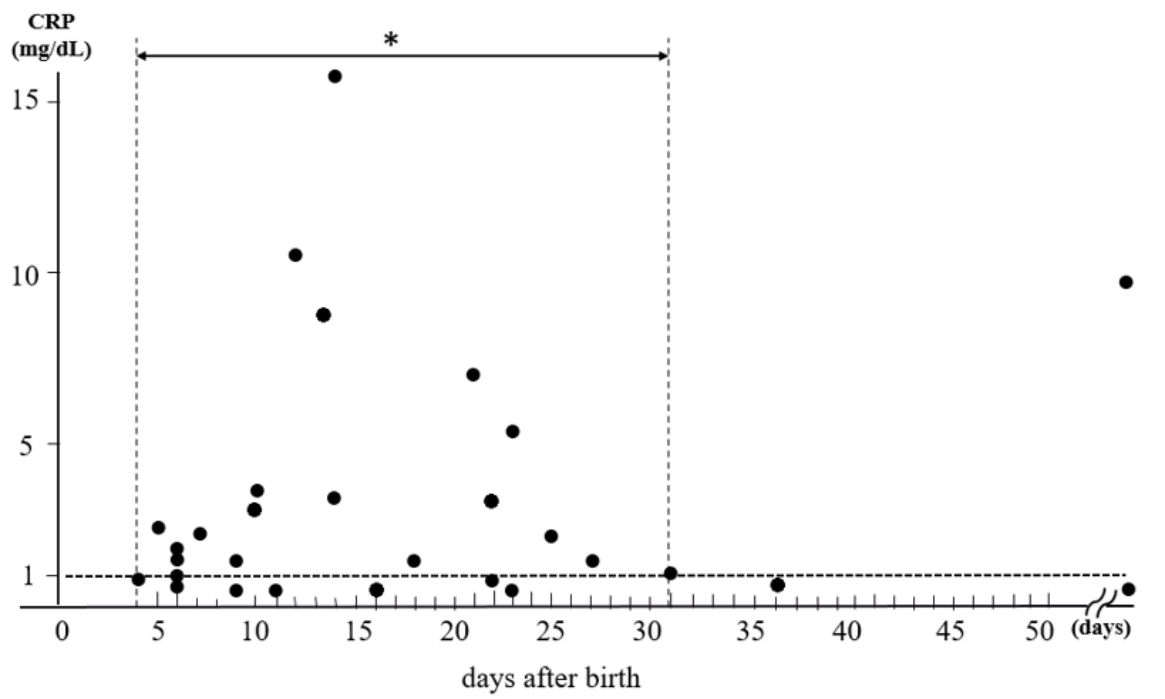

Figure 1: Age at diagnosis of clinical sepsis and CRP value.

Note: Of the 29 cases of delayed sepsis, 26 cases (89.6%) were concentrated within the first month of life. Only 10 cases were CRP-positive at the time of clinical diagnosis.

Postnatal Age and AGP Value at Time of Blood Culture

When blood culture was performed after diagnosis of clinical sepsis, AGP was significantly elevated, and the APR score was 1 point in 26 patients (89.7%), which accounted for the majority (Figure 2).

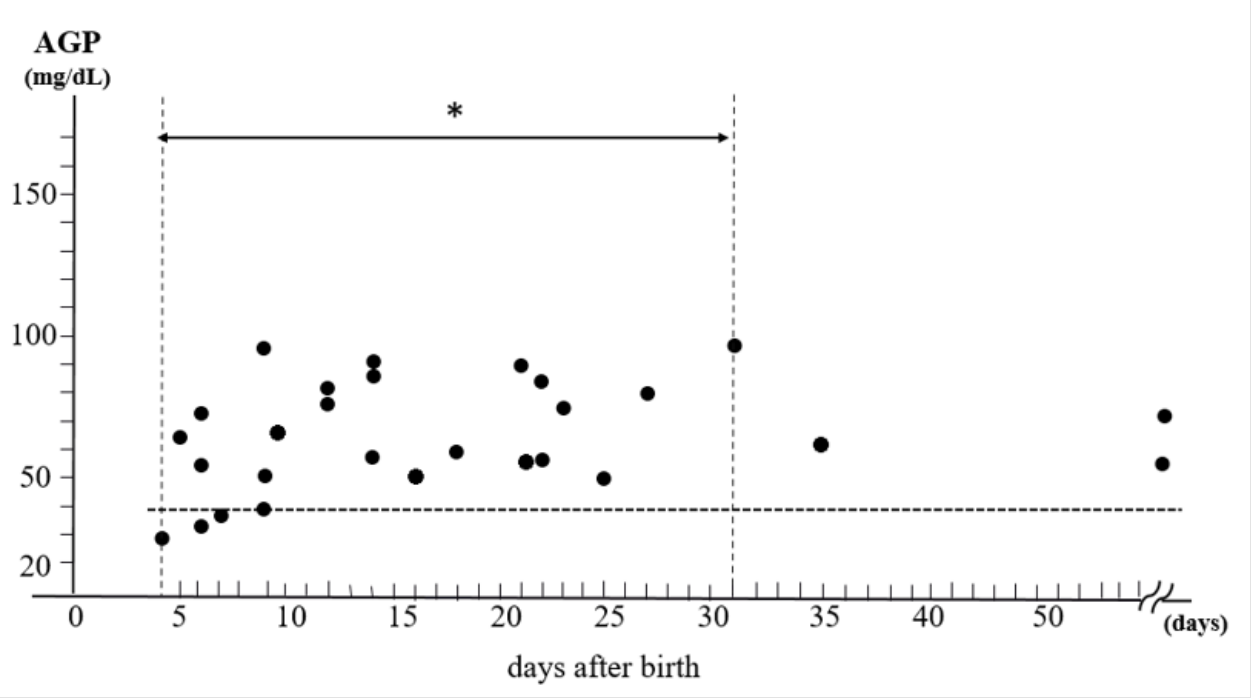

Figure 2: Age at diagnosis of clinical sepsis and AGP value.

Note: At the time of clinical diagnosis, the significant increase in AGP was 89.6% in 26 cases, which was clearly higher than the CRP positive rate.

Presence or Absence of Increase in AGP Prior to Increase in CRP

An increase in AGP preceding an increase in CRP was observed in 26 of 29 patients (89.7%). We have conveniently called this phenomenon in which the AGP rises prior to the rise in CRP as the "Warning Sign" of sepsis. At the time of blood culture, CRP was elevated in 19 patients (65.5%), whereas AGP was not elevated in only 2 patients (10.5%). Only 1 of 10 patients without the rise in CRP (10%) had no elevation of AGP. In other words, only 1 of 29 patients (3.4%) had negative for both CRP and AGP according to the APR score when blood cultures were performed after diagnosing clinical sepsis. A chi-square test revealed that Waring Sign was significantly associated with LOS ($p<0.001$). This warning sign is associated with 89.7% sensitivity and 69.7% specificity in diagnosing sepsis in ELBWI.

Specific Examples of Postnatal APR Score

One of the 19 patients with the "warning sign" who were positive for CRP at the time of blood culture, and 1 of the 19 patients with the "warning sign"' who had elevated levels of CRP and AGP at the time of the clinical sepsis diagnosis are presented in Figures 3 & 4. We also present one patient who had an APR score of 0 from the beginning to end of treatment, and one patient who had the "warning sign" and did not show subsequent elevation of CRP or clinical symptoms suggestive of sepsis in Figures 5 & 6.

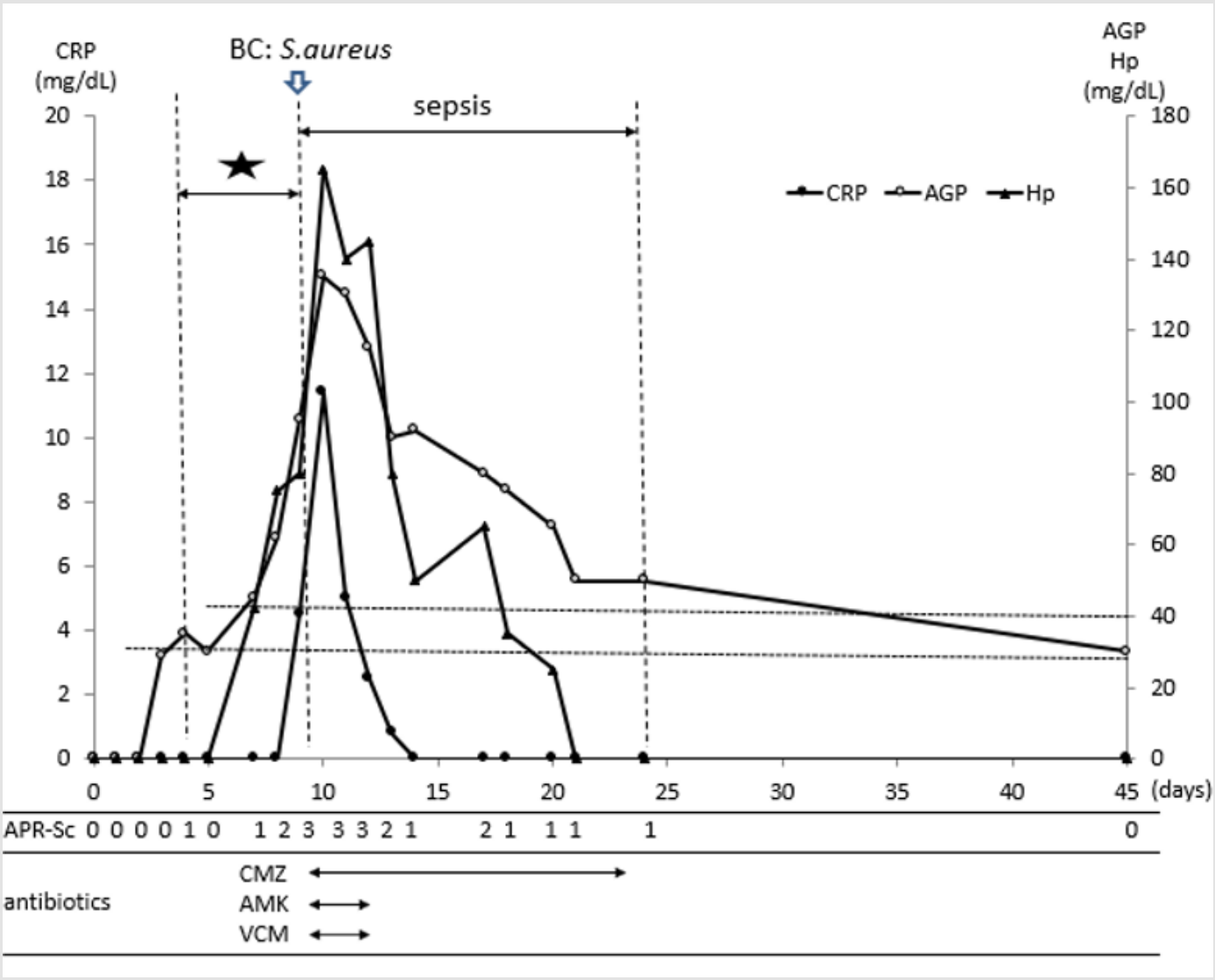

Figure 3: Serial changes in APRs before and after the onset of sepsis.

Note: A case in which an increase in AGP preceded an increase in CRP.

A female with a gestational age of 24 weeks and 5 days and a birth weight of 708 g. She was given artificial lung surfactant (S-TA) after a diagnosis of respiratory distress syndrome (RDS) and continued mechanical ventilation. Abdominal distension increased and AGP increased from day 4 onwards. She was diagnosed with clinical sepsis at 9 days of age. At that time, CRP increased significantly for the first time.

BC: blood culture, S. aureus: Staphylococcus aureus

★ Warning Sign

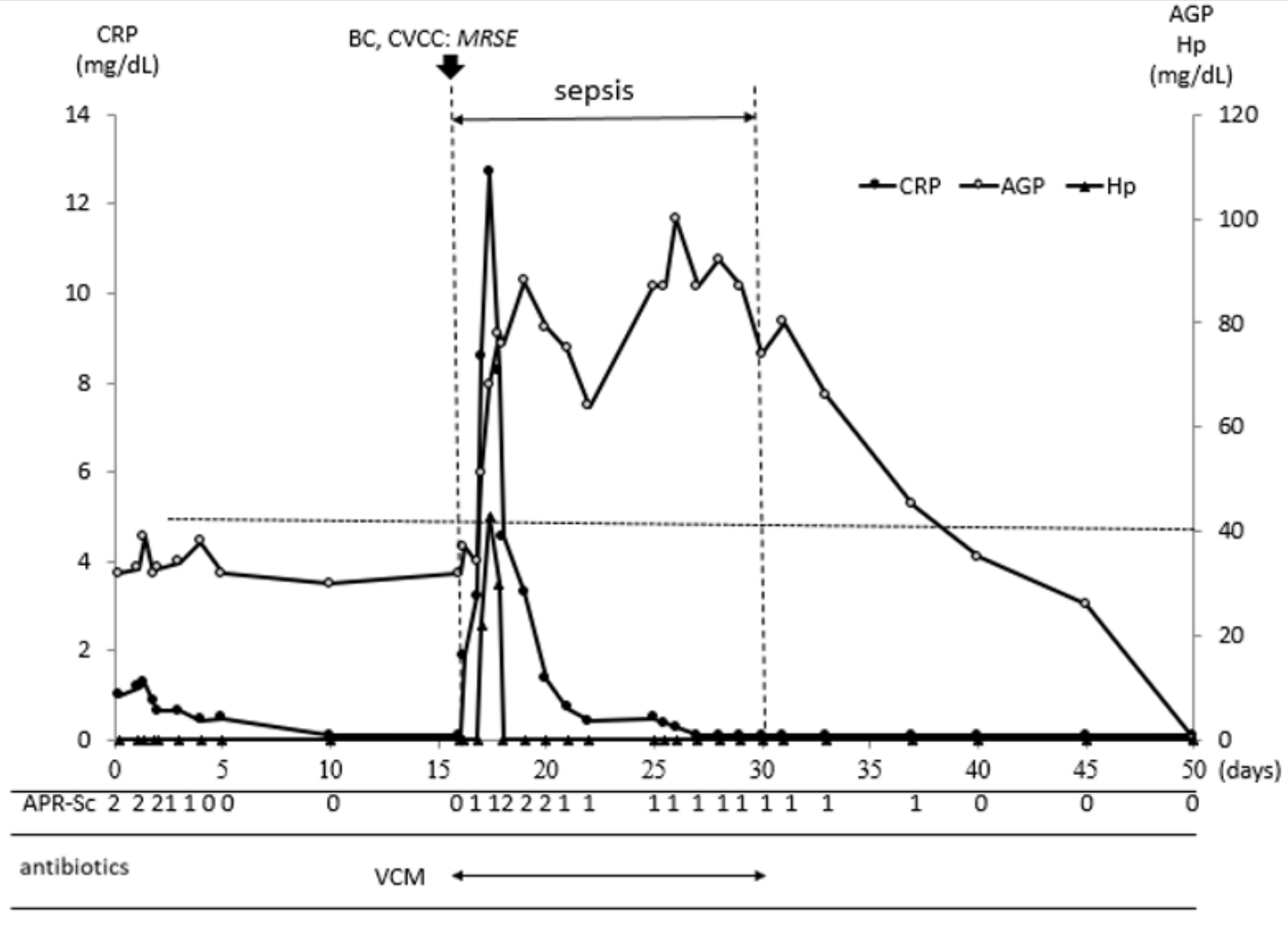

Figure 4: Serial changes in APRs before and after the onset of sepsis.

Note: A case where AGP and CRP increased at the same time.

A female with a gestational age of 27 weeks and 2 days and a birth weight of 912 g. No RDS, continued respiratory management with N-CPAP. He was 16 days old, presented with sudden not doing well, and was diagnosed with clinical sepsis. CRP and AGP increased 6 hours after diagnosis. Methicillin-resistant Staphylococcus epidermidis (MRSE) was isolated from the peripherally inserted central venous catheter (PICC) tip and blood cultures.

DOI: 10.26717/BJSTR.2023.49.007861

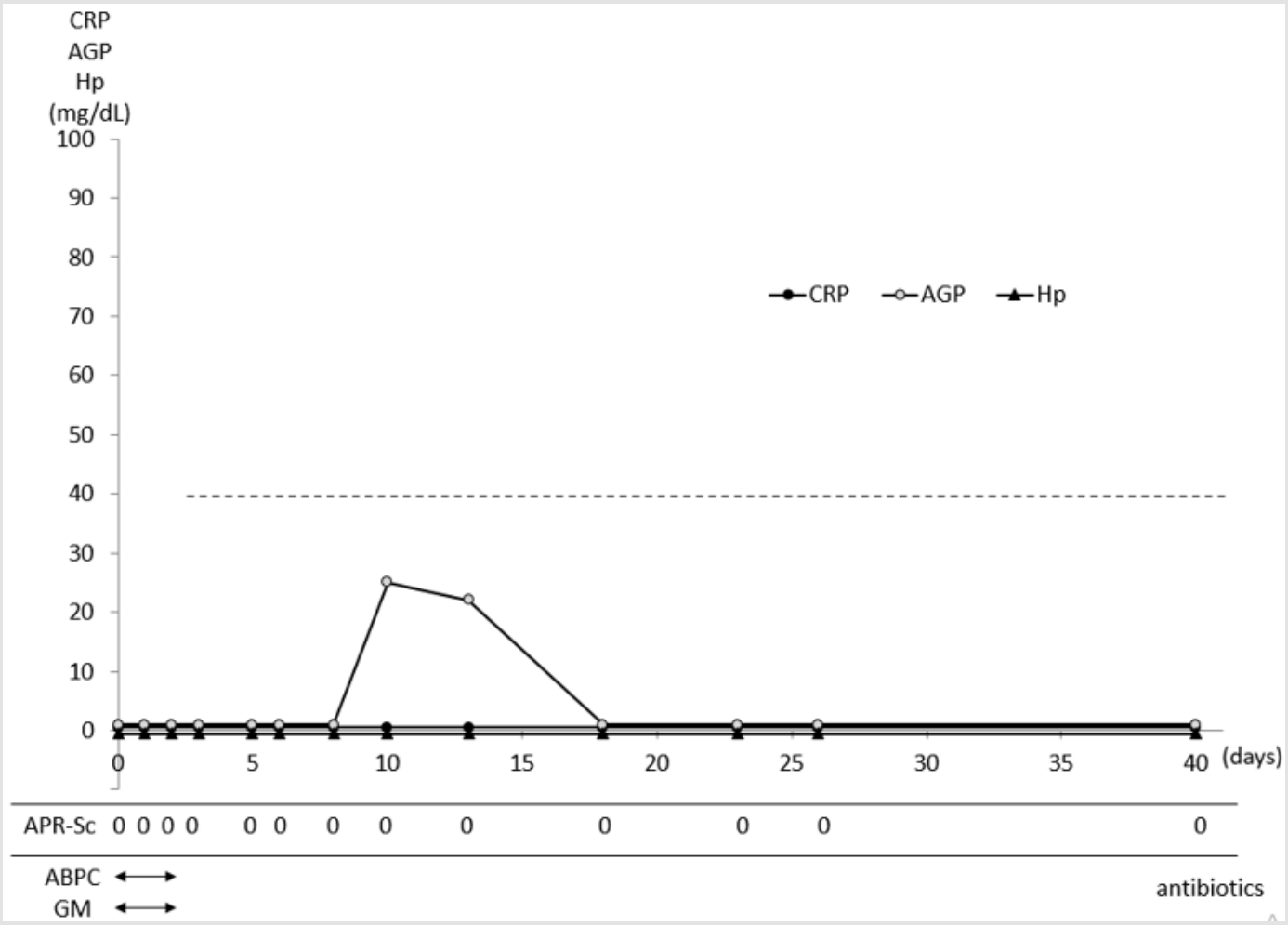

Figure 5: Serial changes in APRs for ELBWIs who did not develop sepsis.

Note: An example that did not show a warning sign.

A female with a gestational age of 26 weeks and 5 days and a birth weight of 834 g. After the diagnosis of RDS, S-TA was administered, and mechanical ventilation therapy was performed until 27 days of age. AGP was slightly elevated from 8 to 13 days of age, but APR-Sc score was 0 from beginning to end.

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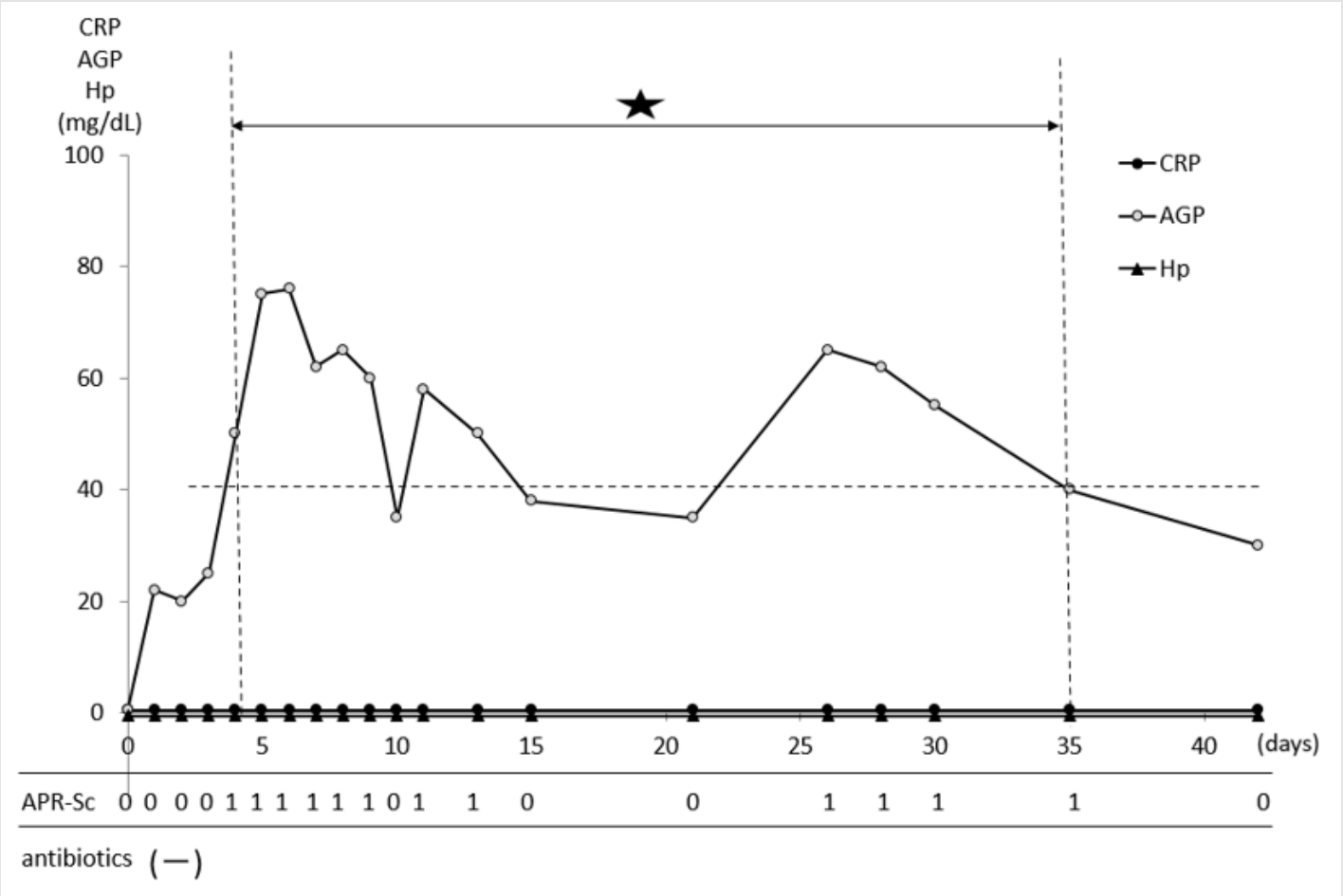

Figure 6: Serial changes in APRs for ELBWIs who did not develop sepsis.

Note: A case that showed a Warning Sign but did not develop sepsis.

A female infant with fatal growth retardation, gestational age 30 weeks 2 days, birth weight 840 g. She had no respiratory problems from birth and continued her systemic management while observing changes in APR-Sc without administration of antibiotics. Only AGP scored 1 point for APR-Sc, but a significant increase was observed from 4 days of age, and a positive period (warning sign) was observed until 35 days of age. During this period, the infant showed no signs of gastrointestinal symptoms or PICC blood stream infection.

★ Warning Sign

Discussion

In the mechanism of the onset of infectious disease, increases in inflammatory cytokines such as IL-1β, IL-6, and TNFα lead to the production and secretion of APRs mainly in the liver, so measurement of these cytokines is superior to that of APRs for early diagnosis [11,12]. In addition, the purpose of the present research was to investigate other new merits of APRs or the APR score in diagnosing LOS in ELBWI. For APR score measurement, just 30 μL of serum is required, and the measurement time is approximately 3 minutes. Focusing on this advantage, we decided to plan research targeting ELBWI. Opinions may be divided as to whether antibiotics should be administered prophylactically during hospitalization when ELBWI are admitted to the NICU and systemic management is initiated. However, examinations for infection, such as bacterial cultures of each site including blood cultures, complete blood counts, biochemical tests, X-rays, and echo examinations must be essential. In addition, blood examination will be performed every day until 72 to 96 hours after birth, even if only small amounts of blood are obtained, so it is possible to incorporate a check for infection into testing because APRs can be measured using a capillary blood sample that has been centrifuged to measure bilirubin.

However, LOS, the subject of this study develops after 72 hours after birth, and it is unknown when each individual will develop sepsis. Further, the clinical symptoms are all non-specific. Thus, determining the timing of blood sampling is not easy, and if sepsis onset can be predicted by a test in advance, that test would be ideal to perform. However, in practice, when we are managing ELBWI, incidental testing has been associated with hyperglycemia, increased acidosis, low white blood cell count, and a left shift of the differential white blood cell, even in the absence of clinical sepsis symptoms. It is not uncommon for sepsis to develop as a result [13,14]. There are many reports on the reaction in vivo when an infectious disease is established, such as studies on improving the accuracy of sepsis diagnosis by combining measurement of inflammatory cytokines such as IL-6, IL-1β, and TNFα, which rise prior to the increase in the APRs, with procalcitonin, which is proved to have high specificity in bacterial infection [15-18].

However, most of the studies on the diagnosis of sepsis have examined specimens obtained by blood collection when clinical sepsis is recognized. At the very least, we have already confirmed that, in theory, inflammatory cytokine measurements show a delayed rise in APRs when diagnosing clinical sepsis at its onset. Inflammatory cytokines that are elevated due to infection rise within a relatively short period of time and then rapidly disappear from the blood. Therefore, although these cytokines are not suitable for judging the timing to end treatment, in contrast, APRs were found to be useful for judging this timing [8]. A major finding of the present study was that when the APR score was followed over time after birth, some infants showed a gradual increase in AGP in the blood, and more of these infants were diagnosed as having clinical sepsis. We judged that an increase in AGP prior to an increase in CRP in these infants corresponds to a warning of the start of LOS and thus identified it as a "warning sign" as mentioned above. As the results showed, there was a high incidence of sepsis in infants with this "warning sign", but there were more than a few cases in which CRP and AGP increased almost simultaneously. In this study, tip culture of a peripherally inserted central venous catheter (PICC) was not mandatory at the time of diagnosis of clinical sepsis. Therefore, it is impossible to distinguish the entrance site of sepsis by the presence or absence of this "warning sign" in this study.

However, the appearance of LOS was concentrated in the first month of life in most cases, and gastrointestinal symptoms such as full stomach, increased stomach residual, and vomiting often occurred before onset. Invasion of bacteria into the bloodstream of the intestinal buffy coat due to disruption of bacterial translocation from the sterile state in the intestinal mucosa due to the immaturity of ELBWI leads to an increase in AGP, which exceeds the upper limit of normal value, and eventual sepsis [19,20]. Catheter-associated bloodstream infections via PICCs have two possible entry routes. In other words, S. epidermidis existing on the skin around the catheter entry site may enter along the outer wall of the catheter or may enter the inner wall of the central venous catheter via the drug injection port or the like. In the former case, erythema and induration are often observed along the course of the subcutaneous vein. Vasculitis is less common in the latter case, and bacteria similar to those isolated from blood cultures are often identified from the catheter tip. The mechanism of the emergence of this "warning sign" is currently under investigation. Among ELBWI, there are cases in which the APR score did not rise even once after birth, so there is a difference in cases that did not develop sepsis, such as the two cases presented here. It will thus be necessary to investigate the mechanism for this occurrence. In the present study, the APR score was followed up over time from the early postnatal period, and infants with isolated AGP elevations should undergo clinical changes and further follow-up of the APR score. Thereafter, if the CRP is elevated or if clinical sepsis is diagnosed, a sepsis work-up should be performed as soon as possible and antibiotics selected and administered as soon as possible based on surveillance culture or other methods. After that, the presence or absence of causative bacteria from various cultures can be confirmed. We consider this method of using the APR score to be one of the best methods for the management of LOS in ELBWI.

Conclusion

Chronological follow-up of the APR score from the early postnatal period is possible at the bedside with a sample using a very small amount of blood and short measurement time. When the so-called "warning sign", in which only the AGP rises prior to the rise in CRP,

is observed, the presence or absence of a subsequent rise in CRP and the appearance of clinical symptoms of sepsis can be noted, thus improving the diagnostic accuracy of the onset of LOS in ELBWI.

Author Contributions

T.N. and H.G. contributed to the study of conception and design. The first draft of the manuscript was written by T.N. S.Y. and J.X. contributed to the interpretation of the results and critically revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.49.007861

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