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Clinical Study

Both Acute and Chronic Placental Inflammation Are Overrepresented in Term Stillbirths: A Case-Control Study

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Objective. To elucidate differences in the frequency and severity of acute chorioamnionitis (CAM) and chronic villitis in placentas from stillborns compared with liveborns at term and to evaluate other risk factors and placental findings. *Design*. Case-control study. *Setting*. All delivery wards in major Stockholm area. *Population or Sample*. Placentas from stillborn/case ($n = 126$) and liveborn/control ($n = 273$) neonates were prospectively collected between 2002 and 2005. *Methods*. CAM was assessed on a three-grade scale based on the presence and distribution of polymorphonuclear leucocytes in the chorion/amnion. The presence of vasculitis and funisitis was recorded separately. Chronic villitis was diagnosed by the presence of mononuclear cells in the villous stroma. Relevant clinical data were collected from a specially constructed, web-based database. The statistic analyses were performed using multivariable logistic regression. *Results*. CAM (especially severe, AOR: 7.39 CI: 3.05–17.95), villous immaturity (AOR: 7.17 CI: 2.66–19.33), villitis (<1 % AOR: 4.31 CI: 1.16–15.98; ≥1 %, AOR: 3.87 CI: 1.38–10.83), SGA (AOR: 7.52 CI: 3.06–18.48), and BMI > 24.9 (AOR: 2.06 CI: 1.21–3.51) were all connected to an elevated risk of term stillbirth. *Conclusions*. We found that CAM, chronic villitis, villous immaturity, SGA, and maternal overweight, but not vasculitis or funisitis are independently associated with risk for stillbirth at term.

1. Introduction

Infection is a common cause of stillbirth, although its incidence varies greatly with gestational age and between developed and developing countries [1]. In an earlier study by our group, infection was associated with stillbirth in 24% of cases [2]. In Sweden, stillbirth is currently defined as ante- or intrapartum death from the 22nd week of pregnancy; its incidence is reported to be 5.3 per 1,000 live births [2].

Different infectious agents are widely presumed to produce distinct patterns of inflammation in the placenta. More specifically, bacterial infections are associated with acute inflammation of the placental membranes, notably acute chorioamnionitis (CAM), whereas in viral infections chronic inflammation of the placental parenchyma (chronic villitis) is described [3–5]. The main focus of this paper will be on histologically diagnosed CAM.

Chronic inflammation in the placenta is primarily directed at the villous parenchyma, that is, villitis with little or no involvement of membranes or vessels. It is seen in connection with viral infections or, alternatively, as villitis of unknown etiology with a presumed immunological background [3–7]. Chronic villitis has been linked to intrauterine growth restriction (IUGR) in several studies [4, 6, 7], although its eventual association with stillbirth has not been adequately investigated.

Most previous studies investigating CAM in stillbirth have compared term and preterm stillborn placentas with placentas from term healthy liveborns, disregarding the multifaceted differences between preterm and term period,

not the least concerning the pathophysiology of placenta. Moreover, few studies have addressed the issue of CAM and villitis in the same stillborn and control placental material.

The aims of this study were to elucidate differences in the frequency and severity of CAM and villitis in placentas from stillborn neonates compared with liveborn controls at term and to evaluate other risk factors and placental findings in the study group.

2. Material and Methods

Placentas from stillborn deliveries were prospectively collected between March 2002 and December 2005 at all delivery units in Stockholm, Sweden. Three hundred and forty-nine infants were delivered stillborn during the study period. Forty-three cases were excluded because of twin pregnancy ($n = 23$) or because the placenta was not sent to the Pathology Department ($n = 20$). As controls, we recruited the first term liveborn infant subsequent to the stillborn case, born in the same delivery ward independent of delivery method. A total of 273 term controls were included. The case and the recruited control were born on the same day in 89% of cases. In the present study, we included only placentas from term singleton pregnancies ($n = 126$ cases and $n = 273$ controls).

Since 1998, data from all cases of stillbirth in Stockholm have been collected in a database (http://www.iufd.se). A group of obstetricians representing all delivery wards in Stockholm and perinatal pathologists has regularly performed clinical audits of all stillbirths in the area. All cases of stillbirth in the major Stockholm area are investigated according to a consensus, structured test protocol validated in an earlier study [2]. The protocol includes maternal blood tests (i.e., hemoglobin concentration (Hb), coagulation tests, etc.) and some immunological tests, together with culture from the amnion and fetal heart blood, PCR analysis for cytomegalovirus, parvovirus B19, and enterovirus in placenta, chromosomal analysis in amnion or placenta, placental examination, and autopsy. The results together with relevant family, medical, and obstetrical history are registered in the database. Cause of stillbirth is ascribed to each individual case according to our previous classification [8]. The most common causes (definite or probable) in the 126 term cases presented in this study were identified as infections (25%), placental insufficiency/intrauterine growth restriction (13%), umbilical cord complications (7%), and placental abruption (6%).

During the study period we also used the database to collect information on the controls, including relevant family, medical, and obstetric history as well as the infant's birth weight. Small for gestational age (SGA) was defined (using the standard definition for SGA in Sweden) as birth weight < 2 standard deviations below mean [9]. Maternal blood tests, culture samples, and PCR analyses of the placenta were not routinely performed in the controls.

The study was approved by the Regional Ethics Committee of the Karolinska Institutet in Stockholm, Sweden (no. 02-012).

2.1. Histopathological Examination of the Placentas. All placentas were sent to the Department of Pathology at Karolinska University hospital, Huddinge. One senior perinatal pathologist (N.P.) performed all of the histological investigations. This design was specifically chosen in order to avoid the well-documented interobserver variability in interpreting placental pathology [10]. Due to logistic circumstances, the pathologist was aware of which group the placenta belonged to. The placentas were received fresh and were subjected to thorough gross morphologic assessment. The trimmed placental weight (after fixation) was compared to the gestational age and was determined to be low (<10th percentile), normal, or high (>90th percentile), as previously described by us [11]. For histological examination, we routinely included two samples from the cord, the membranes and two to three samples from macroscopically normal parenchyma as well as from any sites of focal change. Macroscopic and histological examination was performed according to a structured protocol (see the appendix).

Placental data included in this study are: placental weight in relation to/adjusted for gestational age, immaturity of placental villi, infarction, intervillous thrombosis, fetal thrombosis, presence of CAM, vasculitis in the placenta or umbilical cord and/or funisitis (inflammation of the umbilical cord), and chronic villitis. CAM was defined as the presence of polymorphonuclear leukocytes in the chorion or amnion layers with or without necrosis of the membranes and was initially assessed using a three-grade scale, essentially as described by Rindsjö et al. [12]. Grade 1: presence of polymorphonuclear leucocytes at the subchorionic plate and/or lower third of chorion; grade 2: at least two separate foci of leucocytic infiltration in chorion and amnion; grade 3: extensive leucocytic infiltration in chorion and amnion, together with necrosis of amniotic epithelium and/or microabscess formation. Because of the very small amount of grade 3 cases, grade 2 and 3 groups were studied together in the final statistical analysis. Vasculitis was defined as the presence of polymorphonuclear leukocytes in the vessel wall of chorionic plate or umbilical vessels. Funisitis was defined as the presence of polymorphonuclear leukocytes in the Wharton's jelly. Chronic villitis was defined as the presence of a mononuclear cell (lymphocyte, histiocyte, and plasma cell) infiltrate in the villous stroma, often with destruction/necrosis of the villous parenchyma.

2.2. Statistical Analysis. Analyses were performed using Statistica software 8.0. Data were analyzed using Student's t-test (comparing means between the groups), the Mann-Whitney U-test (comparing medians between the groups) or the chi-squared test or the Fishers exact test (comparing proportion between the groups) as indicated in the tables. P values <0.05 were considered statistically significant. Bivariate analyses of categorical data were computed by univariable logistic regression, and risk factors were expressed as odds ratios (OR) with 95% confidence intervals (95% CI). Statistically significant and borderline significant variables in the univariable logistic regression were entered in a stepwise forward

multivariable logistic regression analysis to obtain adjusted odds ratio (AOR) with 95% CI.

3. Results

A total of 126 term stillbirth pregnancies were compared with 273 term liveborn pregnancies. Mothers of stillborn babies were older and had a higher body mass index (BMI) than the controls; the difference in BMI was not dependent on maternal age (Table 1(a)). There was a higher incidence of a history of miscarriage among the cases but the groups did not differ with respect to smoking habits, primiparity, previous stillbirth, severe illness or pregnancy complications, Hb at admission to maternity health care centre, and sex of the infant or delivery method (i.e., caesarean section or vaginal delivery, Table 1(a)). The rate of labour induction was, not surprisingly, different between the groups (Table 1(a)) since induction is routine in cases of stillbirth in Sweden. The groups differed with respect to birth weight. In the stillbirth group, 21.4% was SGA, while only 2.9% was SGA in the control group (Table 1(a)). Similarly, the stillborn group showed lower absolute placental weight and a higher amount of small placental weight versus gestational age (<10th percentile) when compared to the controls (Table 1(b)). In the histological evaluation, several significant differences were noted; placentas from stillborn cases had significantly more CAM, villitis, immature villi, infarction, fetal thrombosis and vasculitis and/or funisitis (Table 1(b)). Intervillous thrombosis did not differ between the groups (Table 1(b)).

In the stillbirth group, 65.1% had CAM (of any grade) compared with 40.7% in the control group ($P < .001$). CAM was initially graded (grade 1–3), but because there were only two cases of CAM grade 3 in the stillbirth group and none in the control group, CAM grades 2 and 3 were studied together (referred to as severe CAM), yielding a frequency of 33.3% in the stillbirth group and 11.4% in the control group. CAM grade 1 (referred to as light CAM) was found in 31.7% in the stillbirth group and 29.3% in the control group (Table 1(b)).

Villitis was seen more frequently in the stillbirth group than in the control group (18.3% and 5.1%, resp., Table 1(b)). Villitis was subgrouped with respect to extent. Villitis <1% was found in 6.4% of the stillbirth group and 1.8% of the control group; whereas 11.9% in the stillbirth group and 3.3% in the control group had ≥1% villitis (Table 1(b)).

Overweight or obesity (BMI>24.9), history of miscarriage, SGA, low placental weight versus gestational age, immature placental villi, CAM (light and severe), vasculitis and/or funisitis, and villitis <1% and ≥1% were all associated with an elevated risk for stillbirth in the univariable logistic regression (Table 2). Smoking at registration in maternity centre, primiparity and severe illness or pregnancy complication, sex of the baby, and intervillous thrombosis were not associated with an increased risk whereas age >34 years and infarction of placental villi were of borderline significance (Table 2). As shown in Table 1(b), fetal thrombosis was significantly overrepresented in stillborn placentas. However, since histopathological analysis cannot with certainty discriminate between thromboses having occurred before fetal death, and those associated to postmortem changes this finding was not analyzed further statistically.

In the stepwise forward multivariable logistic regression severe CAM, immature placental villi and SGA had the highest risk for stillbirth, but even light CAM, villitis (<1% and ≥1%), and BMI >24.9 were associated with an increased risk. Age >34 years, previous miscarriage, low placental weight versus gestational age, and infarction were not found to be independent risk factors and were therefore not included in the final model of the multivariable logistic regression (Table 3). Vasculitis and/or funisitis did not either show an independent risk but was forced in the model since it has a well-known association with CAM [4, 6, 13] (Table 3).

Amniocentesis was performed in 49 stillborn cases (38.9%), and the bacterial culture was positive in six of those cases (12.2%). Because there were so few analyses, and no cultures were performed in the controls, no further analysis was done. Blood cultures from the stillborns' heart were performed in 102 (81.0%) of the 126 cases and was positive in 27 (26.5%) of the analyzed cases.There was no statistical correlation between positive bacterial culture of heart blood and CAM (independent of grade), vasculitis, funisitis, villitis, or thrombosis (data not presented).

4. Discussion

In this study, we have found that CAM, chronic villitis, immature placental villi, SGA, and maternal overweight/obesity are independently associated with risk for stillbirth at term.

We found CAM in 65.1% of stillborn cases and 40.7% in liveborn controls. Other studies have shown prevalence of CAM in stillbirths ranging between 57% to 96% [14–17] and in term liveborns from 5% to 68% [13–19]. The reason for these discrepancies between different studies is not entirely clear. The fact that the majority of studies claiming high proportions of CAM were from developing countries may suggest some kind of underlying geographical/environmental factor. Other explanations may include differences in the definition of stillbirth and histological CAM, interobserver variability between reporting pathologists, small sample sizes, or selection of material. The high frequency of CAM in our control group might be additionally related to the selection criterion being liveborn, and not uncomplicated ("healthy") pregnancy, in contrast to several other reports. Our study is one of the largest studies in the literature and provides a comprehensive view of the stillbirth population in the major Stockholm area.

A high frequency of CAM, vasculitis and/or funisitis could be due to the duration of established contractions or the time interval between broken membranes and delivery. This information was not available for all in the study

Table 1

(a) Maternal and child demographic data from stillborns and liveborn controls.

Variable	*n*	Stillborns	*n*	Liveborns	*P* value
Gestational age in completed weeks	*126*	39.4 ± 1.6	*273*	39.3 ± 1.2	ns
Maternal age	*126*	31.8 ± 4.8	*273*	30.3 ± 5.3	<0.01
BMI	*114*	24.3 (18.2–43.7)	*259*	23.1 (14.9–47.1)	<0.001
Previous miscarriage	*124*	35 (28.2)	*269*	45 (16.7)	<0.01
Smoking at registration in maternity centre	*122*	13 (10.7)	*266*	17 (6.4)	ns
Smoking at 32 weeks	*104*	7 (6.7)	*221*	10 (4.5)	ns
Primipara	*124*	68 (54.8)	*269*	142 (52.8)	ns
Previous stillbirth (≥1 para)	*56*	2 (3.6)	*127*	0 (0.0)	ns*
Sever illness or pregnancy complication	*126*	13 (10.3)	*273*	26 (9.5)	ns
Hb at registration in maternity centre	*122*	129.0 ± 11.6	*262*	127.5 ± 11.1	ns
Induction of labour	*126*	70 (55.6)	*273*	37 (13.6)	<0.001
Caesarean section	*126*	10 (7.9)	*273*	26 (9.5)	ns
Sex of the infant (male/female)	*126*	60/66 (47.6/52.4)	*273*	153/120 (56.0/44.0)	ns
Birth weight	*126*	3243 ± 585	*273*	3560 ± 509	<0.001
Small for gestational age	*126*	27 (21.4)	*273*	8 (2.9)	<0.001

BMI: body mass index; Hb: hemoglobin concentration; ns: not significant
BMI was not related to age in linear logistic regression
Data are presented as mean ± SD, median (min-max) or *n* (%); Student's *t*-test, Mann-Whitney *U*-test, Chi-squared test, or Fisher's exact test*.

(b) Histopathological findings in placentas from stillborns and liveborn controls.

Variable	*n*	Stillborns	*n*	Liveborns	*P* value
Placental weight	*126*	431.0 ± 107.9	*269*	483.1 ± 97.7	<0.001
Low placental weight versus gestational age	*126*	64 (50.8)	*269*	90 (33.5)	<0.001
Immature placental villi	*126*	18 (14.3)	*273*	7 (2.6)	<0.001
Infarction	*126*	50 (39.7)	*273*	80 (29.3)	<0.05
Intervillous thrombosis	*126*	43 (34.1)	*273*	71 (26.0)	ns
Fetal thrombosis	*126*	80 (63.5)	*272*	44 (16.2)	<0.001
Chorioamnionitis	*126*	82 (65.1)	*273*	111 (40.7)	<0.001
Chorioamnionitis grade 1		40 (31.7)		80 (29.3)	
Chorioamnionitis grade 2-3		42 (33.3)		31 (11.4)	
Vasculitis and/or funisitis	*126*	37 (29.4)	*272*	43 (15.8)	<0.01
Villitis	*126*	23 (18.3)	*273*	14 (5.1)	<0.001
Villitis <1%		8 (6.4)		5 (1.8)	
Villitis ≥1%		15 (11.9)		9 (3.3)	

Data are presented as mean ± SD or *n* (%); Student's *t*-test, Chi-squared test.

group. A subanalysis of half of the vaginally delivered cases and controls did not show any difference concerning time between rupture of membranes and delivery but there was a significant difference regarding time from established contraction to delivery, with longer labour in the control group ($P < 0.05$, data not presented).

Although CAM is generally believed to represent an ascending microbial infection [14, 17, 18, 20], our results of blood culture from the heart of the neonate failed to show a bacterial etiology of this placental inflammation. One reason for this might be that blood samples were only cultured for aerobic and anaerobic bacteria. The presence of other microorganisms, such as ureaplasma or mycoplasma, which have been related to stillbirth in other studies [1], may have eluded detection. Several studies have questioned the efficacy of culture alone for detecting bacteria [21–23] suggesting the need for supplemental molecular techniques (i.e., PCR).

The high risk of stillbirth correlated to severe CAM point and to the importance of clarifying the background of CAM since it is obviously a threat to the fetus in term pregnancy.

Vasculitis and funisitis are histological markers of the fetal inflammatory response syndrome and are risk markers for neurological impairment [4] and decreased psychomotor development [6, 24]. However, the relation between fetal inflammatory response syndrome and stillbirth is less well understood. In a recent work, Gordon et al. [25] found that absence of a fetal inflammatory response was strongly associated with unexplained antepartum death. Our results are consistent with these data. Although vasculitis and/or funisitis were significantly more common in the placentas

Table 2: The odds ratio for maternal and child demographic data and histopathological findings in placentas from stillborns and liveborn controls.

Variable		*Total number*	Stillborns *n* (%)	Liveborns *n* (%)	OR	95% CI
Maternal age	>34 year	*97*	39 (40.2)	58 (59.8)	1.66	1.03–2.68
	<35 year	*302*	87 (28.8)	215 (71.2)	1	reference
Maternal BMI	>24.9	*121*	51 (42.2)	70 (57.8)	2.19	1.38–3.46
	<25.0	*252*	63 (25.0)	189 (75.0)	1	reference
Previous miscarriage	Yes	*124*	35 (28.2)	45 (36.3)	1.96	1.18–3.24
	No	*269*	48 (17.8)	122 (45.4)	1	reference
Smoking at registration in maternity centre	Yes	*30*	13 (43.3)	17 (56.7)	1.75	0.82–3.72
	No	*358*	109 (30.4)	249 (69.6)	1	reference
Primipara	Yes	*210*	68 (32.4)	142 (67.6)	1.09	0.71–1.66
	No	*183*	56 (30.6)	127 (69.4)	1	reference
Severe illness or pregnancy complication during actual pregnancy	Yes	*39*	13 (33.3)	26 (67.7)	1.09	0.54–2.21
	No	*360*	113 (31.4)	247 (68.6)	1	reference
Sex of the infant	Male	*213*	60 (28.2)	153 (71.8)	0.71	0.47–1.09
	Female	*186*	66 (35.5)	120 (64.5)	1	reference
Small for gestational age	Yes	*35*	27 (77.1)	8 (22.9)	9.03	3.97–20.55
	No	*364*	99 (27.2)	265 (72.8)	1	reference
Low placental weight versus gestational age	Yes	*154*	64 (41.6)	90 (58.4)	2.05	1.33–3.16
	No	*241*	62 (25.7)	179 (74.3)	1	reference
Immature placental villi	Yes	*25*	18 (72.0)	7 (28.0)	6.33	2.57–15.60
	No	*374*	108 (28.9)	266 (71.1)	1	reference
Intervillous Thrombosis	Yes	*114*	43 (37.7)	71 (62.3)	1.47	0.93–2.33
	No	*285*	83 (29.1)	202 (70.9)	1	reference
Infarction	Yes	*130*	50 (38.5)	80 (61.5)	1.59	1.02–2.47
	No	*269*	76 (28.2)	193 (71.8)	1	reference
Chorioamnionitis (grade)	No	*206*	44 (21.4)	162 (78.6)	1	reference
	grade 1	*120*	40 (33.3)	80 (66.7)	1.84	1.11–3.05
	grade 2-3	*73*	42 (57.5)	31 (42.5)	4.99	2.82–8.83
Vasculitis and/or Funisitis	Yes	*80*	37 (46.2)	43 (53.8)	2.21	1.34–3.66
	No	*318*	89 (28.9)	229 (72.0)	1	reference
Villitis (%-grade)	No	*362*	103 (28.4)	259 (71.6)	1	reference
	<1%	*13*	8 (61.5)	5 (38.5)	4.02	1.29–12.59
	≥1%	*24*	15 (62.5)	9 (37.5)	4.19	1.78–9.88

CI: confidence interval; OR: odds ratio; BMI: body mass index.

Table 3: The adjusted odds ratio and their 95% confidence intervals for maternal and childe demographic data and histopathological findings in placentas from stillborns and liveborn controls.

Variable	Adjusted OR	95% CI
BMI >24.9	2.06	1.21–3.51
Small for gestational age	7.52	3.06–18.48
Immature placental villi	7.17	2.66–19.33
Chorioamnionitis (graded)		
No	1	reference
grade 1	2.00	1.08–3.70
grade 2-3	7.39	3.05–17.95
Vasculitis and/or Funisitis	0.67	0.30–1.51
Villitis		
No	1	reference
<1%	4.31	1.16–15.98
≥1%	3.87	1.38–10.83

CI: confidence interval; OR: odds ratio. BMI: body mass index.

from stillborn than from liveborn neonates in our study, the risk of stillbirth was not elevated in the multivariate logistic regression analysis. Further studies are needed to elucidate whether fetal inflammatory response, exemplified by the presence of inflammation in fetal vessels and umbilical cord in the placenta, as well as cytokine release, is unrelated to the mechanisms of stillbirth or even confers some kind of protective action.

Chronic villitis has received less attention than CAM, especially in stillbirth. Villitis affects 5–15% of all third trimester placentas [7] and has been associated with adverse neurologic outcomes, IUGR, stillbirth [4, 6, 7], premature delivery, and high maternal BMI [7].

Syridou et al. recently showed [5] that the presence of chronic villitis in stillbirth placentas was associated with increased detection of viral DNA, especially in advanced gestational ages, implicating a link between viral infection and the pathogenesis of stillbirth. However, in the majority of cases, the etiology of chronic villitis remains unknown, often referred to as villitis of unknown etiology (VUE, [7]). Myerson et al. [26] demonstrated that the majority of lymphocytes in VUE are maternal T cells, and the process is associated to significant destruction of syncytiotrophoblast; this may contribute to the breakdown of the local placental barrier and the graft-versus-host-like invasion of maternal cells into the villi. In our study, villitis, independent of the extent, was clearly overrepresented in stillbirth placentas (18.3% compared to 5.1% in controls) and villitis (<1% or ≥1%) had approximately a four-fold risk for stillbirth in term pregnancies. Our data do not provide any clues regarding the underlying mechanism, although it appears to not be a direct effect, that is, not related to extent of reduction of placental parenchyma. Rather, it might involve some subtle immunologic imbalance with possible secretion of regulatory cytokines or other factors in parallel with fetal inflammatory response.

Villous immaturity, otherwise referred to as villous maturation defect, retarded villous maturation or terminal villous insufficiency, mainly involving a defect in villous vascularisation, is an often underrecognized entity [27]. The histopathological diagnosis is generally considered difficult, and a high rate of interobserver variability has been documented [28]. Nevertheless, Stallmach et al. clearly showed that this type of placental defect can be a cause of fetal hypoxia and is linked to highly increased risk for stillbirth, especially during the end of pregnancy [27]. Our results confirm these observations, showing a more than six-fold risk of stillbirth. Villous immaturity has been associated to maternal diabetes [27] and trisomy 21 [29]. Among our stillbirth placentas showing villous immaturity, two (11%) were connected to diabetes, a figure similar to the findings of Stallmach et al. [27], and additional two had trisomy 21; in the remaining cases, the defect must be regarded as "idiopathic."

SGA showed a sevenfold risk of stillbirth. SGA is a known risk factor for stillbirth [30] and can include infants with intrauterine growth retardation and be related to maternal smoking and mothers with illness as for example preeclampsia [31]. In this study, we did not find an elevated risk for stillbirth among smoking mothers or mother with severe illness or pregnancy complication. The lack of difference might be due to the small amount of smokers and mothers with severe illness or pregnancy complications that might lead to a type 2 error related to inadequate power in this study. Another explanation can be that fetuses with these risks die in earlier gestation.

Earlier studies have reported that high BMI is a risk factor for stillbirth [32]. In this study, we found a two-fold risk for stillbirth if BMI >24.9. High BMI is an increasing problem globally and is associated with several diseases as preeclampsia and diabetes [33]. Even in healthy overweight and obese women, the risk for term stillbirth is increased [32]. The reason for that is unclear and needs to be further investigated.

There is always a risk for bias in case-control studies, for example, recall or observer bias. Since the cases in this study were included after the stillbirth was identified and most of the information was collected from the maternity medical records, we do not think that recall bias is a big problem in this study, even though the risk cannot be ignored. The pathologist was not blinded for the groups, implying a risk for observer bias. To decrease that risk, a structured protocol for placental examination was used. By using a graded scale for CAM the risk for inter- and intra-observer variability can be further reduced [10]. We could exclude the risk for interobserver variability since all placental examinations were performed by only one pathologist. A disadvantage of this approach is that the reproducibility of placental findings is not validated, but we considered that outside the scope of the present study.

5. Conclusion

In summary, we found that CAM, chronic villitis, immature placental villi, SGA, and maternal overweight but not vasculitis or funisitis are independently associated with risk

for stillbirth at term. The underlying mechanism for this needs to be further investigated.

Appendix

A. Structured Protocol for Singleton Placental Examination

A.1. Gross Anatomy. Size; largest and smallest measurement (. . . x. . . x. . . cm), thickness (up to. . . cm).

Shape: normal, accessory lobe(s), artifacts, other

Insertion of membranes: if extrachoreal % of circumference, width (cm).

Trimmed weight (after fixation, gram).

Fetal surface: color, consistency of membranes; chorionic vessels; other,

Umbilical cord: site of insertion (if velamentous distance from placental edge), vascular coiling index, length, true knots, other.

Maternal surface (completeness, signs of abruption, other).

Cut surface: focal changes (relation to basal and chorionic plates, color, extent in % of placental volume), other.

Sampling for histology: umbilical cord (fetal and placental end), membrane roll (x2), central and peripheral part of placenta; additional sampling from focal changes in placenta or cord.

A.2. Histology. Villous maturation (related to gestational age).

Villous morphology (inflammation, necrosis, thrombosis, other).

Chorionic plate (inflammation, fetal stem vessels, other).

Basal plate/decidua (completeness, signs of abruption, arteriopathy, other).

Umbilical cord (number of vessels, inflammation, thrombosis, other).

Fetal membranes (inflammation, arteriopathy, other).

Conflict of Interests

There was no conflict of interests.

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References

[1] R. L. Goldenberg and C. Thompson, "The infectious origins of stillbirth," *American Journal of Obstetrics and Gynecology*, vol. 189, no. 3, pp. 861–873, 2003.

[2] K. Petersson, K. Bremme, R. Bottinga et al., "Diagnostic evaluation of intrauterine fetal deaths in Stockholm 1998-99," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 81, no. 4, pp. 284–292, 2002.

[3] K. Benirschke, R. Coen, B. Patterson, and T. Key, "Villitis of known origin: varicella and toxoplasma," *Placenta*, vol. 20, no. 5-6, pp. 395–399, 1999.

[4] R. W. Redline, "Placental inflammation," *Seminars in Neonatology*, vol. 9, no. 4, pp. 265–274, 2004.

[5] G. Syridou, N. Spanakis, A. Konstantinidou et al., "Detection of cytomegalovirus, parvovirus B19 and herpes simplex viruses in cases of intrauterine fetal death: association with pathological findings," *Journal of Medical Virology*, vol. 80, no. 10, pp. 1776–1782, 2008.

[6] R. W. Redline, "Inflammatory responses in the placenta and umbilical cord," *Seminars in Fetal and Neonatal Medicine*, vol. 11, no. 5, pp. 296–301, 2006.

[7] R. W. Redline, "Villitis of unknown etiology: noninfectious chronic villitis in the placenta," *Human Pathology*, vol. 38, no. 10, pp. 1439–1446, 2007.

[8] I. H. Varli, K. Petersson, R. Bottinga et al., "The Stockholm classification of stillbirth," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 87, no. 11, pp. 1202–1212, 2008.

[9] K. Maršál, P. H. Persson, T. Larsen, H. Lilja, A. Selbing, and B. Sultan, "Intrauterine growth curves based on ultrasonically estimated foetal weights," *Acta Paediatrica*, vol. 85, no. 7, pp. 843–848, 1996.

[10] R. W. Redline, O. Faye-Petersen, D. Heller, F. Qureshi, V. Savell, and C. Vogler, "Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns," *Pediatric and Developmental Pathology*, vol. 6, no. 5, pp. 435–448, 2003.

[11] M. T. Vinnars, L. C. D. Wijnaendts, M. Westgren, A. C. Bolte, N. Papadogiannakis, and J. Nasiell, "Severe preeclampsia with and without HELLP differ with regard to placental pathology," *Hypertension*, vol. 51, no. 5, pp. 1295–1299, 2008.

[12] E. Rindsjö, I. Hulthen Varli, M. F. Ofori et al., "Presence of IgE^+ cells in human placenta is independent of malaria infection or chorioamnionitis," *Clinical and Experimental Immunology*, vol. 144, no. 2, pp. 204–211, 2006.

[13] M. L. Houben, P. G. J. Nikkels, G. M. van Bleek et al., "The association between intrauterine inflammation and spontaneous vaginal delivery at term: a cross-sectional study," *PLoS One*, vol. 4, no. 8, Article ID e6572, 2009.

[14] P. A. Quinn, J. Butany, J. Taylor, and W. Hannah, "Chorioamnionitis: its association with pregnancy outcome and microbial infection," *American Journal of Obstetrics and Gynecology*, vol. 156, no. 2, pp. 379–387, 1987.

[15] S. R. Moyo, I. Hägerstrand, L. Nyström et al., "Stillbirths and intrauterine infection, histologic chorioamnionitis and microbiological findings," *International Journal of Gynecology and Obstetrics*, vol. 54, no. 2, pp. 115–123, 1996.

[16] E. Folgosa, C. Gonzalez, N. B. Osman, I. Hägerstrand, S. Bergström, and A. Ljungh, "A case control study of chorioamniotic infection and histological chorioamnionitis in stillbirth," *APMIS*, vol. 105, no. 4, pp. 329–336, 1997.

[17] E. Tolockiene, E. Morsing, E. Holst et al., "Intrauterine infection may be a major cause of stillbirth in Sweden," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 80, no. 6, pp. 511–518, 2001.

[18] L. Maleckiene, R. Nadisauskiene, I. Stankeviciene, A. Cizauskas, and S. Bergström, "A case-referent study on fetal bacteremia and late fetal death of unknown etiology in Lithuania," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 79, no. 12, pp. 1069–1074, 2000.

[19] C. M. Salafia, C. Weigl, and L. Silberman, "The prevalence and distribution of acute placental inflammation in uncomplicated term pregnancies," *Obstetrics and Gynecology*, vol. 73, no. 3, part 1, pp. 383–389, 1989.

[20] R. L. Naeye, "The investigation of perinatal deaths," *New England Journal of Medicine*, vol. 309, no. 10, pp. 611–612, 1983.

[21] D. B. DiGiulio, R. Romero, J. P. Kusanovic et al., "Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes," *American Journal of Reproductive Immunology*, vol. 64, no. 1, pp. 38–57, 2010.

[22] H. S. Seong, S. E. Lee, J. H. Kang, R. Romero, and B. H. Yoon, "The frequency of microbial invasion of the amniotic cavity and histologic chorioamnionitis in women at term with intact membranes in the presence or absence of labor," *American Journal of Obstetrics and Gynecology*, vol. 199, no. 4, pp. 375.e1–375.e1, 2008.

[23] A. Satosar, N. C. Ramirez, D. Bartholomew, J. Davis, and G. J. Nuovo, "Histologic correlates of viral and bacterial infection of the placenta associated with severe morbidity and mortality in the newborn," *Human Pathology*, vol. 35, no. 5, pp. 536–545, 2004.

[24] R. Mittendorf, A. G. Montag, W. MacMillan et al., "Components of the systemic fetal inflammatory response syndrome as predictors of impaired neurologic outcomes in children," *American Journal of Obstetrics and Gynecology*, vol. 188, no. 6, pp. 1438–1446, 2003.

[25] A. Gordon, M. Lahra, C. Raynes-Greenow, and H. Jeffery, "Histological chorioamnionitis is increased at extremes of gestation in stillbirth: a population-based study," *Infectious Diseases in Obstetrics and Gynecology*, vol. 2011, Article ID 456728, 7 pages, 2011.

[26] D. Myerson, R. K. Parkin, K. Benirschke, C. N. Tschetter, and S. R. Hyde, "The pathogenesis of villitis of unknown etiology: analysis with a new conjoint immunohistochemistry-in situ hybridization procedure to identify specific maternal and fetal cells," *Pediatric and Developmental Pathology*, vol. 9, no. 4, pp. 257–265, 2006.

[27] T. Stallmach, G. Hebisch, K. Meier, J. W. Dudenhausen, and M. Vogel, "Rescue by birth: defective placental maturation and late fetal mortality," *Obstetrics and Gynecology*, vol. 97, no. 4, pp. 505–509, 2001.

[28] R. W. Redline, T. Boyd, V. Campbell et al., "Maternal vascular underperfusion: nosology and reproducibility of placental reaction patterns," *Pediatric and Developmental Pathology*, vol. 7, no. 3, pp. 237–249, 2004.

[29] H. Fox, *Pathology of the Placenta*, Elsevier Saunders, London, UK, 3rd edition, 2007.

[30] J. Gardosi and A. Francis, "Adverse pregnancy outcome and association with small for gestational age birthweight by customized and population-based percentiles," *American Journal of Obstetrics and Gynecology*, vol. 201, no. 1, pp. 28.e1–28.e1, 2009.

[31] L. McCowan and R. P. Horgan, "Risk factors for small for gestational age infants," *Best Practice and Research*, vol. 23, no. 6, pp. 779–793, 2009.

[32] O. Stephansson, P. W. Dickman, A. Johansson, and S. Cnattingius, "Maternal weight, pregnancy weight gain, and the risk of antepartum stillbirth," *American Journal of Obstetrics and Gynecology*, vol. 184, no. 3, pp. 463–469, 2001.

[33] D. Mandal, S. Manda, A. Rakshi, R. P. Dey, S. C. Biswas, and A. Banerjee, "Maternal obesity and pregnancy outcome: a prospective analysis," *The Journal of the Association of Physicians of India*, vol. 59, pp. 486–489, 2011.