Blood leucocyte count in the human fetus

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Abstract

Total and differential leucocyte counts were measured in cord blood samples obtained by cordocentesis (n=316) or at elective caesarean section (n=11) from normal fetuses of between 18 and 40 weeks' gestation. The total fetal leucocyte count increased exponentially from $2{\cdot}8\times10^9$/l at 18 weeks to $11{\cdot}8\times10^9$/l at term. The lymphocyte and monocyte counts increased linearly and the number of neutrophils increased exponentially from a mean value of $0{\cdot}2\times10^9$/l at 18 weeks to $0{\cdot}8\times10^9$/l at 31 weeks and then $8{\cdot}5\times10^9$/l at term. Early myeloid cells, eosinophils, and basophils were observed in 24%, 55%, and 15% of the blood films respectively; they contributed less than 2% to the total leucocyte count and there were no significant changes with gestation. The physiological leucopenia observed in fetuses early in the third trimester may partly explain the predisposition of premature neonates to infection.

Knowledge of fetal blood leucocyte counts has been based largely on analysis of cord blood obtained at term or premature delivery (table 1)[1–9] and after spontaneous abortion.[10] However, the assumption that these data represent normal physiological values may not be valid as the stress of delivery or the underlying reason for spontaneous abortion or premature delivery, such as infection or hypoxia, could affect fetal haemopoiesis.

Potentially more reliable, but incomplete, data to allow delineation of changes with gestational age have been provided from the study of fetal samples obtained at hysterotomy, fetoscopy, and cordocentesis.[10–13] The present study was undertaken to establish comprehensive reference ranges for the total and differential leucocyte counts from 18 weeks' gestation to term.

Patients and methods

The fetal total and differential leucocyte counts were measured in umbilical cord blood samples obtained at cordocentesis (n=316) or at elective caesarean section (n=11) from fetuses of between 18 and 40 weeks' gestation. The study was cross sectional and in each case gestation was determined from the menstrual history or by an ultrasound scan in early pregnancy.

The indications for cordocentesis included: (i) prenatal diagnosis of inherited coagulation disorders, for example, haemophilia A (n=29), (ii) maternal rubella or toxoplasmosis infection (n=21), (iii) fetal karyotyping (n=243), and (iv) fetal blood grouping in red cell isoimmunised pregnancies (n=23). Cordocentesis was performed without maternal sedation or fetal paralysis. At the time of cordocentesis, the fetal blood gases and abdominal circumstances were normal for gestational age.[14] All fetuses included in the study were subsequently shown to have a normal karyotype, to be unaffected by the coagulation disorders or infection under investigation or, in cases of suspected red cell isoimmunisation, to be Coombs' negative.

Cord blood samples at delivery were obtained from patients undergoing elective caesarean section at 37–40 weeks' gestation for previous caesarean section (n=6) or breech presentation (n=5); all infants were normal and of appropriate size for gestation.

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Accepted 13 December 1991

Table 1 Summary of data from previous reports on total leucocyte, lymphocyte, and neutrophil counts in samples obtained from babies delivered at different gestations when samples were taken from the umbilical cord, heel, or other peripheral vessels. Also shown are data from hysterotomy, termination of pregnancy (TOP), fetoscopy, and cordocentesis studies. The values given are the means; when a range is given this represents the mean values at the lowest and highest gestation

Author	*No of infants studied*	*Gestational age (weeks)*	*Vessel sampled or sampling method*	*Age at at sampling*	*Counts ($\times10^9$/l)*		
					Total leucocytes	*Lymphocytes*	*Neutrophils*
Manroe *et al*[1]	434	29–40	Heel+other peripheral vessels	>120 Hours	—	—	3·6
Weinberg *et al*[2]	393	29–40	Heel+other peripheral vessels	<60 Hours	—	4·2	—
Coulombel *et al*[3]	132	27–43	Heel+cord	<12 Hours	—	—	6–12
Xanthou[4]	68	>37	Heel	<96 Hours	9·7	3·9	4·1
Washburn[5]	6	>37	Heel	<10 Days	15·2	4·2	9·9
Diaz-Jouanen *et al*[6]	12	>37	Cord	Not given	—	2·1	—
Lippman[7]	42	>37	Heel	<2 Hours	10·1	4·1	5·2
Lucas *et al*[8]	159	Not given	Peripheral vessels	<24 Hours	12·2	—	—
Rosse *et al*[9]	57	>37	Peripheral vessels	<2 Hours	11·5	3·5	6·5
Playfair *et al*[10]	137	8–27	Hysterotomy+TOP		—	≈1–10	≈0·05–10
Thomas and Yoffey[11]	36	10–25	Hysterotomy		—	≈0·4–3	≈0·05–1
Millar *et al*[12]	99	15–21	Fetoscopy		1·6–2·7	1·4–2·3	0·1–0·2
Forestier *et al*[13]	163	18–30	Cordocentesis		4·2–4·4	3·3–3·6	0·2–0·4

Fetal blood samples (180 µl) were collected into 20 µl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l sodium chloride). Maternal contamination was excluded by the acid elution (Kleihauer) method. The total nucleated cell count was determined with a Coulter Stacker Automated Cytometer (Coulter Electronics). Blood films were stained by the May-Grünwald-Giemsa method and after adjustments for the presence of the nucleated red cells (erythroblasts), the differential leucocytes were determined by morphological classification of 100 cells.

STATISTICAL ANALYSIS

Regression analysis was used to derive reference ranges (mean, 5th, and 95th centiles) for the total and differential leucocyte counts with gestation. Normality of the distribution of residuals was examined using the Shapiro-Francia W' test.[15] Where the distribution of the residuals was skewed, logarithmic transformation was used to achieve a normal distribution. To produce the reference ranges with gestation in the original units, the limits of the calculated reference range in logarithmic units were subjected to an antilogarithmic transformation.

Results

The total leucocyte count in the fetus increased exponentially with gestation from a mean of $2{\cdot}27\times10^9$/l at 18 weeks to $9{\cdot}41\times10^9$/l at 40 weeks (fig 1). This increase reflected two components, a linear increase in the lymphocyte count from a mean of $1{\cdot}17\times10^9$/l at 18 weeks' gestation to $3{\cdot}67\times10^9$/l at 40 weeks (figs 2, 3), together with an exponential rise in neutrophil numbers from a mean value of $0{\cdot}77\times10^9$/l at 31 weeks to $8{\cdot}53\times10^9$/l at 40 weeks (figs 2, 3).

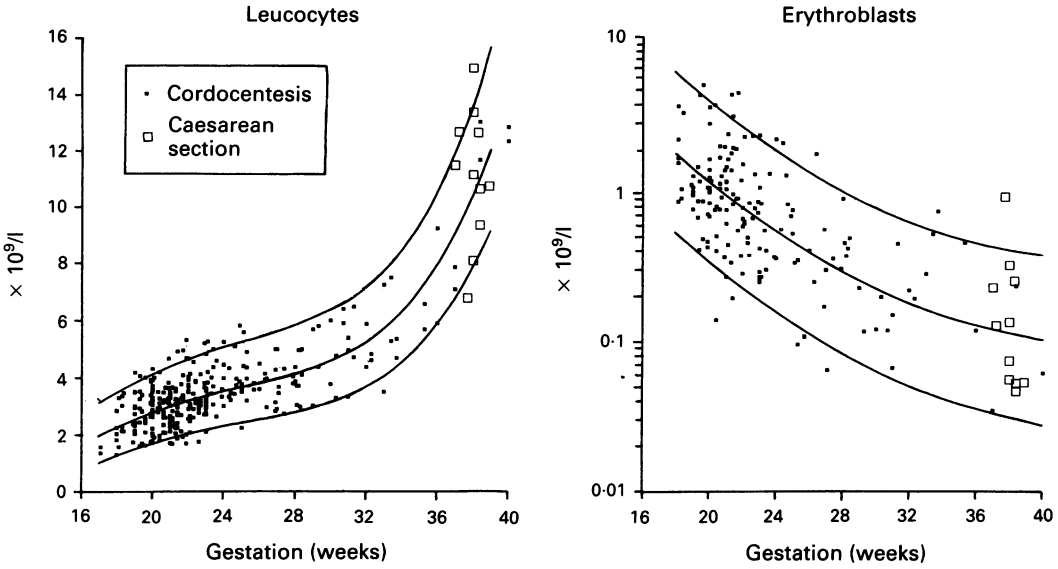

Figure 1 Reference range (mean, 5th, and 95th centile) for total fetal leucocyte (×10^9/l) and erythroblast (×10^9/l) counts with gestation derived from the study of umbilical cord blood samples obtained by cordocentesis or at elective caesarean section.

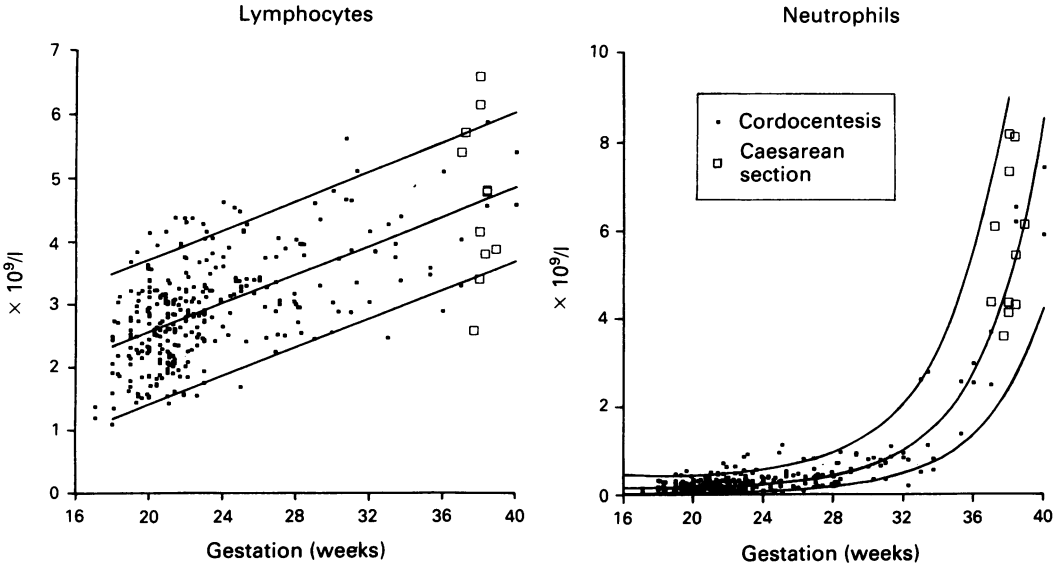

Figure 2 Reference range (mean, 5th, and 95th centile) for the number (×10^9/l) of fetal lymphocytes and neutrophils with gestation derived from the study of umbilical cord blood samples obtained by cordocentesis or at elective caesarean section.

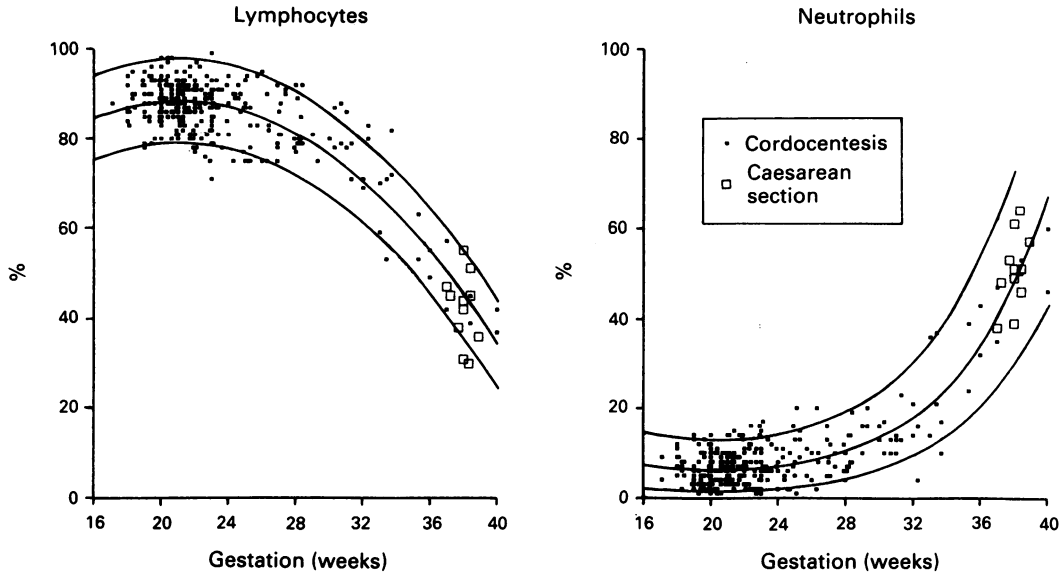

Figure 3 Reference range (mean, 5th, and 95th centile) for the percentage of fetal lymphocytes and neutrophils with gestation derived from the study of umbilical cord blood samples obtained by cordocentesis or at elective caesarean section.

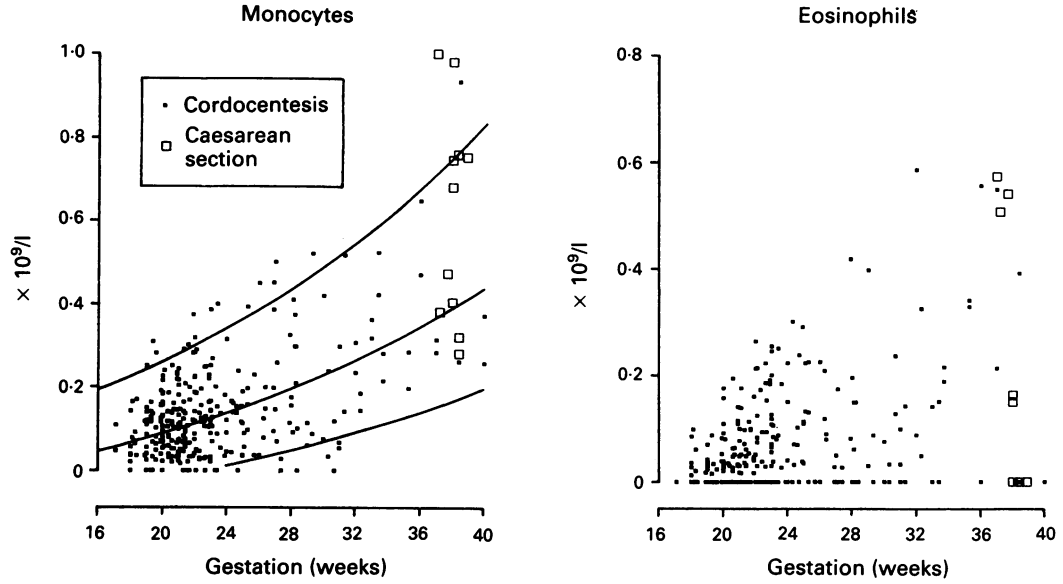

Figure 4 The number (×10⁹/l) of fetal monocytes and eosinophils with gsetation in umbilical cord blood samples obtained by cordocentesis or at elective caesarean section.

Table 2 Reference ranges (mean, 5th, and 95th centile) for fetal total leucocyte, lymphocyte, neutrophil, and monocyte counts (×10⁹/l) with gestational age

Gestational age (weeks)	*Total leucocytes*			*Lymphocytes*			*Neutrophils*			*Monocytes*		
	Mean	*5th*	*95th*	*Mean*	*5th*	*95th*	*Mean*	*5th*	*95th*	*Mean*	*5th*	*95th*
18	2·75	1·45	4·21	2·33	1·17	3·49	0·16	0·03	0·41	0·07	0·00	0·23
19	2·79	1·49	4·25	2·44	1·28	3·60	0·16	0·03	0·41	0·08	0·00	0·24
20	2·86	1·55	4·32	2·56	1·40	3·72	0·16	0·03	0·42	0·09	0·00	0·26
21	2·95	1·63	4·43	2·67	1·51	3·83	0·17	0·04	0·44	0·10	0·00	0·28
22	3·06	1·74	4·56	2·79	1·63	3·95	0·19	0·04	0·47	0·11	0·00	0·30
23	3·21	1·86	4·72	2·90	1·74	4·06	0·21	0·05	0·50	0·13	0·00	0·32
24	3·38	2·02	4·92	3·02	1·86	4·17	0·23	0·07	0·55	0·14	0·01	0·34
25	3·59	2·20	5·15	3·13	1·97	4·29	0·26	0·08	0·62	0·15	0·02	0·36
26	3·82	2·41	5·41	3·24	2·09	4·40	0·31	0·10	0·70	0·17	0·03	0·39
27	4·09	2·64	5·71	3·36	2·20	4·52	0·36	0·13	0·80	0·18	0·04	0·41
28	4·39	2·91	6·05	3·47	2·31	4·63	0·43	0·17	0·94	0·20	0·05	0·43
29	4·72	3·21	6·43	3·59	2·43	4·74	0·52	0·21	1·11	0·21	0·06	0·46
30	5·10	3·54	6·85	3·70	2·54	4·86	0·63	0·27	1·33	0·23	0·07	0·49
31	5·52	3·91	7·32	3·82	2·66	4·98	0·77	0·34	1·61	0·25	0·08	0·52
32	6·00	4·32	7·84	3·93	2·77	5·09	0·97	0·42	1·98	0·27	0·09	0·55
33	6·49	4·77	8·41	4·05	2·88	5·21	1·22	0·57	2·47	0·28	0·10	0·58
34	7·05	5·27	9·05	4·16	3·00	5·33	1·55	0·74	3·12	0·30	0·11	0·61
35	7·67	5·82	9·74	4·27	3·11	5·44	1·99	0·97	4·00	0·32	0·13	0·64
36	8·34	6·41	10·51	4·39	3·22	5·56	2·60	1·28	5·19	0·35	0·14	0·68
37	9·09	7·07	11·36	4·50	3·33	5·67	3·43	1·70	6·83	0·37	0·15	0·71
38	9·90	7·78	12·29	4·61	3·45	5·79	4·59	2·28	9·14	0·39	0·17	0·75
39	10·80	8·56	13·32	4·73	3·56	5·91	6·23	3·09	12·40	0·41	0·18	0·79
40	11·78	9·41	14·58	4·84	3·67	6·02	8·53	4·23	17·09	0·44	0·20	0·83

Table 3 Equations for reference ranges describing normal physiological changes in total leucocyte, erythroblast, lymphocyte, neutrophil, and monocyte counts as well as the percentage contribution of lymphocytes and neutrophils derived from measurements in 327 normal fetuses. For all parameters the normal mean for gestation = intercept + a × gestation + b × gestation^2 + c × gestation^3

Parameter	*Intercept*	*a*	*b*	*c*	*SD*	*Correlation coefficient*
$\log_{10}$ (total leucocyte count+3)	−0·97	0·19	-7×10^{-3}	9×10^{-5}	$0\cdot93\times10^9$/l	0·86**
$\log_{10}$ (erythroblast count)	2·67	−0·17	2×10^{-3}		$1\cdot02\times10^9$/l	0·70*
Lymphocyte count	0·27	0·11			$0\cdot70\times10^9$/l	0·64**
$\log_{10}$ (9×neutrophil count+1)	1·44	−0·12	3×10^{-3}		$0\cdot45\times10^9$/l	0·87*
$\log_{10}$ (5×monocyte count+1)	−0·19	0·02			$0\cdot12\times10^9$/l	0·58**
Lymphocytes (%)	21·75	6·35	0·15		5·66	0·89**
$\log_{10}$ (% neutrophils+9)	1·92	−0·07	2×10^{-3}		4·52	0·82**

*p<0·001; **p<0·0001.

These changes were paralleled by a reciprocal decrease in the erythroblast count (fig 1).

Monocytes were observed in the majority of samples, irrespective of gestational age. Their numbers increased linearly with gestation, from a mean of $0\cdot07\times10^9$/l at 18 weeks to $0\cdot44\times10^9$/l at 40 weeks (fig 4). Eosinophils were present in 55% of samples and there was a non-significant increase in the total number with gestation (fig 4).

Early myeloid cells (metamyelocytes, myelocytes, promyelocytes, and blasts) and basophils were observed in 24% and 15% of the blood films respectively. They contributed less than 2% to the total white count and there was no significant change with gestational age. Reference ranges for the total and differential leucocyte counts at different gestational ages between 18 and 40 weeks are shown in table 2. Equations describing the observed changes with gestation in the total leycocyte, lymphocyte, neutrophil, and monocyte counts are summarised in table 3.

Discussion

In normal human pregnancy the fetal leucocyte count increases with gestational age. Until 37–38 weeks' gestation lymphocytes predominate. However, from 32 weeks onwards the proportion of neutrophils increases to become the commonest leucocyte at term.

In the second trimester, the total and differential leucocyte values observed in this study concur with those found in fetal blood obtained at hysterotomy and fetoscopy,[10–12] but they are lower than those observed after spontaneous abortion.[10] This may reflect the effects on leucopoiesis of those factors underlying spontaneous abortion or the process of abortion itself. The observed progressive rise in the fetal leucocyte count contradicts the reported absence of a significant change from 18 to 30 weeks' gestation in a previous study, which also examined samples obtained by cordocentesis.[13] The most likely explanation for this discrepancy is that in the study of Forestier *et al* no adjustments were made for the presence of nucleated red blood cells.[13]

In the third trimester, the leucocytes count was lower than that reported in studies which examined cord blood after preterm or term delivery.[1–9] These findings suggest that either delivery itself and/or the causes of preterm delivery affect the number of circulating leucocytes. Additionally, the majority of neonatal studies have utilised heel prick capillary samples, which yield values approximately 20% higher than those obtained from a vein or artery.[16]

The observed changes in the number of circulating leucocytes as well as changes in other fetal haematological values with gestation[17 18] presumably reflect alterations in the pattern of differentiation of pluripotent haemopoietic stem cells to meet changing fetal physiological priorities during gestation. The first primitive oxygen carrying cells in the human fetus appear around day 14 in the yolk sac, and throughout the hepatic phase of haemopoiesis the majority of haemopoietic cells are erythroid.[19 20] The increase in lymphocyte number in mid-gestation may be reconciled with the need to acquire immunological tolerance and antigen recognition functions that are demonstrable by 20 weeks.[21] This is of potential importance in relation to viral infections, which can be placentally transmitted. In contrast, the placenta acts as an effective barrier to most bacteria,[22] and therefore the acquisition of host defence mechanisms directed against bacterial infection is only necessary in preparation for extrauterine life. This may explain the dramatic rise in the number of circulating neutrophils during the late third trimester.

The factors that regulate the alterations in the pattern of differentiation of pluripotent haemopoietic stem cells are uncertain. Although hepatic haemopoiesis is primarily erythroid,[20] fetal liver haemopoietic stem cells are capable of reconstituting all haemopoietic lineages in primates.[23] Moreover, both pluripotent and committed haemopoietic progenitors are plentiful in cultures from human fetal liver.[24] One explanation for the predominance of erythroid activity in the hepatic phase of fetal haemopoiesis may be the influence of locally produced erythrogenic growth factors such as erythropoietin.[25] Similarly, the production of leucopoietic colony stimulating factors by marrow stromal cells may partly explain the expansion in fetal leucopoiesis, which follows the switch from liver to marrow haemopoiesis.[26 27]

The findings of this study suggest that one factor responsible for the vulnerability of premature neonates to bacterial infection,[28] which is a significant cause of perinatal mortality[29] and morbidity,[30] is physiological neutropenia. In patients receiving myelosuppressive drugs or radiotherapy the risk of infection is directly related to the degree of neutropenia.[31] Leucocyte values in neonates should be interpreted in the light of in utero values at the corresponding

gestational age. A clearer understanding of the mechanisms mediating leucocyte maturation in the fetus, which may include specific haemopoietic growth factors, could provide a basis for therapeutic manipulation in utero that was aimed at the prevention of bacterial infection in premature neonates.

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