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Extra-hepatic anomalies in infants with biliary atresia: results of a large prospective North American multi-center study

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Abstract

Background and aims—The etiology of biliary atresia (BA) is unknown. Given that patterns of anomalies might provide etiopathogenetic clues, we utilized data from the North American

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Childhood Liver Disease Research and Education Network to analyze patterns of anomalies in infants with BA.

Methods—Two hundred eighty-nine infants who were enrolled into the prospective database prior to surgery at any of 15 centers participating were evaluated.

Results—Group 1 was non-syndromic, isolated BA (without major malformations) (n = 242, 84 %), Group 2 was BA and at least one malformation considered major as defined by the National Birth Defects Prevention Study but without laterality defects (n = 17, 6%). Group 3 was syndromic, with laterality defects (n = 30, 10%). In the population as a whole, anomalies (either major or minor) were most prevalent in the cardiovascular (16%) and gastrointestinal (14%) systems. Group 3 patients accounted for the majority of subjects with cardiac, gastrointestinal and splenic anomalies. Group 2 subjects also frequently displayed cardiovascular (71%) and gastrointestinal (24 %) anomalies; interestingly this group had genitourinary anomalies more frequently (47%) compared to Group 3 subjects (10%).

Conclusions—This study identified a group of BA (Group 2) that differed from the classical syndromic and non-syndromic groups and that was defined by multiple malformations without laterality defects. Careful phenotyping of the patterns of anomalies may be critical to the interpretation of both genetic and environmental risk factors associated with BA, allowing new insight into pathogenesis and/or outcome.

Keywords

birth defects; laterality defects; cholangiopathy; embryonic; nonsyndromic

Introduction

The etiology of biliary atresia (BA) is unknown. In a large series of European infants reported by Davenport (1), infants with BA were catalogued by two different presentations: acquired/perinatal/non-syndromic (~90%) vs. embryonal/syndromic (~10%). Infants with splenic malformation (SM) and associated laterality defects were placed in the latter category, implying that the pathogenetic and developmental features of the two types of BA probably differed. In contrast, investigators from Taiwan described the BASM syndrome in only 0.7% of BA infants whereas a total of 15.4% had other major congenital anomalies, suggesting different etiopathologies (2).The number of potential etiologies that explain the pathogenesis of BA has expanded as the sophistication of scientific methods to detect them has evolved. The viral etiology hypothesis has been supported by a number of reports, such as the finding of cytomegalovirus in the livers of BA infants (3) and characterization of the rotavirus-induced murine model of BA (4, 5). Other investigators have suggested an important role for primary immunologic dysfunction, possibility secondary to maternal microchimerism (6). One hypothesis unifying the viral and immune dysfunction concept is that an *in utero* or perinatal viral infection may trigger an autoimmune attack on the biliary epithelium (7). Still other groups have utilized new technologies to examine genetic susceptibility to BA. Leyva-Vega et al (8) reported overlapping heterozygous deletions of chromosome 2q37.3 in two BA patients; the etiologic significance of these abnormalities is unclear. A genome wide association study demonstrated a BA susceptibility locus on chromosome10q24 (9). Recent animal and human evidence support a role for epigenetic regulation of interferon-gamma signaling in BA (10). Kohsaka et al (11) found human *JAG1* missense mutations in about 10% of their BA patients and noted an association of these mutations with a severe phenotype. Hartley et al (12) suggested that the most likely etiopathogenetic explanation of BA is that there are multiple mechanisms of biliary injury leading ultimately to the one common phenotype of obliterative cholangiopathy.

Given that there well may be more than two forms of BA, we believe that a critical reappraisal of the anomalies associated with BA could provide useful clues as to the etiopathogenesis of the disease and have followed the guidelines which the Center for Disease Control utilized in the National Birth Defects Prevention Network. This Network was established in 1997 in order provide uniform reporting of birth defects which might then be linked to a common etiology. Major birth defects were defined by the following criteria: "a) considered to be a major defect (affecting survival, requiring substantial medical care, or resulting in marked physiological or psychological impairment); b) usually identifiable in the first 6 weeks of life (may be extended for some defects); and c) consistently classifiable."(13) In an attempt to identify environmental causes of a given disease process (such as toxins or viral infections) as well as genetic causes, the Center for Disease Control has followed the principle that careful homogenous case definition is the optimum way to identify risk factors and that etiologies of disease conditions such as biliary atresia are likely to be distinct for isolated cases without other major birth defects (our Group 1 – no major anomalies), cases associated with other major birth defects but not syndromes (our Group 2 – major anomalies without laterality defects) vs. cases associated with stereotypical anomalies (our Group 3 – Major anomalies with laterality defects (14).

The purpose of this study was to utilize data from the large prospective multi-center study of BA of the North American Childhood Liver Disease Research Network (ChiLDREN) to perform a detailed analysis of congenital anomalies associated with BA. A sub-aim was to determine if certain demographic variables were associated with the subgroups of BA.

Patients and methods

Subjects

Infants with suspected BA were enrolled into a prospective longitudinal study of cholestasis in infancy (PROBE: Clinicaltrials.gov NCT00061828) prior to diagnostic surgery at any of 15 centers participating in ChiLDREN. The diagnosis of BA was confirmed by intraoperative cholangiogram and surgical exploration prior to Kasai hepatoportenterostomy. In addition the central Pathology Committee of the network supported the diagnosis of BA by blinded review of liver biopsies, coupled with examination of the biliary remnants in cases where the biopsy was uncertain. Determination of each associated anomaly was made from information gathered at the time of surgery, by review of imaging and other clinical studies and by physical examination. When a discrepancy was identified (e.g. no mention of polysplenia made on ultrasound vs. polysplenia noted at the time of surgery), a three-person adjudication committee determined the credibility of evidence. After review of all the data collected on a patient, infants were assigned to one of three groups. Group 1 was isolated BA (without major malformations and with a single spleen), Group 2 was BA without laterality defects but with other congenital malformations, including at least one malformation considered major as defined by the National Birth Defects Prevention Study (13). Group 3 was BA with one or more laterality defects. These defects included splenic abnormalities (asplenia, polysplenia, right sided spleen, or a double spleen), cardiovascular anomalies (dextrocardia, mesocardia, total or partial anomalous pulmonary venous return [TAPVR/PAPVR], absent or interrupted inferior vena cava [IVC], anomalous/bilateral superior vena cava [SVC], and/or preduodenal portal vein and gastrointestinal anomalies ("abdominal heterotaxy," midline/transverse liver, right sided stomach, intestinal malrotation, and anomalous or annular pancreas) (15).

All children in this study were enrolled between May 29, 2004 and November 1, 2010. Written informed consent was obtained from the parent/legal guardian of each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval by the institutional review committees at each site.

Demographic and Clinical Variables

Extensive demographic information was collected prospectively for each subject. This information included maternal age, paternal age, parity and fetal exposure to drugs (including prescribed, over-the-counter, recreational and herbal preparations) and gestational diabetes. Location of the home during the pregnancy was categorized as rural, urban or suburban. Family history included the presence of autoimmune diseases among the primary family and 1st degree relatives and the frequency of autoimmunity in family members was calculated (percent of patients with at least one first degree relative with autoimmune disease.) Both mothers and fathers were asked if any first degree relatives had one or more of the autoimmune diseases listed in Table 1. Information collected about the child included birth weight, birth length and sequential laboratory tests from the time of presentation to the evaluation by the specialist. All laboratory tests are reported as measured except that globulin was inferred by subtraction of albumin from total protein.

Analytic Methods

Descriptive data were summarized by means and standard deviations (sd) for continuous variables and as percentages for categorical variables. The data were summarized overall, as well as within each of the 3 BA groups. In addition to the descriptive analyses, several factors were evaluated for differences across the BA groups. For the continuous variables, analysis of variance was utilized to assess overall differences amongst the groups. Where the F-test reached statistical significance ($p < 0.1$), pairwise comparisons were made for the 3 BA groups to ascertain specific differences. The categorical variables were assessed by chi-square tests where evidence of general association ($p < 0.1$) was further explored through pairwise comparisons of the 3 groups. All analyses were performed using SAS (SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc).

Results

Three distinct BA groups identified

The majority of patients with BA were within Group 1, isolated BA without associated major malformations (242/289, 84%). Group 2, BA without laterality defects but with at least one major malformation, encompassed 17 of the 289 BA patients (6%) and Group 3, BA with one or more laterality defects, encompassed 30 of 289 patients (10%). Table 2 summarizes the most common major and minor anomalies reported by system in all 289 subjects and in each of the 3 groups. Overall, anomalies were most prevalent in the cardiovascular (16% of subjects), and gastrointestinal (14%) systems and splenic anomalies (7%). Group 3 patients with laterality defects accounted for the majority of subjects with cardiac, gastrointestinal and splenic anomalies. Splenic anomalies were noted in 70% of Group 3 patients.

Group 2 subjects, while also displaying significant cardiovascular (71%) and gastrointestinal (24%) anomalies, also had significant genitourinary (47%) anomalies which were uncommon in Group 3 subjects. The most common genitourinary defects found in this group were cystic kidney and hydronephrosis. Four of the Group 2 patients had vertebral and rib anomalies but only one had a major musculoskeletal anomaly (longitudinal limb deficiency.) The cardiovascular anomalies in Group 2 included aortic arch abnormalities, aortic coarctation, atrial septal defects, patent ductus arteriosus, patent foramen ovale, pulmonary artery stenosis, pulmonary valvular stenosis, Tetralogy of Fallot, transposition of the great vessels and ventricular septal defect. Gastrointestinal anomalies included duodenal/jejuna atresia, esophageal atresia, and imperforate anus. Supplementary Table S1 summarizes the distribution of the systems with at least one reported anomaly for the 47 individual patients

in Groups 2 and 3. Supplementary Table S2 summarizes the distribution of specific genitourinary anomalies across all three Groups.

Demographic variables associated with BA groups

Analysis of demographic variables between groups revealed significant differences in the age at first evaluation, with Group 1 having a later age at evaluation compared to Group 3 (Table 3). Recreational drug use during pregnancy was reported more commonly in Group 3 compared to Group 1. There was no difference between the 3 groups for mother's or father's age, gender, race, history of familial autoimmune disease, z-scores for birth weight or length, or rural vs. urban location. For gestational age, the difference across the three groups was significant (F test p=0.0912). Subsequent pairwise comparison revealed Group 1 infants tended to be slightly older than Group 3 infants (p = 0.0512). The mean maternal age was 29.2 +/− 6.0 years and the mean paternal age was 31.9 +/− 7.0 years. The incidence of gestational diabetes was increased in Group 3 compared to Group 1. Interestingly, the incidence of an autoimmune disease in first degree relatives was substantial – 44% overall, with no difference between groups. Sixty-three percent of the whole population of BA infants was white, without differences between the three groups. The race/ethnicity distribution was relatively even across groups but the small sample size makes it difficult to compare anything other than white vs. non white.

Clinical laboratory variables associated with BA groups

Table 4 reports select clinical and laboratory variables that were prospectively collected. While total bilirubin did not differ across the three groups, there was a difference in direct bilirubin across groups (F test p=0.0693). Group 1 infants tended to have a higher direct bilirubin values compared to Group 2 and Group 3 though neither of these pairwise comparison reached significance at the p=0.05 threshold (p=0.0999 and p=0.0654, respectively). Gamma-glutamyl transpeptidase (GGTP) was similar across the groups. Alkaline phosphatase was significantly higher in Group 1 compared to Group 2. After adjusting for age at first evaluation, these laboratory differences across the groups remained (data not shown).

Total protein and albumin levels were higher in Group 1 compared to Group 3. Alanine aminotransferase was lower in Group 2. Group 3 was characterized by higher white blood cell counts and platelet counts vs. the other two groups.

Discussion

In this prospective North American multi-center study of BA, we identified 3 groups of BA patients. The most common was isolated BA, the perinatal or acquired form of BA without associated major malformations (Group 1). A second group was identified whereby not only gastrointestinal and cardiac anomalies were associated with BA in the absence of laterality defects, but also findings of genitourinary anomalies (Group 2). The most frequent renal anomalies reported in Group 2 were cystic kidneys or hydronephrosis. The observation that as many as 16% of children with BA may have heart disease and 3% may have renal anomalies, makes differentiation from Alagille Syndrome difficult. Likewise, the fact that infants with biliary atresia may occasionally have cystic kidneys may make differentiation from infants with polycystic liver-kidney disease a bit of a challenge, although cholestasis is rare in the latter condition The incidence of clinically significant hydronephrosis in otherwise healthy newborns is approximately 1 in 600 live births (0.17%) (16, 17). The incidence of hydronephrosis in BA patients in this study (all within Group 2) was 3 in 289 (1%), an almost 10-fold greater incidence compared to the general population. There is scant recent literature on genitourinary and musculoskeletal abnormalities associated with BA. A

case report described an infant with BASM, sacro-coccygeal agenesis, clubfoot, and ano-urinary incontinence (18). A BA patient with anorectal agenesis and a complicated urogenital malformation was also described (9). It is known that many genitourinary anomalies are associated with concurrent vertebral segmentation anomalies (20).

In our study of Group 2 patients with genitourinary and musculoskeletal abnormalities, a similar association to that previously reported in the literature is suggested. In addition some of the Group 1 had clinically insignificant rib or vertebral defects. Almost twenty years ago Carmi et al (21) reported that one-third of their 51 BA patients with major anomalies had laterality defects but two-thirds had cardiac, genitourinary and musculoskeletal defects not associated with laterality defects. Our report confirms their findings, extends the spectrum of renal anomalies observed, and also strongly reinforces the authors' suggestion that there is etiologic heterogeneity in BA.

In a large study from England the incidence of splenic anomalies was 10.2% (1), almost identical to the incidence identified in this study. The investigators from England also reported similar rates of intestinal malrotation, absent or interrupted IVC and preduodenal portal vein in patients with splenic anomalies. Fifteen percent of the patients in their series with laterality defects were born to mothers with diabetes and this association was not found in their BA patients without laterality defects. Gestational diabetes was observed in 9.9%, 11.8%, and 23.3% of our infants in Groups 1, 2, and 3. Interestingly, the English study also found a female predominance of 2:1 in patients with splenic anomalies, a finding that was not identified in our North American cohort. As noted in the paper by Davenport et al (1), in addition to "BASM," another term for infants with BA and stereotypical syndromic abdominal and vascular anomalies is "biliary atresia laterality sequence." Given that only 70% of our patients with laterality defects actually had splenic anomalies, the latter term might be preferable in the future to "BASM" to describe this stereotypical group of infants.

The Canadian Pediatric Hepatology Research group has recently reported their analysis of 382 infants with BA and the associated anomalies (22). Forty-four (13%) had associated anomalies, only 25 (6.5%) of which were associated with SM. The authors concluded that BA infants with anomalies demonstrated a spectrum of laterality defects and suggested that the meaning of the acronym BASM be modified to "biliary atresia structural malformation." Our conclusions are somewhat similar in that a total of 16% of our infants were in the anomaly Groups 2 and 3. On the other hand, the main difference between our observations and those of the Canadian group was that Group 2 infants frequently exhibited major birth defects of the genitourinary and/or gastrointestinal systems, not considered part of defective lateralization, suggesting that this group may represent a different etiopathogenesis than Groups 1 and 3.

Group 3 infants were younger at the time of initial evaluation compared to Group 1. The associated anomalies in Group 3, especially the cardiac lesions associated with murmurs or cyanosis, probably brought the patient to medical attention sooner than the infants with isolated cholestasis.

An unexpected finding was the high incidence of autoimmunity in first degree relatives of all BA groups (average 44%). The occurrence of autoimmune diseases in relatives provides circumstantial evidence that a candidate disease (i.e. BA) may be autoimmune in nature (23). The incidence of autoimmunity in first degree relatives is much higher than that found in the general population, where autoimmunity rates vary from 2.5-9% (26,27). Importantly, the incidence of autoimmunity in first degree relatives of BA patients was similar to the rate of 37-43% identified in autoimmune hepatitis (26) and 25.5% in type-1 diabetes mellitus (25). This intriguing finding of autoimmunity in first degree relatives of BA patients

warrants further investigation. The fact that there was no difference in autoimmunity rates between the three groups suggests that the autoimmune hypothesis of BA may be relevant to the pathogenesis of all types of BA and is a clue to be pursued in further studies. It is also possibley that the high incidence simply resulted from our rigorous questionnaire containing a long list of autoimmune diseases and not being of pathogenetic signficance. We agree that the lack of differences between groups is not only at variance to explain the autoimmune hypothesis of Group 1 but also at variance to explain the genetic, non-immune hypothesis of syndromic BA, in which lower familial rates of autoimmune disease would have been expected. Possible explanations include the possibility that this hypothesis is incorrect vs. the immune dysregulation hypothesized for Group 1 BA (27) being atypical from the usual types of familial autoimmune diseases.

The analysis of laboratory tests revealed no difference in total bilirubin across the Groups though infants in Group 1 had higher alkaline phosphatase levels and they also tended to have higher direct bilirubin values. The significance of this observation is uncertain. Group 1 infants tended to be older at time of initial evaluation and thus could be hypothesized to have a longer duration of obstruction. We explored this possibility by adjusting for age at first evaluation and the laboratory differences across the groups remained, suggesting age alone was not responsible. Group 1 infants had higher total serum albumin levels compared to Groups 2 and 3. It has been reported that newborns have lower albumin levels that increase with age (28). Both Groups 2 and 3 were younger at the time of evaluation compared to Group 1 and the younger age at presentation may explain the lower albumin levels. Furthermore, it is possible that increased protein and albumin losses could be associated with some of the anomalies present in Groups 2 and 3. Specifically, intestinal atresias could lead to intestinal protein loss and renal anomalies could result in urinary protein loss. Finally, higher total white cell counts and platelet counts were identified in Group 3 compared to the others. The hemodynamics within the spleen in polysplenia are most likely altered and it is theorized that decreased filtration through the splenic venules would be associated with decreased trapping and removal of white cells and platelets.

In summary, BA is a heterogeneous disease that is composed of at least three subgroups. This study identified a group that was defined by multiple malformations including genitourinary anomalies, reinforcing a similar report by Carmi et al in 1993 (21). Future investigations are indicated to determine if each of these subtypes is associated with unique predisposition or etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of abbreviations

BA	(biliary atresia)
SM	(splenic malformation)
IVC	(inferior vena cava)
SVC	(superior vena cava)
PHTN	(pulmonary hypertension)
AV	(atrioventricular)
AVSD	(atrioventricular septal defect)
LV	(left ventricle)
RV	(right ventricle)
TAPVR/PAPVR	(total anomalous pulmonary venous return/partial anomalous pulmonary venous return)
TGA	(transposition of the great arteries)
VSD	(ventricular septal defect)
TEF	(trachea-esophageal fistula)
CNS	(central nervous system)
KG	(kilograms)
CM	(centimeters)
GGTP	(gamma-glutamyl transpeptidase)
ALT	(alanine aminotransferase)
WBC	(white blood cells)

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Table 1

Autoimmune diseases in first degree relatives about which parents were queried

Autoimmune liver disease
Primary biliary cirrhosis
Primary sclerosing cholangitis
Autoimmune hepatitis
Autoimmune and connective tissue disease
Systemic lupus erythematosus
Raynaud's syndrome
Rheumatoid arthritis
Multiple sclerosis
Sjogren's syndrome
Polymyositis
Autoimmune endocrine diseases
Insulin dependent diabetes in subjects < 30 years old
Thyroid disease including hypothyroid, goiter, thyrotoxicosis, and thyroid disease type unknown
Autoimmune gastrointestinal diseases
Ulcerative colitis
Crohn's disease
Other unspecified autoimmune disease

Table 2

Frequency of Congenital Anomalies in 289 Biliary Atresia patients by Group

Any Anomaly	TOTAL		GROUP 1 (without major anomalies)		GROUP 2 (major anomalies without laterality defects)		GROUP 3 (laterality defects)	
	N	%	N	%	N	%	N	%
	289		**242**		**17**		**30**	
Cardiovascular	47	16.3	11	4.5	12	70.6	24	80.0
Pulmonary	4	1.4	1	0.4	1	5.9	2	6.7
Gastrointestinal	40	13.8	9	3.7	4	23.5	27	90.0
Genitourinary	15	5.2	4	1.7	8	47.1	3	10.0
Splenic anomaly	21	7.3	0	0	0	0	21	70.0

Table 3

Clinical and Demographic Characteristics of 289 Infants with Biliary Atresia

Parameter	N	Mean +/− SD or N (%)	P-value vs. Group 2	P-value vs. Group 3
Gender (Male vs. Female) (p=0.4063)	289	135 (46.7%)	-	-
BA Group 1	242	109 (45.0%)	-	-
BA Group 2	17	10 (58.8%)	-	-
BA Group 3	30	16 (53.3%)	-	-
Birth Weight KG (p=0.4079)	269	3.15 +/− 0.57	-	-
BA Group 1	223	3.16 +/− 0.54	-	-
BA Group 2	16	3.25+/−0.56	-	-
BA Group 3	30	3.03+/−0.78	-	-
Birth Length CM (p=0.2858)	252	49.8+/−3.6	-	-
BA Group 1	211	49.9+/−3.6	-	-
BA Group 2	15	49.2+/−2.6	-	-
BA Group 3	26	48.8+/−4.0	-	-
Gestational Age in Weeks (p=0.0912) *	271	38.2+/−2.2	-	-
BA Group 1	226	38.3+/−2.2	0.2448	0.0512
BA Group 2	17	37.6+/−2.1	-	0.7471
BA Group 3	28	37.4+/−2.3	0.7471	-
Age at First Evaluation (Days) (p=0.0397*)	288	68.1+/−36.3	-	-
BA Group 1	242	70.4+/−37.6	0.2181	0.0204
BA Group 2	16	58.9+/−27.8	-	0.6688
BA Group 3	30	54.2+/−24.7	0.6688	-
Recreational Drug Use During Pregnancy (p=0.0964 *)	276	10 (3.6%)	-	-
BA Group 1	230	6 (2.6%)	0.4325	0.0321
BA Group 2	17	1 (5.9%)	-	0.6041
BA Group 3	29	3 (10.3%)	0.6041	-
Rural vs. Urban (p=0.4650)	280	230 (82.1%)	-	-
BA Group 1	234	191 (81.6%)	-	-
BA Group 2	26	22 (84.6%)	-	-
BA Group 3	20	17 (85.0%)	-	-
Gestational Diabetes (Yes vs. No) (p <0.0948*)	279	32 (11.5%)	-	-
BA Group 1	232	23 (9.9%)	0.8064	0.0298
BA Group 2	17	2 (11.8%)	-	0.3328

Parameter	N	Mean +/− SD or N (%)	P-value vs. Group 2	P-value vs. Group 3
BA Group 3	30	7 (23.3%)	0.3328	-

* Significant at $\alpha = 0.1$ for *differences across BA groups*.. Pairwise comparisons were assessed

BA Group 1, n=242 – infants without major anomalies

BA Group 2, n =17 – infants with major anomalies without laterality defects

BA Group 3, n=30 – infants with laterality defects

Table 4

Selected Laboratory Characteristics of 289 Infants with Biliary Atresia

Parameter	N	Mean +/− SD	P-value vs. Group 2	P-value vs. Group 3
Total Bilirubin (p=0.4777)	277	8.43+/−3.48		
BA Group 1	231	8.53+/−3.54		
BA Group 2	17	8.29+/−3.44		
BA Group 3	29	7.70+/−3.01		
Direct Bilirubin (p=0.0693) *	167	5.68+/−2.29		
BA Group 1	138	5.87+/−2.22	0.0999	0.0758
BA Group 2	12	4.74+/−2.49		0.9184
BA Group 3	17	4.83+/− 2.51	0.9184	
GGTP (p=.3921)	262	686.8+/−533.4		
BA Group 1	217	692.2+/−530.6		
BA Group 2	16	802.0+/−669.5		
BA Group 3	29	582.4+/−470.2		
Alkaline Phosphatase (p=0.0010) *	272	589.6+/−315.5		
BA Group 1	226	608.3+/−316.6	0.0007	0.0654
BA Group 2	17	342.5+/−131.6		0.1056
BA Group 3	29	495.6+/−316.0	0.1056	
Total Protein (p=0.0140) *	213	6.00+/−0.82	-	-
BA Group 1	176	6.07+/−0.81	0.1121	0.0094
BA Group 2	15	5.73+/−0.96	-	0.6263
BA Group 3	22	5.60+/−0.59	0.6263	-
Albumin (p=0.0003) *	268	3.56+/−0.54	-	-
BA Group 1	224	3.62+/−0.51	0.0026	0.0034
BA Group 2	17	3.22+/−0.66	-	0.5895
BA Group 3	27	3.30+/−0.57	0.5895	-
ALT (p=0.0299) *	276	150+/−112	-	-
BA Group 1	230	153+/−101	0.0117	0.5534
BA Group 2	17	83+/−50	-	0.0141
BA Group 3	29	166+/−189	0.1041	-
WBC (p=0.0121) *	267	13.67+/−4.54	-	-
BA Group 1	223	13.41+/−4.33	0.7998	0.0034
BA Group 2	16	13.11+/−3.91	-	0.0369
BA Group 3	28	16.06+/−5.76	0.0369	-
Platelets (p=0.0416) *	266	432.0+/−178.1	-	-
BA Group 1	224	426.1+/−165.2	0.3511	0.0238
BA Group 2	15	382.1+/−172.9	-	0.0278

Parameter	N	Mean +/− SD	P-value vs. Group 2	P-value vs. Group 3
BA Group 3	27	508.0+/−256.0	0.0278	-

* Significant at $\alpha = 0.1$. Pairwise comparisons were assessed

BA Group 1, n=242 – infants without major anomalies

BA Group 2, n =17 – infants with major anomalies without laterality defects

BA Group 3, n=30 – infants with laterality defects