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LEUKOCYTE BLOOD PICTURE IN EARLY NEONATAL PERIOD IN ANTALYA REGION OF TURKEY*

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In two hundred neonates, white cell counts and peripheral blood smears were evaluated at birth, and after the 24th, 48th and 72nd hours of delivery to compare the differential counts of leukocytes with the values of other investigators. Parity between neutrophils and lymphocytes was observed on the third day, which was earlier than the time reported previously by other investigators. The total white cell counts were similar to those generally observed, but absolute total neutrophil counts, absolute total immature neutrophil counts and immature-to-total neutrophil ratio were found to be higher than those values previously reported in the literature.

Environmental factors during delivery and in early postnatal life may play a role in the dynamics of leukocytes. These differentials should be taken into account when the diagnosis of early neonatal infections is considered. *Key words: leukocyte counts, neonate, reference values.*

Peripheral white blood cell (WBC) counts and differentials display wide variations in the first few days of postnatal life. The patterns of sequential total neutrophil counts, immature neutrophil counts, immature-to-total neutrophil ratio and calculated absolute cell counts are becoming more valuable indices in the early diagnosis and outcome of bacterial infections in neonates[1-8].

There are several factors affecting the blood picture at birth and thereafter. The local, hygienic, environmental and even genetic factors may affect the neonate's response.

This study was planned to determine whether neonates in the Antalya region have different reference values for WBC counts and differentials than the previously reported results.

Material and Methods

The study population consisted of 91 female and 109 male, full-term, healthy neonates consecutively delivered at Antalya Maternity Hospital in Antalya, Turkey.

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The healthy neonate was defined as one without maternal complications (fever during the last 24 hours of delivery, use of intrapartum antibiotics, presence of hypertension or diabetes, prolonged labor, severe pain), intrapartum complications (early membrane rupture, use of oxytocin, midforceps rotation, fetal bradycardia, meconium stained amniotic fluid), neonatal complications (hyaline membrane disease, transient tachypnea, sepsis, TORCH infections, hemolytic disease, nonphysiologic hyperbilirubinemia, use of phototherapy, suspected or confirmed periventricular or subarachnoid hemorrhage, hypoglycemia, seizures, gastrointestinal complications, meconium aspiration syndrome, pneumothorax, pneumonia, meningitis) and also with normal physical examination at the time the blood samples were collected.

Out of 200 neonates, 23 were delivered by cesarean section. The birth weight, postnatal age (hours) and gestational age (determined by Dubowitz Score) were recorded on each subject.

Samples for hematologic analysis were obtained from a free-flowing heel prick at birth and in the postnatal 24^{th}, 48^{th} and 72^{nd} hours of life. On sectio-delivered babies, samples from the 120^{th} hour were added to the study. WBC count was measured by the classical method[9]. On Wright-stained blood smears, two sets of 100 cells were counted by the same physician (BG). A neutrophil was defined as having lobes connected by a thread or filaments. A band was defined as a neutrophil in which the isthmus between the lobes was wide enough to reveal two distinct margins with nuclear material in between.

All bands and cell forms less mature than bands were classified together as immature neutrophils. The absolute number of total neutrophils (ATN) included both mature and immature forms and was calculated by the following formula;

$$ATN = \frac{\text{Total WBC Count/Percent of Total Neutrophils}}{100}$$

The absolute number of total immature neutrophils (ATIN), total lymphocytes (ATL), total monocytes (ATM) and total eosinophils (ATE) were calculated using the same formula. A proportion expressing the fraction of immature to total neutrophilic forms (I/T) was calculated by the following formula:

$$I/T = \frac{ATIN}{ATN}$$

Statistical Analysis :

All values were evaluated according to the postnatal age and sex and the mode of delivery. The median, mean 1 SD, 5^{th} percentile and 95^{th} percentile values were

estimated. Nonparametric Mann-Whitney U test was used to compare the data on Amstrad 6128.

Probabilities of <0.05 were considered significant.

Results

The mean gestational age was found to be 39 ± 1.1 weeks and the birth weight was 3300 ± 509 g.

The WBC counts were shown in Fig. 1. The peak value was detected at birth, and the median, 5th and 95th percentile values were found to be decreased during the first five days. Daily decrement was statistically significant in the first three days ($p<0.05$).

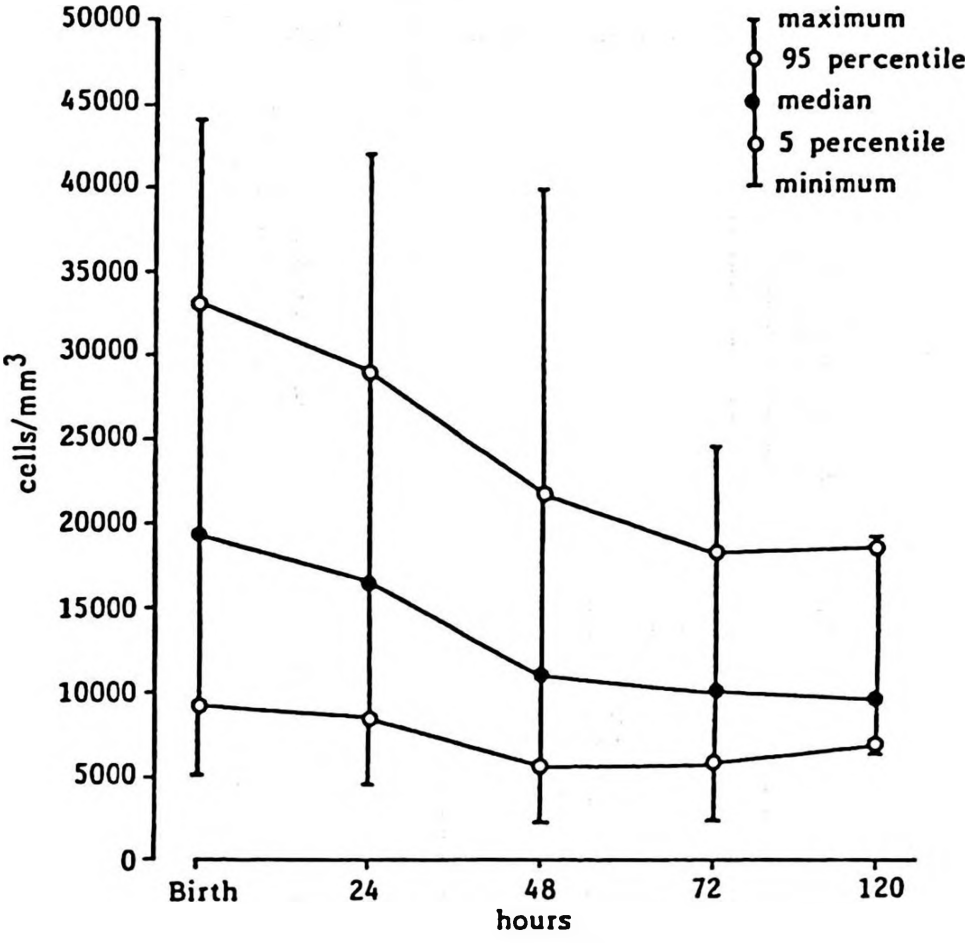

Fig. 1: Total white blood cell counts.

The ATN counts also gradually decreased and reached the lowest values in the 5th day of life (Fig. 2).

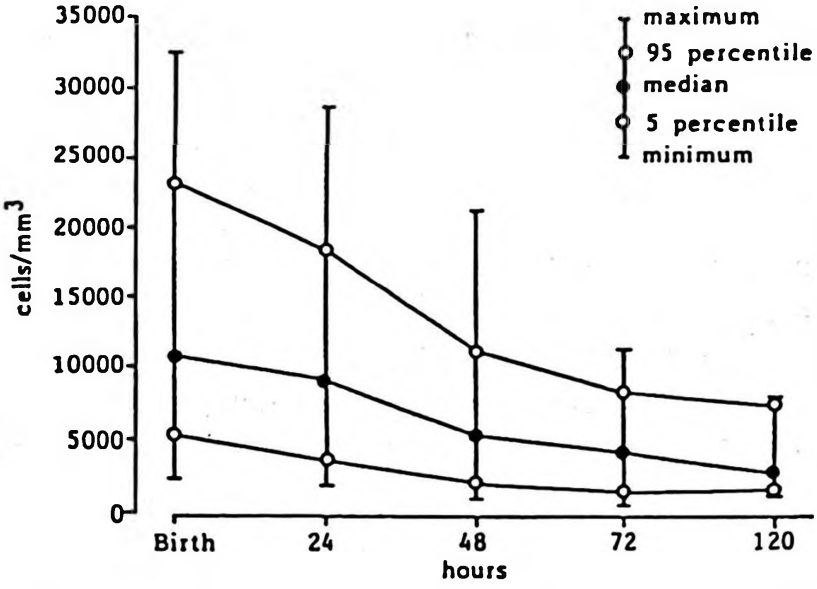

Fig. 2: Absolute total neutrophil counts.

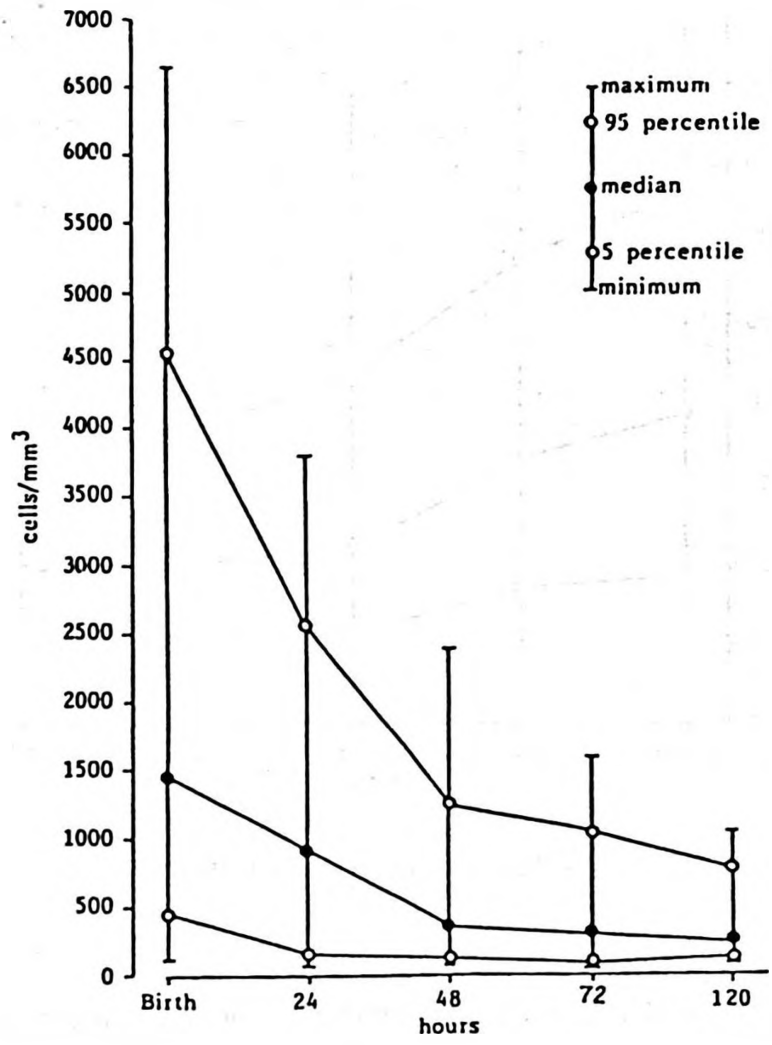

Fig. 3: Absolute total immature neutrophil counts.

For ATIN counts (Fig. 3), the most impressive decrease was observed the first day. This parameter showed stable values after 48 hours of life.

The I/T ratios showed a rapid decrease in the first 48 hours and a slide increase thereafter (Fig. 4). The progression in the ATL profile was very similar to I/T ratios (Fig. 5).

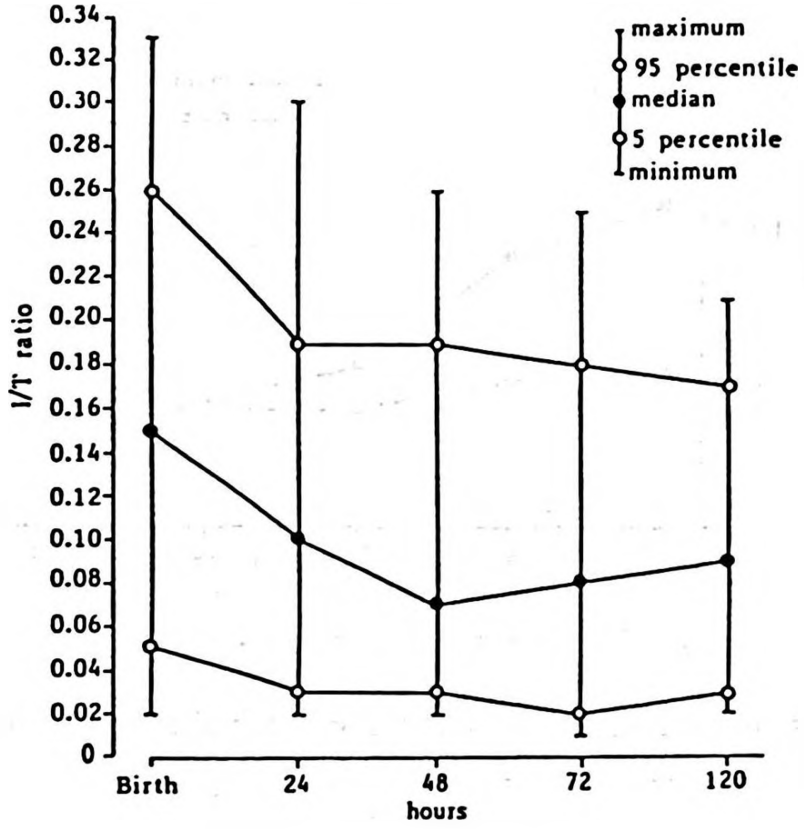

Fig. 4: Pattern of I/T ratios.

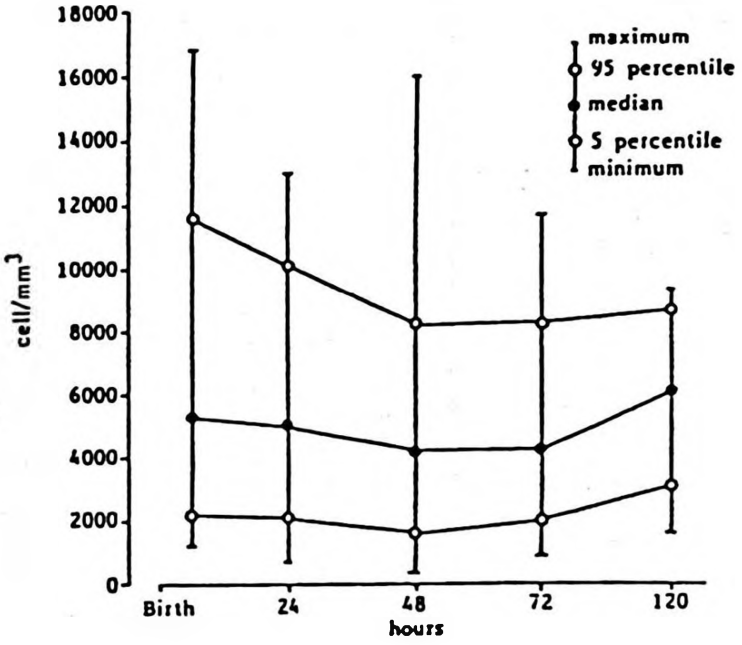

Fig. 5: Absolute total lymphocyte counts.

The parity between neutrophils and lymphocytes was observed on the third day (Fig. 6). The percentage of lymphocytes was higher than the neutrophils in 65% of subjects in the 72nd hour and in 87% of subjects in the 120th hour of life.

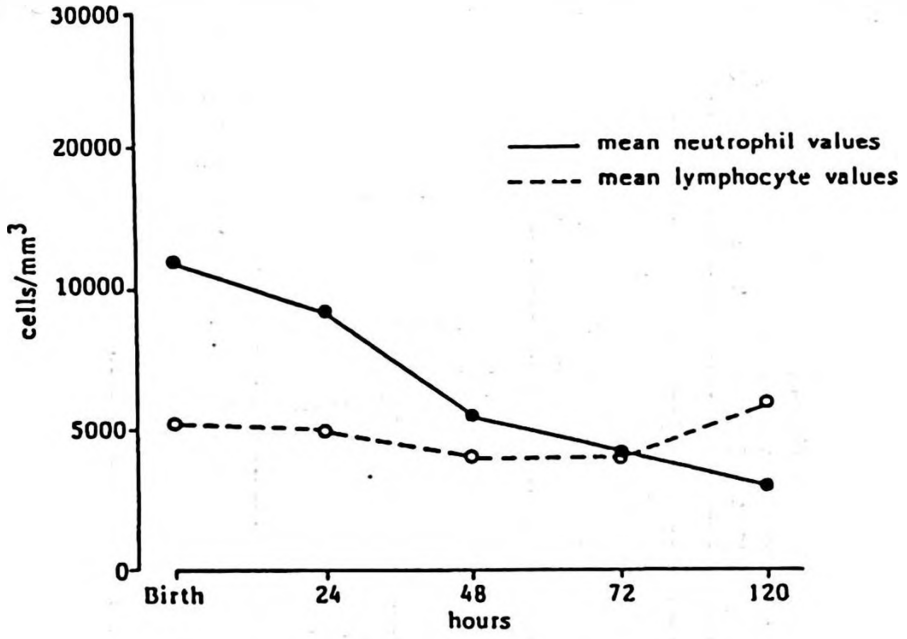

Fig. 6: The parity between neutrophils and lymphocytes.

Absolute total monocyte (ATM) counts were higher in the first 24 hours and remained stable thereafter (Fig. 7).

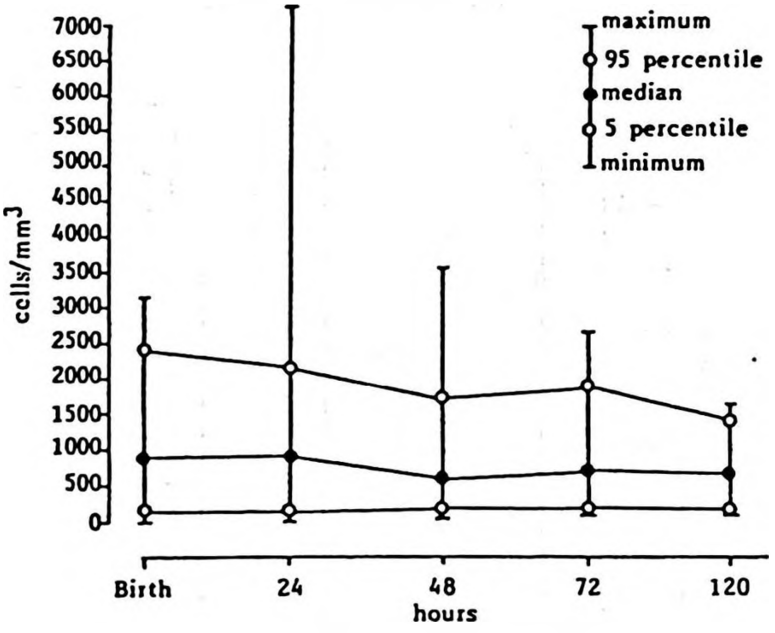

Fig. 7: Absolute total monocyte counts.

Absolute total eosinophil (ATE) counts showed a wide range among subjects, but the median values were constant during the study period (Fig. 8).

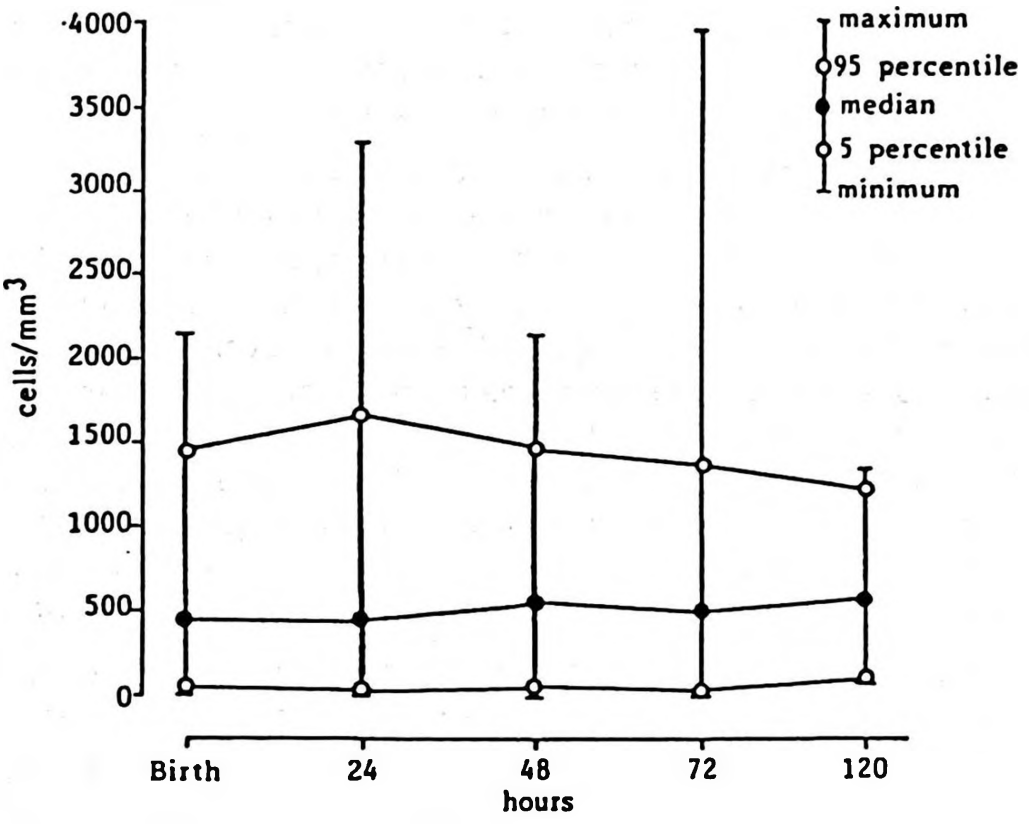

Fig. 8: Absolute total eosinophil counts.

In all these parameters, there was no significant difference between girls and boys, and between the babies who were delivered by sectio and were not. Sectio babies presented significantly higher monocyte counts in the 72nd hour and lower eosinophil counts in the 48th hour ($p<0.05$).

Discussion

It is generally accepted that the neutrophil profile is a useful screening technique for the early diagnosis of neonatal sepsis[4,6,10-14]. Although the reference ranges for the neutrophil values in normal neonates have been extensively documented in the literature, whether the different ethnic groups or regions present different patterns for sequential analysis of leukocytes in the early neonatal period is not known. In our study, total WBC counts showed a peak value in the first 24 hours and rapidly decreased after the first day. Fifth percentile values of 5423/mm^3 and 90th percentile values of 33080/mm^3 were similar to the values published in the literature[1,2,5].

The absolute total neutrophils also showed a gradual decrease in median values after birth. The peak value of 32560/mm^3 occurred at birth and the lowest value of 720/mm^3 in the third day of life. The reference values of ATN counts were found to be 1243/mm^3 for the 5th percentile and 2341/mm^3 for the 95th percentile in our study. Our results were different from Manroe's[5] and Xanthou's[2] studies and were similar to the results of Kuchler[8].

Maternal neutrophils gradually increase due to high estrogen levels and increased muscle function during the gestational period and reach a high level in labor, so the WBC and ATN counts obtained soon after birth generally project the maternal peripheral blood cell picture[15-17]. The neutrophil counts were found to be higher in babies whose mothers had prolonged labor and/or severe pain. This temporary increase in neutrophils may be due to the releasing of cells from the vascular bed to the circulation[18]. The etiopathology of those changes is explained by the sum of the effects of hormonal factors such as adrenalin and hydrocortisone colony stimulating factors, and marginal pool-bone marrow pool interactions of the mother. Neonates with a history of prolonged labor and/or severe maternal pain were excluded from our study in order not to change the accuracy of the values.

Soon after birth, a variety of perinatal and postnatal problems, mostly infections, may be associated with an abnormal neutrophil profile[3-5,19,20].

The increases in immature neutrophil counts and in the immature-to-total neutrophil ratio have generally been accepted as sensitive markers for neonatal sepsis[3-6,10,12]. Knowledge of the upper-normal limits of immature total neutrophil values is important. As far as the 95th percentile of ATIN values is concerned, our study showed the counts of 4531/mm^3 at birth, 2560/mm^3 in the 24th, 1249/mm^3 in the 48th, 1031/mm^3 in the 72nd and 765/mm^3 in the 120th hour of life (Fig. 2). Our results were higher than the results of Manroe[5] and Akenzua[4] but very similar to the results of Kuchler[8]. In our study, the I/T ratios were higher than others' reference values[2,5]. Our 95th percentile values were 0.26, 0.19, 0.19, 0.185 and 0.17 respectively (Fig. 4). The patterns of ATIN and the I/T ratio can be considered as the reference values for us.

As far as the ATL values are concerned, the significant decrease was reported on the 3rd day by Xanthou et al[2], on the 5th day by Weinberg et al[21] and more than 5 days by others[1]. In our study, the median lymphocyte values were 5298/mm^3 in the 24th, 4193/mm^3 in the 48th, 4216/mm^3 in the 72nd and 6032/mm^3 in the 120th hour of life (Fig. 5). A significant decrease in ATL values was observed in the 48th hour of lifeşearlier than was cited in previous reports.

The crossing between neutrophils and lymphocytes was generally reported on the 5th-7th days of life[2,5,22]. In our infants, this occurred on the 3rd day. This was the most important observation of our study. It may show the earlier accomodation and stabilization of leukocyte dynamics in our neonates.

In our infants, the median ATM values were relatively stable and were similar to the observations of Xanthou et al[2].

Absolute total eosinophil counts were 450/mm^3 at birth and slightly increased thereafter (Fig. 7). Our results were lower than the results of Xanthou[2], but higher than Weinberg[21] and Barbero[23].

Considering the range of leukocyte dynamics boys and girls had values similar to those reported by other investigators[2,11,21,24].

In conclusion, we believe that it will be more suitable to establish the reference values for our country by doing similar investigations in different regions of Turkey and to use these reference values in the diagnosis of sepsis and other infectious diseases.

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