Usefulness of Acute Phase Reactant Score in the Neonatal Period

Usefulness of Acute Phase Reactant Score in the Neonatal Period:

Managing Neonatal Infections

By

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TABLE OF CONTENTS

PREFACE

To All Who Have Obtained this Book

This book covers the Acute Phase Reactant Score (APR-Sc). The APR-Sc test method reflects the desire of neonatologists to detect neonatal infectious diseases as early as possible, as easily as possible, and as quickly as possible. In writing this book, the contract with Cambridge Scholars Publishing stipulates that the content must have already been accepted and published by peer review, rather than revealing new findings. Therefore, we carefully selected articles that have been accepted and published in overseas peer-reviewed professional journals, as well as academic journals and specialized journals in Japan. I have revised some of the articles to fit the format of this book.

The former part features a review of the APR score for the diagnosis of neonatal infections. We selected only three of the many APRs: C-reactive protein (CRP), α1-acid glycoprotein (AGP) and haptoglobin (Hp). If these three APRs exceed the reference values for each neonate, the score is considered positive and a score of 1 is given. If they are within the reference values, the score is considered negative and a score of 0 is given. If all three parameters are positive, the score is 3, while if all three parameters are negative, the score is 0. The results of studies focused on inflammatory cytokines involved in the hepatic synthesis of these three proteins are presented. Next, fetal inflammatory response syndrome (FIRS), which has recently attracted attention as a pathological condition associated with infectious diseases, is discussed. Among the pathologies associated with FIRS, we investigated the mechanism underlying the onset of the leukemoid reaction in the early postnatal period in neonates, especially preterm infants, and the mechanism underlying the onset of Wilson-Mikity syndrome, a chronic lung disease that frequently occurs in premature births is discussed from the perspective of APR-Sc. Furthermore, sepsis, which greatly affects the prognosis of neonates, was divided into early-onset sepsis and late-onset sepsis, and the usefulness of the APR score in each diagnosis is described.

In the latter part, I would like to look at various neonatal infectious diseases with specific cases. In most cases, the APR score and cytokines were measured at the same time, and the advantages of the APR score, which is excellent for making a bedside diagnosis, were described. The

cases include diseases that are rare, even in newborns. I hope that readers will feel that the APR score can play an active role precisely because of its rarity.

Neonatal medicine is a site of life-saving emergency care. In fact, it is often the case that normal examinations cannot keep up with the instantaneous diagnosis of the pathology of a sick neonate who cannot describe the condition that they are facing. Ultimately, it cannot be denied that much depends on the eyes of the medical staff. Is the treatment that is initiated optimal or not? Should we continue or not? When faced with such choices, I think that the APR score has incomparable advantages over cytokines.

I completed this book in the hope that there will be readers of this book who can introduce the APR score to neonatal intensive care units in facilities outside of Japan.

Toshihiko Nakamura, MD, PhD
Haruo Goto, MD, PhD

CHAPTER 1

PROLOGUE

The path to the convenience of the acute phase reactant score (APR score) was a so-called “gift of chance”.

In 1987, Mr. Noguchi, who was working at Shino-Test in Sagamihara City, Kanagawa Prefecture in Japan at the time, recommended the introduction of the bedside inspection device "Quick Turbo™," which he had developed (Figure 1-1). It was put into practice when he visited Dr. Seki, who is in charge of the laboratory department at Nagoya City University, a public university in Nagoya City, Aichi Prefecture, Japan. At that time, it was possible to measure CRP as APR with this inspection machine. On seeing it, Dr. Seki remarked, “It would be good to visit Dr. Goto, head of the pediatric department at Nagoya Municipal Higashi Municipal Hospital. This is exactly the kind of device he was looking for!" After making an appointment with Dr. Goto on the phone in front of Dr. Seki, Mr. Noguchi rushed to Higashi Municipal Hospital. Dr. Goto, a coauthor of this book, used three types of APR—namely, C-reactive protein (CRP), α1-acid glycoprotein (AGP), and haptoglobin (Hp)—to increase reliability in the diagnosis of neonatal infections. At the same time, measurements were taken, and a score of 1 point was given when the upper limit of each substance was exceeded, and a score of 0 points was given when the upper limit was not exceeded.

Figure 1-1 1st generation device for APR score with notebook PC connected.
(This photo is provided by Shino-test corporation).

Once the usefulness of this APR score is realized, physicians involved in neonatal medicine become "addicted". Unfortunately, however, doctors who have never experienced the use of the APR score may consider it to be unnecessary because CRP alone is sufficient for the diagnosis of infectious diseases.

We herein present what we have learned from our clinical practice thus far. We have found the APR score to be a meaningful and very useful diagnostic tool for the clinical interpretation of neonatal pathology.

I would like to start in the hope of inspiring as many fans of the APR score as possible.

CHAPTER 2

THE THREE ACUTE PHASE REACTANTS THAT FORM THE APR SCORE

① **C-Reactive Protein (CRP):** CRP is a member of the pentraxin family that has a pentamer structure in which five subunits are linked in a ring. The molecular weight is 105,000. It is a protein that is produced when inflammatory reactions and tissue destruction occur in the body and is called C-reactive protein because it binds to pneumococcal C-polysaccharide. It is mainly synthesized in hepatocytes by the induction of inflammatory cytokines, such as IL-1β, IL-6, and TNFα, which are produced by the activation of immune system cells such as macrophages. Therefore, it takes approximately 6 hours for CRP to increase in the blood after inflammation occurs. Doubling occurs within approximately 8 hours, and peaks in 2-3 days. When inflammation occurs, the rate of CRP increase reaches several tens to several thousand times and rapidly decreases as the inflammation subsides. It has a half-life of approximately 19-27 hours and is frequently used as a guideline for starting and ending antimicrobial therapy. An opsonic effect causing the accumulation of macrophages and neutrophils was previously pointed out as a physiological activity of CRP. However, it has since been proven to suppress the activation of platelets and neutrophils (Khreiss 2004, 2713-2720). In other words, the increase in blood as APR has a purposeful action to prevent excessive inflammatory reactions.

② **α 1-Acid Glycoprotein (AGP):** AGP belongs to the lipocalin family, and although research on its structure as a protein and its drug transport function is progressing, little is known about its involvement in inflammation. Many researchers are more familiar with the name orosomucoid (ORM). AGP is mainly produced in the liver, but it is said to be produced in cell membranes such as neutrophils, lymphocytes, and monocytes. It has also been reported

that the increase in the acute phase of myocardial infarction is mainly derived from neutrophils. Acidic and neutral drugs bind to basic albumin, but drugs administered to newborns, such as furosemide and phenobarbital, are basic drugs and bind to acidic AGP and lipoproteins, thus playing an important role in drug metabolism. Regarding the physiological activity of this substance, it has been reported that it suppresses the activation of neutrophils, prostaglandin production, vascular permeability enhancement, and the like (Matsumoto 2007, 1226-1230).

③ **Haptoglobin (Hp):** Hp is a glycoprotein composed of an H(β) chain that binds to hemoglobin (Hb) and a genetically polymorphic L (α) chain that has an immunosuppressive effect and which is mainly produced in the liver. When Hb is released into the blood by hemolysis, Hp rapidly and strongly binds to Hb to form an Hp–Hb complex. After that, it is quickly taken up via receptors in reticuloendothelial cells and degraded. As a result, a system that neutralizes the angiopathy toxicity of free Hb and reduces Hb loss from renal glomeruli is obtained. Inflammation and infection promote the production of Hp, resulting in high levels of Hp. In hemolysis, uremia, and liver disease, Hp consumption increases and production decreases, resulting in a decrease in blood levels of Hp. Among the inflammatory cytokines, IL-6 has been confirmed to be the strongest production stimulus. Furthermore, TNFα and IL-1β are also weakly stimulated in comparison to IL-6. IFNγ had no such effect. Synergy was confirmed with IL-6 and TNFα, and INFγ showed a negative synergistic effect on IL-6. IL-1β is believed to have a production-promoting effect through a different pathway from IL-6 (Baumann 1988, 277-292). It should be noted that from January 1998, the standard value was changed to match the IFCC (International Conference) unit. The standard value for adults is 40-270 mg/dL, while the new standard value is 17–169 mg/dL, which is approximately half of the conventional value. Since the APR score is the same value as before, when using the new reference value, it is necessary to double the measured value and evaluate the APR score. The current Latessier™ uses the conventional value, so it can be used without correction.

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CHAPTER 3

SETTING THE REFERENCE VALUES FOR APR

Preface

The three APRs adopted as APR scores include CRP, which gradually increases and peaks from the early postnatal period, then gradually decreases, and AGP and Hp, which gradually increase and peak, and then are maintained at a certain level without decreasing. The APR was found to exhibit unique daily changes, including changes in type. Therefore, normal values in adult specimens cannot be used as they are. Even in term neonates, vaginal delivery is a devastating event that is comparable to a form of ischemia–reperfusion injury. The hemodynamics associated with elective cesarean delivery may differ from those associated with a vaginal delivery because there is less stress in the early postnatal period. In addition, there are different reasons for premature birth. In particular, fetal inflammatory response syndrome (FIRS) has a considerable influence on the APR score (see Chapter 4.1). Taken together, we set the standard values for three APRs: CRP, AGP and Hp.

The content of this chapter is modified from an article published in *Neonatal Care（in Japanese）in 2006 19; 234-242,* which was translated in English.

Abstract

To establish a reference value for the APR score, we observed changes over time in infants that did not affect APR production. Among the infants admitted to the NICU, the subjects were infants with pathological conditions that were not related to APR production. Premature infants without severe respiratory distress, infants with neonatal asphyxia who recovered with only mild resuscitation, and infants with jaundice requiring treatment were included in the present study. We excluded infants with obvious infections, infants who experienced a strong inflammatory reaction in the uterus, infants with respiratory disorders with amniotic fluid that were difficult to

distinguish from infections, and infants with obvious malformation syndromes or chromosomal abnormalities. The reference value for CRP was set at 1.0 mg/dL, and a significant increase above the reference value was defined as an APR score of 1 point. AGP was analyzed separately for term infants and preterm infants. The reference value was 30 mg/dL for term infants up to 24 hours after birth and 50 mg/dL thereafter. The baseline value for preterm infants was 20 mg/dL for up to 72 hours after birth, then the reference values were 30 mg/dL from 72 hours to 96 hours, and 40 mg/dL thereafter. For infants weighing ≥1000 g, the reference value was 30 mg/dL until 72 hours after birth and 40 mg/dL thereafter. Hp was also categorized into term and preterm infants. For term infants, the reference value was 20 mg/dL up to 24 hours after birth, 50 mg/dL from 24 hours to 48 hours after birth, 100 mg/dL thereafter that (until 7 days of age). Thereafter, it decreased again and the reference value was set to 50 mg/dL. For preterm infants, the reference value was 20 mg/dL for up to 72 hours after birth and 50 mg/dL thereafter. The presence or absence of the elevation of these three APRs was scored with a maximum value of 3 points and a minimum value of 0 points. This was an advantageous test for predicting the presence or absence of infectious diseases and their progression in infants.

Introduction

The diagnosis of neonatal infectious diseases is important; however, the diagnostic value and credibility of tests is in question. APRs themselves are mainly produced in the liver and secreted into the blood. Inflammatory cytokines produced from macrophages and vascular endothelial cells during infection promote its production. It can be said that measurement of these cytokines is superior to determination of the APRs in terms of their ability to make an early diagnosis. However, it is not easy to measure cytokines in the clinical setting, and simple bedside measurements of cytokines are not possible. Therefore, we selected three (CRP, AGP, and Hp) of the many APRs. These APRs are scored as 0 or 1 depending on the presence or absence of an increase in their value, and the transition of the pathology was examined. This chapter describes the setting of reference values for these three APRs.

Cases covered by reference values

Ideally, the reference values for serum APR would be determined for healthy term infants, normal preterm infants, or low birth weight infants by postnatal age and birth weight. However, preterm infants and low birth weight infants are treated as sick newborns. Such reference values are difficult to create because even full-term infants often show ischemia–reperfusion stress and mild tissue damage during delivery. Therefore, these factors inevitably affect blood APR levels in newborns, even though they do not exert as much stress as infectious diseases. Therefore, in our analysis to set the standard values, we first excluded children with clear infection. We also excluded children with obvious chromosomal abnormalities and congenital malformation syndromes. Then, we selected children with pathological conditions that showed a minimal association with the APR value, including: neonatal asphyxia that required only mild resuscitation, transient tachypnea, respiratory distress syndrome, premature infants who completed respiratory management in < 2 weeks, and hyperbilirubinemia. Patients with meconium aspiration syndrome that was indistinguishable from infection, placental findings of chorioamnionitis, Hirschsprung's disease with enteritis and related diseases, meconium disease, necrotic enteritis, and two infants requiring respiratory control for more than two weeks were excluded.

Table 3-1. Reference values of C-Reactive Protein [CRP]

[BW≧2,500g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	0.000	0.407	0.490	0.215	0.181	0.043	0.138				
SD	0.000	0.524	0.562	0.266	0.336	0.117	0.256				
N	23	15	21	20	26	28	8				

[2,000g≦BW<2500g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	0.014	0.154	0.100	0.087	0.018	0.035	0.106	0.062	0.000		
SD	0.076	0.324	0.207	0.189	0.073	0.109	0.175	0.150	0.000		
N	28	24	15	16	17	20	17	13	5		

[1,500g≦BW<2,000g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	0.014	0.164	0.111	0.021	0.000	0.000	0.067	0.000	0.000	0.000	
SD	0.076	0.290	0.188	0.092	0.000	0.000	0.174	0.000	0.000	0.000	
N	28	22	18	19	22	21	21	16	14	3	

[1,000g≦BW<1,500g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	0.000	0.000	0.033	0.000	0.019	0.015	0.000	0.000	0.024	0.000	0.000
SD	0.000	0.000	0.033	0.000	0.019	0.015	0.000	0.000	0.0097	0.000	0.000
N	21	17	18	10	16	27	9	14	17	10	4

[BW<1000g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	0.000	0.125	0.031	0.050	0.033	0.000	0.000	0.000	0.000	0.000	0.000
SD	0.000	0.349	0.111	0.187	0.115	0.0000	0.000	0.000	0.000	0.000	0.000
N	14	12	13	14	12	14	12	10	10	7	4

Table 3-2. Reference values of α_1-acid glycoprotein [AGP]

[BW≧2,500g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M	
Mean	8.913	36.933	41.762	44.200	41.385	37.643	35.250					
SD	13.990	17.186	07.447	13.976	14.988	11.852	4.400					
N	23	15	21	20	26	28	8					

[2,000≦BW＜2,500g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	4.214	18.708	21.667	27.750	23.294	25.750	31.059	26.000	0.000		
SD	10.751	18.289	16.616	17222	17.153	11.295	16.192	10.247	0.000		
N	28	24	15	16	17	20	17	13	5		

[1,500g≦BW＜2,000g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	1.071	11.773	17.882	18.444	22.143	23.048	25.524	21.688	24.071	30.333	
SD	5.669	13.825	16.643	16.400	20.917	14.496	13.148	16.520	15.204	9.452	
N	28	27	17	18	21	21	21	16	14	3	

[1,000g≦BW＜1,500g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	1.095	11.000	6.000	12.800	10.125	19.789	16.333	21.29	29.176	34.100	41.250
SD	5.019	15.004	14.994	22.075	13.875	19.205	16.439	15.629	13.956	12.004	10.436
N	21	17	18	10	16	19	9	14	17	10	4

[BW<1,000g]

	~24h	~48h	~72h	~96h	~7d	~14d	~21d	~28d	~2M	~3M	~4M
Mean	0.000	0.125	0.031	0.050	0.033	0.000	0.000	0.000	0.000	0.000	0.000
SD	0.000	0.349	0.111	0.187	0.115	0.000	0.000	0.000	0.000	0.000	0.000
N	14	12	13	14	12	14	12	10	10	7	4

Tables 3-1, -2, and-3 show the standard values of CRP, AGP, and Hp for the above subjects by age and birth weight. The lower limit of measurement for each measurement item was 0.3 mg/dL for CRP, 20 mg/dL for AGP, and 20 mg/dL for Hp. The baseline values for APR from birth to 24 hours after birth were <0.3 mg/dL, <20 mg/dL, and <20 mg/dL for all weight groups, which were below the limit of detection. At 24 hours after birth, AGP gradually increased with age and peaked at 96 hours to 1 week after birth. After that, AGP gradually decreased. The peak AGP of term infants did not exceed 50 mg/dL, but there was a wide range of variation from 0 to 77 mg/dL. In low birth weight infants, AGP showed lower values in lower weight infants and was less sensitive in very low birth weight infants. CRP was also less sensitive in most measurements, although there were a few term infants in whom CRP exceeded 1 mg/dL in the 24–48 hours after birth. In most cases, the Hp levels were below the limit of detection, but some term infants showed high levels of 72–92 mg/dL at 96 hours to 1 week after birth.

Setting the APR Score

1) In clinical practice, it is necessary to determine the presence or absence of infectious diseases as quickly as possible. Therefore, we set the normal upper limit for each of the three APRs so that we could determine whether it increased or decreased. CRP was considered to be significantly elevated at levels of ≥1 mg/dL, AGP and Hp, were measured separately in term infants and low birth weight infants, with the upper limit of normal set according to postnatal age. Values that exceeded these upper limits were considered to represent a significant increase, and these increases were counted to determine the APR score (Figure 3-1and Figure 3-2).

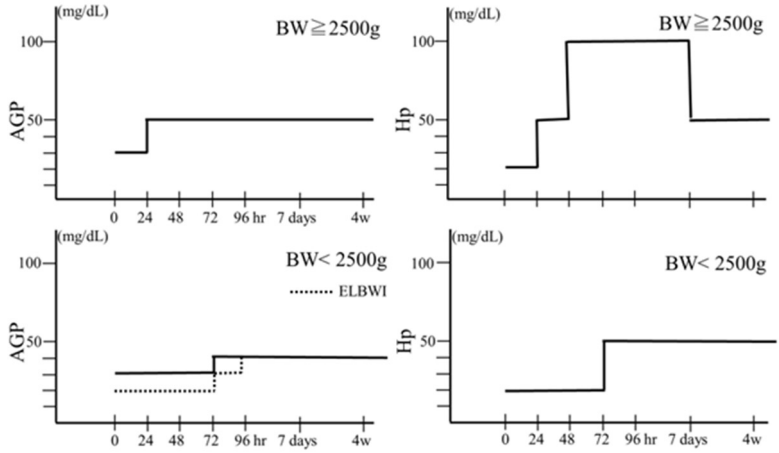

Figure 3-1. Setting normal limits for AGP and Hp.

APR score	3	2	2	2	1	1	1	0
CRP	↑	↑	—	↑	↑	—	—	—
AGP	↑	↑	↑	—	—	↑	—	—
Hp	↑	—	↑	↑	—	—	↑	—

↑：CRP≧1.0mg/dL, AGP and Hp ＞upper limit of reference values.
—：≦ upper limit of reference values.

Figure 3-2. Points of APR score.

APR score	Evaluation
3 CRP, AGP, Hp	Acute infection
2 CRP, AGP	
2 AGP, Hp	Subacute infection, Viral infection
1 AGP	Pre-status of complication during ventilation, Waring sign of late onset sepsis
1 CRP Hp	Non-infection, mild inflammation
0	Denial of infection

Figure 3-3. Evaluation of APR score.

2) CRP, AGP, and Hp surged in parallel during the early acute phase of infection, indicating an acute infection pattern (APR score: 3 points: Figure 3-3). In this pattern, the value of CRP increases, becoming significant after 12 hours, peaking at 24–48 hours, and then rapidly disappearing as the infection improves. In contrast, with an APR score of 2 points, AGP shows a significant increase at 24–48 hours after the onset of infection, but disappears very slowly thereafter. Hp is often not elevated in the early stage of infection, although CRP and AGP are elevated. As the infection improves, it gradually rises and then disappears. An APR score of 2 is often found in low birth weight infants or in full-term infants with severe infection. Hp rarely increases during infection in very low birth weight infants. When the infection improves, CRP rapidly disappears, but AGP and Hp remain high, and the APR score is 2 points, indicating a subacute pattern. As will be described in detail in other chapters, when CRP is not elevated and only AGP and Hp are elevated, viral infection is often present.

3) If the APR score remains at 0 from the beginning, infection can be ruled out. However, these APRs require 12–24 hours from the onset of infection to exhibit significant elevation in the blood. First, there is the production and increase of inflammatory cytokines, and then APR is produced and secreted for the first time as a result of message transmission in the liver; thus, the time lag between them is 12–24 hours.

The APR showed a false-negative result in approximately half of infants with early-onset sepsis that develops immediately after birth. However, the next day, these infants also showed an infection pattern with an APR score of 2–3 points. Although empiric antibiotics are often administered to newborns, it is effective to use an APR score of 0 to determine when to stop antibiotics. For the relationship between these cytokines and APR, refer to Chapter 4-2.

4) As shown in Figure 3-3, it is considered that the APR score can be used to evaluate the presence or absence of infection in the clinical setting. An APR score of 3 points and CRP-positive and AGP-positive APR scores of 2 points strongly suggest the presence of infection, regardless of clinical symptoms. AGP-positive and Hp-positive APR scores of 2 points should be considered to represent postinfectious or preinfectious states, and careful follow-up should be subsequently conducted. Note that viral infections, especially enterovirus infections, may yield similar scores. In addition, if the APR score is 0–1 point and there is no increase in subsequent follow-up, there is no problem the result is considered to be negative for infection.

Actual change in APR score

1) The APR decreases rapidly with successful treatment of infection, but the trends are not necessarily parallel. Figure 3-4 shows the changes in APR scores over the course of treatment for various bacterial infections. CRP disappears fastest, followed by a gradual decline in AGP and Hp. The APR score becomes 1 point or 0 points when the infection is almost cured. Figure 6.5-2 shows an example of a case of severe infection in which the patient could not be saved. The APR score remained at 2–3 points (CRP- and AGP-positive) and the score for AGP and Hp positivity never declined to 2 or 0–1 points.

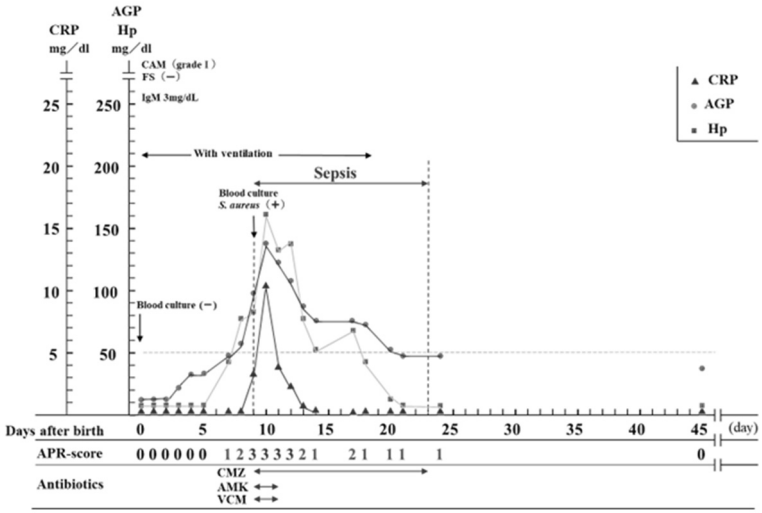

Figure 3-4. Extremely low birth weight infant with late onset sepsis, birth weight 708gr and 24 weeks 5 days of gestational age.

2) The diagnosis of infection during mechanical ventilation: There are many cases in which prolonged mechanical ventilation management is unavoidable, including very preterm infants. It is very difficult to distinguish between abnormal shadows (e.g., atelectasis and fibrosis) and the presence of pneumonia on chest radiographs during long-term mechanical ventilation. In such cases, the dynamics of the APR score can be important data for the diagnosis. Figure 3-5 shows the transition of the APR score during artificial ventilation. When artificial ventilation is prolonged, the APR score of 0 becomes AGP-positive (an APR of 1), AHP- and Hp-positive (an APR of 2), and the amount of airway secretions increases in parallel. This probably suggests a mild infection of the upper respiratory tract. At this time, none of the blood cultures were positive. After this condition, when the APR score becomes 3 points or CRP and AGP are positive (2 points), it is judged that a strong infectious complication is present. The prompt administration of antibiotics from this point reduces the APR score, but this condition often continues while mechanical ventilation is continued. The APR score becomes 0 points for the first time after extubation and artificial ventilation are completed. Thus, the trends in APR scores during mechanical ventilation can contribute not only to the diagnosis of

concomitant infections but also to predicting the onset of infection.

3) Infants with fetal inflammatory response syndrome (FIRS) often show an elevated APR score immediately after birth as a result of infant hypercytokinemia, even if intrauterine infection does not actually occur. Since FIRS theoretically resolves at birth, it typically peaks with hypercytokinemia in postnatal specimens or cord blood, as described in Chapter 4.1. The APR then peaks and then falls. In this case, AGP and Hp are unlikely to remain high, as is observed in infectious diseases.

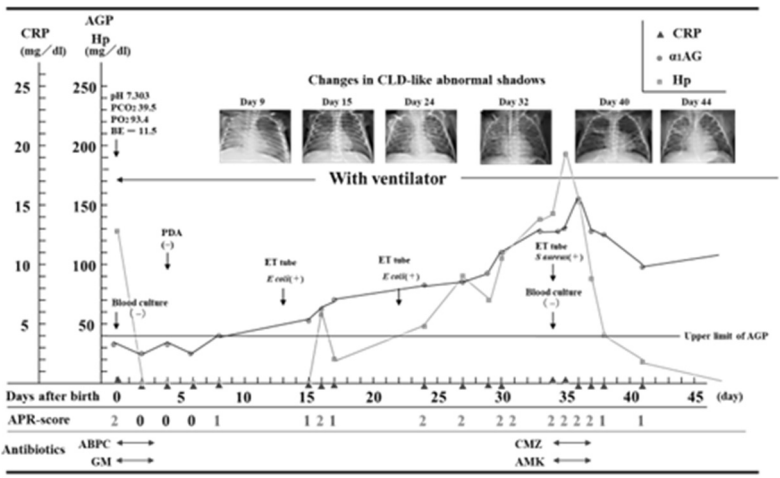

Figure 3-5. A case of ventilator-associated pneumonia (VAP) complicated with bronchopulmonary dysplasia. A female infant with a gestational age of 24weeks and 0 day, a birth weight 562 grams, and chorioamnionitis

4) Unlike RDS and TTN, patients with meconium aspiration syndrome often show elevated APR. However, it is difficult to distinguish from complications of pneumonia, and it is not possible to distinguish whether the increase in APR is due to meconium aspiration syndrome itself or due to complicating infections. These conditions can only be distinguished by a comprehensive diagnosis based on clinical symptoms and chest X-ray findings. Even in meconium aspiration syndrome without the administration of antibiotics, the increase in APR is transient and then disappears spontaneously (Figure 3-6). Recently, one member of our group, Dr. Yokoi, found a significant

relationship between meconium aspiration syndrome and chorioamnionitis (Yokoi, 2019, 2021). In other words, meconium aspiration syndrome can be said to be a condition with one aspect of FIRS. Defecation in the uterus occurs as the result of transient ischemic injury in the fetus (specifically FIRS), and ischemic injury is added to it, resulting in a marked inflammatory response and a noninfectious APR score. Fetal defecation in the uterus occurs as a result of transient ischemic injury in the fetus. The ischemic injury is added to the original inflammatory reaction called FIRS, and the fetal inflammatory reaction is remarkably strong, and as a result, the APR score is 3 points even though there is no infection.

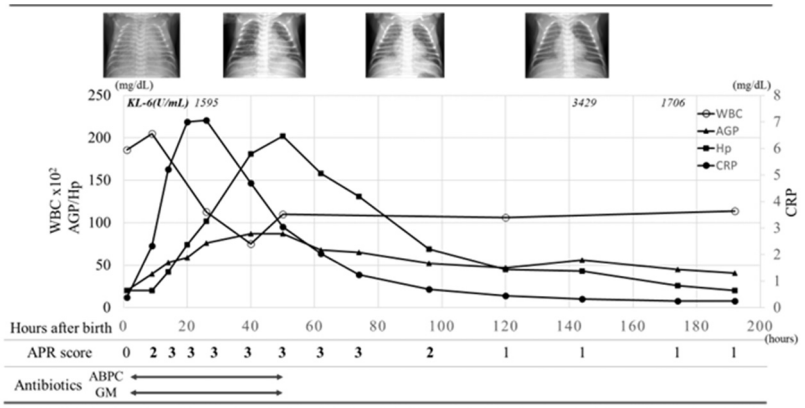

Figure 3-6. Meconium aspiration syndrome (MAS). A male infant with gestational age of 40 weeks and 3 days, a birth weight 3232 gram, and Apgar score of 4 points at 1 min and 7 points at 5 min.

5) The early stages of Hirschsprung's disease, meconium-associated intestinal obstruction in low birth weight infants (meconium disease), and necrotizing enterocolitis are easy to diagnose as the respective diseases progress, it is important to understand how they should be managed in the early stages. We found interesting trends in APR when these diseases coexisted with any enterocolitis. There is a gradual increase in AGP from the early stage of onset. The further exacerbation of the disease condition is followed by an increase in CRP. We called the increase in AGP that precedes the increase in CRP the "***Warning sign***" and reported that it is effective for the early detection of these diseases (Chapter 4.4). In meconium disease in low

birth weight infants, the onset can be prevented by prompt enema without the intragastric injection of Gastrographin® or other agents, as soon as AGP elevation is observed to promote defecation (Figure 3-7).

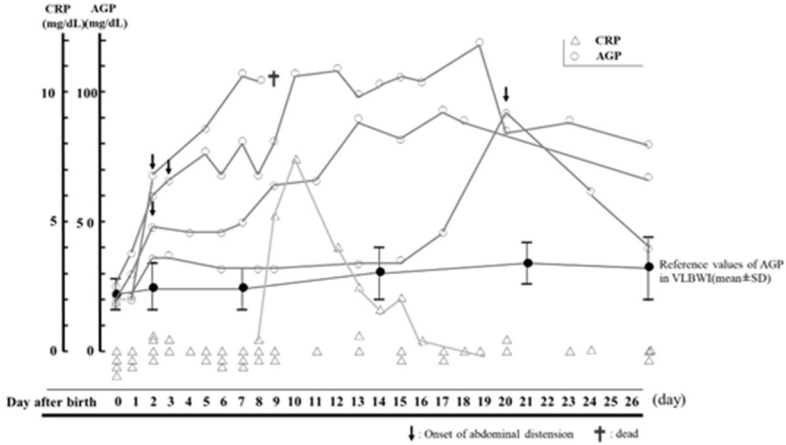

Figure 3-7. 4 cases of very low birth weight infants complicated with Meconium Disease.

References

Yokoi K, Iwata O, Kobayashi S, Muramatsu K, Goto H. (2019) Influence of foetal inflammation on the development of meconium aspiration syndrome in term neonates with meconium-stained amniotic fluid. Peer J. DOI: 10.7717/peerj.7049.

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CHAPTER 4

DIAGNOSIS OF NEONATAL INFECTIONS USING APR SCORES

Chapter 4.1: Usefulness of APR Score in Diagnosing Early-Onset Neonatal Sepsis

The content of this chapter is modified from an article published in ***Japanese Journal of Practical Pediatrics*** *(in Japanese) 2009 9; 1595-1599.*

Introduction

Needless to say, the purpose of making an accurate diagnosis of neonatal sepsis is to save the patient's life without any sequelae. The following two items are considered to be clinically important. In other words, (1) how can antibiotics be started in a timely manner (rule in sepsis), and how can antibiotics be discontinued when necessary (rule out sepsis)? (Richard 2003, 3-4) (2) Is it possible to accurately determine whether it is necessary to switch to another antibiotic during treatment?

However, there are not many diagnostic tests for sepsis that accurately reflect the general condition of the infant at the time of testing (blood sampling). This is because all of these measurement items are parameters (cytokines, acute phase reactant proteins, etc.) produced secondary to bacterial infection. Unfortunately, there is still no gold standard for diagnosing sepsis today. At present, the two items of "clinical symptoms of sepsis (not doing well)" and "positive blood culture" are accurate diagnostic criteria for sepsis. But it takes at least 48 hours for blood culture results to be obtained, and waiting until treatment is started may make it difficult to save the life of the infant.

In this chapter, I will introduce some of the recent findings on how clinical hospitals (community hospitals) that do not have laboratories like ours facilitate clinical diagnosis. The research was funded by the Kawano Pediatrics Foundation, and part of the content was presented at the 52nd

Annual Meeting of the Japanese Society of Premature Neonatology in 2006).

Definition of sepsis

In recent years, sepsis has been defined as a systemic inflammatory response syndrome (SIRS) caused by infection, and it has become a feature that the identification of bacteria in blood cultures is not an essential requirement. As an indication for SIRS in children, a diagnostic method has been proposed in which standard values for the three items of heart rate, respiratory rate, and systolic blood pressure are set according to age (Goldstein 2005, 2-8). Even in the field of neonates, the term ``clinical sepsis'' has been used to define the diagnosis of sepsis based on clinical symptoms, even if the bacterium is not identified. This method is a pathophysiologically attractive interpretation. However, the clinical problem is that it is necessary to determine whether antibiotics are effective even if they are started at good timing. Continuing to administer ineffective drugs to premature infants in the early postnatal period can actually do more harm than good. In that case, identification of the bacteria by blood culture and identification of drug susceptibility of the bacteria are essential. In other words, blood culture has been, is, and will not be omitted in the diagnostic examination of sepsis.

Testing for sepsis

1. Testing for early-onset sepsis

Most are included in the patient's admission examination. Early-onset sepsis is possible if prenatal information (presence or absence of signs of maternal infection, premature rupture of membranes, presence or absence of preterm PROM, etc.) and birth information (degree of amniotic fluid turbidity, presence or absence of foul odor in amniotic fluid). Bacterial culture and blood tests are performed on blood and other sites that may become infected.

2. Testing for late-onset sepsis

It is no exaggeration to say that the ability to quickly recognize changes in the neonatal course determines the prognosis of the neonate. I will omit the details of “not doing well” (Remington and Klein, 2006, 266-275) here, but I think that “somehow” can only be understood by those involved in clinical practice. It is most important for the medical staff to investigate the stable

daily condition of each patient through their own eyes and feeling it with their hands.

Examinations in practice

It seems that the majority of facilities carry out blood cultures together with various types of blood collection for examinations upon admission to the NICU. Most of the blood collection sites are arterial routes, and the radial artery or the posterior tibial artery is suitable for safety considerations. The selection of the hip artery and vein may also be included in old textbooks, but we believe that it should not be used due to the possibility of contamination and complication of hip arthritis. Recently, it has become possible to perform visual arterial puncture using a compact LED built-in transilluminator, which has made it possible to perform blood collection in a shorter time and more accurately than blood collection by palpable arterial puncture. As for skin disinfection, when puncturing after applying 10% povidone-iodine solution or 1% Chlorhexidine Gluconate Ethanol and drying, the permeated artery can be directly seen without palpation, so disinfection of unnecessary parts for palpation is unnecessary. According to the guidelines for blood culture tests in the United States, the volume of blood collected is recommended to be calculated based on the total volume of blood (Baron, 2007, 12-18). For extremely low birth weight infants, 2 mL of blood is required at one time, and for very low birth weight infants, the same amount of blood culture from two sites is recommended. However, in neonatal blood culture, he found that collecting samples from multiple sites only increased the detection rate by a few percent. In addition, the amount of blood collected is often less than the recommended amount (Table 4.1-1). In that case, it is necessary to respond according to the patient's condition. In addition to blood culture, we used peripheral blood leukocyte count (total leukocyte count of 5,000/μL or less or 25, 000/μL or more) and hemogram (immature neutrophil count (I)/total neutrophil count (T) ratio > 0.25) and platelet count (10 x 10^4/μL or less) are examined. For newborns, 250 μL of these are sent to the laboratory as a blood count. It often takes several hours for a hemogram to be reported, during which whole blood is centrifuged in two capillary tubes (approximately 80 μL x 2) and plasma is removed from APR (acute phase reactants). It goes without saying that this APR score is the life work of co-author Dr. Goto. In our hospital, we consulted with Dr. Goto and measured the APR score over time. Differential diagnosis of infection and inflammation in the early neonatal stage is being performed by focusing on the changes in the condition.

Table 4-1. Recommended blood collection volume for blood cultures in neonates

birth weight (g)	total blood volume (mL)	1st culture	2nd culture	total blood culture volume (mL)	vs. total blood volume (%)
500 ~999	50 ~99	2	not done	2	4
1000 ~1999	100 ~199	2	2	4	4
2000<	200<	4	2	6	3

APR Score

Among the acute reaction proteins, we selected three that met the conditions of rapid response and change as large as possible, that is, CRP (C-reactive protein), AGP (α1 acid glycoprotein), and Hp (haptoglobin), and added 10 μL of serum. SHINO-TEST's Quick Turbo C™ (Figure 4.1-5) is equipped at the bedside and is capable of calculating results in a very small amount and in a total measurement time of 3 minutes. Prior to the release of this medical device, immunodiffusion using Partigen™ was used, and it took at least 48 hours to make a judgment. Quick Turbo C™ is extremely convenient for NICU hospitalized infants, as it allows the use of residual serum that should be discarded after bilirubin measurement with a capillary tube. Regarding the interpretation and treatment of APR scores of 0 to 3 points, please refer to Dr. Goto's original papers (Goto 1988, 1709-1717) (Goto 1987, 277-281). Figure 4-1. shows changes in the APR score for bacterial infections (for which the causative bacteria have been identified), including sepsis. APR is also positive in fetal inflammatory response syndrome (FIRS), a state in which various inflammations in the uterus spread to the fetus and the fetus falls into hypercytokinemia, and the transition is similar to that of bacterial infections. (Figure 4.1-2.). Looking only at changes in the APR score, the average time spent in the infectious state is longer in the infectious disease group, but it is difficult to judge individual cases. Therefore, I would like you to observe the transition of only CRP, which has the shortest half-life among the three proteins of APR, in a further illustration. CRP levels in bacterial infections peaked after 48 hours after birth (Figure 4.1-3.), whereas in FIRS and other groups (Figure 4.1-4.), CRP peaked by 48 hours after birth. It was found that it can be confirmed by Table 2 shows the usage of antibiotics at our hospital using the APR score