

Evaluation of IgA measurements as a method for detecting maternal blood contamination of cord blood samples

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Several studies have evaluated the value of cord blood IgE concentrations for predicting the risk of allergic disease in children. In all of these studies it is necessary to exclude cord blood samples in which the IgE may be falsely elevated due to admixing of maternal and fetal blood during parturition. The most common method for detecting mixing of fetal and maternal blood is measurement of cord blood IgA concentrations. We have examined the theoretical basis of IgA measurements for detecting maternal blood contamination and reexamined our own data to evaluate IgA measurements. Our data suggest that the average IgA concentration of 28.2 µg/ml used in previous studies may not adequately exclude contaminated cord blood sample. Failure to reject contaminated cord blood samples would reduce the positive predictive value of cord blood IgE measurements.

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Measurement of cord blood IgE concentration has been proposed as a method for assessing the allergic risk of a child (1–4). While initial studies showed that the cord blood IgE concentrations were relatively good predictors of allergic disease, even better than family history of atopy (3, 5–8), more recent studies have questioned the predictive value of cord blood IgE concentrations (9, 10). In theory cord blood IgE concentrations represent the basal genetic predisposition toward IgE production and hence should be related to the genetic risk of atopy. The predictive value of cord blood IgE measurements would be decreased if cord blood became contaminated with maternal blood at the time of delivery, producing falsely elevated cord blood IgE concentrations. Several approaches have been used to reduce the potential problem of maternal blood contamination. Serum could be collected when the infant was several days of age or concentrations of allergen specific IgE could be measured to detect contamination. These methods, unfortunately, are relatively difficult and expensive (11). The most commonly used method for detecting contamination of cord blood with maternal blood has been measurement of the IgA concentration in cord blood (10–18). This method is based on the premise that IgA is not normally produced *in utero*; therefore if IgA is detectable in cord blood, the most probable source is maternal blood.

Even though a number of investigators have used IgA measurement to detect maternal blood contamination of cord blood, we are not aware of any studies which have carefully evaluated the theoretical basis of IgA in terms of the amount of maternal blood necessary to produce detectable changes in cord blood IgA and the corresponding change in cord blood IgE. It is also necessary to reexamine the assumption that IgA is not normally produced *in utero* since very sensitive IgA assays are now available and used for cord blood IgA measurements.

The purpose of this study was to use current information concerning immunoglobulin concentrations and blood volumes to mathematically evaluate the relationship between the quantity of maternal blood detectable by IgA measurement and the quantity needed to produce falsely elevated IgE concentrations. The calculated values are contrasted with the values used in previous studies. Cord blood IgE and IgA concentrations from a large birth cohort of children were also examined to further evaluate the relationship between IgE and IgA in cord serum.

Patients and methods

The selection of children for this study has previously been described (19). Briefly, all pregnant women living in an area of northern, suburban Detroit, defined by contiguous zip codes, and belong-

ing to the Health Alliance Plan HMO, were eligible for recruitment if their babies were due between April 15, 1987 and August 31, 1989. Women meeting eligibility criteria were invited to participate during prenatal visits. If the woman agreed to participate, demographic, health and lifestyle-related data were collected by a study nurse. At delivery, cord blood specimens were obtained and assayed for total IgE concentration by ELISA. IgA concentrations were also measured by ELISA. The study population consisted of 847 infants who met initial eligibility criteria, including having only one child per family admitted to the study.

IgE was measured in the cord blood samples using an enzyme linked immunosorbent assay (ELISA) as previously described (19). Briefly, cord blood samples were obtained at the time of delivery from the cord prior to delivery of the placenta. The cord was wiped to reduce the potential of maternal blood contamination during collection. The cord blood serum was stored frozen at -20°C until assayed. The ELISA used was biotin-avidin amplified, with a fluorescent substrate. The anti-IgE antibody used in both the capture and detecting steps was affinity-purified antihuman IgE (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). When used as the detecting antibody, the anti-IgE was biotin-labeled. Standard curves of IgE were constructed from appropriate dilutions of a patient serum that had been standardized against the U.S. reference standard. The average net fluorescence produced by triplicate wells of the unknown samples were converted to units of IgE (international units, IU) by calculation from the computer fitted standard curve. The typical sensitivity of the assay was 0.02 IU/ml of IgE with an average interassay coefficient of variation of 17.6%.

The IgA concentration was measured, using an ELISA, in the first 150 cord blood samples and then in all samples with IgE concentrations of greater than or equal to 0.56 IU/ml. The IgA assay had a sensitivity of 1 $\mu\text{g/ml}$ and an interassay coefficient of variation of 9.8%.

Calculation of detectable blood volumes

To calculate the volume of maternal blood which would produce a detectable increase in cord blood IgE or IgA, the plasma volume of a newborn must be estimated. The birth weight was available for 808 of the 847 infants initially admitted to the study. The average birth weight was 3,427 gm. According to Usher et al. the average plasma volume of a full-term infant at birth is 70 ml/kg (20). Multiplying 3.4 kg by 70 ml/kg produces an estimated average plasma volume of 240 ml at birth. The geometric mean cord IgE concentration in the 841 infants included

Table 1. Relationship of maternal serum IgE concentration and the volume of maternal blood producing an elevated cord blood IgE concentration

Maternal IgE (IU/ml) (Population percentile)*		Volume of blood (ml)** producing a cord blood IgE concentration of:	
		>0.6 IU/ml	>0.9 IU/ml
17	(50%)	5.9	10.4
45	(75%)	2.2	3.8
100	(90%)	0.97	1.7
180	(95%)	0.54	0.94

*IgE concentrations and population percentile values of 15–34 year old, non-atopic, women, from Klink et al. (21).

**Volume of blood in milliliters, assuming a plasma volume of 240 ml for a 3.4 kg infant having an initial serum IgE concentration of 0.20 IU/ml.

in this study was 0.20 IU/ml which is similar to the mean values observed in several other studies (4, 10, 12). Using the estimated plasma volume allows calculation of the quantity of maternal blood needed to increase the cord blood IgE from the mean level, to an “elevated level”, for any given maternal serum IgE concentration. The value used to define the IgE concentration as “elevated” is arbitrary and varies between studies, but values between 0.6 and 0.9 IU/ml are commonly used (10–18).

Results

Table 1 presents the results of calculating the amount of maternal blood required to increase cord blood from a value of 0.20 IU/ml to either 0.6 or 0.9 IU/ml for several different maternal serum IgE concentrations, assuming that the infant's plasma volume was 240 ml. For example, with a relative high maternal IgE of 180 IU/ml, only 0.54 ml of maternal blood are required to increase the cord blood concentration from 0.20 to 0.6 IU/ml in an infant with a birth weight of 3.4 kg.

Table 2 presents the relationship between the IgA concentration used to classify a cord blood sample as contaminated and the volume of maternal blood required to produce the same IgA concentration. As the IgA concentration used to classify a sample as contaminated is decreased from 39 to 10 $\mu\text{g/ml}$, the volume of detectable admixed maternal blood also decreases, from 3.16 to 0.80 ml. These calculations are based on the following assumptions: 1) that the maternal serum IgA concentration was 300 mg/dl (the mean value reported for normal women); 2) that the initial cord blood IgA concentration was zero; and 3) that the infant's plasma volume is 240 ml at birth.

Table 3 presents a summary of ten published studies which have used IgA as a method for excluding maternal blood contamination of cord blood samples. The geometric mean of the IgA values used for excluding maternal blood contamination, in

Table 2. Relationship between the critical value of IgA used to detect cord blood contamination and volume of maternal blood needed to produce the IgA concentration

IgA concentration used as the critical value ($\mu\text{g/ml}$)	Volume of maternal blood detectable by IgA measurement (ml)*
10	0.80
14	1.13
18	1.45
28.2	2.28
32	2.59
39	3.16

*Assuming: 1) maternal serum IgA concentration of 300 mg/dl; 2) an infant plasma volume of 240 ml; and 3) an initial cord serum IgA concentration of 0 $\mu\text{g/ml}$.

these 10 studies, was 28.2 $\mu\text{g/ml}$. According to Table 2, a cord blood concentration of 28.2 $\mu\text{g/ml}$ would result from a maternal blood exchange of 2.28 ml, and interpolating from data in Table 1, a blood volume of 2.28 ml would result in a cord blood IgE concentration of greater than 0.6 IU/ml, if the maternal IgE concentration were ≥ 43 IU/ml. Table 1 also shows the population percentiles for total serum IgE concentrations in nonatopic, 15 to 34 year old, women residing in the United States (21). Using the population percentile data, we estimated that serum IgE of 43 IU/ml or greater would be found in approximately 25% of nonatopic women. This means that for approximately 25% of nonatopic women, and for a higher percentage of atopic women, a blood exchange of 2.28 ml or less would elevate the infant's IgE to greater than 0.6 IU/ml. Admixing of less than 2.28 ml of maternal blood would not be detected using an IgA value of 28.2 $\mu\text{g/ml}$, but volumes of less than 2.28 ml of maternal blood could result in IgE concentrations of more than 0.6 IU/ml. The consequence of missing increased cord IgE from maternal blood admixing would be to falsely classify an infant as one at high risk of atopy. False classifications would reduce the predictive value of the IgE measurement. Reducing the critical IgA value to 10 $\mu\text{g/ml}$ would allow detection of as little as 0.8 ml of contaminating maternal blood, if maternal IgA concentration was at least 300 mg/dl. This in turn would reduce the risk of failing to detect maternal blood contamination to mothers with IgE concentrations of greater than 121 IU/ml or, from Table 1, about 10% of nonatopic women (21). The actual percentage of infants misclassified in a study would depend upon the true frequency of maternal-fetal blood admixture.

Of the 847 cord blood samples we examined, 141 samples had IgE concentrations >0.56 IU/ml. When the IgA concentrations of these 141 samples were measured, six (4.3%) samples had IgA concentrations of >39 $\mu\text{g/ml}$. If we had used an IgA concentration of 10 $\mu\text{g/ml}$, in an attempt to reduce the risk

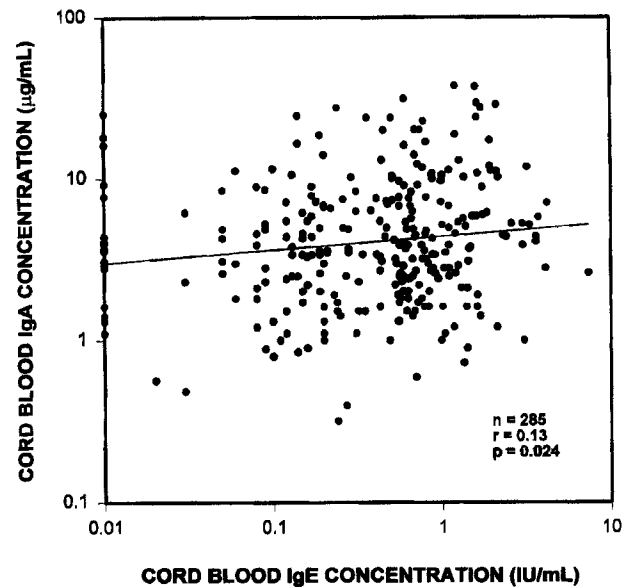


Fig. 1. The relationship between IgE and IgA concentrations in 285 cord blood samples ($r=0.13$, $p=0.024$).

of false positive misclassification, 34 (24%) of the 141 cord blood samples would have been considered contaminated, a 5.7-fold increase in the number of contaminated samples.

Fig. 1 illustrates the relationship between cord blood IgE and IgA concentrations in 285 cord blood samples from our study. The 285 samples in the figure, include all of the 141 samples with IgE concentrations of greater than or equal to 0.56 IU/ml and 145 samples with IgE concentrations of less than 0.56 IU/ml. As shown in the figure, there is a small but statistically significant correlation ($r=0.13$, $p=0.024$) between the logarithmically transformed IgE and IgA concentrations. If IgA concentrations of greater than 10 $\mu\text{g/ml}$ are excluded from the analysis, the correlation is no longer significant ($r=0.08$, $p=0.21$).

Discussion

At least 10 previous studies of the relationship between cord serum IgE concentrations and the risk of allergic disease in children have used measurement of IgA as a method of detecting cord blood samples contaminated with maternal blood. The concentration of IgA used to categorize a cord blood sample as probably contaminated has ranged from 10 to 100 $\mu\text{g/ml}$. Some studies have used IgA as a criterion, but failed to mention the IgA value used for classification (11, 13). Based on estimates of neonatal blood volume and maternal serum IgA and IgE concentrations, we feel that the IgA concentrations used

Table 3. Studies using measurement of IgA to detect maternal blood contamination of cord blood

Reference	Year	N	Critical IgA value ($\mu\text{g/ml}$)	% Increased IgA	Mean IgA ($\mu\text{g/ml}$)
Kimpen et al. (12)	1987	5512	32.3*	2.8	NR**
Haus et al. (13)	1988	163	NR**	NR**	26
Tseng et al. (14)	1989	340	100	2.4	NR
Zeiger et al. (15)	1989	288	39	2.4	NR
Lilja et al. (16)	1990	83	14.1*	10.8	5.3
Ruiz et al. (11)	1991	61	NR	7	NR
Halonen et al. (17)	1991	1074	18	0	NR
Hide et al. (10)	1991	1111	10	6.7	NR
Ownby et al. (19)	1991	847	39	0.71	NR
Bergmann et al. (18)	1995	6401	32	1.0	NR
Geometric means		425	28.2	3.04	11.7

*Critical values established as the mean plus three standard deviations.

**NR = Not Reported; the value was not reported and could not be calculated from the information in the report.

in some studies may have been too high to adequately detect some contaminated samples. The resulting misclassification of low risk infants as high risk infants would be expected to reduce the positive predictive value of cord blood IgE measurements for allergic disease. This may partially explain why some studies have found, and others studies have failed to find, a relationship between cord blood IgE concentrations and the risk of allergic disease in children.

The estimates we have made assume that each infant starts with an average serum IgE concentration and also assume that infants do not produce any IgA *in utero*. The consistent finding of low, but measurable, concentrations of IgA in all 285 of the cord blood samples we tested (Fig. 1) suggests that low levels of IgA are normally produced by infants prior to birth. Most previous studies have used radial diffusion or nephelometry to measure cord blood IgA and these methods are not sensitive enough to measure the small quantities of IgA detected by the ELISA used in this study. The modest correlation found between cord blood IgE and IgA concentrations may be the result of a common mechanism controlling both IgE and IgA production. Alternatively, the correlation could be the result of relatively frequent contamination of the cord blood by small quantities of maternal blood. If maternal blood contamination was common, a correlation would be expected between maternal and cord blood IgE values. Studies have found correlations between maternal but not paternal IgE and cord blood IgE (5, 23). Businco et al., however, failed to find a correlation between maternal and cord blood IgE in 101 infants of atopic parents (1).

There are several alternative approaches to the problem of detecting maternal blood contamination. One approach would be to measure maternal serum

IgA and IgE at the time of delivery and to combine this information with measurements of the infant's blood volume and cord blood IgA and IgE concentrations to calculate the probability that a sample was contaminated. The major disadvantages to this approach are the necessity of obtaining and measuring IgA and IgE in the maternal sample and of measuring or estimating the infant's blood volume, in addition to measuring IgE and IgA in the cord blood sample. Lilja et al. measured IgE in maternal serum, cord blood and serum obtained from the infant at four to five days of age (16). They interpreted the significant correlation between maternal and cord blood IgE concentrations as evidence of maternal blood contamination of the cord blood. They also noted a tendency for serum IgE concentrations, obtained when the infants were four to five days of age, to be lower than cord IgE concentrations, but they did not exclude the possibility that the IgE concentrations at four to five days were lowered by drawing capillary, rather than venous, blood samples. Duchateau and Casimir also measured serum IgE concentrations on the fifth day of life and stated, but did not offer evidence, that maternal blood contamination did not influence IgE concentrations this many days after delivery (22). A concern about using serum IgE concentrations from infants older than four days of age is the difficulty of obtaining suitable specimens and the difficulty of determining the half-life of maternal IgE in the infant's serum. Lilja et al. also suggested that an association between allergen specific IgE in the cord blood and maternal blood could be used to indicate maternal blood contamination (16). The disadvantages of this strategy are requiring a maternal blood sample, the large number of specific IgE assays needed, and the low probability of detecting specific IgE in cord blood, given the very low total IgE concentration.

In conclusion, measurement of IgA concentrations in cord blood using sensitive assays, such as ELISAs, may be the most practical method for detecting contamination of fetal blood with maternal blood, but the critical value used to decide if a sample is contaminated should be relatively low to adequately detect potentially contaminated samples. The IgA concentrations used in many previous studies may have allowed modest numbers of contaminated samples to be considered noncontaminated, resulting in the misclassification of infants and lowering the positive predictive value of cord blood IgE measurements. The risk of using a lower IgA value is the risk of rejecting true positive samples.

Based on our calculations, we would suggest that a cord blood IgA value of approximately 10 µg/ml is more appropriate than the higher values used by ourselves and others for detecting maternal blood contamination of cord blood samples. The value of 10 µg/ml is similar to the value of the mean IgA plus three standard deviations (14.1 µg/ml) reported by Lilja et al. (16). Based on the information presented in this study some investigators may wish to reanalyze their data and future investigators may wish to evaluate the effects on the predictive value of IgE using several different IgA values to accept or reject cord blood IgE values.

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