

Neonatal IgE: a poor screen for atopic disease

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Summary

Screening for atopic disease using neonatal serum IgE has been advocated on the basis of the predictive value of elevated levels. However, this is only one measure of validity. The test was validated fully in 92 infants with a bi-parental history of atopy using 0.7 IU/ml as the cut-off. All infants were assessed prospectively for evidence of atopic disease (eczema, recurrent wheezing or food reactions) and skin-prick test positivity in the first year. Total serum IgE was measured by ultrasensitive ELISA on 61 cord blood samples and 92 samples taken at 7 days. All cord samples were re-analysed by PRIST and the first 33 by ultrasensitive RIA giving, respectively, 82% and 94% concordance (regarding undetectable, detectable and elevated levels) with ELISA. Maternal contamination was indicated in 7% of cord samples by high serum IgA. Ninety-five per cent of cord/7-day IgE pairs showed no change or minor rises at 7 days. Forty-nine per cent of the infants had evidence of atopic disease. Only 5% had elevated 7-day IgE. The positive and negative predictive values of the 7-day test were 60% and 52%, respectively, and specificity 96% but the sensitivity was only 7%. High levels did not distinguish the infants with the most unequivocal evidence of disease, i.e. eczema with a positive skin test. In conclusion IgE at 7 days is comparable to and more reliable than cord IgE. However, neonatal IgE screening is too insensitive to have clinical application.

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Introduction

High neonatal serum IgE predicts atopic disease which is often manifested in infancy [1-7]. IgE screening has been proposed in neonates with an intermediate risk of atopic disease based on family history to help select those for preventive measures [2]. Some advocate screening all at-risk neonates [5], or even all neonates regardless of any risk [7]. Simply comparing the predictive capacity of high and normal neonatal IgE as percentages can be misleading if the widely differing denominators in either group are not emphasized. The usefulness of IgE screening in clinical practice needs to be judged on its sensitivity and specificity as well as the predictive value, which varies with the prevalence of disease in the study population.

The original cut-off for elevated cord blood IgE, 1.3

IU/ml [1], was the geometric mean + 2 s.d. in 24 normal Swedish neonates [8]. In fact, it has been lowered repeatedly [2-7] to improve the sensitivity. In a recent series of cord blood IgE levels in 5305 unselected neonates [9], only 6% had levels over 0.7 IU/ml (1.68 µg/l). Even if the positive predictive value of the test using this cut-off were 100%, over half of the children who manifest atopic disease would be missed, assuming a prevalence of about 15% [10]. Could normal neonatal IgE be a false assurance?

This study aimed to answer the question. Neonatal IgE screening was validated in a group of infants with a bi-parental history of atopy. This maximized atopic disease prevalence to give both an optimal positive predictive value and sufficient numbers for a reasonable calculation of sensitivity. A re-evaluation of the initial data on 7-day sera [11] with larger numbers is presented here together with a justification of its preference to cord blood estimations and an appraisal of the method of IgE measurement.

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Materials and methods

Subjects

Selection of potential subjects was based on a bi-parental history of atopy from antenatal questionnaires. All parents were skin tested, except for two fathers. Infants entered the study if informed parental consent was obtained and atopy confirmed in at least one parent by a positive skin-prick test. Ninety-two (47 girls and 45 boys) term neonates born between September 1987 and July 1989 in the Camberwell Health Authority were studied: 38 had uniparental and 52 bi-parental atopy according to skin tests; 75 (82%) were Caucasian, seven were Black, six were Black/Caucasian, three were Eurasian and one was Chinese. There were two sets of non-identical twins.

Blood sampling

Cord blood was obtained in 61 of the infants. All 92 had blood samples taken by heel-prick during routine screening for phenylketonuria and hypothyroidism on day 7 of life. Maternal blood samples were collected with the routine haemoglobin estimation usually on day 3 post-partum. The sera were stored at -20°C and assayed blind at the end of clinical follow up.

Laboratory methods

Total IgE was measured in cord and 7-day sera in duplicate by an ultrasensitive ELISA as described previously [12]. The 7-day sera were diluted 1/5. Briefly, the microtitre plates were coated with mouse monoclonal anti-IgE, the second antibody was alkaline phosphatase (AP) labelled Fab' anti-IgE and the optical density of the substrate was measured by an automated plate reader (Multiskan Titertek, Flow Laboratories, Irvine, U.K.) at 405 nm. The assay could measure down to 0.02 IU/ml. The dilution factor made the effective lower limit of detection in 7-day sera 0.1 IU/ml. For consistency, levels below 0.1 IU/ml in cord sera were also considered undetectable. An elevated neonatal IgE level was regarded to be 0.7 IU/ml or above.

All cord samples were re-analysed using the Phadebas IgE paper radioimmunosorbent test, PRIST (Pharmacia, Uppsala, Sweden) used in most previous studies. A third measurement was made in the first 33 cord samples using a newer ultrasensitive radioimmunoassay (Pharmacia IgE RIA Ultra). For both assays, sera were undiluted and the detection limits were 0.5 IU/ml and 0.2 IU/ml, respectively.

Maternal total serum IgE was measured by a conventional ELISA which used AP-labelled anti-IgE as the second antibody and measured down to 0.5 IU/ml [12].

Total serum IgA was measured by ELISA in the cord samples which had elevated IgE levels on ELISA estimation. This was a check for possible contamination with maternal blood as IgA is not transmitted to the fetus as a rule, and very little is normally detectable in the neonate [13].

Clinical assessment

The same examiner saw every infant at 3, 6 and 12 months in hospital to document evidence of atopic disease and perform skin-prick tests. Additional home assessments were made whenever coughs or suspicious rashes were reported by the parents. The following were regarded as evidence of atopic disease: (i) eczema which was diagnosed if criteria based on those of Hanifin and Rajka [14] but modified for infants [15] were fulfilled and if graded 2 or 3 on the scale of Matthew *et al.* [16]. Thus, as far as possible seborrhoeic dermatitis was excluded; (ii) wheezing on at least two occasions; (iii) history of an immediate-type reaction to a particular food on repeated occasions. In exceptional cases, if parents declined rechallenging because of the severity of a reaction, one adverse response was accepted if the history were clear-cut. The examiner was unable to diagnose allergic rhinitis in infancy with confidence.

The skin-prick tests were done to cows' milk, hens' eggs, mixed grass pollens, *Dermatophagoides pteronyssinus*, cat fur (Bencard), mixed moulds (Dome/Hollister-Stier), and histamine 1 mg/ml and diluent control solutions (Bencard). The forearm was used in parents and the back in children. A weal after 15 min, at least 3 mm in adults, or at least 2 mm in infants, larger than that caused by the diluent control, was taken to be positive.

Approval from the hospital's Ethical Committee was obtained.

Results

Evidence of atopic disease occurred in 45 out of 92 (49%) of the infants; 26 had eczema, 13 had recurrent wheezing, and 21 had food reactions (Fig. 1). The latter included three without repeated challenge. Twenty-two infants gave positive skin-prick tests at some time during infancy, of which 18 had disease manifestations, mostly eczema (Fig. 2).

IgE was detected by ELISA in 11 out of 61 cord samples. Only six had elevated levels. Using PRIST 17 cord samples had detectable IgE levels of which 11 were elevated. Table 1 gives a qualitative comparison of cord IgE results using the two assay methods. The results agreed in 50 out of 61 instances giving 82% concordance. All six with elevated levels by ELISA were elevated by

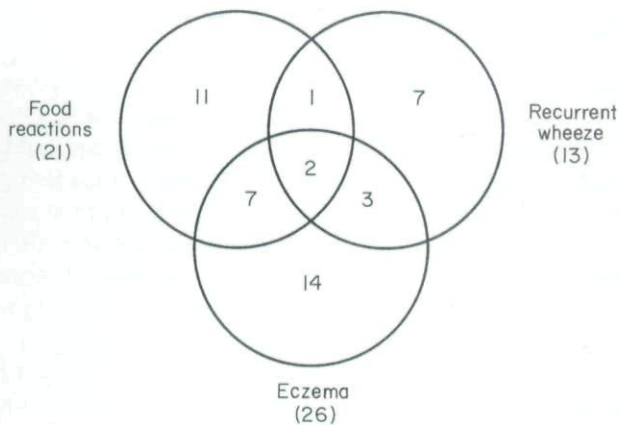


Fig. 1. Numbers of infants with various types of atopic disease manifestation in the first year ($n=45$).

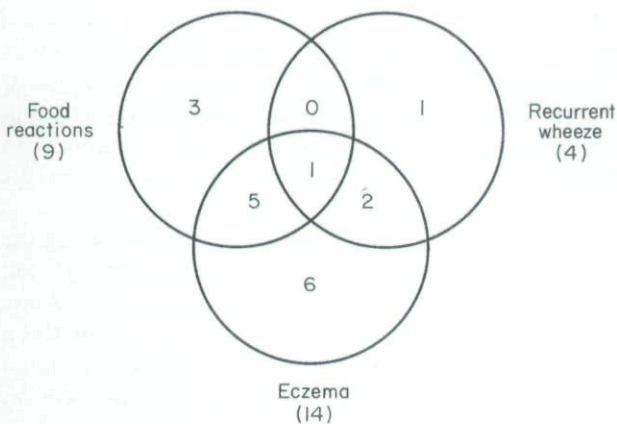


Fig. 2. Numbers of infants with positive skin-prick tests and various types of atopic disease manifestation in the first year of life ($n=18$).

Table 1. Comparison between total serum IgE measured by ELISA and by PRIST in 61 cord blood samples

	ELISA		
	Undetectable	<0.7 IU/ml	≥0.7 IU/ml
Undetectable	42	2	0
PRIST <0.7 IU/ml	4	2	0
PRIST ≥0.7 IU/ml	4	1	6

PRIST. PRIST values were slightly higher than ELISA for values below 10 IU/ml, although above this range they were consistently lower.

There was 94% concordance between ELISA and RIA on 33 cord samples. Concordant results included 23 undetectable and eight detectable levels, of which four were elevated. The two discrepancies had undetectable and 0.49 IU IgE/ml by ELISA but 1.76 IU/ml and 1.36 IU IgE/ml, respectively, by RIA.

Serum IgA was measured on the six cord samples with elevated IgE by ELISA. High levels were found in four which were therefore considered contaminated. None of the four had corresponding elevated 7-day levels; two had considerably lower and two had undetectable IgE at 7 days. One cord sample with an IgE level of 25.0 IU/ml had a low serum IgA, but contamination was nevertheless suspected to explain the wide discrepancy with the corresponding 7-day IgE which was undetectable. The remaining sample with elevated cord IgE (1.1 IU/ml) and low IgA was associated with an even higher IgE at 7 days (4.2 IU/ml).

Rejecting the contaminated cord samples left one infant with an elevated level by ELISA and six by PRIST, although five of the latter would not have been formally tested for contamination. The infant with high cord IgE by ELISA had a food reaction and positive skin test and was among the three with elevated levels by PRIST who had evidence of atopic disease. Disease manifestations were present in 28 out of 56 infants with uncontaminated cord samples.

The uncontaminated cord values are compared with the respective 7-day levels in Table 2. Three showed very minor falls in IgE over 7 days and 12 demonstrated small rises but the majority (41) had undetectable IgE at both times.

Table 2. Comparison between cord and 7-day serum IgE in 56 paired samples, both measured by ELISA

	Cord IgE		
	Undetectable	<0.7 IU/ml	≥0.7 IU/ml
Undetectable	41	3*	0†
7-day			
<0.7 IU/ml	6	1	0‡
IgE			
≥0.7 IU/ml	3	1	1

* Actual cord values: 0.14 IU/ml, 0.14 IU/ml, 0.18 IU/ml.

† Three and ‡ two sample pairs excluded due to presumed maternal contamination of cord blood.

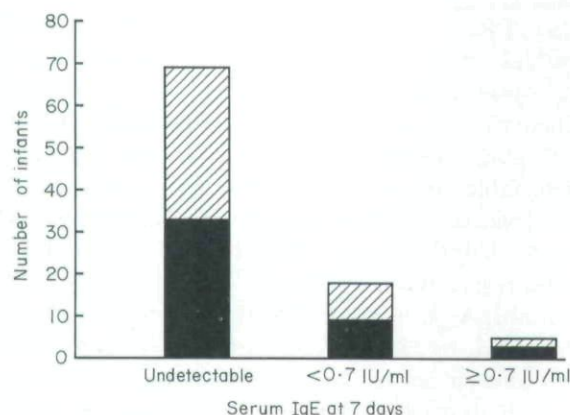


Fig. 3. Numbers of infants in three categories of 7-day serum IgE concentration, with indication of atopic disease status (■, present; ▨, absent).

Table 3. Atopic manifestation categories related to serum IgE at 7 days

	7-day IgE		
	Undetectable	<0.7 IU/ml	≥0.7 IU/ml
Eczema	21	4	1
Wheeze	8	4	1
Food reaction	15	5	1
Eczema/wheeze/food reaction	2	0	0
Skin test ⁺	16	4	2
Eczema/skin test ⁺	12	2	0
Eczema/Wheeze/skin test ⁺	2	1	0

Only five infants (5.4%) had high 7-day IgE (Fig. 3). Eighteen (19.6%) had levels below 0.7 IU/ml but still detectable, and 69 (75.0%) had undetectable levels. Atopic disease manifestations were present in three out of the five with high neonatal IgE. However the majority of those with evidence of disease had undetectable levels (33 out of 69) or levels below 0.7 IU/ml (nine out of 18). Considering each disease manifestation separately still yielded a preponderance of infants with undetectable 7-day IgE in every category (Table 3). Furthermore, neither the two infants with all three manifestations nor any of the 14 with a positive skin test as well as eczema had elevated neonatal IgE.

There was no correlation between maternal and cord or infant 7-day total serum IgE.

Discussion

The prevalence of atopic disease in 7-year-old Swedish children with a history of bi-parental atopy was 42.9% [17]. The 49% prevalence of disease manifestations in the present study after 1 year without even considering rhinitis may therefore appear to be an overestimation. However, basing parental atopy on skin testing rather than mere history and actively seeking evidence of atopic disease prospectively instead of by retrospective questionnaire may have contributed to a higher prevalence.

Most previous studies of neonatal IgE have used cord blood. Spuriously elevated IgE due to contamination with maternal blood was found in 4% of cord samples in one study on the basis of IgA measurement [13]. This compared with four out of 61 (7%) in the present series. In an additional sample, although the serum IgA was low, contamination was the most likely explanation for a remarkable discrepancy with the corresponding 7-day level. A precipitous fall in IgE in the first 7 days may be theoretically possible if cord IgE were passively acquired maternal antibody. However, the lack of association between maternal and cord IgE found here and by others [18] implies that free transfer of IgE does not occur across the placenta and that the minute quantities sometimes detectable in neonates should be intrinsic. Contamination without high serum IgA may occur if maternal serum IgA is low.

In the majority of infants serum IgE levels stayed the same or rose modestly in the first seven days. IgE fell slightly in only three infants. Conclusions based on 7-day blood should therefore give a good reflection of those based on cord blood, but avoid the problems associated with collection of the latter. Attention will now focus on the 7-day data.

The fact that only 5.4% of the present study group had elevated IgE while 49% had evidence of atopic disease would argue against any reassurance from normal neonatal IgE in predisposed infants. A formal appraisal of the test performance using the 0.7 IU/ml cut-off, gave a sensitivity of only 7%, meaning that 93% of affected infants would not have been picked up by the test (Fig. 3). However, the specificity was 96% since 45 out of 47 unaffected infants were correctly predicted. The high prevalence of affected infants should have ensured a relatively low number of false positives. In fact, the positive predictive value was 60%, although based only on five children and liable to improve with longer follow-up. Forty-five out of the 87 infants with levels below the cut-off had evidence of disease, giving a negative predictive value of 52%. Lowering the cut-off level to 0.5 IU/ml improved the sensitivity to a mere 18%. The sensitivity became 32% if 'detectability' were used as the cut-off.

IgE levels at 7 days below 0.1 IU/ml occurred in 75% of the infants. This was only slightly more than the findings of a large study from a Belgian group [19]. They also used an ELISA technique and found 70% of 5353 unselected cord blood samples had IgE concentrations below 0.2 IU/ml, of which the majority were less than 0.1 IU/ml. Thirteen per cent had levels above 0.4 IU/ml compared with 15% (14 out of 92) in the present study. However, in other studies 32% of unselected cord samples [4] and 43% of cord samples from infants with atopic parents [5] had IgE levels over 0.5 IU/ml. Indeed, 12.6% of unselected cord IgE determinations exceeded 0.9 IU/ml in the Swedish study [2] compared with 3% (three out of 92) here. The Belgian study had 3% above 1.0 IU/ml [19].

Possible causes for the discrepancies would include falsely elevated levels due to contamination with maternal blood. These were avoided here by using 7-day sera and in the Belgian study by measuring IgA concentrations, but do not appear to be accounted for in the other studies.

Secondly, the latter studies used the PRIST instead of ELISA. In this study the PRIST gave slightly greater values in the low range and lower values in the high range than the ELISA. Thus for a given set of samples a few more might be expected to exceed the cut-off with PRIST than with ELISA as was found here. It is pertinent that cord measurements with ultrasensitive RIA agreed more closely with ELISA than with PRIST values, suggesting that the latter may be less reliable with very low levels of IgE.

However, it must be emphasized that where levels were undetectable there was excellent concordance between all assays. Since the vast majority of results were undetectable, the present findings cannot be attributed to the assay method. Indeed the data here allow a validation of cord blood IgE screening by PRIST if reservations over the assay and cord sampling are overlooked. Using the same 0.7 IU/ml cut-off, the positive predictive value of this test would be only 50% and sensitivity 11%. Even in the Swedish study the sensitivity of the test using a 0.9 IU/ml cut-off and calculated on over 200 unselected children followed up for 7 years was just 40% [2]. The sensitivity increased to 93% if only children who had had atopic disease throughout the 7-year study period were considered, but this excluded 80% of the children diagnosed 'definite atopic.'

The duration of follow-up period in any prospective study is bound to influence the number of children with disease. However, extending the follow up beyond 1 year in the present study would only tend to reduce the numbers of false-positives and increase the false-negatives. It may therefore improve the positive predictive value but could only give an even poorer sensitivity.

Two studies have shown less atopic disease in breast-

fed infants with high cord IgE than their counterparts on cows' milk [5,6]. Ideally, the significance of neonatal IgE should be studied in isolation from measures such as breast-feeding which may be used to alter outcome. In practice it would be unethical to ensure an unmodified disease prevalence in the study population. Allergy-preventive measures would tend to increase the false-positives. The two false-positives in this study happened to have been breast-fed for over 6 months, but so were two of the three true-positives.

A potential cause of discrepancy between studies of atopic disease is the difficulty in making unequivocal diagnoses particularly in infancy. The various clinical manifestations may therefore be more accurately described as 'evidence' of atopic diseases than as actual 'atopic dermatitis,' 'asthma' or 'food allergy'.

The criteria proposed by Hanifin and Rajka [14] are widely accepted diagnostic guidelines for atopic eczema [20]. However, many of the minor features do not apply to infancy. Other investigators, including one of the original proposers, have set out modified infant criteria [13] which were felt to be more appropriate for the present study. Indeed, eczema was probably the most reliable evidence of atopic disease since wheezing in infancy may not have an allergic basis and food reactions were based on history alone. Nevertheless, none of the infants who fulfilled the strictest criteria for evidence of atopic disease (eczema with a positive skin test) had elevated neonatal serum IgE. Screening thus failed to separate those with most obvious atopic disease in the first year.

Neonatal IgE estimation using cord blood had serious drawbacks and 7-day samples provided comparable and more reliable data. High neonatal IgE in predisposed infants had a positive predictive value of 60% in the first year. It may distinguish a subgroup with particularly increased IgE responsiveness, although this was not reflected clinically after 1 year of follow up. Its application as a screening test in clinical practice must be limited by its infrequency among infants with atopic disease as a whole and thus its poor sensitivity. It is arguable whether the encouragement of relatively simple measures such as breast-feeding or delayed introduction of solids in predisposed infants should ever need to depend on a measure of serum IgE, quite apart from the false assurance this test is likely to engender. Certainly a means of selecting predisposed infants for more complex measures such as maternal avoidance diets would be useful. However, neonatal IgE measurement does not provide this means.

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