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Fetal bacterial infections in antepartum stillbirth: A case series

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ABSTRACT

Objectives: This study aims to assess the frequency of fetal bacterial infections in stillbirth (SB) and to evaluate the best samples for the diagnosis of infection-related SB.
Study design: Consecutive cases of antepartum SB were enrolled. Vaginal and placental swabs, as well as heart blood cultures and surface swabs from the neonate, were collected. Histological examinations were performed by the same examiner. Immunohistochemistry for leukocyte common antigen was performed in the placenta and fetus. Each case was discussed in a multidisciplinary audit.
Results: One hundred and nine cases were enrolled. Fetal blood cultures were positive in 20/95 cases (21%). Significant histological findings in the placenta/cord and in at least one fetal organ were observed in 8 cases of them (4 *Group B Streptococcus* GBS, 2 *Listeria monocytogenes*, 1 Coagulase negative *Staphylococcus*, 1 *Pseudomonas aeruginosa*). Neither tissue damage nor inflammatory infiltrate was found in the 12 remnant cases. Funisitis while not histological chorioamnionitis was associated with microbiological findings. Positive findings in maternal/placental/fetal swabs occurred in 18–32% of cases with both negative fetal blood cultures and histopathological findings. With the exception of GBS, no other bacteria agent could be detected by any of the swabs.
Conclusions: Eight cases (8.4%) fulfilled both microbiological and histology criteria allowing the diagnosis of SB-related fetal infection demonstrating that search for infections is essential in SB evaluation. Fetal blood culture, placenta swab for GBS and search for histological funisitis are mandatory actions within the SB work-up in order to guide pathology examination and reach clinical conclusions.

1. Introduction

Stillbirth (SB) is a common adverse pregnancy outcome with a rate of 3–8 per 1000 live births in developed countries and approximately 20–40 per 1000 births in developing countries. Rates are conditioned by gestational age definition which ranged 20–28 weeks, according to different countries [1,2]. Although several conditions and specific risk factors have been associated with SB, in most cases, it is difficult to identify the definitive pathway causing death, and a significant number of SBs remain unexplained [3,4]. Every SB classification includes infectious disease as a possible cause of death [5,6]. Several case series have estimated that 10 to 25% of SBs are attributed to fetal infections [7–9]. Moreover, several maternal infection that require hospitalization, placental infection that involves extensive placental damage or infection that leads to preterm labor at pre-viable gestations can be considered probable cause of death [10].

The bacteria that have been most commonly associated with SB include the following: *Treponema pallidum*, *Borrelia burgdorferi*, *Listeria monocytogenes*, *Leptospira*, *Pseudomonas aeruginosa*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Group B Streptococcus*, *Escherichia coli*, *Klebsiella*, *Enterococcus* and *Bacteroides* [6,11]. Some bacteria cause transplacental infections, such as *Treponema*, *Borrelia*, *Leptospira*, *Listeria* and *Pseudomonas*. For example, *Treponema* can cross the placenta, resulting in a reduction of blood flow to the fetus, and can lead to a direct infection of the fetus [12]. *Borrelia* instead causes death, especially in the second trimester, and crossing the placenta infects the fetus. *Borrelia* spirochetes have been found in fetal liver, spleen, kidney and brain [13].

Listeria and *Pseudomonas* also cause transplacental infections. During bacteremia, these organisms are transported to the placenta and can cause villous necrosis and abscesses. The occurrence of SB is caused by both placental dysfunction and fetal infection. Other bacteria, such as *Group B streptococcus*, *E. coli*, *M. hominis*, *U. urealyticum*, *Enterococcus*, *Bacteroides* and *Klebsiella*, cause ascending infection. These organisms enter the amniotic fluid either through intact choriodecidual membranes or after membrane rupture. The most common organ infected is the fetal lung, and the most frequent autoptic finding in these fetuses is pneumonia [14].

The relationships between maternal infections and SB are often unclear, and causality is difficult to prove. Although there are some

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organisms, such as the *Mycoplasmas*/*Ureaplasmas*, that are not easy to identify, finding an organism in the placenta or in the fetus does not prove causality per se.

Moreover, histologic chorioamnionitis has been frequently reported in cases of SB. This finding lacks specificity and it is also reported in live births [7–9].

The aim of this study is twofold. First, to assess the prevalence of SB-related fetal bacterial infections in a multilevel study, including microbiological and histological findings. Second, to determine the best fetal and/or maternal samples for the diagnosis of infection-related SBs.

2. Materials and methods

2.1. Subjects

This study is part of a comprehensive national program sponsored by the Italian Ministry of Education, University and Research to investigate the epidemiology, risk factors, causes and management of SB. Consecutive cases of antepartum SB were collected in the period between June 2005 and December 2011 in the Maternal-Fetal Medicine Unit of our University-Hospital in Modena, Italy. The university's Ethics Committee approved the study. Sensitive information was treated according to the Italian Privacy Act.

SB was defined as neonates of ≥22 complete weeks of gestation or >500 g (if gestational ultrasound age was not available) whose Apgar scores were 0 at the 1st and 5th minutes and who were not actively resuscitated. Such definition was suggested for high-income countries [15]. Ninety-five percent of the pregnancies were dated by a first trimester ultrasound; the fetal weight criterion was utilized in only 3 cases.

To classify the causes of death, the work-up protocol included the following: careful obstetric history and circumstances of SB diagnosis collected in an “ad hoc” form attached to a maternal diary, placenta histology, stillborn autopsy, microbiology evaluation, chromosome analysis (from either amniotic fluid, blood or fascia lata), and neonate inspection.

We defined a SB-related fetal bacterial infection those cases with the simultaneous presence of histological findings (placental/fetal organ damage) and positive fetal microbiological cultures.

2.2. Methods

Each case was thoroughly investigated according to the diagnostic protocol previously explained. Specimens for aerobic cultures and yeast were obtained from the vagina and endocervix using three sterile cotton-tipped swabs, at diagnosis, before labor induction. In only 6 cases women were referred from outside antenatal services. The swabs were inoculated into solid transport media. The bacteria assessed included *L. monocytogenes*, *Neisseria gonorrhea*, *Trichomonas vaginalis*, *aerobic bacteria*, *Gardnerella vaginalis*, *Mycetes*, *M. hominis*, *U. urealyticum* and *Chlamydia trachomatis*. An additional maternal vaginal–rectal sample was obtained to test for *Streptococcus agalactiae* (GBS). Each mother was screened early in pregnancy for *Rubella*, *HBV*, *HCV*, *HIV*, *T. pallidum* (VDRL, RPR), and *Toxoplasma gondii* (IgG, IgM) as part of their routine antenatal care. Maternal serology samples were specifically collected at entry in the hospital if non-immune (e.g. *Toxoplasma*) or not screened. In addition, serology samples from all mothers were tested for *Cytomegalovirus* and *Parvovirus B19*.

At delivery, the stillborn fetus and placenta were placed in sterile towels, and the physicians obtained cultures from both. The cultures from placenta were obtained by the obstetrician by incising through the chorionic plate with a sterile scalped blade and taking two sterile samples from both fetal and maternal sides. The samples from the placental tissue were taken with care to avoid contact with the decidua basalis, and both aerobic and anaerobic bacteria were assessed. The two neonatal surface swabs, one in the nose and throat and one in the ears, were performed by the neonatologists to test for aerobic and anaerobic bacteria. Last, a blood culture was obtained by a puncture of the right ventricle of the stillborn fetus. The neonatal blood was placed into 2 bottles, 1 for anaerobic bacteria and 1 for aerobic bacteria and yeasts.

Fetal autopsy and pathological examination of the placental tissue were performed with the parents' oral, informed consent. The autopsy of the fetus and the placental examination were performed in the Department of Pathology by the same expert perinatal histopathologist, according to international guidelines [16]. Three membrane rolls from different sites, one segment of umbilical cord and two full-thickness blocks of the placenta (1 from the site of cord insertion and 1 from the placenta edge) were isolated and fixed in a 10% formaldehyde solution. The tissue specimens were embedded in paraffin, sectioned and stained by hematoxylin–eosin by light microscopy. Histopathological examinations were performed on the encephalon, heart, lungs, liver, spleen, kidneys, thymus, bone marrow and placenta. The histologic criterion of chorioamnionitis was the presence of polymorphonuclear leukocytes in ten nonadjacent microscopic fields of the fetal membrane roll examined at 400× magnification. This identification was performed using immunohistochemistry for myeloperoxidase and for leukocyte common antigen (CD45). Stillborn fixed tissues, namely the lung and liver, were also subjected to immunohistochemistry. PCR examination was performed in placenta and fetal tissues to confirm the presence of bacteria agents.

All cases with positive blood culture and 10 of those with histologic chorioamnionitis and negative blood culture were reviewed by a second pathologist, in a blind fashion. Disagreement occurred in one case and discussed till a final diagnosis was reached. Statistical analyses were performed with software SPSS 18. Chi-square test was used to compare frequencies. A value of $P < 0.05$ was considered significant.

3. Results

One hundred and nine (109) cases of antepartum SB were enrolled in our study. During the same period 2 intrapartum cases occurred, and were excluded. In 35 cases (32.1%) a cause of death was identified using the CoDAC classification [17]. They were as follow: 5 malformations, 4 genetic disorders, 2 viral infections, 2 immune hydrops and 22 placental vascular disorders (10 placenta abruptio, 8 severe fetal growth restriction <3rd centile, with extensive villous infarcts or perivillous parenchymal fibrin deposition, and 4 severe preeclampsia with large placenta infarctions).

Table 1 described the demographic features of SB population compared with those of general population attending the hospital in the

Table 1
Demographic features of the population (number with % in brackets).

Demographic features	Stillborn 109	Live born 22,700	OR (95% CI)
Mean age (years)	31.1 (17–42)	34 (16–53)	NS
Nulliparity	45(41.3%)	11,881 (52.3%)	0.64 (0.43–0.95)
Ethnicity			
Caucasian	64 (58.7%)	16,989 (74.8%)	NS
Maghreb	16 (14.7%)	2442 (10.7%)	NS
Sub-Saharan Africa	11 (10.1%)	1397(6.2%)	NS
South East Asia	14 (12.8%)	1557(6.8%)	2.0 (1.09–3.6)
Others	4 (3.6%)	315 (1.4%)	NS
Low education (<8 years)	45(41.3%)	7644 (34%)	NS
Overweight–Obesity (BMI > 25/30)	39(9.2%)	1589 (7%)	NS
Gestational age			
$<33^{+6}$ weeks	65(59.6%)	799 (3.5%)	40.5 (26.9–60.8)
34–36^{+6} weeks	13(11.9%)	1669 (7.3%)	NS
>37 weeks	31(28.4%)	21,183 (89%)	0.03 (0.02–0.04)
Males	50 (45.9%)	11,706 (51.5%)	NS
Smokers in pregnancy (since at least 5 years)	18 (16.5%)	2368 (10.4%)	NS