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Problems and Solutions in the Perinatal Management of Intrauterine Infection/Inflammation

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Abstract

Intrauterine infection/inflammation, a major cause of preterm birth, is known to be an exacerbating factor for perinatal mortality and morbidity, as well as childhood neurological morbidity. Treatment of intrauterine infection/inflammation is crucial in the prevention of preterm birth; however, the prevention of preterm birth using antimicrobial therapy is not recommended, amniocentesis for the diagnosis of intra-amniotic infection/inflammation is not commonly performed, and therapeutic intervention for intrauterine infection/inflammation is only provided when intra-amniotic infection/inflammation is confirmed by clinical findings. Thus, to date, intrauterine infection associated with preterm birth is still considered untreatable during pregnancy. However, several recent studies reporting successful treatment using multiple broad-spectrum antimicrobial drugs have led to disagreements regarding the necessity of treatment for intra-amniotic infection/inflammation during pregnancy. In recent years, technological innovations such as next-generation sequencing and quantitative polymerase chain reaction have led to the development of comprehensive methods for bacterial quantification. This has opened up the possibility of grasping the overall picture of pathological conditions occurring in human tissue and bacterial flora that may also be useful in general clinical settings. Thus, reassessment of several issues involved in the perinatal management of intrauterine infection/inflammation including the 1) selection of antimicrobial agents, 2) evaluation of therapeutic efficacy, and 3) selection of patients to be treated, as well as the establishment of a new approach to this clinical practice, are required. Therefore, we aim to conduct a randomized controlled trial (phase II of the specified clinical research, jRCTs071210114) to address some of these issues.

Keywords: Preterm Birth; Intrauterine infection; Inflammation; Amniocentesis; Microbiome; Randomized Controlled Trial (RCT)

Introduction

Preterm birth, which occurs in 5%-18% of pregnancies, is a contributing cause of perinatal mortality and morbidity, as well as childhood neurological morbidity [1-3]. Furthermore, it is the leading cause of neonatal death, accounting for an estimated 1 million deaths annually [1-4]. Preterm birth prevention is one of the most important issues for human society as a whole because of the significant medical and public costs (especially the long-term costs of preterm birth in childhood) associated with the healthcare management of preterm infants [5].

Preterm birth is thought to be the result of various causes, among which, intrauterine infection/inflammation is the most predominant [6-11]. It is estimated that at least 40% of preterm infants are born to pregnant women with intrauterine infection/inflammation, and the incidence is even higher in very early preterm births [12-16].

Intrauterine infection/inflammation reportedly not only induces fetal systemic inflammatory response, but also causes adverse developmental outcomes, especially in the central nervous system, lungs, and heart [17-25]. More recently, an association between maternal infections, including intrauterine infections, and autism spectrum disorder in children has been reported [26-33].

As shown in experimental studies using model organisms, intrauterine infection/inflammation is considered a potentially preventable and treatable cause of preterm birth [34,35]; however, Randomized Clinical Trials (RCTs) have failed to yield any effective results [36,37]. As such, specialized institutions, including major academic societies, do not recommend antimicrobial agents as treatment for preterm labor. However, in recent years, studies reporting successful treatment of intra-amniotic infection/inflammation with multiple broad-spectrum antimicrobial agents have led some researchers to argue about a shift in the traditional paradigm that preterm birth cannot be prevented in women with intra-amniotic infection [16,38-40].

On the other hand, with the recent introduction of next-generation sequencing technologies and droplet digital Polymerase Chain Reaction (PCR), comprehensive and quantitative identification of bacteria has become a reality [41-45]. This may lead us to grasp the overall picture (e.g., quorum sensing or multi-omics analysis) of the pathological conditions affecting human tissues and bacterial flora, an accomplishment that was thought to be impossible to achieve with conventional testing methods [46-50]. We believe that the time has come to find an answer to the problems associated with the perinatal management of intrauterine infection/inflammation and to develop a new approach to this clinical practice. Therefore, we are conducting an RCT (jRCTs071210114) to find a solution to some of these problems.

Literature Review

Because of the difficulties in establishing a correct diagnosis, intrauterine infection has often been referred to as chorioamnionitis; histological findings in the delivered placenta have long been known as the "gold standard" for diagnosing this pathological condition [51-54]. However, recent advances in evaluation methods have allowed us to clearly differentiate between infection and inflammation [10]. As a result, intrauterine infection and intrauterine inflammation are now regarded as two separate conditions (Figure 1).

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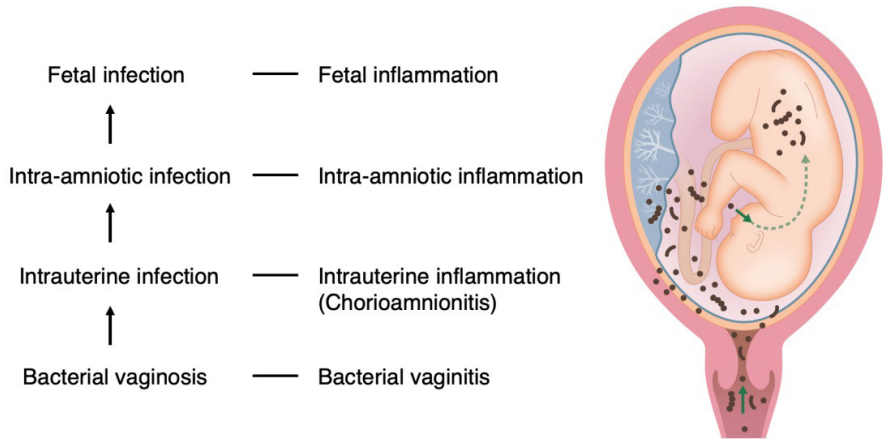

Figure 1: Schema of vaginal ascending infection and intrauterine infection/inflammation. Vaginal ascending infections are the most frequent route of intrauterine infections. There should be a clear distinction between intrauterine and intra-amniotic infection and inflammation.

It also became possible to further classify intrauterine into intra and extra-amnion. In this context, histopathological diagnosis of chorioamnionitis can be used as a method to evaluate intrauterine inflammation (Figure 1). One method to evaluate intra-amniotic inflammation is to measure the levels of cytokines (i.e., interleukin [IL]-6), chemokines (i.e., IL-8) and microRNA (miRNA). An elevated amniotic fluid IL-6 concentration (≥ 2.6 ng/dL), one of the most commonly used indicators, is used to define intra-amniotic inflammation [55-57]. We have recently focused our attention on miR-4535 and miR-1915-5P, two miRNA biomarkers present in the amniotic fluid and have found that they are highly correlated not only with intrauterine inflammation but also with fetal infection [58,59]; further developments are expected in the future.

Diagnostic tests that can directly evaluate intrauterine infection include the Gram stain test and culture test, and PCR and metagenomic analysis that directly measure bacterial DNA. However, because of its high specificity and very low sensitivity, the Gram stain test shows positive results only when large amounts of bacteria are present in the amniotic fluid. The culture test is clinically irrelevant because of its low sensitivity and the time required to obtain results due to the large number of difficult-to-culture bacteria that can be found among the causative organism of intrauterine infection. Despite high expectations for a technique that can directly capture bacterial DNA, the large amount of human DNA in the amniotic fluid makes metagenomic analysis (e.g., whole-genome shotgun sequencing) impracticable. Therefore, PCR is considered the most effective method to directly detect infection. The most common detection method makes use of the 16S ribosomal RNA gene that can be found only in bacteria [60-63]. In this respect, in recent years, the sequencing of bacterial 16S rDNA amplicon using next-generation sequencing technology has been widely reported [43,64]. However, the clinical significance of the test has not yet been fully established as there are still many difficulties in interpreting the results, such as contamination of cell-free DNA from bacteria found in the environment [43,57,65,66]. Moreover, further research and development of clinical applications are expected to contribute to the implementation of more accurate medical care, such as in the case of changing the antimicrobial agents of a selected drug based on the combination of bacteria detected.

Past attempts at preterm birth prevention

Past studies focusing on preterm birth prevention cover a wide range of interventions including antimicrobial administration and supplementation, hormone therapy (mainly progesterone), cervical pessary, cervical cerclage, pro/prebiotics, and bacterial flora transplant [67]. However, none of these studies have yielded clear results due to various confounding factors associated with preterm birth. A 2018 Cochrane review evaluating previous interventional studies clearly suggested that screening for lower genital tract infection is considered beneficial; however, information on the methodologies used was not clear [67].

To date, several large double-blind studies have used antimicrobial agents to prevent intrauterine bacterial infection [36,37,68,69]. However, while some meta-analyses have suggested a potential preventive effect of these agents on preterm birth [68], a large number of trials have failed to yield satisfactory results [36,37,69]. The failure of RCTs to demonstrate the effectiveness of microbial agents in preventing it is thought to be due to the fact that only 10% of the patients enrolled in the RCTs had intra-amniotic infection or inflammation; the remaining 90% had no infection or inflammation and thus did not benefit from the antimicrobial therapy [16]. Therefore, as suggested by the aforementioned Cochrane review, if the patient is appropriately selected by noninvasive methods, antimicrobials are appropriately administered, and their effectiveness is appropriately assessed, we may be able to establish an effective method for preventing preterm birth in women with intrauterine infection.

Selection of antimicrobial agents

Amniotic fluid was traditionally considered sterile until 1927 when bacteria were detected in amniotic fluid obtained in a sterile fashion during a cesarean section [70]. Notably, the assumption that amniotic fluid was not always necessarily sterile was already put forward in the past. While it is obvious that, as shown in subsequent studies, the spread of infection/inflammation into the womb is a major factor in preterm birth [9,13], the presence of bacteria in the womb does not necessarily cause preterm labor, as reported by a study showing that bacteria were detected in 70% of fetal membranes obtained in a sterile fashion by cesarean section at full term [71]. In addition, another study showed that the meconium and umbilical cord blood of term-born infants delivered by cesarean section immediately after birth were not sterile [72-74]. The detection rate of bacteria in amniotic fluid obtained by culture is higher in cases of preterm labor at earlier weeks of gestation [12,13,55]. After aggregating data with low bias from the main four studies [60-63], 40 bacterial types, at the genus level, of those usually found in amniotic fluid

of women with preterm birth with or without preterm premature rupture of membranes, were identified (Table 1). At the genus level, the most commonly detected bacteria were: Ureaplasma (especially *U. parvum, U. urealyticum), Fusobacterium* (especially *F. nucleatum*), Sneathia (especially *S. sanguinegens*), *Streptococcus* (especially *S. agalactiae*), *Bacteroides*, *Leptotrichia, Haemophilis, Mycoplasma* and *Prevotella*. The detection rate of bacteria in amniotic fluid of women with preterm labor differed according to whether premature rupture of membranes occurred or not, with 10%-22% in the case of unruptured membranes, and about 50% in the case of ruptured membranes. Subsequently, in 2017, we analyzed the bacterial flora of amniotic fluid using next-generation sequencing technologies and droplet digital PCR and performed a comparison against a control group appropriately selected to assess contamination [43]. We then put forward the concept of microbiomic chorioamnionitis (miCAM) and identified 11 bacterial species that were considered to be the main causative organisms *(Ureaplasma parvum, Streptococcus agalactiae, Gardnerella vaginalis, Streptococcus anginosus, Sneathia sanguinegens, Eikenella corrodens, Prevotella bivia, Lactobacillus jensenii, Bacteroides fragilis, Porphyromonas endodontalis,* and *Mycoplasma hominis*), which are detailed in Table 1 [60-63]. Furthermore, Jung, et al. [57] assessed the relationship between intra-amniotic inflammation and the bacteria detected in amniotic fluid using the results of previous reports [60-62, 75-80], and suggested that *Ureaplasma parvum, Mycoplasma hominis, Sneathia sp., Candida albicans, Fusobacterium nucleatum, Staphylococcus aureus, Gardnerella vaginalis, Haemophilis influenzae,* and *Streptococcus agalactiae* were microorganisms indicated as true pathogens [57], which is generally consistent with our findings [43,80-87]. The causative organisms responsible for intrauterine infections include both aerobic and anaerobic bacteria, although they are almost impossible to identify. Therefore, it may be necessary, at this time, to select antimicrobial agents that cover a broad spectrum of bacterial species with a high tissue distribution rather than conventional administration of antimicrobials that target highly pathogenic bacterial organisms. However, the sequencing of 16S rDNA amplicon libraries using the aforementioned next-generation sequencing technologies may allow for the optimization of the selection of antimicrobial agents based on individual cases (e.g., changing the selected antimicrobials depending on the combination of bacteria detected). Further research will be needed in the future as the interpretation of the results is still hampered by the considerable influence of contamination of cell free DNA from bacteria in the environment [43,57,65,66].

Genus name	Total		Combs 2014 [63]		DiGiulio 2010 [62]		Han 2009 [61]		DiGiulio 2008 [60]	
	16S*	Culture	16S*	Culture	16S*	Culture	16S*	Culture	16S*	Culture
Ureaplasma †	65	68	11	11	49	49	2	5	3	3
Fusobacterium	22	15	5	4	5	2	7	4	5	5
Streptococcus †	19	15	3	3	12	7	2	3	2	2
Sneathia †	13	0	4	0	3	0	2	0	4	0
Bacteroides †	11	2	5	2	2	0	3	0	1	0
Leptotrichia	8	0	1	0	3	0	2	0	2	0
Haemophilis	6	2	1	1	5	1	0	0	0	0
Mycoplasma †	6	10	0	1	3	8	2	0	1	1
Prevotella †	5	3	0	0	2	0	1	2	2	1
Clostridiaceae	4	1	0	0	3	0	1	0	0	0
Bergeyella	3	0	2	0	0	0	1	0	0	0
Enterococcus	3	0	0	0	3	0	0	0	0	0
Gardnerella †	3	3	1	1	2	0	0	0	0	2
Lactobacillus †	3	1	0	0	2	0	0	0	1	1
Bifidobacterium	2	0	0	0	2	0	0	0	0	0
Listeria	2	1	1	0	1	1	0	0	0	0
Neisseria	2	0	0	0	1	0	0	0	1	0
Peptoniphilus	2	0	0	0	2	0	0	0	0	0
Shigella	2	0	0	0	0	0	2	0	0	0
Staphylococcus	2	6	1	1	1	4	0	0	0	0
Atopobium	1	0	0	0	1	0	0	0	0	0
Brachybacterium	1	0	0	0	1	0	0	0	0	0
Campylobacter	1	0	0	0	1	0	0	0	0	0
Citrobacter	1	1	0	0	0	0	1	1	0	0
Coprobacillus	1	0	0	0	1	0	0	0	0	0
Delftia	1	0	0	0	0	0	0	0	1	0
Dialister	1	0	0	0	1	0	0	0	0	0
Filifactor	1	0	0	0	1	0	0	0	0	0
Kingella	1	0	0	0	1	0	0	0	0	0
Myroides	1	0	0	0	1	0	0	0	0	0
Peptostreptococcus	1	4	0	1	0	1	1	0	0	2
Rothia	1	0	0	0	1	0	0	0	0	0
Actinomyces	0	2	0	1	0	1	0	0	0	0
Bacillus	0	1	0	0	0	0	0	0	0	1
Diphtheroides	0	1	0	1	0	0	0	0	0	0
Eikenella †	0	1	0	0	0	0	0	1	0	0
Escherichia	0	2	0	0	0	0	0	2	0	0
Klebsiella	0	1	0	0	0	0	0	1	0	0
Mobiluncus	0	2	0	1	0	1	0	0	0	0
Propionibacterium	0	2	0	0	0	2	0	0	0	0

Note: *16S ribosomal DNA analysis by using broad-range end-point and real-time PCR assays;†: likely to cause chorioamnionitis [43]

Table 1: Demographic variables and co-morbidities.

Evaluation of therapeutic efficacy

Amniocentesis is the most effective and common method to evaluate intrauterine infection/inflammation (especially intra-amniotic infection/inflammation) during pregnancy [88-92]. However, since both clinical doctors and patients tend to think that inserting a needle through a pregnant woman's abdomen is risky, this kind of examination is rarely performed in general clinical practice. In recent years, however, the risk of complications from amniocentesis has been reduced due to improved image resolution in ultrasound tomography and the development of safer puncture needles. In this respect, recent studies have reported abortion rates of 0.06%-0.13% [88,89]. Similarly, a high rate of success and safety have been reported for amniocentesis performed after the middle pregnancy period and in cases of premature rupture of membranes [90,91]. In fact, numerous reports have suggested the usefulness of limited implementation of this procedure in some tertiary care facilities and in clinical studies [16,38,39,43,58,59,92,93].In addition, amniocentesis may be useful in cases of infection or inflammation extending into the amniotic membrane. Rapid Matrix Metalloproteinase (MMP)-8 and IL-6 tests may be effective methods to assess intra-amniotic inflammation [94]. On the other hand, estimation of 16S rDNA copy number is probably the most effective method to directly assess intra-amniotic infection [43,58,59]. As with other infectious diseases, it is obvious that a method that directly assesses infection rather than inflammation (e.g., absolute quantification of 16S rDNA copy number by droplet digital PCR or other means, at the current stage) is preferable as a method for evaluating therapeutic efficacy. However, one of the drawbacks of the quantification of 16S rDNA copy number by digital droplet PCR is that it cannot differentiate between bacterial species. Despite the problems posed by contamination and costs mentioned above, it may be necessary, in the future, to develop methods for evaluating therapeutic efficacy using next-generation sequencing technologies.

Discussion

Selection of patients to be treated

Most women who experience preterm birth associated with intrauterine infection/inflammation do not develop any clinical signs or symptoms of infection (e.g., fever, uterine tenderness) [16,52]. Since non-invasive diagnostic methods have not yet been developed, establishing a diagnosis of intrauterine infection/inflammation in pregnant women is very difficult. Many studies focusing on amniotic fluid, including ours, have allowed the assessment of intra-amniotic infection/inflammation with a fairly high degree of accuracy [43,55-59,94]. However, intra-amniotic infection cannot be prevented unless the patient is selected at a pre-infection stage. Moreover, since the tissue distribution of antimicrobials becomes extremely poor once the infection reaches the amnion, it is necessary to diagnose patients at an early stage when the infection has not yet spread into the amniotic membrane. Many previous studies have suggested that vaginal ascending infections are the most frequent route of intrauterine infections (Figure 1) [13,43,64,95,96]. Therefore, we examined whether analyzing the vaginal bacterial flora at the time of hospital admission for preterm labor was a predictor of chorioamnionitis [44]. While it is already known that chorioamnionitis is characterized by increased vaginal bacterial diversity, the identification of bacterial species with a low composition ratio is crucial. To objectively narrow down our choice, we used a machine learning algorithm called random forest to extract bacterial species that are strongly associated with chorioamnionitis. Then, we identified the 20 bacterial species most strongly associated with chorioamnionitis and created a new scoring method called PCAM (predictive chorioamnionitis) based on their detection pattern, and obtained an area under the curve for the predictive accuracy of chorioamnionitis of 0.849 (95% Confidence Interval: 0.765-0.934), with a sensitivity of 71.4% and specificity of 82.4%. Moreover, the PCAM score was significantly correlated with prolonged pregnancy and developmental disorders in 3-year-old infants. In other words, if the values of patients in the PCAM group were aligned with those of the non-PCAM group by administering antimicrobials and prebiotics, for example, we may be able to prolong the gestation period and improve the poor prognosis of at-risk children. Despite the lack of research focusing on this kind of risk assessment method, if patients at high-risk can be accurately diagnosed, our method may be useful in clarifying the potential therapeutic targets and help develop means of prevention and methods of treatment. We were also able to confirm the practical utility of next-generation sequencing technologies (e.g., Nanopore sequencing), with the main advantages including speed and reduced costs and decided to utilize them in our ongoing RCT (phase II of the specified clinical research; jRCTs071210114). Next-generation sequencing technologies and machine learning/artificial intelligence will likely be applied in medical care and general clinical practice in the near future.

Establishment of new preterm birth prevention and treatment strategies

To date, in the field of amniotic fluid research, we have developed a method to assess the status of intrauterine infection [43]. We have also found that the risk of developing intrauterine infection can be assessed by integrating machine learning techniques with data from vaginal bacterial flora analysis [44]. Subsequently, through joint research with business companies, we have established a system that can be implemented in clinical settings quickly and at a low cost. Accordingly, we are currently selecting high-risk cases using next-generation sequencing technologies to conduct an RCT (phase II of a specified clinical research; jRCTs071210114) assessing the 16S rDNA copy number in amniotic fluid at approximately 1 week after initiation of therapy as a primary endpoint to establish which treatment would be more effective between a comprehensive antimicrobial therapy (e.g., meropenem and azithromycin) and a conventional targeted antimicrobial therapy (e.g., azithromycin). Strategies to suppress inflammation, as well as infection, will also be important. The anti-inflammatory action of lactoferrin, which has recently attracted attention as a treatment for endometritis in infertile women, may also be effective in preventing preterm birth [97-103]. In addition, regenerative medicine is receiving considerable attention for anti-inflammatory treatment in areas other than the perinatal field [104-112]. Recently, we reported that adipose-derived stem cells suppressed inflammation in a preclinical murine model of endometriosis [113]. In addition to using antimicrobials to eradicate the infection, the initiation of such an anti-inflammatory treatment for pregnant women would be ground-breaking and would also set high expectations for the treatment of newborns immediately after birth.

Conclusion

Since medical developments focusing on intrauterine infection have fallen behind due to difficulties in clinical research, a large number of mothers and children are currently exposed to the risk of sequelae of intrauterine infection/inflammation. Given the current situation in developed countries, where the number of births is declining and the number of high-risk pregnancies is increasing, research should be undertaken to prevent premature births and developmental disorders in children using the latest technological advances in science. We are currently conducting an RCT (jRCTs071210114) to investigate the effect of antimicrobial therapy in high-risk cases of chorioamnionitis. The

accumulation of findings obtained from this RCT are expected to yield a breakthrough in the field of perinatal management of intrauterine infection/inflammation.

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