Neonatal IgE levels and three different blood sampling techniques

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Comparison was made of IgE and IgA levels in aspirated and gravity-collected cord blood (CB) from the umbilical vein and in capillary blood samples collected on the 4–5th day of life among 21 infants with atopic heredity. The IgA levels, but not the IgE levels, were significantly ($p < 0.001$) lower at days 4–5 of life than at delivery. Further, there were significantly ($p < 0.05$) more infants with decreasing IgA levels (20/21; 95%) than IgE levels (9/15; 60% of those with detectable IgE, i.e., ≥ 0.125 kU/l). These observations, together with the highly significant correlation observed between IgE in aspirated CB samples and at 4–5 days of age, suggest active IgE synthesis during the prenatal and postnatal periods. Contamination of CB with maternal blood, defined as an increased CBIgA level (≥ 14.1 μg/ml), was found in 3 (14%) CB samples, all of which were gravity-collected. Of 4 CB samples (gravity-collected) with elevated IgE (i.e., ≥ 0.9 kU/l), 2 had suspected maternal contamination. Therefore, aspiration of CB or capillary collection at 4–5 days of age should be preferred for allergy prediction. If gravity collection is used, contamination should be investigated by determining IgA in all CB samples with IgE concentrations exceeding the cut-off point.

Clinical aspects

Determination of cord blood IgE (CBIgE) has been proposed as a method for predicting the development of atopic diseases during early childhood. However, this study suggests that the neonatal IgE measurements are influenced by the blood sampling technique. This should affect the interpretation of CBIgE and IgE determinations during the postnatal period of life as allergy predictors.

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The prevalence of atopic diseases during infancy and adolescence has increased during recent decades (2, 26). Interest has therefore focused on the possibility of preventing and predicting the development of atopy during childhood (15). Increased levels of IgE in cord blood (CB) (3, 5, 16, 21, 22) and in blood samples collected during early infancy (6, 14) have been suggested as good predictors of infant atopy. However, the results concerning the predictive capacity of CBIgE are contradictory (10, 18, 23). This may be ascribed partly to differences in the populations studied and to the influence on CBIgE of factors like atopic heredity (11, 21, 22), ethnicity (11, 19), sex (13, 19), maternal smoking (20) and medication (1, 22). Another factor affecting CBIgE is the contamination of CB by maternal blood, as suggested in several studies (5, 8, 11–13, 16–18, 21, 25). Therefore, the sampling of CB by aspiration from the umbilical vein (11, 22), or of capillary blood when the child is a few days old (4, 6, 18, 24), has been proposed as an alternative procedure to minimize the risk of maternal influence on the perinatal IgE levels.

The aim of the present investigation was to compare the IgA and IgE levels obtained by 3 different sampling techniques: aspiration and gravity collection of CB and capillary collection at 4–5 days of age among infants with atopic heredity.

Subjects and methods

Twenty women (mean age 30; range 24–44 years) attending the maternity ward at Nacka Hospital, Stockholm, participated in the study. In a questionnaire they all reported allergy towards animal danders (cat or dog) and/or pollens (tree or grass). Forty percent of the women had bronchial asthma, 90% had allergic rhino-conjunctivitis and 43% atopic dermatitis. One-third of the women were smokers.

In addition, a paternal atopic heredity was also reported in 6 (30%) of the families. An older sibling with atopic disease was reported in 6 (30%) cases.

Twenty-one infants, 12 boys and 9 girls, including one pair of twins, were born fullterm and none had any perinatal illness.

Immunoglobulin determinations

A blood sample was collected from all women at delivery for the determination of IgE. After careful cleaning of the umbilical cord, CB was collected first by aspirating blood from the umbilical vein with a needle and then by letting the blood flow freely into a tube (gravity collection). At 4–5 days of age, a capillary blood sample was collected from each child by heel puncture. The serum samples, one from the mother and 3 from each child, were stored at −20 °C pending analyses. All 3 samples from each child were simultaneously analysed in duplicate to minimize intraindividual variation.

The maternal IgE levels were determined by PRIST (Pharmacia Diagnostics, Uppsala, Sweden), according to the instructions of the manufacturer. A modified PRIST method, with higher sensitivity (i.e., 0.125 kU/l), was used to determine IgE levels in the CB and 4–5-day samples (17). A value below the threshold of sensitivity was given the value of 0.125 kU/l in the statistical analysis. A neonatal IgE level exceeding 0.9 kU/l was regarded as elevated (16). The presence of IgE antibodies to inhalant allergens in the maternal serum samples was determined by Phadiatop® (Pharmacia Diagnostics, Uppsala, Sweden; 9).

All serum IgA concentrations were determined by a sandwich ELISA, as previously described (17). The upper normal limit of the CBIgA level was set at 14.1 μg/ml, calculated as the geometric mean plus 3 standard deviations (SD) in 20 newborn infants with maternal atopic heredity who had CBIgE <0.125 kU/l (17). An IgA value above this limit was taken as an indication of a possible contamination of CB with maternal blood.

Ethics

The study was approved by the Human Research Ethics Committee at Söder Hospital, Stockholm, Sweden. Informed consent was given by the women before entering the study.

Statistical analysis

Fisher's exact test, Wilcoxon's paired rank sum and Spearman rank correlation tests were used to analyse the results. A p-value <0.05 (two tailed) was considered significant.

Results

Infant IgA

The individual IgA levels from the 3 sampling occasions are presented in Table 1 and Fig. 1. The geometric means (GM) for IgA were 7.7 μg/ml in gravity-collected CB ($CBIgA_{grav}$), 5.3 μg/ml in aspirated CB ($CBIgA_{asp}$) and 3.6 μg/ml in capillary-collected blood at 4–5 days of age (IgA_{4-5}).

An increased CBIgA level, defined as ≥ 14.1 μg/ml, was found in 3 (14%) CB samples (Nos. 1, 4 and 6), all 3 of which were gravity-collected (Fig. 1). The corresponding $CBIgA_{asp}$ values were all below 14.1 μg/ml (Table 1). For unknown reasons the degree of contamination was exceptionally high in sample No. 1, with a $CBIgA_{grav}$ of 257 μg/ml. When the 3 CB samples with suspected maternal contamination were omitted, the GM of $CBIgA_{grav}$ was 5.7 μg/ml, which is about the same ($p = 0.10$; Wilcoxon) as the GM of $CBIgA_{asp}$ (Table 1). Moreover, $CBIgA_{grav}$ and $CBIgA_{asp}$ values were also highly correlated ($n = 18$, $p < 0.001$; $r_s = 0.84$). The levels of IgA_{4-5} were significantly ($p < 0.001$; Wilcoxon) lower than $CBIgA_{asp}$. However, despite these significant changes, IgA_{4-5} and $CBIgA_{asp}$ levels were highly correlated ($n = 21$; $p < 0.001$; $r_s = 0.85$). These results indicate that CB samples taken by aspiration are free from major maternal contamination and therefore

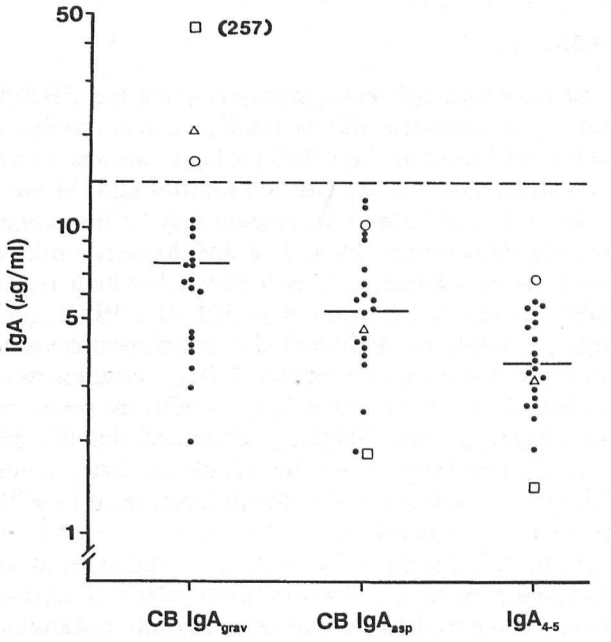

Fig. 1. Serum IgA levels (μg/ml) in gravity-collected cord blood ($CBIgA_{grav}$), in aspirated cord blood ($CBIgA_{asp}$) and in capillary-collected blood samples at 4–5 days of age (IgA_{4-5}) among 21 infants. Horizontal bars indicate the geometric means. The horizontal interrupted line corresponds to 14.1 μg/ml (upper normal limit). Symbols represent infants No. 1 (□), No. 4 (△) and No. 6 (○).

Table 1. Maternal IgE levels (Mat. IgE) in 21 cases (Nos. 1–21) and the corresponding infant IgA (μg/ml) and IgE (kU/l) levels in gravity-collected cord blood ($CBIgA_{grav}$; $CBIgE_{grav}$), in aspirated cord blood ($CBIgA_{asp}$; $CBIgE_{asp}$) and in capillary-collected blood at 4–5 days of age (IgA_{4-5}; IgE_{4-5})

No.	Mat. IgE	$CBIgE_{grav}$	$CBIgE_{asp}$	IgE_{4-5}	$CBIgA_{grav}$	$CBIgA_{asp}$	IgA_{4-5}
1	568	230	0.415	<0.125	257	1.83	1.42
2	252	1.690	1.460	1.750	3.87	3.86	3.48
3	12.3	0.825	0.863	1.020	7.56	6.07	4.88
4	225	2.130	0.272	0.236	20.10	4.55	3.18
5	69.7	0.275	0.127	0.356	4.03	3.99	3.29
6	137	0.406	0.235	0.161	16.80	10.10	6.93
7	<5	<0.125	<0.125	<0.125	6.38	6.56	5.87
8	56.3	2.920	3.170	3.840	6.78	12.20	3.64
9	321	0.359	0.347	0.290	3.00	2.51	2.67
10	85.1	<0.125	<0.125	<0.125	3.44	3.59	1.89
11	351	0.800	0.771	0.612	6.28	5.91	4.36
12	63.1	0.314	0.314	0.252	9.44	9.30	5.72
13*	46.8	<0.125	<0.125	<0.125	8.16	5.31	2.94
14*	46.8	<0.125	<0.125	<0.125	7.81	5.26	2.57
15	5.7	0.229	0.229	0.217	10.80	11.60	5.57
16	13.3	<0.125	0.132	0.156	10.20	9.46	5.28
17	274	0.521	0.507	0.291	7.57	7.37	4.70
18	18.9	<0.125	<0.125	<0.125	4.31	4.41	3.34
19	275	0.524	0.499	0.243	6.60	5.80	3.78
20	8.5	<0.125	<0.125	<0.125	4.52	4.20	2.71
21	133	0.360	0.330	0.447	2.00	1.87	2.41
GM	66.2	0.481	0.300	0.278	7.66	5.27	3.57
Median	69.7	0.359	0.272	0.236	6.78	5.31	3.48
GM + 1SD	284.1	2.707	0.754	0.720	20.33	9.02	5.33

GM = geometric mean. * pair of twins.

more suited for comparisons than are gravity-collected CB.

Infant IgE

The individual IgE levels in gravity-collected CB ($CB\ IgE_{grav}$), aspirated CB ($CBIgE_{asp}$) and capillary-collected blood at days 4–5 (IgE_{4-5}) are presented in Table 1 and Fig. 2. The corresponding GM were 0.48, 0.30 and 0.28 kU/l, respectively. If the 3 contaminated samples (Nos. 1, 4 and 6) were omitted the GM of $CBIgE_{grav}$ was 0.32 kU/l, which is not different ($p > 0.10$) from the GM of $CBIgE_{asp}$ or IgE_{4-5} (Table 1). Although the correlation became stronger if the 3 contaminated CB_{grav} samples were excluded ($n = 18$; $p < 0.001$; $r_s = 0.97$), the levels of all $CBIgE_{grav}$ and $CBIgE_{asp}$ correlated significantly ($n = 21$; $p < 0.001$; $r_s = 0.89$; Table 2). IgE_{4-5} and $CBIgE_{asp}$ levels were also highly correlated ($n = 21$; $p < 0.001$; $r_s = 0.84$).

Four CB samples (Nos. 1, 2, 4 and 8) had an elevated $CBIgE_{grav}$. Two of these (Nos. 1 and 4) were considered to be due to maternal contamination, an interpretation that was also corroborated by the $CBIgE_{asp}$ and IgE_{4-5} values, which were below 0.9 kU/l. The other 2 cases (Nos. 2 and 8), on the contrary, showed a persistently elevated IgE (≥ 0.9 kU/l) irrespective of the sampling technique, which indicated no major maternal contamination of

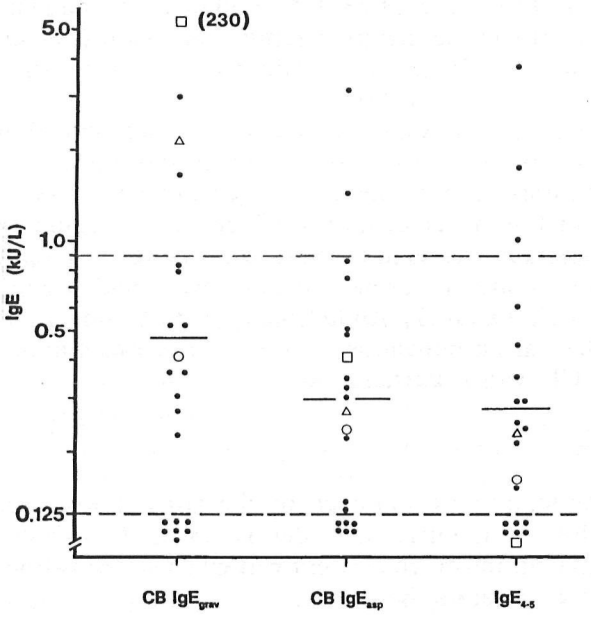

Fig. 2. Serum IgE levels (kU/l) in gravity-collected cord blood ($CBIgE_{grav}$), in aspirated cord blood ($CBIgE_{asp}$) and in capillary-collected blood at 4–5 days of age (IgE_{4-5}) among 21 infants. Horizontal bars indicate the geometric means. The lower and upper horizontal interrupted line represents the lower sensitivity of the test (0.125 kU/l and the cut-off limit of an increased CBIgE level (0.9 kU/l), respectively. Symbols refer to infants No. 1 (□), No. 4 (△) and No. 6 (○).

Table 2. Spearman rank correlations (r_s) between IgE levels in maternal blood at delivery (Mat. IgE), in gravity-collected cord blood ($CBIgE_{grav}$), in aspirated cord blood ($CBIgE_{asp}$) and in capillary-collected blood samples on days 4–5 (IgE_{4-5}) in 21 cases

	Mat. IgE	$CBIgE_{grav}$	$CBIgE_{grav}$*	$CBIgE_{asp}$	IgE_{4-5}
Mat. IgE		0.63	0.54	0.55	0.33
$CBIgE_{grav}$	<0.01		–	0.89	0.66
$CBIgE_{grav}$*	<0.05	–		0.97	0.94
$CBIgE_{asp}$	<0.05	<0.001	<0.001		0.84
IgE_{4-5}	0.14	<0.01	<0.001	<0.001	

* Correlation on 18 cases after omission of 3 gravity-collected cord blood samples having elevated $CBIgA_{grav}$ (≥ 14.1 µg/ml) and suspected to be maternally contaminated. The right triangle represents the rank correlation values and the left triangle the corresponding p-values.

CB. There were no significant correlations between IgA and IgE levels on any sampling occasion (data not shown).

Postnatal changes in infant IgA- and IgE levels

The IgA levels decreased in 20 (95%) infants from birth up to days 4–5; this meant a reduction of about 32% in GM. This decline was highly significant ($p < 0.001$; Wilcoxon). In contrast, the IgE levels decreased in only 9 (60%) and increased in 6 (40%) infants considering the 15 samples with detectable IgE (i.e., ≥ 0.125 kU/l). This postnatal difference in the frequency of decreasing/increasing IgA and IgE levels was significant ($p < 0.05$; Fisher's exact test). Interestingly, all 3 samples with elevated IgE (≥ 0.9 kU/l) at days 4–5 showed continuously increasing values from delivery to 4–5 days of age (Table 1).

Maternal and infant IgE

The GM of maternal IgE was 66 kU/l (range <5–568 kU/l, Table 1) and a positive Phadiatop was found in 85% of the women.

As shown in Table 2, there were significant correlations between the levels of maternal IgE at delivery and the level of $CBIgE_{grav}$ ($n = 21$; $p < 0.01$; $r_s = 0.63$) and $CBIgE_{asp}$ ($n = 21$; $p < 0.05$; $r_s = 0.55$). The stronger relation between maternal IgE and $CBIgE_{grav}$ weakened and became similar to that with $CBIgE_{asp}$ after omission of the 3 $CBIgE_{grav}$ samples that were thought to be contaminated with maternal IgE ($n = 18$; $p < 0.05$; $r_s = 0.54$). It is noteworthy that there was no significant correlation ($n = 21$; $p > 0.10$) between the levels of maternal IgE and infant IgE at days 4–5 (Table 2).

Discussion

This study shows that the measured levels of IgE and IgA during the perinatal period of life are influenced by the sampling technique. Even after careful cleaning of the umbilical cord string before allowing the CB to flow freely into a tube (gravity collection), contamination with maternal blood occurred in at least 3 (14%) of the CB samples, as judged by CBIgA analyses. This is in agreement with a previous study by our group (18) but the figure is higher than those reported by others (12, 25). With regard to allergy prediction, it is pertinent that of 4 gravity-collected CB samples with elevated IgE (i.e., ≥ 0.9 kU/l), 2 were attributed to maternal contamination. In agreement with previous observations (11, 22), such contaminations were minimized by the aspiration of CB. This sampling procedure, although preferable, is laborious and less convenient for routine sampling of CB. However, the very good correlation between IgE levels of aspirated CB and capillary-collected blood at 4–5 days of age suggests that the latter sampling technique may also be an alternative (4, 6, 18, 24). Moreover, it totally avoids maternal contamination, reduces the risk of other maternal influences, and the collection of neonatal blood could be performed on filter-paper at the same time as the routine screening test for inherited metabolic diseases (7).

Determination of the IgA level in CB has been suggested as one way to study possible maternal contamination of CB (5, 8, 11–13, 16–18, 21, 25). However, opinions vary as to whether IgA is appropriate for this purpose (8, 18). In the present study CBIgA distinguished well between 3 maternally contaminated CB samples, all gravity-collected, and the others. The assumption that the gravity-collected CB was contaminated was confirmed by the IgA and IgE assays in aspirated CB. In this study and in previous ones (17, 18) we used 14.1 µg/ml as the upper normal limit of IgA in cord blood, while Kimpen (13) and Zeiger (25) used 32 and 39 µg/ml, respectively. Using these latter limits we would have missed one case (No. 4) with an elevated $CBIgE_{grav}$, which was obviously due to contamination. This suggests that the limits proposed by Kimpen and Zeiger are too high or that the calibrations of the IgA analyses may vary. It could also explain the lower incidence of contamination found in these studies (12, 13, 25). Zeiger (25) suggests that determination of the IgA level should always be performed when CBIgE is > 1.0 kU/l. However, since the cut-off limits vary between studies, i.e., 0.3 kU/l (Høst, personal communication), 0.5 kU/l (25), 0.9 kU/l (16) and 1.2–1.3 kU/l (5, 21), an evaluation of the risk of maternal contamination should be performed on all CB samples with an elevated IgE and be guided by the cut-off point chosen for prediction.

Besides the contamination problems, other interesting findings emerged from this study. In contrast to IgE, the IgA levels were significantly ($p < 0.001$) lower at days 4–5 days than at delivery. The IgA

levels decreased in 95% of the cases, while the IgE levels showed both decreasing (60%) and increasing (40%) values. The difference between isotypes is interesting, since this period of life is characterized by large haemodynamic changes that ought to affect the levels of various immunoglobulin isotypes in a similar fashion. The explanation may not lie in the half-life, since it is longer for IgA (5–6 days) than for IgE (2–3 days). It is more likely that the significant drop in IgA is partly due to the capillary sampling technique. We observed significantly lower IgG levels in capillary-collected blood than in venous blood collected simultaneously at 4–5 days of age (Lilja & Magnusson; unpublished data). It therefore seems plausible that the high frequency of cases with increasing postnatal IgE levels indicates an active IgE synthesis which is overcoming both a putative haemodynamic and a technical drop. Moreover, it is interesting that the IgE levels in aspirated CB correlated significantly with the IgE levels in both capillary-collected blood at 4–5 days of age and maternal IgE, while there was no correlation between maternal IgE and infant IgE at days 4–5. Taken together, all these data suggest that besides an active pre- and postnatal IgE synthesis there is also a discernible prenatal maternal influence.

Thus, besides the need to establish convenient, reliable and standardized blood sampling procedures during the neonatal period that are suitable for allergy prediction, we also require more information about the physiological changes that seem to occur in the immunoglobulin levels in early life.

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