HSOA Journal of Neonatology and Clinical Pediatrics

Research Article

Revisiting the Preterm Gastric Aspirate: Potential for Use as an Additional Predictor for Chorioamnionitis, Early-Onset Sepsis, and Complications of Inflammation

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Abstract

Objective: To evaluate the potential role of Gastric Aspirate (GA) analysis in diagnosing infection and guiding decisions relating to the use of antibiotics and prevention of complications of prematurity associated with infection.

Methods: This is a prospective observational single-center cohort study of 184 preterm infants less than 33 weeks gestation who underwent GA analysis at birth. It includes a subset of 170 (92%) of these infants whose placentas were examined for Histopathological Chorioamnionitis (HPCA). Comparison of amniocentesis and GA's in a small subset of 6 of these cases was made to confirm that GA was similar to swallowed amniotic fluid.

Results: Gastric aspirates were positive for culture, Gram stain, and/or more than 5 white blood cells per high power field in 59 (32%) of 184 study infants. Eleven percent (21) had proven (2) or suspected sepsis. A positive "Rapid" GA (RGA) including Gram stain and WBC occurred in 25%. The RGA predicted sepsis or SS with sensitivity 71%, specificity 82%, positive predictive value 33%, negative predictive value 96%, or 11.08 (95% CI 2.46-18.52). Full GA including culture predicted the 60 (35%) with HPCA with sensitivity 75%, specificity 91%, positive predictive value 82%, negative predictive value 87%, and OR 30. Forty-one percent had either HPCA or positive GA or both.

Conclusion: Addition of PH (Placental Histology) and GA to the initial septic work up is beneficial. These added tests provide, with reasonable certainty, identification of the approximately 40% of preterm infants born prematurely who had intra-uterine infections.

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GA is helpful in identifying infants who may be septic and need to be treated with antibiotics as well as assisting in the decision to withhold or stop early antibiotics. It also has the potential to help prevent some of the neonatal complications of inflammation.

Abbreviations

BPD: Bronchopulmonary Dysplasia; CBC: Complete Blood Count; CI: Confidence Interval; CRP: C - Reactive Protein; FIR: Fetal Inflammatory Response; GA: Gastric Aspirate; GBS: Group B Strep; HPCA: Histopathological Chorioamnionitis; IVH: Intraventricular Hemorrhage; LR: Bayesian Likelihood Ratio; NEC: Necrotizing Enterocolitis; OR: Odds Ratio; NICU: Neonatal Intensive Care Unit; PCR: Polymerase Chain Reaction; PDA: Patent Ductus Arteriosus; PH: Placental Histology ; PIH: Pregnancy Induced Hypertension; PPHN: Prolonged Pulmonary Hypertension; PROM: Prelabor Rupture of Membranes; PTL: Preterm Labor; PVL: Periventricular Leukomalacia; RGA: Rapid Gastric Aspirate; SS: Suspected Sepsis; WBC/HPF: White Blood Cells per High Power Field

Introduction

Over the past two decades, there has been a surge of obstetrical interest in Histopathological Chorioamnionitis (HPCA) and related biochemical mechanisms involved in triggering Preterm Labor (PTL) as summarized by Romero et al., [1]. The neonatal literature discusses the involvement of similar biochemical pathways in nearly all the major complications of prematurity. These include developmental delays and cerebral palsy [2-10] related to Periventricular Leukomalacia (PVL) and Intraventricular Hemorrhage (IVH) [11-15], Patent Ductus Arteriosus (PDA) [16,17], Retinopathy of Prematurity (ROP) [18,19], Persistent Pulmonary Hypertension (PPHN) [20], Bronchopulmonary Dysplasia (BPD) [21-30], and Necrotizing Enterocolitis (NEC) [31-34]. It is apparent that HPCA, involving inadequately regulated inflammatory responses influenced by genetic factors [35,36] is frequent and complex while acute treatable early onset neonatal infections such as pneumonia and sepsis are relatively infrequent.

Most HPCA presents insidiously [37-41] and may have various outcomes for the fetus ranging from stillbirth [42] to the "Fetal Inflammatory Response" (FIR) [1,8,43] to immunity against late onset sepsis [44,45]. Antibiotics administered to the mother have an uncertain effect on the fetus. They may alter the types of organisms that colonize or infect the premature infant or change the infants' inflammatory responses [23,46,47].

There is serious concern over excessive use of broad spectrum antibiotics in these newborns who receive them without identification of infecting organisms and their microbial sensitivities. Some infants, given the diagnosis of "Suspected Sepsis" (SS) or "culture negative sepsis and pneumonia," receive variable courses of antibiotics, which damage their microbiomes and the surrounding Neonatal Intensive Care Unit (NICU) environment [33,48,49].

While the obstetric literature attributes 25 to 40% [1] of prematurity to infection or inflammation, just 1-2% [50] of newborns experience blood culture positive early onset sepsis. We theorized that these differing frequencies could be reconciled by expanding the initial sepsis investigation to include more than the currently-recommended blood culture with adjunct Complete Blood Counts (CBC's) and serial C - Reactive Protein (CRP) [51,52].

We studied a cohort of preterm infants less than 33 weeks gestation, theorizing that reintroduction of the previously used Gastric Aspirate (GA) examination would: first, correlate favorably with amniocentesis fluid samples performed in a small subset; second, be predictive of early onset infection in the newborn; and third, assist in antibiotic management due to identification of infecting organisms. We also theorized that the GA would correlate with a diagnosis of HPCA and that investigation for infection would benefit from routinely including Placental Histopathology (PH). In addition, we theorized that, if the GA correlated with HPCA, it has the potential to be useful in earlier identification of infants at higher risk for BPD, PDA, ROP, PPHN, NEC, IVH and PVL, at least until routine cytokine measurements become available [53].

Patients and Methods

We performed a prospective observational cohort study, approved by our Institutional Review Board, analyzing the results of blood cultures, GA, PH, and other maternal and newborn data on all infants admitted to the Neonatal Intensive Care Unit (NICU) who were less than 33 completed weeks gestation at birth. Infants were excluded who did not have a GA performed and those who had lethal congenital anomalies. GA testing had been used routinely by some attending neonatologists prior to the study and had been included in standing admission orders for all infants less than 33 weeks gestation prior to the study. Stomach contents were obtained as soon as possible during stabilization in the delivery room or in the first few hours (always prior to the first feeding) in the NICU, secured in a DeLee trap, and sent to the lab. The Becton Dickinson BACTEC Peds Plus blood culture bottles requiring a minimum of one milliliter of blood were used for infants. BACTEC plus aerobic and Lytic/anaerobic/F bottles were used for mothers. PH exams were done by general pathologists in the hospital laboratory and reported in about 5 days.

The "Rapid" GA (RGA) test was ordered on a STAT basis. It included White Blood Cell count per High Power Field (WBC/HPF) and Gram stain for bacteria or fungi. The full GA included the RGA plus aerobic, anaerobic and *Mycoplasma* cultures. A positive RGA was defined as positive Gram stain for organisms and/or more than 5 WBC/ HPF similar to amniocentesis counts reported by Romero et al., [54] a positive (full) GA was defined as one or more of the following: positive Gram stain, more than 5 WBC/HPF, or one or more positive cultures. Anaerobic and *Mycoplasma* cultures were sent to referral labs and results were available after 7 to 9 days. Bacteria considered contaminants were coagulase negative *Staph., Proprianobacteria acnes*, and any single colonies of non-pathologic organisms. Diphtheroids reported on aerobic culture or Gram stain were considered pathogens because they often grew Gram positive rods on anaerobic culture. No differentiation between heavy and light growth was made. If one of multiples had ruptured membranes, all were included in the Prelabor Rupture of Membranes (PROM) group. Suspected Sepsis (SS) was defined as infants who, at the discretion of the attending physician who evaluated the infants' lab results and length and degree of illness, received a 7 or more day course of antibiotics beginning at birth.

Missing data occurred due to clerical error or inability to obtain a sample. Included infants had, at minimum, aerobic cultures, Gram stain and WBC. Those missing anaerobic cultures and/or cultures for *Mycoplasmas* were included in the results.

Test versus disease statistical comparisons for sensitivity, specificity, positive and negative predictive values, positive and negative Bayesian likelihood ratios, odds ratios and P values were calculated to evaluate both the RGA and full GA for prediction of proven or suspected sepsis. In a large subset of study infants who had both GA and PH, calculations for prediction of HPCA were performed. The online calculator www.medcalc.org/calc/diagnostic_test.php was used for these calculations.

Results

A total of 209 newborns less than 33 0/7 weeks gestation were admitted during the study period from December, 2010, through March, 2013. Excluded were three with lethal anomalies and 22 who had no GA performed. Thus, 184 (89%) of the 206 had at minimum Gram stain, WBC and aerobic cultures, and were included in the study. Table 1, lists numbers of study infants and excluded infants and the comparative percentages several categories. The excluded group was significantly different only in the percent of patients who died. Thirty-seven (20%) infants who were missing anaerobic cultures and 30 (16%) missing *Mycoplasma* cultures were included in the results. A subset of 170 (92%) of the 184 infants had PH completed and were included in analysis of the correlations between RGA and GA and PH. Seventy-eight percent of patients were begun on antibiotics after birth. A group of 21 (11%) with proven or Suspected Sepsis (SS) received 7 or more days of antibiotics. There were 117 males and 67 females in the study.

	Study patients (184)		Excluded (22)		P value
Birth Weight					
470-1000 grams	47	25%	8	36%	0.23
1001-1500 grams	62	34%	6	27%	0.72
1501-2000 grams	46	25%	5	23%	0.92
2001-2370 grams	29	16%	3	14%	0.93
Gestational Age					
23-24 weeks	20	11%	5	23%	0.39
25-26 weeks	29	16%	1	4%	0.74
27-29 weeks	48	26%	4	18%	0.72
30-32 weeks	87	47%	12	55%	0.58
Other Categories					
Died	2	1%	3	14%	0.02*
One of Multiples	55	30%	6	27%	0.87
Vaginal delivery	43	23%	7	32%	0.57
C-Section	141	77%	15	68%	0.41
PROM	71	39%	10	45%	0.57
Preterm labor, no PROM	55	30%	8	36%	0.71

Table 1: Study patients divided into categories and compared to the proportion of patients in the same categories who were excluded from the study due to lack of gastric aspirate sample reports.

PROM: Prelabor Rupture of Membranes; *p<0.05

Among 156 mothers in the study, a small subset of 6 underwent amniocentesis to rule out infection prior to delivery of a preterm newborn in whom a GA was done. See results in table 2. The GA

correlated well with amniocentesis findings in view of the interval time periods during which antibiotics were usually given.

Fifty-nine (32%) of the 184 study infants had a positive full GA and 25% had a positive RGA. Table 3 is a listing of positive results for the full and RGA by categories. As we expected, positive full GA increased with decrease in gestational age. The percent with positive GA in infants born by vaginal deliveries and those with PROM were high as expected. In the 10 study infants born after cervical cerclage, all had at least one positive test when PH was included. In those 54 (29%) of the study patients delivered by C/S with no labor and no PROM, only 2 (4%) had a positive GA. The 2 were discordant twins with 7 and 10 WBC/HPF in their RGA, and negative PH, GA culture, and Gram stain.

Case No.	Gestation in weeks	C-Section Vaginal	Birth Weight	Amniocentesis results	Time Interval	Gastric Result	Placenta
1	31	C-Section	2110 grams	9 WBC gluc 25 rare GBS	1 day	1 WBC rare GBS	+chorio-.-funiitis, hvygm+cocci
2	28 6/7	C-Section	1000 grams	2 WBC No growth	2 ½ months	3-5WBC	+chorio +funisitis, yellow-green
3	29	Vaginal	1385 grams	160 WBC Gluc<20 Heavy GBS	<1 day	5-10WBC Light GBS	+chorio, +funisitis
4	28 4/7	C-Section	970 grams	6 WBC, Gluc<20 No growth	10 days	*Eubacteria, Mycoplasma*	+chorio +funisitis
5	27 5/7	Vaginal	1030 grams	98 WBC Heavy *AlphaStrep*	<1 day	20-50WBC Heavy *Strep Anginosus*	+chorio +funisitis, yellow
6	25 2/7	Vaginal	750 grams	1000 WBC *Ureaplasma urealyticum*	1 day	100 WBC Moderate Gm neg rods No growth	+chorio -funisitis

Table 2: Results of a subgroup of 6 amniocenteses done in women in preterm labor compared to the gastric aspirate results of their infants who were in the study born after varying time intervals.

WBC: White Blood Cells per high power field; GBS: Group B Strep; Chorio: Chorioamnionitis; Hvy: Heavy; Gm: Gram; Neg: Negative; Gluc: Glucose

Categories	Number Infants	Positive Gastric Aspirate (%)	Positive Rapid Gastric Aspirate (%)
Total infants	184	59 (32%)	45 (25%)
Gestational Age			
23-24 weeks	20	10 (50%)	8 (40%)
25-26 weeks	29	11 (38%)	6 (21%)
27-29 weeks	48	15 (31%)	15 (31%)
30-32 weeks	87	23 (26%)	16 (18%)
Other Categories			
Cerclage	10	7 (70%)*	4 (40%)
Multiple	55	12 (22%)	7 (13%)
PROM	71	37(52%)	28 (39%)
PTL-No PROM	55	16 (29%)	12 (22%)
C-Section delivery	140	30(21%)	27 (19%)
Vaginal delivery	44	29 (66%)	18 (41%)
No labor, ROM at C-S	54	2 (4%) **	2 (4%)

Table3: Percent positive (full) gastric aspirates and rapid gastric aspirates in the study by categories.

PROM: Prelabor Rupture of Membranes; PTL: Preterm Labor

*8 (80%) had positive PH and all 10 had at least one + factor, 3 had 2 factors and 3 had 3. In the one set of twins one had one factor and one had 2 factors but they were the 2 with negative PH.

**Discordant twins with 7 and 10 WBC/HPF, negative GA cultures and Gram stain, and negative PH.

See table 4 for calculations of test predictability for disease results including numbers of patients with positive and negative results. Both the RGA and GA proved to correlate well with the group of 21 (11.4%) with either proven sepsis (2) or SS (19). The RGA is more useful for deciding whether to begin antibiotics after birth because it can easily be reported on a STAT basis. The full GA requires 48 hours for aerobic culture results and 7 to 9 days for *Mycoplasma* and anaerobic results. It is, thus, more useful for later management of antibiotics after 48 hours. In the subset analysis which included 170 patients who had both PH and GA performed, the full GA was more predictive than the RGA for HPCA with many fewer false negatives.

See table 5 for combined results of tests for the 2 infants with proven sepsis, the 21 infants with sepsis or SS and for those with positive RGA in the 15 infants with and the 30 infants without sepsis or SS. Those with sepsis or SS had very high positive rates of all tests and no negative tests. Those without sepsis or SS also had high rates of positive GA and PH, although not as high as those with sepsis or SS.

Combined PH and GA results from the subset of 170 patients could be divided into 4 groups: see table 6. Seventy (41%) had at least one positive test (PH, GA or both) for intrauterine infection. Funisitis was present in 25 (42%) of those with HPCA and those infants were generally sicker.

Two (1%) of the 184 study newborns had proven early onset sepsis. Both had positive GA Gram stains. Their GA cultures grew the same organisms as their blood cultures. In the first of the septic infants, the blood culture grew *Strep. Pneumonia* and his RGA as well as the placenta showed "too numerous to count" WBC's. Delivery of the

	+RGA/ Sepsis or SS	+RGA/+PH	+GA/Sepsis or SS	+GA/+PH
Disease Prevalence	11.41%	35.29%	11.41%	35.29%
#Patients + for disease	21	60	21	60
#Patients tested				
Total number	184	170	184	170
a (true+)	15	27	18	45
b (false+)	30	7	41	10
c (false neg)	6	33	3	15
d (true neg)	133	103	122	100
a + b (total+)	45	34	59	55
c + d (total neg)	139	136	125	115
% Sensitivity	71.43	45.00	85.71	75.00
95% CI	47.83-88.65	32.13-58.39	63.63-96.78	2.14-85.27
% Specificity	81.60	93.64	74.85	90.91
95% CI	74.78-87.22	87.32-97.39	67.46-81.30	83.91-95.55
+ Likelihood Ratio	3.88	7.07	3.41	8.25
95% CI	2.55-5.92	3.28-15.27	2.48-4.68	4.49-15.16
Neg Likelihood Ratio	0.35	0.59	0.19	0.28
95% CI	0.18-0.69	0.46-0.74	0.07-0.55	0.18-0.43
% + Predictive Value	33.33	79.41	30.51	81.82
95% CI	20.01-48.95	62.09-91.26	19.19-43.87	69.09-90.91
% Neg Predictive Value	95.68	75.74	97.60	86.96
95% CI	90.84-98.39	67.64-82.67	93.14-99.47	79.40-92.51
Odds Ratio	11.08	12.03	17.85	30.00
95% CI	3.97-30.93	4.80-30.18	5.00-63.73	12.51-71.90
P value	<0.0001	<0.0001	<0.0001	<0.0001

Table 4: Test/disease predictability correlations for +RGA and + (full) GA versus + for Sepsis or Suspected Sepsis and +PH.

SS: Suspected Sepsis; PH: Placental Histology

	# Patients	+RGA	+GA culture	+PH	All +All	Neg
+Sepsis, proven	2	2	2	1	1	0
+Sepsis or SS	21	15/21 (71%)	19/21(90%)	17/20(85%)	12/21(57%)	0
+RGA, not septic or SS	30	-	20/30(67%)	20/30(67%)	15/30(50%)	6 (20%)
+RGA/ +septic or SS	15	-	15/15(100%)	12/14(86%)	12/14(86%)	0

Table 5: Test results for patients with proven sepsis, proven or suspected sepsis, and + RGA with and without sepsis or suspected sepsis.

second septic baby was induced for Pregnancy Induced Hypertension (PIH) after artificial PROM. Blood and GA grew both *E. Coli* and Group B Strep (GBS). Polymicrobial sepsis cases such as this have been documented [55,56]. GA Gram stain showed organisms but no WBC's.

Another case of interest was an infant whose mother had a positive anaerobic blood culture for *Prevotella*. The infant was one of 26 week twins with PROM for 9 weeks who developed septic shock after C-section. Twin A's GA showed more than 100 WBC/HPF, heavy

	+ GA	-GA
+PH	45 (26%) Acute infection.WBC, bacteria or both.	15 (9%) Past or cured infection or poor swallowing.
-PH	10 (6%) Acute but too early for WBC response	100 (59%) No apparent infection

Table 6: Numbers of patients in 4 groups with combinations of +/- PH and +/- GA.

Gram negative coccobacilli on gram stain, and grew *Prevotella*. She required inhaled nitric oxide and ventilation. Twin B had a negative GA and benign course. PH was negative.

Forty-nine (27%) of the 184 study infants had one or more positive cultures of the GA. Table 7 lists the organisms by categories. There were 15 cultures omitted as contaminants. Each positive culture grew 1 to 3 organisms. There were 27 with one, 21 with two and one with three organisms. There were 46 aerobic organisms cultured and 24 (34%) anaerobic organisms. The most common organism, found in 11 infants, was *Ureaplasma urealyticum*. The organisms found in the study GA's are similar to organisms found in amniotic fluid from amniocentesis from women in PTL with intact membranes [37,38,40,57-60]. All four infants noted to be foul-smelling grew anaerobes.

Due to concern over possible contamination of the GA in the vaginal deliveries, we looked at other factors in those 26/43 (60%) who had positive GA cultures. Half of these had both positive placentas and WBC's, most more than 20/HPF. The others had at least one other factor including positive placenta, positive WBC, foul smell, and twins who grew the same organisms in both of their GA's and had high CRP's. Thus in none of these positives was the GA the only factor consistent with infection.

Discussion

Historically, discovery of a rapid test to diagnose early onset neonatal sepsis while we await the "gold standard" blood culture result has eluded us [61-63]. This has resulted in extreme caution to avoid missing infants whose blood cultures eventually prove positive or falsely negative. During the two decades prior to 1996, when prophylactic antibiotics during labor were begun for GBS colonization, fulminant early-onset GBS sepsis was seen much more frequently than it is now [64]. During that time, the GA was commonly used to screen for GBS sepsis and pneumonia. It was known that gastric acid was not present in the newborn's stomach until after the first feeding [65]. Seeing Gram positive cocci with or without WBC's in the GA was used to decide whether a baby should receive ampicillin, without the delay of awaiting CBC's or improvement in transient respiratory distress [66]. Some of those with a negative GA could be left off antibiotics to see if they improved. The GA as a reflection of swallowed lung organisms in non-ventilated babies was also considered useful, as was the GA culture confirming GBS when an infant had blood culture negative pneumonia [67,68]. Although GBS remains a fulminant, although less frequent, cause of sepsis [55], the GA Gram stain and culture have fallen out of favor [69,70], being considered of no value by some [71]. Rather, the focus is now on developing a highly technical method of testing for infection in the amniotic fluid and gastric aspirates, the 16s rDNA PCR (PCR). Two such recent studies of PCR's done on GA's prompted our desire to re-examine the usefulness of GA sampling [72,73]. Although the development of a rapid PCR would be beneficial, much study will be needed before the meaning of results in newborns can be fully understood and readily available.

Organism	Number of Infants with + cultures
Mycoplasmas	
Ureaplasma urealyticum	11
Mycoplasma hominis	3
Fungi	
Candida	3
Aerobic bacteria	
Aerobic gram positive cocci	
Alpha *Strep*	8
Strep.mitis	1
Strep.anginosus	2
Strep.agalactiea (GBS)	7
Non-hemolytic *Strep.*	2
Strep.pneumoniae (Pneumococcus)	1
Enterococcus	1
Staph.aureus (mom had purulent salpingitis)	1
Aerobic Gram negative rods	
E. coli	5
Morganella morganii (facultative)	3
Aerobic Gram positive rods	
Gardenerella	1
Anaerobic bacteria	
Anaerobic Gram positive cocci	
Peptostreptococcus	8
Anaerobic Gram positive rods	
Clostridium	3
Eubacteria	1
Anaerobic Gram negative rods	
Prevotella	5
Fusobacterium	3
Bacteroides	2
Campnocytophagia (facultative)	1
Eikenella corrodes (facultative)	1

Table 7: Classification of organisms and number of infants with positive gastric aspirate cultures.

Currently, determining which babies are actively infected and may benefit from antibiotics remains dependent on blood cultures, degree of illness, and adjunct lab results [61]. The results of our study seem to justify considering reinstitution the GA as an adjunct laboratory test in the initial newborn septic work up. Although the positive predictive value of RGA is only 33%, this correlation is similar to the positive predictive findings in Benitz' 1998 statistical analysis of the use of early and serial CRP testing for diagnosing of sepsis [74]. In spite of that, the CRP is now used routinely because the positive predictive values are still higher than the relatively low incidence of sepsis.

The most urgently sought objective of studies investigating laboratory tests for infection is to identify methods to decrease both the routine initiation and prolonged administration of broad spectrum antibiotics [75,76]. Antibiotics were started at birth in78% of the infants in our study. This large number is similar to other recent studies, including the one by Stoll et al., in 2011 [55], in which 82% of ELBW infants were treated. More timely than the CRP, which is usually done only after 12 hours of age, the RGA potentially improves

our ability to narrow down the numbers of infants initially given antibiotics from the current about 80% to closer to 30-40%. While the RGA's positive predictive value of 33% means we will treat 3 times the 11% at risk, that would be an improvement over treating 80%, more than 7 times too many. The negative predictive value of the RGA is 96%, which likely further supports the decision not to treat in many patients. Certainly, there are some with false negatives, likely those in high risk groups and who have clinical signs and symptoms at birth, who will need to be treated initially. Subsequently, a negative full GA with aerobic culture results and a negative predictive value of 98% can be used in the decision along with negative or equivocal CBC and CRP to stop antibiotics. This will likely bring down the numbers of those treated for a lengthy course. Infants with a positive full GA alone must not be automatically treated, but again evaluated along with other adjunct tests. If their other adjunct tests are negative, they likely have intra-uterine infection without neonatal infection. The 29% of study infants in our low risk category of C/S, no PROM and no labor, and negative PH, 96% of whom have negative RGA, will need antibiotics at birth very rarely. A larger study using the GA, which includes more infants with proven sepsis, would likely provide a more exact number of infants who could reasonably be spared early antibiotic courses.

Our findings of 4 groups of GA and PH seem to support the idea of a somewhat complex continuum of HPCA and/or intra-uterine infection with variable implications in individual patients, a concept also requiring further investigation. It appears that, in the 41% with either a positive GA, PH or both, infection has likely triggered the cascade leading to PTL. We speculate, as in table 6, as follows: In Group 1(26%) with both GA and PH positive, there is likely an active, acute process. This infants' GA may show either a good WBC response or bacteria or both. In Group 2 (9%) with positive PH and ("false") negative GA, a sub-acute or resolved process may have occurred due to antibiotics or an effective maternal immune response. This may still eventually result in preterm delivery as in our amniocentesis case number two. Those in this group may do well in the NICU or they might have unexpected PVL on an early head ultrasound with later cerebral palsy. In Group 3 (6%) with ("false") positive GA and negative PH, there is likely a rapidly progressive acute intra-uterine infection which lacks the time for mother or infant to produce a robust WBC response before delivery. This group would seem to have the potential to be sicker as in our second septic infant, and also, perhaps, more likely to have an ascending infection beginning after PROM. In Group 4 (59%) there is no evidence of infection.

Obtaining a GA also aids patient management by identifying specific bacterial pathogens in sick infants. As seen in table 7, there are multiple species of pathogens that may be present for any one morphologic Gram stain result. Knowing results of the early Gram stain from the RGA, when positive in a sick infant, has potential to greatly improve early antibiotic management. Although, ampicillin and gentamicin are the broad spectrum antibiotics of choice, resistance to ampicillin is increasing [77,78] and gentamicin sometimes does not penetrate well into the lungs and spinal fluid [79]. The RGA result can improve antibiotic management, for example, when the Gram stain shows a Gram negative rod. If the Gram negative rod is found in the GA of a foul-smelling infant who doesn't respond to the routine antibiotics, it may be a resistant anaerobe and require coverage with meropenem or metronidazole. If the infant who is not responding to antibiotics is not foul-smelling, the infection may be caused by a resistant aerobe which would respond better to Cefotaxime. Occasionally Gram positive cocci are eventually

identified as a resistant *Staph. aureus* or *Strep. mitis* which would also respond better to a change in antibiotic coverage.

Further potential use for the RGA occurs early when high numbers of WBC's are reported, likely indicating significant inflammation. Also, since positive GA correlates well with HPCA, we can use the GA to help diagnose placental inflammation. Although our study was not large enough to investigate this, the literature indicates that inflammation or HPCA results in an increased incidence of CLD, PDA, PPHN, ROP, and NEC. Thus, in cases of inflammatory WBC response in RGA, PH or both, we speculate that these patients can be designated as high risk and in need of particular emphasis on prevention of these debilitating diseases. Preventive measures include, for example, earlier trials of extubation to prevent BPD, increased attention to avoiding hypoxia and excessive oxygen saturation swings to prevent PPHN and ROP, and exclusive use of breast milk feeding to prevent NEC.

A higher number of polymicrobial and anaerobic cultures than expected was found in our study. There is evidence that anaerobes found in periodontal disease may be seeded hematologically to the amniotic cavity [80]. In particular, Bearfield [81] found that *Fusobacterium, Eikenella corrodes, Campnocytophagia, Eubacteria, Peptostreptococcus* and *Prevotella,* all grown in our study, are found in the oral cavity from an early age. She concluded that they carry a high probability of a non-genitourinary and non-gastrointestinal source when found in amniotic fluid. In addition, Aagaard [82] has also more recently found, by studying 16S ribosomal DNA, that the aggregate placental microbiome is most akin to the human oral microbiome. In her study, there were also associations with a remote history of antenatal infection, such as urinary tract infections in the first trimester, as well as with preterm birth. Our study and Dr. Aagaard's call attention to the possibility that improving maternal dental health may be more important in prematurity prevention than we have previously thought.

The fact that we can predict HPCA with the GA has an additional potential for use, along with PH when available, in the medico-legal defense of obstetrical and neonatal cases of unexpected cerebral palsy. Children with positive GA have evidence of risk for FIR in-utero, therefore increasing the chances that the central nervous system insult, most often due to PVL, occurred prior to PTL.

The limitations of our study include the unlikely possibility of false positives in vaginal deliveries, false negatives due to fastidious organisms and viruses (especially cytomegalovirus), antibiotics, and occasional problems obtaining samples. Treatment with prolonged antibiotics in some of the 19 infants with SS may have been influenced by knowledge of results of GA rather than other infant adjunct labs alone, although the number getting long antibiotic courses in the study was relatively few. In addition, some babies may have been too sick in utero to swallow amniotic fluid. In some with oligo hydramnios and/or PROM, there may not have been enough fluid to swallow. Inability to produce an acute WBC response in the face of overwhelming infection may have resulted in low or negative GA WBC counts. Inclusion of patients missing anaerobic and *Mycoplasma* cultures likely means that several positives were missed. We also know that the majority of bacteria in the human microbiome are too fastidious to grow in currently available culture media [1,83].

Conclusion

Expansion of the early-onset sepsis evaluation at birth by addition of the GA (with anaerobic and *Mycoplasma* cultures), and PH to the routine blood culture, CBC's and CRP's can provide important useful information. Judicious interpretation of the easily available GA test done after birth, when added to degree of clinical illness, PH, blood culture, CBC and CRP has the potential to significantly improve the understanding and treatment of infection in preterm infants. Larger studies involving more infants with proven sepsis in infants of all gestational ages are needed. Development of micro-methods to culture blood for anaerobic sepsis and rapid screening for *Mycoplasmas* are also needed to aid in decreasing the needless use of broad spectrum antibiotics.

Acknowledgements

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