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Amniotic Infection Syndrome: Nosology and Reproducibility of Placental Reaction Patterns

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ABSTRACT

Clinically responsive placental examination seeks to provide useful information regarding the etiology, prognosis, and recurrence risk of pregnancy disorders. The purpose of this study was to assemble and validate a complete set of the placental reaction patterns seen with amniotic fluid infection in the hope that this might provide a standardized diagnostic framework useful for practicing pathologists. Study cases (14 with amniotic fluid infection, 6 controls) were reviewed blindly by six pathologists after agreement on a standard set of diagnostic criteria. After analysis of initial results, criteria were refined and a second, overlapping set of cases were reviewed. Majority vote served as the gold standard. Grading and staging of maternal and fetal inflammatory responses was found to be more reproducible using a two- versus three-tiered grading system than a three- versus five-tiered staging system (overall agreement 81% vs. 71%). Sensitivity, specificity, and efficiency for individual observations ranged from 67–100% (24/30 > 90%). Reproducibility was measured by unweighted kappa values and interpreted as follows: < 0.2, poor; 0.2–0.6, fair/moderate; > 0.6, substantial. Kappa values for the 12 lesions evaluated in 20 cases by the six pathologists were: acute chorioamnionitis/maternal inflammatory response (any, 0.93; severe 0.76; advanced stage, 0.49); chronic (subacute) chorioamnionitis (0.25); acute chorioamnionitis/fetal inflammatory response (any, 0.90; severe, 0.55; advanced stage, 0.52); chorionic vessel thrombi (0.37); peripheral funisitis (0.84); acute villitis (0.90); acute intervillositis/intervillous abscesses (0.65), and decidual plasma cells (0.30). Adoption of this clearly defined, clinically relevant, and pathologically reproducible terminology could enhance clinicopathologic correlation and provide a framework for future clinical research.

Key words: amniotic infection syndrome, chorioamnionitis, nomenclature, placenta, reproducibility

INTRODUCTION

The general sequence of pathologic changes accompanying amniotic fluid infection has been recognized for some time [1, 2]. Other clinically relevant histologic patterns have been added to this basic framework [3–9]. Despite a fairly extensive

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literature on this topic, there has been little change in the prevailing diagnostic terminology which has basically been "acute chorioamnionitis with or without funisitis." Recent data has suggested a relationship between placental inflammation and important clinical outcomes such as neurologic impairment and chronic lung disease [10–12]. This has coincided with a more active clinical approach to the management of chorioamnionitis and preterm labor [13]. These changes have led to the need for more detailed description, more specific diagnostic criteria, and greater standardization of diagnostic terminology in this area.

As in other organ systems, questions have been raised regarding the reliability of placental diagnosis, i.e., the ability of expert placental pathologists to agree with each other and with their general pathology colleagues [14–16]. With renewed interest in the clinical significance of placental pathology, the time seems appropriate to introduce more precision and uniformity in placental diagnosis. With this in mind, the Perinatal Section of the Society for Pediatric Pathology has undertaken an initiative to review, define, and validate diagnostic criteria for a number of different placental reaction patterns. The current report presents the results of the Amniotic Fluid Infection Nosology Committee.

METHODS

A study set of 20 cases was assembled from the files of University Hospitals of Cleveland. Three slides from each case (umbilical cord, placental membrances, and one full-thickness section of placental parenchyma) were selected. Gestational age and placental weight were provided for each case. Fourteen placentas were originally diagnosed by one of the pathologists (R.W.R.) with one or more findings consistent with amniotic fluid infection. The remaining six placentas had other pathologic diagnoses, but lacked signs of amniotic fluid infection. Placental reaction patterns relevant to amniotic fluid infection were chosen for evaluation. These patterns are described below, summarized in Table 1, and illustrated in Figures 1–3. All cases were examined in a blinded fashion by the six members of the study group using a previously agreed upon set of diagnostic criteria (round 1). Terminology was chosen to correspond as closely as possible to that proposed by the College of American Pathologists consensus group on placental diagnosis [17]. Tabulated results from the preliminary round (round 1) were circulated and discussed. Representative photomicrographs of each lesion were reviewed at a meeting of the Society for Pediatric Pathology—Perinatal Section. After the initial review a second revised set of diagnostic criteria were proposed and agreed upon. Twelve of the original cases were retained, eight new cases were added (five new cases and three new controls), and the numerical order was changed. This second overlapping set of 20 cases was then circulated for reexamination followed by an analysis of individual performance and reproducibility (round 2).

The revised diagnostic criteria as agreed upon by the study participants before the final evaluation (round 2) were as follows:

Maternal inflammatory responses

Stage/progression of disease

Stage 1 (acute subchorionitis/early acute chorionitis) = patchy-diffuse accumulations of neutrophils in the subchorionic plate fibrin (Fig. 1a) and/or membranous chorionic trophoblast layer (a few scattered neutrophils in the lower half of chorionic plate and/or the membranous chorionic connective tissue are allowed) (Fig. 1b). Stage 2 (acute chorioamnionitis) = more than a few scattered neutrophils in the chorionic plate or membranous chorionic connective tissue and/or the amnion (Fig. 1c). Stage 3 (necrotizing chorioamnionitis) = degenerating neutrophils (karyorrhexis), thickened eosinophilic amniotic basement membrane, and at least focal amnionic epithelial degeneration/sloughage (Fig. 1d).

Grade/intensity

Grade 1 (mild–moderate) = individual or small clusters of maternal neutrophils diffusely infiltrating amnion, chorionic plate, chorion laevae, and/or subchorionic fibrin (Fig. 1c). Grade 2 (severe) = three or more chorionic microabscesses (microabscess = confluent neutrophils measuring at least 10×20 cells in extent) between chorion and decidua in the membranes and/or under the chorionic plate (Fig. 1e) or a continuous band of confluent chorionic polymorphonuclear leuko-

Table 1. Placenta reaction patterns related to amniotic fluid infection: nomenclature and definitions

Diagnostic categories	Suggested diagnostic terminology	Definitions
Maternal inflammatory response		
Stage		
1—Early	Acute subchorionitis or chorionitis	PMN in subchorionic fibrin and/or membrane trophoblast (Fig. 1a,b)
2—Intermediate	Acute chorioamnionitis	Diffuse-patchy PMN in fibrous chorion and/or amnion (Fig. 1c)
3—Advanced	Necrotizing chorioamnionitis	PMN karyorrhexis, amniocyte necrosis, and/or amnion basement membrane thickening/hypereosinophilia (Fig. 1d)
Grade		
1—Mild–moderate	No special terminology required	Not severe as defined below
2—Severe	Severe acute chorioamnionitis *or* with subchorionic microabscesses	Confluent PMN ($\geq 10 \times 20$ cells in extent) between chorion and decidua; ≥ 3 isolated foci or continuous band (Fig. 1e)
Other	Chronic (or subacute) chorioamnionitis	Subamnionic mononuclear cell infiltrate with occasional PMN (meconium and hemosiderin-laden macrophages excluded) (Fig. 1f)
Fetal inflammatory response		
Stage		
1—Early	With chorionic vasculitis *or* umbilical phlebitis	Intramural PMN-chorionic vessels and/or umbilical vein (Fig. 2a,b)
2—Intermediate	With umbilical vasculitis (one or two arteries ± vein) *or* umbilical panvasculitis (all vessels)	Intramural PMN-umbilical artery or arteries (± umbilical vein) (Fig. 2c)
3—Advanced	With (subacute) necrotizing funisitis *or* with concentric umbilical perivasculitis	PMN ± associated debris in concentric bands-rings-halos around one or more umbilical vessels (Fig. 2d)
Grade		
1—Mild–moderate	No special terminology required	Not severe as defined below
2—Severe	With a severe fetal inflammatory response *or* with intense chorionic (umbilical) vasculitis	Near confluent intramural PMN-chorionic and/or umbilical vessels with attenuation/degeneration of VSMC (Fig. 2e,f)
Other	With associated fetal vessel thrombi	Recent thrombosis associated with intramural PMN (Fig. 2f)
Other specific features	Peripheral funisitis	Focal aggregates of PMN at the umbilical cord surface (Fig. 3a)
	Acute villitis	PMN in villous stroma (or between trophoblast and stroma) (Fig. 3b)
	Acute intervillositis with intervillous abscesses	Patchy-diffuse PMN in intervillous space (Fig. 3c)
	Decidual plasma cells	Unequivocal plasma cells in decidua basalis or capsularis (Fig. 3d)

PMN, polymorphonuclear leukocyte; VSMC, vascular smooth muscle cell.

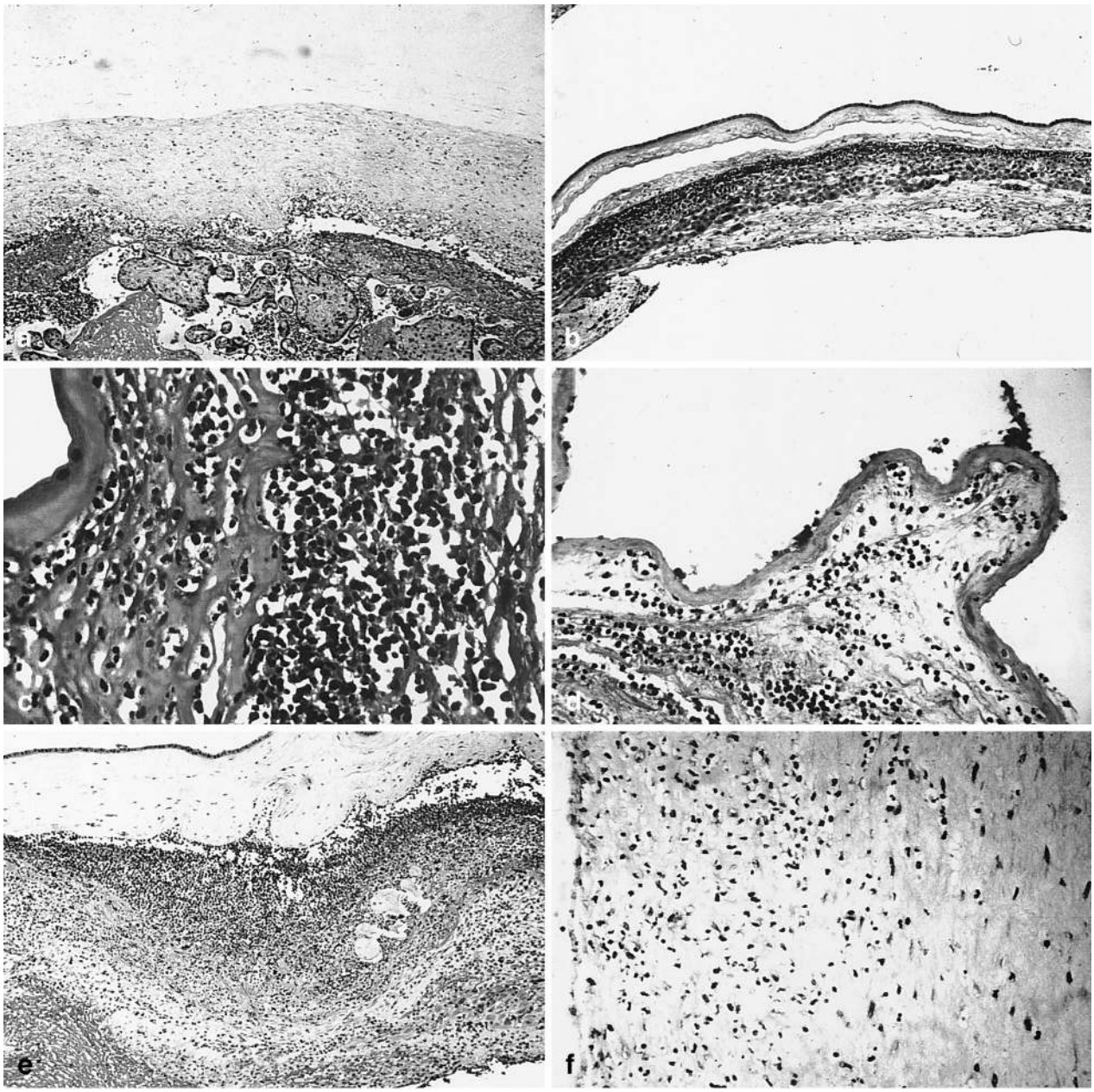

Figure 1. Histologic features of reaction patterns related to the maternal inflammatory response to amniotic fluid infection. **a.** Acute subchorionitis (maternal stage 1): neutrophils emanating from the intervillous space are diffusely scattered in the subchorionic fibrin. **b.** Acute chorionitis of chorion laevae (maternal stage 1): a band of neutrophils emanating from decidual small venules are gathered at the chorion laevae trophoblast layer without spread to the overlying amnion. **c.** Acute chorioamnionitis, not otherwise specified (maternal stage 2, grade 1): neutrophils are seen in both chorionic and amnionic connective tissue in the placental membranes. **d.** Necrotizing (maternal stage 3) chorioamnionitis: degenerating subamnionic neutrophils, thickened amnionic basement membrane, and desquamation of amnionic epithelial cells are seen in placental membranes. **e.** Severe (maternal grade 2) acute chorioamnionitis: (sub)chorionic microabscesses are depicted comprised of confluent aggregates of maternal neutrophils, at least 10 × 20 cells in extent. **f.** Chronic (subacute) chorioamnionitis: patchy maternally derived histiocytic infiltrate of the chorionic plate concentrated immediately below the amnionic surface (left). Occasional neutrophils are also present.

cyte (PMN) more than 10 cells in width occupying more than half of the subchorionic fibrin or one revolution of the membrane role. Chronic (or subacute) chorioamnionitis = more than occasional maternally derived mononuclear cells (usually macrophages) in the chorionic plate (most frequently below the amnion) in cases without exogenous pigment deposition (i.e.,

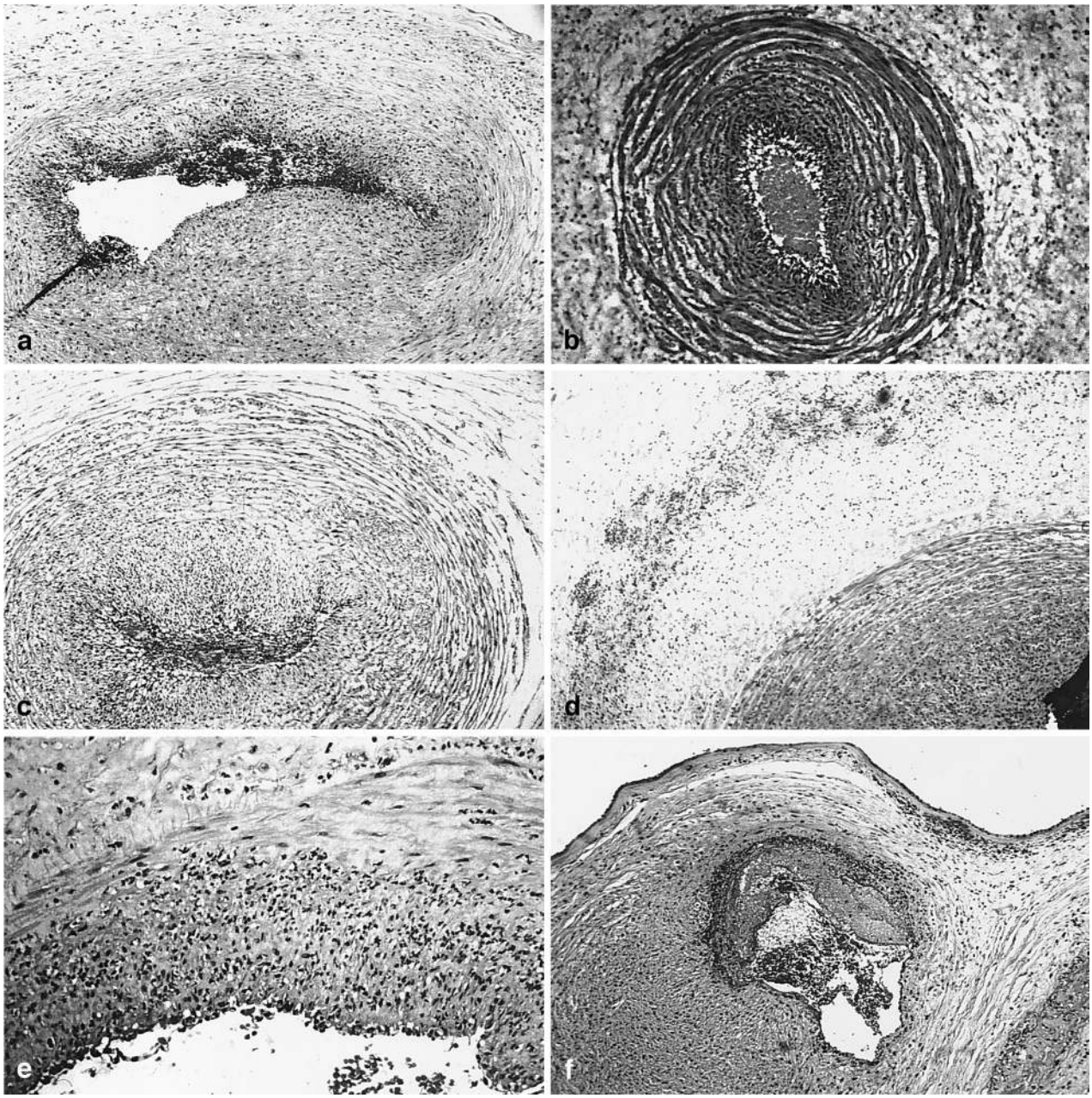

Figure 2. Histologic features of reaction patterns related to the fetal maternal inflammatory response to amniotic fluid infection. **a.** Acute chorionic vasculitis (fetal stage 1, grade 1): neutrophils are scattered throughout the upper amnionic aspect of a major chorionic plate vessel. **b.** Umbilical phlebitis (fetal stage 1): neutrophils are identified between the widely separated smooth muscle fascicles of the umbilical vein. **c.** Umbilical arteritis (fetal stage 2): neutrophils infiltrate the subendothelial cushion and tightly packed smooth muscle cells of the umbilical artery. **d.** Subacute necrotizing funisitis (fetal stage 3): an arc of degenerating neutrophils and mineralized cell debris in Wharton's jelly surrounds an umbilical vessel. **e.** Intense chorionic vasculitis (fetal grade 2): near confluent fetal neutrophils infiltrate the outer (amnionic) wall of a chorionic vessel. **f.** Chorionic vasculitis with adherent fetal vessel thrombus: a recent nonocclusive thrombus adheres to the outer (amnionic) wall of an acutely inflamed chorionic vessel.

meconium, hemosiderin). Neutrophils may be rare or abundant, but a coexistent acute chorioamnionitis should be present in at least one section (Fig. 1f).

Fetal inflammatory responses

Stage/progression of disease

Stage 1 (chorionic vasculitis/umbilical phlebitis) = neutrophils in the wall of any chorionic plate vessel

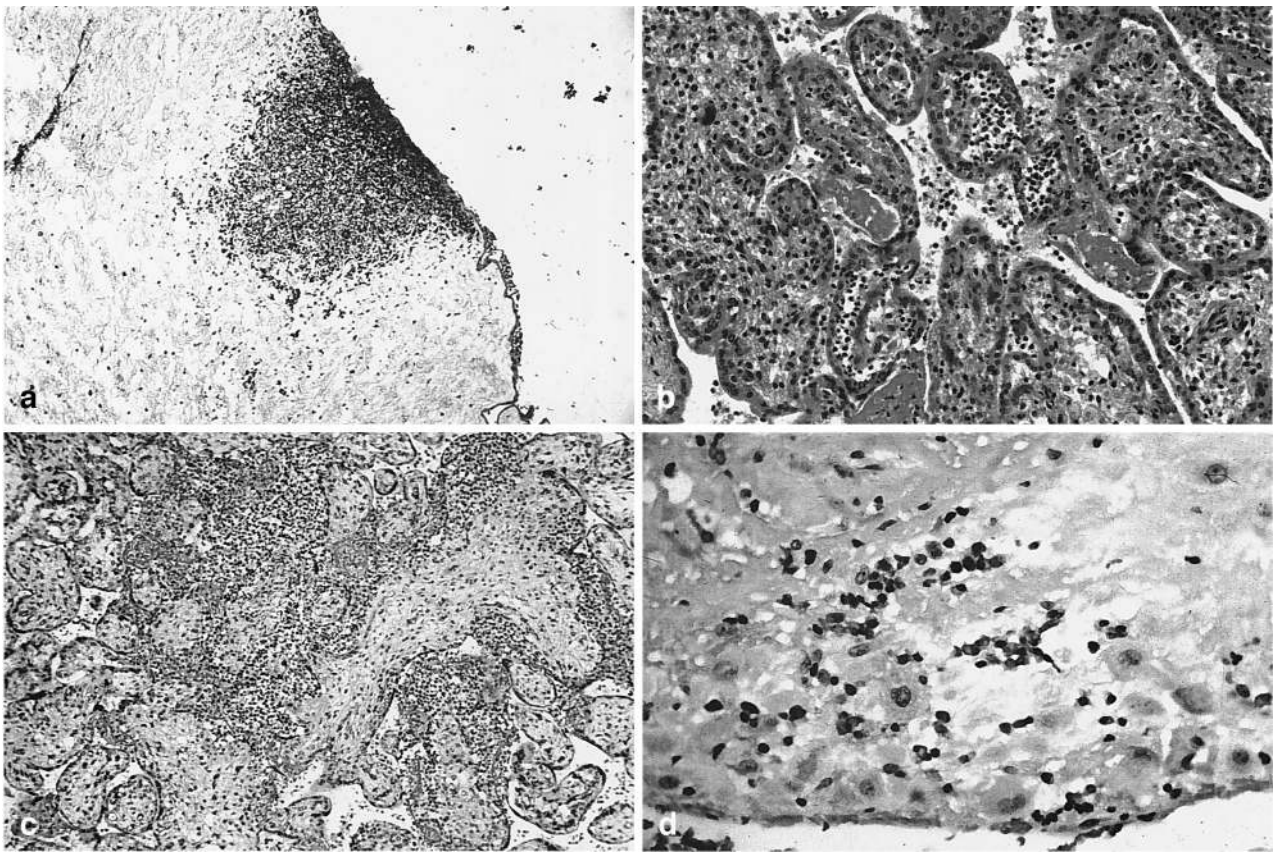

Figure 3. Histologic features of other specific features of amniotic fluid infection. **a.** Peripheral funisitis: a triangular neutrophilic microabscess is seen just below the umbilical surface epithelium. **b.** Acute villitis: fetal neutrophils are present in the villous stroma and subtrophoblastic space of terminal villi. Occasional neutrophils are also present in the intervillous fibrin. **c.** Acute intervillositis/intervillous abscesses: near confluent neutrophils are noted in the intervillous space with only focal involvement of adjacent villous stroma. **d.** Decidual plasma cells: small aggregates of plasma cells are present in the decidua basalis.

(Fig. 2a) or the umbilical vein (Fig. 2b). Extension of neutrophils into Wharton's jelly is allowed if not aggregated in a concentric band, ring, or halo around the umbilical vein. Stage 2 (umbilical vasculitis) = neutrophils in one or both umbilical arteries ± vein (Fig. 2c). Extension of neutrophils into Wharton's jelly is allowed if not aggregated in a concentric band, ring, or halo around the umbilical vessel(s). Stage 3 (necrotizing funisitis or concentric umbilical perivasculitis) = neutrophils, cellular debris, eosinophilic precipitate, and/or mineralization arranged in a concentric band, ring, or halo around one or more umbilical vessels (generally most severe on the side nearest the periphery of the cord) (Fig. 2d).

Grade/intensity

Grade 1 (mild–moderate) = scattered neutrophilic infiltrate in the subendothelial or intramural portions of any chorionic (or umbilical) vessel (Fig. 2a). Grade 2 (severe) = chorionic plate (or umbilical) vessels with near confluent neutrophils plus attenuation and/or degeneration of vascular smooth muscle cells on the side facing the amniotic cavity (Fig. 2e). Associated fetal vessel thrombi = recent thrombosis of chorionic plate (or umbilical) vessel(s) in the context of a fetal inflammatory response (Fig. 2f).

Other specific features

Peripheral funisitis = small punctate microabscesses with neutrophils on the outer surface of cord (Fig. 3a). Acute villitis = neutrophils in the villous stroma and/or the adjacent subtrophoblastic space (Fig. 3b). Acute intervillositis/intervillous abscesses = neutrophils within perivillous fibrin or the intervillous space (Fig. 3c). Decidual plasma cells = unequivocal plasma cells in either the decidua basalis or capsularis (Fig. 3d).

Data analysis

The gold standard for the presence or absence of a diagnosis in each case was the group consensus. The diagnosis from the original institution was to be used as the "tie breaker" in split cases, but was not required for any diagnosis in any case. Individual variations from consensus grades and stages were tabulated for rounds 1 and 2 and the percent agreement was determined. The final analysis (round 2) evaluated the following 12 reaction patterns: any maternal of fetal inflammatory response (grade and stage not = 0), severe maternal or fetal inflammatory response (grade 2), advanced stage maternal or fetal inflammatory response (stage 3), and the six other features listed above. Collective performance of the group was assessed by combining the individual diagnoses to derive specificity (true negatives/true negatives plus false positives), sensitivity (true positives/true positives plus false negatives), and efficiency (true positives plus true negatives/total observations) relative to the gold standard. Interobserver reliability was assessed by unweighted kappa analysis [18]. Kappa analysis is a measurement of agreement between observers on a case to case basis. Kappa values can vary from -1 to $+1$, with -1 indicating perfect inverse correlation; 0, no correlation; and $+1$, perfect positive correlation. Interpretation of kappa values can vary. We used the following guidelines: < 0.2, poor agreement; 0.2–0.6, fair/moderate agreement; > 0.6 substantial agreement [18].

RESULTS

Preliminary analysis

The initial study set of 20 cases, 14 with features associated with amniotic fluid infection and 6 controls, was circulated to group members for blinded review and coding on standardized forms (round 1). Analysis of the first round results revealed unacceptable interobserver variation. A revised and simplified set of definitions was circulated and agreed upon and a second overlapping set of study cases was circulated for review (round 2). The lesions, terminology, and definitions used in the final round are described in the Methods section, summarized in Table 1, and illustrated in Figures 1, 2, 3.

Grading and staging

All possible histologic gradations of intensity (grade) and progression of disease (stage) for maternal and fetal inflammatory responses are potentially relevant to clinical outcome. However, excessive subclassification in the absence of documented clinical significance is one cause of unacceptable interobserver variability amongst pathologists. Table 2 compares the group performance using two different classification schemes, and shows the results from round 1 using a slight modification of a previously described classification system used in research studies correlating placental diagnosis with clinical outcome [19]. This scheme utilized three-tiered grading and five-tiered staging of both maternal and fetal inflammatory responses. Overall agreement using this system was modest (71%) and particular problems existed in separating intermediate and low grades of inflammation (Table 2). These distinctions are of no documented clinical significance. Most of disagreement was the result of minor disagreements ($\pm$ 1) suggesting that a simplified scheme might yield better reproducibility without sacrificing the clinical significance associated with high grade and stage. For round 2, the number of grades was reduced to two, retaining the high grade categories because of their established clinical significance (see Discussion). The number of stages was reduced to three, distinguishing only those cases with findings suggestive of early and advanced stage infection from those without these characteristics. The results shown in Table 2 showed a substantial improvement in overall agreement (81%).

Final group survey results (round 2)

A complete analysis of the group's performance is shown in Table 3. Twelve reaction patterns were evaluated: presence of any maternal or fetal inflammation, presence of severe (grade 2) maternal or fetal inflammation, presence of advanced (stage 3) maternal or fetal inflammation, chronic (subacute) chorioamnionitis, fetal vessel thrombi, peripheral funisitis, acute villitis, acute intervillositis/intervillous abscesses, and decidual plasma cells. The gold standard for the presence or absence of each condition (prevalence) in the 20 study cases was group consensus. Group consensus was unanimous for 43/60 (72%) diagnoses and near unani-

Table 2. Stage and grade of infection, preliminary and final rounds, comparing expanded versus contracted scales

Stage/grade	No. of cases consensus positive	Individual deviations from consensus[a] (% of observers)				
Initial evaluation (round 1): three-tiered grading/five-tiered staging		-2	-1	0	+1	+2
Maternal inflammatory response						
Grade						
1	2	—	8	67	8	17
2	2	0	8	50	42	—
3	10	3	17	80	—	—
Combined	14	2	14	74	8	2
Stage						
1	1	—	17	83	0	0
2	4	0	25	67	8	0
3	5	0	7	70	17	6
4	3	0	17	67	16	—
5	1	16	17	67	—	—
Combined	14	1	16	68	13	2
Fetal inflammatory response						
Grade						
1	4	—	8	54	17	21
2	4	0	13	54	33	—
3	5	0	3	97	—	—
Combined	14	0	8	68	17	7
Stage						
1	4	—	8	88	4	0
2	1	0	50	50	0	0
3	2	0	0	83	17	0
4	4	3	11	83	3	—
5	1	0	0	100	—	—
Combined	14	1	10	83	6	0
Overall performance (total observations)	(324)	1	14	71	11	3
Maternal inflammatory response						
Grade						
1	5	—	3	84	13	—
2	9	0	11	89	—	—
Combined	14	0	8	87	5	—
Stage						
1	2	—	8	59	25	8
2	8	0	6	86	8	—
3	4	0	17	83	—	—
Combined	14	0	10	81	8	1
Fetal inflammatory response						
Grade						
1	8	—	7	67	26	—

(*continued*)

Table 2. (*Continued*)

Stage/grade	No. of cases consensus positive	Individual deviations from consensus[a] (% of observers)				
2	6	0	14	86	—	—
Combined	14	0	10	76	14	—
Stage						
1	4	—	17	83	0	0
2	7	0	11	79	10	—
3	3	0	28	72	—	—
Combined	14	0	16	79	5	0
Overall performance (total observations)	(336)	0	11	81	8	< 1

[a]Positive and negative numbers specify the number of stages or grades over or under the consensus score for each individual observer.

mous (5/6) for another 12%. Overall agreement between group prevalence and a priori prevalence (the original diagnosis of the submitting pathologist, R.W.R.) for the 10 lesions in the 20 cases was 87%. The range of individual prevalences for each lesion among the 20 cases was generally narrow. Group performance was evaluated in two ways. First, each individual response was classified as true or false positive, or true or false negative, relative to the group consensus and overall sensitivity, specificity, and diagnostic efficiency. Overall diagnostic efficiency was 84% or greater for all lesions studied. Sensitivity and specificity were also high for the majority of diagnoses. Problem areas included relatively low sensitivity (< 85%) for advanced (stage 3) fetal inflammatory response, fetal vessel thrombi, decidual plasma cells, and chronic chorioamnionitis, and relatively low specificity (< 85%) for decidual plasma cells. The second measure of individual performance was to evaluate overall agreement using the unweighted kappa (k) statistic. For this analysis, κ-values were interpreted as follows: 0.00–0.20, poor; 0.21–0.60, fair/moderate; 0.61–1.00, substantial/near perfect [18]. By these criteria, none of the diagnoses fell into the poor category. Reproducibility for presence of any maternal inflammatory response, presence of any fetal inflammatory response, severe (grade 2) maternal inflammatory response (Fig. 1e), peripheral funisitis (Fig. 3a), acute villitis (Fig. 3b), and acute intervillositis/intervillous abscesses (Fig. 3c) was substantial to near perfect. Reproducibility for the remaining diagnoses—advanced (stage 3) maternal inflammatory response (Fig. 1d), severe (grade 2) fetal inflammatory response (Fig. 2e) advanced (stage 3) fetal inflammatory response (Fig. 2d), chronic (subacute) chorioamnionitis (Fig. 1f), fetal vessel thrombi (Fig. 2f), and decidual plasma cells (Fig. 3d)—was fair to moderate.

DISCUSSION

Premature delivery is the major cause of perinatal morbidity and mortality in the United States accounting for 70% of deaths, nearly half of cerebral palsy, and a substantial proportion of other disorders including chronic lung disease, mental retardation, and sensorineural impairment [20]. Infection of the amniotic fluid is either the initiating event or the final common pathway leading to delivery in more than a third of these patients [21, 22]. The term amniotic infection syndrome was originally coined to encompass the circumstances in which microorganisms enter the normally sterile amniotic sac, the sequence of maternal and fetal inflammatory reactions these organisms elicit, and the adverse consequences of infection and inflammation for the mother and fetus [1]. Pathologists can provide important information related to all three components. In terms of predisposing conditions, pathologists contribute by recognizing bacterial vaginosis on pap smear, detecting cervicovaginal group B streptococci by culture, and by the diagnosis of subacute or chronic deciduitis (endometritis) in the delivered placenta [20, 23, 24]. With respect to the sequence of maternal and fetal

Table 3. Final group survey results, round 2

	Lesion prevalence for the 20 study cases			Group consensus			Collective individual performance (20 cases scored for 12 lesions)			Interobserver reproducibility
Placental lesion reaction pattern	A priori	Group	Individual range	6/6	5/6	4/65	Sensitivity	Specificity	Efficiency	Kappa
Maternal inflammatory response										
Any	0.70	0.70	(0.65–0.70)	14	0	0	100	94	98	0.93
Grade 2 (severe)	0.20	0.45	(0.25–0.55)	7	1	1	88	95	92	0.76
Stage 3 (advanced)	0.15	0.15	(0.15–0.30)	2	1	0	93	87	88	0.49
Chronic (subacute) chorioamnionitis	0.10	0.10	(0–0.20)	0	0	2	75	94	91	0.25
Fetal inflammatory response										
Any	0.65	0.70	(0.65–0.70)	12	1	1	96	100	98	0.90
Grade 2 (severe)	0.30	0.30	(0.25–0.55)	4	1	1	92	85	87	0.55
Stage 3 (advanced)	0.15	0.10	(0–0.20)	0	2	0	83	94	93	0.52
Fetal vessel thrombi	0.15	0.20	(0.15–0.35)	0	2	2	75	87	84	0.37
Other specific features										
Peripheral funisitis	0.10	0.10	(0.10–0.20)	2	0	0	100	98	98	0.84
Acute villitis	0.10	0.10	(0.05–0.10)	1	1	0	92	100	98	0.90
Acute intervillositis/abscesses	0.05	0.05	(0.05–0.15)	1	0	0	100	97	97	0.65
Decidual plasma cells	0.05	0.05	(0.05–0.15)	0	0	1	67	79	91	0.30

inflammation, histologic chorioamnionitis has repeatedly been shown to be both sensitive and specific for infection and is considered the gold standard against which other clinical predictors of infection should be measured [25–28]. Finally, as described below, certain reaction patterns such as severity of inflammation, associated fetal vascular thrombosis, patterns suggestive of a specific causative organism, and estimation of the risk for neonatal sepsis may identify infants at increased risk for specific perinatal complications.

Most attempts to subclassify the inflammatory responses to amniotic fluid infection have built upon the observations of Blanc who described what he believed to be the anatomic sequence of events [29]. In this scheme, stage 1 was defined by neutrophils in the subchorionic fibrin, stage 2 by neutrophils in the chorionic plate, and stage 3 by neutrophils in the amnion. Van Hoeven and colleagues later found that amnionic necrosis (necrotizing chorioamnionitis), a late complication of inflammation in the amnion, was associated with an increased risk of perinatal death and preterm delivery [9]. For this reason, they argued that this feature should be specified in the final pathologic diagnosis. Mueller-Heubach and coworkers also described associations of necrotizing chorioamnionitis with preterm labor, premature rupture of membranes, and decreased gestational age in very low birthweight infants [7].

Severity of the maternal inflammatory response has been estimated in a couple of ways. keenan and colleagues in 1977 showed that severity, as determined by the formation of subchorionic microabscesses, was associated with an increased risk of presumed or documented fetal sepsis [4]. Muller-Heubach and colleagues proposed an alternative numerical system in which $>$ 30 neutrophils in the upper half of the chorionic plate was considered severe [7]. However, severity defined in the latter fashion has not been predictive of clinically significant complications [10, 11, 30]. This numerical definition was also poorly reproducible in our study (round 1). Better reproducibility and the clinical significance described by Keenan et al. [4] lead us to recommend this as a more useful criterion for severity.

In Blanc's original formulation, a fetal inflammatory response was indicated by the presence of neutrophils in the walls of large fetal vessels in the chorionic plate and umbilical cord [1, 29]. The presence of umbilical cord inflammation has been shown to be a risk factor for clinical manifestations of the fetal inflammatory response syndrome including intraventricular hemorrhage and CNS echolucencies in preterm infants [6, 31]. A greater association with complications in preterm infants may relate in part to an increased threshold for mounting fetal inflammatory responses in the less mature fetus [32]. It has generally been observed that chorionic plate vessels and/or the umbilical vein are involved prior to the umbilical arteries. Support for this sequence has recently been published by two recent studies demonstrating increased fetal cytokine levels and increased morbidity in patients with inflammation in one or both umbilical arteries compared to those with involvement of the vein alone [33, 34]. Keenan et al. also demonstrated the additional clinical significance of arterial inflammation by showing that umbilical panvasculitis (veins plus arteries) was an independent risk factor for neonatal sepsis [4]. In view of this data, it seems important to differentiate between involvement of the umbilical vein alone (phlebitis) and umbilical vasculitis with arterial involvement. Use of the generic term "funisitis" is not sufficiently specific and should be discouraged.

Nararro and Blanc were also the first to describe a later stage of fetal inflammation, subacute necrotizing funisitis [3]. In this lesion, fetal neutrophils migrate into the umbilical stroma (Wharton's jelly), arranging themselves in a circular are around the vessel. This pattern has been attributed to the precipitation of immune complexes formed by microbial antigens diffusing in from the amniotic fluid and maternal antibodies diffusing out from the umbilical vessels [5]. Such immune complexes are chemotactic for fetal neutrophils. Matsuda and coworkers later showed that infants with this lesion are at significantly increased risk for chronic lung disease [35]. These observations define three temporal stages of fetal involvement: stage 1, neutrophils in chorionic and/or umbilical vein, stage 2, neutrophils in an umbilical artery, and stage 3, concentric arcs of degenerating neutrophils in Wharton's jelly.

Severity of fetal inflammation was found by Spong and coworkers to be a risk factor for severe variable decelerations and increased circulating NRBC in very low birth weight infants [36]. Other studies by Redline et al. have shown that severe fetal inflammation is a risk factor for neurologic impairment in both very low birth weight and term infants [11, 19]. While severity is more easily assessed and of better documented significance in chorionic plate vessels, umbilical vessels can also be assessed in cases where the chorionic plate has not been sampled. Much of the effect of severe fetal inflammation in the second group of studies cited above was attributable to coexisting chorionic vessel thrombosis. Reproducibility for the diagnosis of fetal vessel thrombi was somewhat disappointing in the present study, largely owing to ambiguity in cases with small foci of endothelial fibrin deposition. For diagnostic purposes, we recommend close adherence to the classic criteria for the identification of true thrombi (glassy red-blue staining, lamination, and adhesion to the vessel wall) when making this diagnosis (Fig. 2f).

Peripheral funisitis, the formation of superficial umbilical cord abscesses, was a highly reproducible finding (kappa = 0.84). This pattern, when diffusely distributed over the cord, is virtually pathognomonic for candida infection [5, 37]. When the peripheral inflammatory response is focal and located near the placental cord insertion, the association with candida is less specific. Our study set contained one case of each type and both were recognized by 100% of observers. Because of this overlap, a final diagnosis of candidal chorioamnionitis requires demonstration of fungal hyphae in tissue sections.

The presence of neutrophils in the villous stroma may be found in two distinct patterns. The first pattern, acute villitis with a minimal intervillous component, is believed to reflect overwhelming fetal sepsis, often caused by gram negative bacilli such as *E. coli* [17]. The histologic findings are acute capillaritis with emigration of fetal neutrophils to villous stroma. Clusters of neutrophils in acute villitis often accumulate between villous stroma and the villous trophoblast basement membrane (Fig. 3b). Bacteria are usually evident by routine hematoxylin and eosin (H&E) stain. The second pattern is acute villitis accompanied by intervillositis and intervillous abscess formation (Fig. 3c). Acute villitis in these cases is less extensive than the intervillositis and is presumed to represent secondary spread to villi. This pattern is most commonly seen in infections caused by *Listeria monocytogenes* [38]. *Campylobacter fetus* and a variety of rare infections, such as coccidiomycosis, psittacosis, tularemia, and brucellosis, may also manifest some or all of these findings [39, 40]. In the present study, the shared feature of acute villitis was easily recognized and agreed upon by virtually all observers (kappa = 0.90). The additional finding of acute intervillositis/intervillous abscesses was recognized by all observers, but was occasionally overdiagnosed in cases of acute villitis with occasional perivillous neutrophils leading to a lower kappa value (0.65).

The final two patterns, decidual plasma cells and chronic (subacute) chorioamnionitis, were less reproducible and often missed in consensus-positive cases. Plasma cells in decidua, like those in nonpregnant endometrium, are notoriously difficult to detect. Overdiagnosis of cells lacking one or more of the classic morphologic features can also be a problem. Chronic (subacute) chorioamnionitis requires the observer to recognize an underlying histiocytic infiltrate in membranes already suffused with neutrophils. It is possible that special stains such as syndecan-1 for plasma cells and CD68 for histiocytes could improve reproducibility. Plasma cells in particular, and B-lymphocytes in general, are always abnormal in the endometrial cavity [41]. Their presence is an indicator of inappropriate antigenic exposure. One source of antigen is bacterial subacute endometritis, which may lead to recurrent acute chorioamnionitis in subsequent pregnancies [20]. Uterine plasma cells in the absence of acute inflammation are also associated with infertility, spontaneous abortion, and chronic villitis [42–44]. We believe that the presence of plasma cells should always be noted in the final diagnosis [45]. Chronic chorioamnionitis, on the other hand, is a heterogeneous entity that can accompany either acute chorioamnionitis or chronic villitis [46, 47]. This lesion overlaps with a very recently characterized lesion known as subacute chorioamnionitis [48]. In the latter report, published after completion of our study, subacute chorioamnionitis when combined with subacute ne-

crotizing funisitis (our fetal Stage 3 chorioamnionitis) was found to be a significant risk factor for chronic lung disease. In view of its evolving status and the lack of reproducibility in the present study, we recommend that the terms "chronic" or "subacute" chorioamnionitis be used sparingly and as descriptive adjuncts rather than primary diagnoses [49].

Reliability of placental diagnosis has not been extensively examined. In one study, it was found that interobserver agreement between subspecialty perinatal pathologists was better than that between general surgical pathologists and perinatal subspecialists [14]. Discrepancy rates in the latter situation ranged from 32–59%. Some reliability studies have been performed with other primary goals, such as demonstrating that gestational age cannot be specified based on placental histology alone and developing a working definition of chronic deciduitis [15, 50]. Statistical measures of reliability were not performed in these studies. To our knowledge, only two studies have directly measured reproducibility. Both were performed by epidemiologists for the purpose of validating the use of archived placental material in retrospective studies [16, 51]. Both concluded that reliability is excellent for many placental lesions including the various stages of the maternal response in acute chorioamnionitis and the presence or absence of a fetal response. While other diagnoses were less reproducible, it was shown that reliability could be improved through the use of a standardized study protocol. Our study is the first designed by pathologists for the purpose of developing and standardizing diagnostic criteria that included statistical evaluation of reproducibility. Furthermore, it is the first to comprehensively address all pathologic findings related to a single perinatal process, in this case amniotic fluid infection. Additional studies are in progress to apply the same procedure to two other processes—maternal vascular underperfusion and fetal thromboocclusive disease.

In conclusion, we have proposed and validated a system for describing the maternal and fetal inflammatory responses to amniotic fluid infection plus a number of other reaction patterns useful for defining the etiology and clinical consequences of infection. We have attempted to utilize terminology and criteria that conform as closely as possible to previously reported schema. We strongly advocate the use of this, or a similarly constructed, system to help standardize diagnoses between hospitals, provide useful information to clinicians, and serve as a valuable tool for conducting future studies relating placental risk factors to adverse perinatal outcome.

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