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Original Article

A comparison of inflammatory indicators in twins on the first postnatal day

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Abstract

Aim: To investigate markers of sepsis/inflammation in twin neonates according to placental, demographic, neonatal, and twin-related complications.

Materials and Methods: In this retrospective study, we compared these parameters in twins with suspected sepsis for whom laboratory data on infection markers were available on the first postnatal day. Sepsis was later ruled out in all the neonates. After obtaining institutional ethics committee approval, twin pairs who were admitted to the neonatal intensive care unit of Hacettepe University IhsanDogramacı Children's Hospital between 2005 and 2017 were screened for inclusion. Data on the twins' sepsis markers, complete blood counts, immature to total neutrophil (I/T) ratios, procalcitonin and CRP levels, and blood culture results in the first 24 h of life were recorded.

Results: In total, 194 twin pairs (388 neonates) were included in the study. Of the 194 twin pairs, 50 were monochorionic twins (25.8 %), and the remaining 144 (74.2 %) were dichorionic twins. Monochorionic and dichorionic twins, twin-to-twin transfusion syndrome with twins and discordant twins showed no significant differences in I/T ratio, procalcitonin, or CRP values in the first 24 h ($p>0.05$).

Conclusion: Based on our findings, chorionicity (monochorionic/dichorionic placenta), twin-to-twin transfusion syndrome, and size discordance were not related to any significant differences in the CRP level, procalcitonin level, or I/T ratio.

Keywords: C-reactive protein (CRP); dichorionic twin; monochorionic twin; newborn; procalcitonin

INTRODUCTION

Although the incidence of twin pregnancy varies according to ethnic origin and geographical region, it has increased over the last 30 y due to assisted reproductive technologies, such as ovulation induction and in vitro fertilization (1). Perinatal mortality and morbidity are more frequent in twins than in singletons at the same gestational age. Twin pregnancies are also associated with an elevated risk of prematurity, twin-to-twin transfusion, twin growth discordance, and congenital anomalies (2).

In multiple pregnancies, chorionicity is determined by the number of chorionic (outer) membranes surrounding the fetuses. In monochorionic twin (MT) pregnancies, there is only one placental membrane, whereas there are two membranes in dichorionic twin (DT) pregnancies (3).

In MT pregnancies, twin-to-twin transfusion syndrome (TTTS) and subsequent growth discordance (i.e., a large difference in the birth weight of the twins can result in serious adverse perinatal and neonatal outcomes, including death. This syndrome is caused by an imbalance in transfusion through placental vascular anastomoses, wherein one twin is the donor and the other is the recipient (4,5).

The most commonly used laboratory markers in neonatal sepsis are C-reactive protein (CRP) and procalcitonin. CRP may also be elevated in noninfectious conditions,

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such as trauma, hypoxia, meconium aspiration syndrome, and hemolysis (6,7). The procalcitonin level increases physiologically in the first hours of life (8). It may also be elevated in noninfectious conditions, such as intracranial hemorrhages or perinatal asphyxia (8).

No studies have investigated whether these commonly used sepsis/inflammation markers vary in twin neonates based on placental, demographic, and neonatal characteristics. In this retrospective study, we compared these parameters in twins laboratory data on infection markers were available on the first postnatal day. Sepsis was later ruled out in all the neonates.

The aim of this study was to investigate whether infection parameters increased in the first day of life in twins and whether multiple pregnancies were effective on infection parameters.

MATERIALS and METHODS

Patients

After obtaining institutional ethics committee approval, 394 twin pairs who were admitted to the neonatal intensive care unit of Hacettepe University Ihsan Dogramacı Children'sHospital between 2005 and 2017 were screened for inclusion in this retrospective study.

Data on the twins' sepsis markers, complete blood counts, immature to total neutrophil (I/T) ratios, procalcitonin and CRP levels, and blood culture results in the first 24 h of life were recorded. The following exclusion criteria were applied: infants with a history of a difficult/traumatic birth, perinatal hypoxia, hypoxic ischemic encephalopathy, the presence of meconium at birth, premature rupture of membranes (PROM), chorioamnionitis, active maternal infections (sepsis and urinary tract infections), and a history of intrauterine infections. Accordingly, 41 twin pairs were excluded due to PROM, congenital anomalies, chorioamnionitis, and active maternal infections. Another 13 pairs were excluded due to incomplete medical records, 89 pairs were excluded due to missing laboratory parameters, and 57 pairs were excluded due to a diagnosis of early neonatal sepsis. Thus, the final study was completed with the remaining 194 twin pairs.

Studies and meta-analyses have used different thresholds (15, 20, and 25%) to define twin discordance (9). In this study, a weight difference between twins of 20% or greater was accepted as growth discordance. Discordance was calculated using the following formula: (larger twin weight - smaller twin weight)/larger twin weight × 100.

Sepsis markers

Serum procalcitonin levels were measured by a homogeny immunoassay method, TRACE (Time Resolved Amplified CryptateEmission,Kryptor; Brahms, Germany) method, with a high limit of detection (0 ng/mL) and a linear range of 0.02-100 ng/mL. Serum CRP levels were measured by rate nephelometry (Beckman Coulter Inc,.CA, USA) method with a linearity of 5-300 mg/dL.

Chiesa et al. (10) reported for the term and preterm neonates, the predicted CRP levels were, at birth, 0.1 mg/L. In this study we have used this value. In a study evaluating newborns of Fendler et al. (11) they found the cut-off value of procalcitonin to be 0.99 ng/mL in the diagnosis of sepsis. In this study we have used this value. The I/T ratio was determined by counting 100 leukocytes in a peripheral smear with Wright staining (12).

Statistical methods

The Statistical Package for the Social Sciences version 23.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used in the analysis of the data. Independent samples t-test was used to compare two independent groups for normally distributed variables, and Mann–Whitney U test was used for comparison of non-normally distributed variables. Pearson chi-square test and Fisher's exact test was used to compare groups for differences in categorical variables. Arithmetic mean, standard deviation, median, and minimum-maximum values were given as descriptive statistics for quantitative data. Qualitative data were summarized using frequency and percentages. A p value of less than 0.05 was considered as statistically significant difference.

RESULTS

In total, 194 twin pairs (388 neonates) were included in the study. The demographic and neonatal characteristics of the infants are presented in Table I.

Of the 194 twin pairs, 50 were MTs, and the remaining 144were DTs. There were 202 (52%) males and 186 (48%) females. The median maternal age was 30 (18–46) y. The mean gestational age at birth in the entire study group was 32.1 (± 3.2) wk. Assisted reproductive techniques (ARTs) had been used in 130 (67%) of the pregnancies. The birth weights and gestational ages of the MT pairs were significantly lower than those of the DT pairs ($p = 0.007$ and $p = 0.005$, respectively). There was no statistically significant difference between the MT and DT groups in terms of sex, types of delivery, antenatal steroid rates, 5-min Apgar scores, SGA ratios, the need for postnatal resuscitation, diagnoses of respiratory distress syndrome (RDS), intubation rate, necrotizing enterocolitis(NEC), bronchopulmonary dysplasia (BPD) or survival rates ($p = 0.345$, $p = 0.051$, $p = 0.451$, $p = 0.078$, $p = 0.809$, $p = 0.419$, $p = 0.182$,$p= 0.901$, $p=0.945$ and $p = 0.061$, $p=0.211$, respectively).

The birth weights were equal in 14 of the MT pairs and 16 of the DT pairs (these twins are not included in Table II and III). In pairs with different birth weights, the infants were classified as the larger twin and the smaller twin. The demographic and neonatal characteristics of the larger and smaller twins are shown in Table II (n=358).

There were no statistically significant differences between the larger and smaller twins in either the MT or DT group in terms of mortality rates or 5-min Apgar scores ($p = 0.336$ and $p = 0.827$; $p = 0.846$, and $p = 0.246$, respectively).

Table 1. Demographic and neonatal characteristics

	Monochorionic n = 100 (25.8%)	Dichorionic n = 288 (74.2%)	Total N = 388 (100%)	p
Sex (M/F) n (%)	48/52 (48/52)	154/134 (53.5/46.5)	202/186 (52/48)	0.345
Gestational age+ (wk)	31.4 ± 3.3	32.4 ± 3.2	32.1 ± 3.2	0.005
Method of conception	48/52	80/208	128/260	< 0.001
Spontaneous/ART,n/n(%/%)	(48/52)	(27.8/72.2)	(33/67)	
Cesarean/NSVD, n/n (%/%)	94/6 (94/6)	282/6 (97.9/2.1)	376/12 (96.9/3.1)	0.051
Antenatal steroids, n (%)	46 (46)	120 (41.7)	166 (42.8)	0.451
Birth weight+ (g)	1578 ± 651	1764 ± 576	1716 ± 601	0.007
Apgar score (5 thmin)*	8 (2-10)	9 (2–10)	9 (2–10)	0.078
SGA, n (%)	18 (18)	55 (19.1)	73 (18.9)	0.809
Neonatal resuscitation, n (%)	41 (41)	105 (36.5)	146 (37.6)	0.419
Intubation rate	45 (45)	124 (43.1)	169 (43.6)	0.901
RDS, n (%)	40 (40)	94 (32.6)	134 (34.5)	0.182
NEC	21 (21)	60 (20.1)	81 (20.9)	0.945
BPD	15 (15)	15 (5.2)	30 (7.7)	0.061
Discharge/death, n/n (%/%)	87/13 (87/13)	263/25 (91.3/8.7)	350/38 (90.2/9.8)	0.211

*median (minimum-maximum); +mean ± standard deviation; ART: assisted reproductive technique; RDS: respiratory distress syndrome; SGA: small for gestational age; NSVD: normal spontaneous vaginal delivery; NEC: Necrotizing enterocolitis; BPD: Bronchopulmonary dysplasia

Table 2. Demographic and neonatal characteristics of the larger and smaller twins (based on birth weight, n=358)

	Monochorionic, n (%)				Dichorionic, n (%)			
	Smaller twin n = 43	Larger twin n = 43	Total n= 86	p1	Smaller twin n = 136	Larger twin n = 136	Total n = 272	p2
Sex (M/F) n (%)	20/23 (46.5/53.5)	18/25 (41.9/58.1)	38/48 (44.2/55.8)	0.664	66/70 (48.5/51.5)	77/59 (56.6/43.4)	143/129 (52.6/47.4)	0.182
Gestational age+ (wk)	1393 ± 620	1756 ± 693	1575±679	0.012	1616 ± 530	1914 ± 568	1766 ± 569	<0.001
Method of conception	8 ± 1.8	8 ± 1.6	8±1.7	0.846	8.3 ± 1.7	8.5 ± 1.7	8.4 ± 1.7	0.264
Spontaneous/ART,n/n(%/%)	15 (34.9)	3 (7.0)	18 (20.9)	0.001	40 (29.4)	14 (10.3)	54 (19.9)	<0.001
Cesarean/NSVD, n/n (%/%)	14 (32.6)	17 (39.5)	31 (36.0)	0.500	51 (37.5)	48 (35.3)	99 (36.4)	0.705
Antenatal steroids, n (%)	15 (34.9)	16 (37.2)	31 (36.0)	0.822	48 (35.6)	42 (31.1)	90 (33.3)	0.439
Birth weight+ (g)	8 (18.6)	5 (11.6)	13(15.1)	0.366	12 (8.8)	11 (8.1)	23 (8.5)	0.827

RDS: respiratory distress syndrome; SGA: small for gestational age

Table 3. Complete blood count parameters and sepsis markers (n=358)

	Monochorionic n = 86 (24.0%)				Dichorionic n = 272 (76.0%)				
	Smaller twin	Larger twin	Total	p1	Smaller twin	Larger twin	Total	p2	p3
Hb (g/dL)	16.2 ± 2.7	16.9 ± 2.8	16.6 ± 2.8	0.227	16.7 ± 2.8	77/59 (56.6/43.4)	16.8 ± 2.7	0.942	0.124
Htc (%)	48.5 ± 8.4	50.4 ± 8.5	49.5 ± 8.4	0.298	50.3 ± 8.6	1914 ± 568	50.2 ± 8.3	0.798	0.261
WBC count* (/uL)	11,450 (4,300–58,600)	11,400 (2,900–32,000)	11,400 (2,900–58,600)	0.493	11,900 (1,700–43,000)	8.5 ± 1.7	10,900 (1,200–49,800)	0.997	0.701
Platelet count* (/uL)	206,500 (11,000–445,000)	235,000 (22,000–572,000)	223,000 (11,000–572,000)	0.434	206,000 (24,000–554,000)	14 (10.3)	221,000 (24,000–672,000)	0.018	0.742
I/T ratio*	0.1 (0–0.3)	0.1 (0–0.4)	0.1(0–0.4)	0.379	0.1 (0–0.8)	48 (35.3)	0.1 (0–0.8)	0.685	0.523
PC* (ng/mL)	0.3 (0.1–8.7)	0.4 (0.1–12.4)	0.3 (0.1–12.4)	0.098	0.4 (0–57.8)	42 (31.1)	0.4 (0–57.8)	0.795	0.919
CRP* (mg/dL)	0.1 (0–1.1)	0.2 (0.1–5.9)	0.1 (0–5.9)	0.512	0.1 (0–4.7)	11 (8.1)	0.1 (0–4.7)	0.264	0.523

*: median (minimum-maximum); mean ± standard deviation; Hb: hemoglobin, Htc: hematocrit, WBC: white blood cell; I/T: immature to total neutrophil, PC: procalcitonin, CRP: C-reactive protein

Table 4. Comparison of blood values in neonates with twin-to-twin transfusion syndrome (TTTS) (n=18)

	Recipient twin(n = 9)	Donor twin (n = 9)	p
Hb (g/dL)*	20.2 ± 3.5	13.3 ± 2.2	0.004
Htc (%)*	61.4 ± 10.6	41.1 ± 6	0.003
WBC count* (/uL)	10350 (2,900–32,000)	11,400 (4,300–17,100)	0.959
Platelet count (/uL)	216,000 (112,000–305,000)	184,500 (11,000–287,000)	0.161
I/T ratio (%)	0.06 (0–0.17)	0.17 (0.06–0.35)	0.190
PC (ng/mL)	1.49 (0.01–4.95)	0.31 (0.1–0.61)	0.489
CRP (mg/dL)	0.17 (0.1–0.33)	0.22 (0.1–1.1)	0.105

*: mean ± standard deviation; Hb: hemoglobin, Htc: hematocrit, WBC, white blood cell; I/T: immature to total neutrophil, PC: procalcitonin, CRP: C-reactive protein

The results of the complete blood counts and sepsis markers in the twins in the first 24 h are presented in Table III (n=358).

There were no statistically significant differences between the larger and smaller MTs in hemoglobin (Hb), hematocrit (Htc), white blood cell (WBC), platelet, I/T ratio, procalcitonin, and CRP values in the first 24 h (p = 0.227, p = 0.298, p = 0.493, p = 0.434, p = 0.379, p = 0.098, and p = 0.512, respectively). There were also no statistically significant differences between larger and smaller DTs in Hb, Htc, WBC, I/T ratio, procalcitonin, or CRP values in the first 24 h (p = 0.942, p = 0.798, p = 0.997, p = 0.685, p = 0.795, and p = 0.264, respectively). However, the platelet counts of the larger twins were significantly higher than those of the smaller twins in the DT pairs (p = 0.018).

TTTS was observed in nine MT pairs (18 infants). The complete blood count parameters and sepsis marker results of these infants are shown in Table IV. In cases of TTTS, the neonates were recorded as being either the donor or recipient twin.

Of the infants with TTTS, the Hb and Htc values were significantly higher in the recipient twin than the donor twin (p = 0.004 and p = 0.003, respectively). There were no statistically significant differences in WBC, platelet, I/T ratio, procalcitonin, or CRP values (p = 0.279, p = 0.195, p = 0.959, p = 0.161, p = 0.190, p = 0.489, and p = 0.105, respectively).

Table 5. Comparison of serum parameters in the twins with size discordance (n=100)

	Monochorionic discordance n = 36 (32.7%)			Dichorionic discordance n = 74 (67.3%)			
	Smaller twin	Larger twin	p1	Smaller twin	Larger twin	p2	p3
Hb (g/dL)*	16.3 ± 2.3	16.8 ± 1.9	0.488	16.3 ± 2.6	16.6 ± 2.7	0.656	0.109
Htc (%)*	49.5 ± 6.9	50.2 ± 6	0.745	48.8 ± 8	48.8 ± 7.9	0.981	0.499
WBC count (/uL)	8,550 (43,00–22,000)	9,000 (2,900–15,800)	0.365	10,900 (1,700–42,500)	10,300 (3,300–30,600)	0.681	0.048
Platelet count (/uL)	193,000 (94,000–445,000)	229,500 (56,000–383,000)	0.567	167,000 (5,300–310,000)	243,000 (58,000–432,000)	<0.001	0.559
I/T ratio (%)	0.14 (0–0.34)	0.11 (0–0.24)	0.243	0.12 (0–0.3)	0.11 (0–0.8)	0.838	0.683
PC (ng/mL)	0.31 (0.08–8.68)	0.3 (0.1–12.42)	0.683	0.39 (0.04–54.79)	0.34 (0.01–43.55)	0.589	0.816
CRP (mg/dL)	0.11 (0.1– 0.78)	0.12 (0.1– 2.3)	0.402	0.12 (0–3.16)	0.1 (0–3.16)	0.151	0.449

*: mean ± standard deviation; Hb, hemoglobin; Htc, hematocrit; WBC, white blood cell; I/T: leae; PC: procalcitonin; CRP: C-reactive protein

Table 6. Characteristics of the twins with elevated acute phase reactants (n=388)

	Twins with elevated acute phase reactantsn = 117	Twins with low/normal acute phase reactant levelsn = 271	p
Gestational age+ (wk)	31.4 ± 3.1	32.8 ± 2.6	<0.001
Birth weight, (g)+	1561 ± 571	1870 ± 503	<0.001
Sex (male/female), n/n	67/50	135/136	0.186
Spontaneous/ART, n/n	40/77	88/183	0.814
Antenatal steroids, n	47	119	0.505
NSVD/cesarean, n/n	3/114	9/262	1.000
Chorionicity (mono/di), n/n	30/87	70/201	1.000
Discordance, n	29	81	0.328
TTTS, n	4	14	0.602
Apgar score (5 min)*	8 (4–10)	9 (2–10)	0.012
Resuscitation, n	37	109	0.112
RDS, n	36	98	0.352
Death, n	8	30	0.074
SGA, n	17	56	0.202
Htc (%)+	49.0 ± 7.6	50.3 ± 8.7	0.158
White blood cell count (/uL)*	12500 (1700–58600)	10200 (1200–49800)	0.133
I/T ratio*	0.11 (0.0–0.8)	0.11 (0.0–0.5)	0.500

*median (minimum-maximum); +mean ± standard deviation; ART: assisted reproductive technique; RDS: respiratory distress syndrome; SGA: small for gestational age; Htc, hematocrit; NSVD: normal spontaneous vaginal delivery; TTTS: twin-to-twin transfusion syndrome; I/T: immature/total neutrophil

There were 18 discordant twin pairs (n = 36) in the MT group and 37 discordant twin pairs (n = 74) in the DT group. The comparison of complete blood parameters of the discordant twins (i.e., a weight difference of at least 20%) is presented in Table V (n=100).

Discordant MTs showed no significant differences as compared with discordant DTs in terms of Hb, Htc, platelet, I/T ratio, procalcitonin, or CRP values in the first 24 h (p = 0.109, p = 0.499, p = 0.559, p = 0.683, p = 0.816, and p = 0.449, respectively). Discordant DTs had significantly

higher WBC values than discordant MTs (p = 0.048). In addition, there were no statistically significant differences between the larger and smaller discordant MTs in Hb, Htc, WBC, platelet, I/T ratio, procalcitonin, or CRP values in the first 24 h (p = 0.488, p = 0.745, p = 0.365, p = 0.567, p = 0.243, p = 0.683, and p =0.402, respectively). There were also no statistically significant differences between the larger and smaller discordant DTs in Hb, Htc, WBC, platelet, I/T ratio, procalcitonin, or CRP values in the first 24 h (p = 0.656, p = 0.981, p = 0.681, p = 0.838, p = 0.589, and p = 0.151, respectively). In discordant DT pairs, the platelet counts of the smaller DTs were significantly lower than those of the bigger twins (p< 0.001).

There were 117 infants with a CRP value over 1 mg/dL and/or a procalcitonin level over 0.99 ng/mL. The characteristics of these twins as compared with those of twins with CRP and procalcitonin levels below these thresholds are shown in Table VI (n=388).

The group with high CRP and/or procalcitonin levels had significantly lower birth weights, gestational ages, and 5-min Apgar scores as compared those of the group with low levels (p < 0.001, p < 0.001, and p = 0.012, respectively). There were no differences in the other analyzed parameters.

DISCUSSION

The increasing use of ARTs in recent years has resulted in a higher incidence of multiple pregnancies. In the U.S., the twinning rate increased from 1/53 pregnancies in 1980 to 1/29 in 2014 (13). The risk of neonatal mortality and morbidity is higher in multiple pregnancies as compared with that in singleton pregnancies (2).This is primarily due to prematurity, but maternal morbidities associated with multiple pregnancies and delivery-related problems also contribute. Twin pregnancies carry a greater risk of perinatal, neonatal, and postneonatal mortality as compared with singleton pregnancies, and these risks are higher in MTs than in DTs (3). Various studies on the neonatal outcomes of twin pregnancies have been conducted (14,15). A meta-analysis of 32 twin studies by Cheong-See et al. (16) examined 29,685 dichorionic and 5486 monochorionic pregnancies. Of these pregnancies, 17,830 (60.1%) in the dichorionic group and 2,149 (39.2%) in the monochorionic group were born after 34 wk of gestation. In the present study, 118 (30.4%) of the neonates were born after 34 wk of gestation: 24 (24%) in the MT group and 94 (32.6%) in the DT group.The mean gestational age of the twins in our study was 32 (± 3) wk (23–40 wk). As in the review by Cheong-See et al. (16), most of the twins were born before reaching full term. In our study, the gestational age of the group with MT was statistically lower than those with DT (p=0.05)

A previous long-term study (approximately 6 y) based on data from a university hospital also reported that the gestational aged and birth weights of MTs were lower than those of DTs (17). In the same study, the mortality rates of MTs were higher than those of DTs. However, a study conducted in Denmark found no difference in neonatal mortality of MTs versus DTs (18). In our study, the mortality rates of the MT and DT groups were similar; 13% and 8.7% respectively (p=0.211) In both groups, the mortality rates of the bigger and smaller twins were also similar.

In the study of Verbeek et al. (19), incompatibility of hematological parameters (Hb and platelet) was detected in twins with twin to twin transfusion syndrome, in our study, a statistical difference was found only between hemoglobin values. (p = 0.004). There were patients with thrombocytopenia among our patients, but there was no statistical difference in terms of platelet values between the groups (p = 0.161). We thought that the reason for this was that patients had a good obstetric follow-up in the antenatal period.

Twin discordance studies in the literature are mostly related to twins that are monochorionic. (20,21). In our study, since both the mt and dt ones were found in the discordant twins, the anemia-polycythemia sequence seen in the discordant twins reported in the literature (20,21) was not determined in our study.

No previous studies investigated whether commonly used sepsis/inflammation markers varied in twin neonates based on placental and demographic and neonatal characteristics. According to our findings, there were no statistical differences in the CRP level, procalcitonin level, or I/T ratio based on chorionicity (monochorionic/dichorionic), TTTS, or birth weight discordance (> 20%). The WBC values were lower among the discordant MTs than the discordant DTs (p = 0.048). However, the other comparisons revealed no differences in WBC counts. Based on our data, we concluded that having a mono- or dichorionic placenta, growth discordance, or TTTS did not initiate an inflammatory process necessary for the release of acute phase reactants.

Bellieni et al. (22) analyzed CRP levels of singleton newborns based on the delivery method and found that those delivered by normal spontaneous vaginal delivery had higher CRP levels as compared with those delivered by a cesarean section. In our study, only 12 of the 388 (3.1%) patients were born by normal spontaneous vaginal delivery, thus precluding such a statistical comparison (since our hospital was the tertiary reference hospital, cesarean delivery rate was very high). Moreover, nearly all the twins in the study group were born by a cesarean section, we can assume that normal birth had no effect on the results of the present study.

Stocker et al. (23) determined a procalcitonin cut-off value of 0.5 ng/mL for singleton babies on the first postnatal day. Fendler et al. (11) determined the cut-off value of procalcitonin to be 0.99 ng/mL in the diagnosis of sepsis in newborns for singleton babies. Chiesa et al. (10) reported that prematurity affected CRP levels in singleton infants. Lee et al. (24) found that respiratory problems, low gestational age, and low Apgar scores were

associated with high procalcitonin levels in singleton infants. In the present study, the group of twins with high CRP and/or procalcitonin levels had significantly lower birth weights, gestational ages, and 5-min Apgar scores as compared with those of the group with low CRP and/or low procalcitonin levels ($p< 0.001$; $p< 0.001$; and $p = 0.012$, respectively). There were no difference in terms of other neonatal features and demographic data.In a study of healthy term singleton infants, Perrone et al. (25) determined that a cut-off value of 10 mg/L (1 mg/dL) can be used for serum CRP values analyzed in the presence of various factors, such as meconium staining, a history of PROM, or maternal prophylactic antibiotic use, that cause elevated acute phase reactants. We excluded these factors when selecting the patient sample for the current study. Despite the absence of early neonatal sepsis, high procalcitonin and CRP levels (up to 57.8 ng/mL and 5.9 mg/dL, respectively) and a high I/T ratio (up to 0.8) were observed. Such high levels of infection markers among twins without clinical or proven sepsis suggests that reasons other than infection may be responsible for elevations in infection markers in twins.

In the study of Perrone et al., it was determined that CRP values were higher in the first 48 hours in single healthy babies, it was emphasized that this has something to do with postnatal age and mode of delivery (25)In our study, mean CRP values of infants without sepsis findings were found 0.1 (0-5.9) mg / dL in monochorionic twins and 0.1 (0-4.7) in dichorionic twins in the first 24 hours. In the study of Perrone et al., the average CRP value in the first 24 hours of life was found to be 0.15 (0.3-6.9) mg / L(25). In the study of Bellieni et al., mean CRP values of 0.112 (0.02-1.54) mg / L were determined at the 24th hour of 1012 single healthy babies. (22). In our study, mean CRP values were higher However, since our study was retrospective desing and the study group did not include single healthy babies whose CRP values were examined within the first 24 hours of life, no comparison was made between CRP values of twin and single babies.

There are studies showing that Procalcitonin values may increase without infection in single healthy babies similar to CRP. (26,27). In our study, we showed that procalcitonin and CRP values may increase in twin babies on the first day of life without infection-sepsis The high procalcitonin and CRP values we found in twin babies did not show statistically significant difference between twin siblings. This is the first study to investigate markers of sepsis/inflammation in twin neonates according to placental, and twin-related complications. Based on our findings, chorionicity (monochorionic/dichorionic placenta), TTTS, and size discordance were not related to any significant differences in the CRP level, procalcitonin level, or I/T ratio. Thus, we conclude that these factors alone do not induce an inflammatory response in twin neonates. We detected no factors that may be associated with elevated acute phase reactants. No statistically significant results were obtained.

A limitation of this study is the small number of twin pairs with size discordance and TTTS. In addition, we could have evaluated twins with and without signs of infection and determined cut-off values for acute phase reactants in twin neonates.

One of the weak point of our study is that we did not compare healthy twin and single infant groups that are demographically similar in terms of infection parameters. Studies with larger patient numbers are needed.There was no statistical difference in terms of infection markers among the twin siblings without clinical or culture-proven infection-sepsis, even in the presence of a discordance between twins with monochorionic, dichorionic, or twin-to-twin transfusion syndrome. This result suggested that routine infection markers can also be realiable for twin babies.

CONCLUSION

Based on our findings, chorionicity (monochorionic/dichorionic placenta), twin-to-twin transfusion syndrome, and size discordance were not related to any significant differences in the CRP level, procalcitonin level, or I/T ratio.

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