

Cut-off values for total serum immunoglobulin E between non-atopic and atopic children in north-west Croatia

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Abstract

Background: The aim of this study was to determine cut-off values for total serum immunoglobulin E (IgE) between non-atopic and atopic children with respiratory symptoms. Children of 0–16 years of age were evaluated for respiratory symptoms of >4-week duration.

Methods: Children were divided into two groups: non-atopic children (n=3355) who were non-IgE-sensitized with undetectable allergen-specific IgE (<0.35 kIU_A/L), and atopic children (n=4620) who were sensitized to ≥1 allergens (specific IgE ≥0.35 kIU_A/L). Upper and lower centiles were determined and cut-off values calculated using receiver operating characteristic (ROC) analysis.

Results: Serum total IgE increased with age in both groups, although at a variable level and rate, and reached a plateau at 9 and 10 years in non-atopic and atopic children, respectively. Atopic children had on average 14-fold higher serum total IgE compared to non-atopic children. In both groups, the median was lower than the corresponding mean and the distribution skewness was always positive (group I, 0.87; group II, 0.91). In almost all age groups, the 95th percentile for non-atopic children corresponded to the calculated cut-off values, whereas the 10th percentile for atopic children corresponded to the respective cut-off values only until the age of 8 years, after which greater differences between the cut-off values and the 10th percentile were recorded. Cut-off values for total serum IgE in children up to 16 years were determined with diagnostic sensitivity, specificity and area under the ROC curve of 96%, 91% and 0.950, respectively.

Conclusions: The 95th percentile for total IgE in non-atopic children and the 10th percentile in atopic children could be taken as cut-off values in children up to 8 years of age, after which significant percentile

discrepancies between non-atopic and atopic children were recorded. Since atopic subjects show a more irregular centile distribution, cut-off values are best determined by ROC analysis.

Keywords: child; cut-off values; hypersensitivity; immunoglobulin E (IgE); receiver operating characteristic (ROC) curve.

Introduction

Determination of the total immunoglobulin E (IgE) concentration in serum is a simple and automated diagnostic procedure included in the algorithm for the detection of IgE-mediated allergic diseases in children (1). Unless a laboratory has determined its own reference values, the so-called “normal values” or “reference values” provided by reagent manufacturers or reported by other authors are generally used in interpreting total serum IgE concentrations in childhood (2, 3). Normal values determined by manufacturers vary according to the sensitivity and specificity of the method employed and the population tested.

Serum IgE concentration is influenced by genetic and environmental factors (allergen exposure, exposure to parasites, etc.) (4–8). It is widely held that the Western lifestyle has imposed a plateauing relationship between atopy and low-grade allergen exposure on the one hand, and increased IgE-mediated allergic respiratory disease in childhood on the other hand (6, 9). A recent attempt to set the atopy/allergy record straight has stated that both atopy and atopic allergy need to be proved by allergen-specific IgE (10). In practice, this means that the gold standard for separating atopic from non-atopic individuals may be reduced to the determination of specific IgE. Subjects defined as atopic/non-atopic by positive/negative findings for specific IgE may then be investigated and compared with regard to their respective differences in genetic, laboratory and clinical complexities of atopic hypersensitivity. Therefore, investigation of a variable such as serum total IgE in the context of IgE-sensitized and IgE-non-sensitized populations is justified.

It is quite difficult to collect authentic data on normal subjects or subjects of the general population to determine reliable ranges for the normal population. Because of the numerous problems in defining normal values for the young population in general, including serum IgE (11), we wanted to determine the reference range for total serum IgE in non-atopic children (IgE-non-sensitized children) compared to atopic children (IgE-sensitized children). In addition, we examined the usefulness of calculated cut-off values

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for serum total IgE in predicting sensitization to common allergens in children with prolonged respiratory symptoms.

Subjects and methods

Subjects

Study subjects were a cross-sectional cohort of children aged 1 month to 16 years, stratified by age, referred by primary care physicians to the Pediatric Hospital Srebrnjak in Zagreb between August 2001 and December 2004 for evaluation of upper and lower respiratory tract symptoms of >4-week duration. Diagnostic procedures were in accordance with the ethical standards of the Hospital Ethics Committee. Standard work-up included history, physical examination, pulmonary function testing (in children aged >5 years), complete blood count, C-reactive protein, immunoglobulins, skin prick test, and total and specific IgE. Prick testing included a panel of standard inhaled and food allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, animal dander, grass and cereal pollen, weed, giant ragweed and tree pollen, and cow's milk protein and egg white). If the skin prick test showed a weal reaction greater than the saline control, specific IgE determination for that specific allergen was undertaken. In children <4 years of age who were negative for skin prick tests, specific IgE was still determined if first-degree relatives had atopy or if the child showed clinical evidence of eczema, wheezing or allergic rhinoconjunctivitis. In these cases the minimum panel included *Dermatophagoides pteronyssinus*, *Dactylis glomerata*, *Ambrosia artemisiifolia*, *Corylus avellana*, *Betula verrucosa*, cat dander, egg white and cow's milk. The panel was expanded as needed to reflect clinical suspicions for individual patients. Atopy was considered as the genetic tendency to develop allergic disorders such as allergic rhinitis, asthma, and atopic dermatitis, which are mediated by increased IgE response to environmental allergens. For analysis, subjects were separated into two groups:

- Non-atopic children, who were IgE-non-sensitized (n=3355) and with undetectable allergen-specific IgE (<0.35 kIU_A/L, i.e., class 0); and
- Atopic children, who were IgE-sensitized (n=4620) with a measurable level of allergen-specific IgE (≥0.35 kIU_A/L, i.e., classes 1–6).

Both groups were stratified by sex and age into 1-year age groups, from <1 year to up to 16 years of age. Non-atopic children were selected for determination of serum total IgE reference values.

Serum samples

Blood samples were obtained by venipuncture and serum was separated after 10-min centrifugation. Serum was stored at +4°C until analysis, which was within 3 days.

Total IgE determination

The concentration of total IgE was determined by the micro-particle enzyme immunoassay (MEIA) method and reagents (Abbott, Abbott Park, IL, USA) using mouse monoclonal anti-IgE antibodies. Measurement of fluorescence was on an IMx autoanalyzer (Abbott). Analysis is based on the sandwich technique and antigen-antibody complex labeling (12). The method sensitivity is 0.048 kIU/L (data from package insert).

Specific IgE determination

The concentration of allergen-specific IgE was measured by the UniCAP method, a second-generation in vitro method (Pharmacia, Uppsala, Sweden) in which high sensitivity is achieved by use of a three-dimensional cellulose carrier (13), on a selective UniCAP 100 autoanalyzer (Pharmacia-LKB-Kabi, Uppsala, Sweden). The confidence interval is from 0.35 (class 1) to 100 kIU_A/L (class 5). Class 0 is the default for values up to 0.35 kIU_A/L, and class 6 is the default for values over 100 kIU_A/L. Calibrators for determination of total and specific IgE were calibrated according to the World Health Organization Second International Reference Preparation for Human IgE (WHO 2nd IRP 75/502).

Statistics

Data were stored using Microsoft Excel 2000 software (Microsoft, Redmond, WA, USA). MedCalc statistical software was used for statistical analysis (14). Frequency distribution tables were used for description of qualitative variables, whereas quantitative variables were described by the arithmetic mean ($\bar{x}$), standard deviation (SD), median (M), minimum (min) and maximum (max) values. For calculation of the standard deviation in atopic children, serum total IgE concentrations exceeding 4000 kIU/L (29 children) were not considered. The significance of age and sex differences for the concentration of total IgE was tested by the Wilcoxon non-parametric test, with the level of significance set at $p < 0.05$. Since the IgE distribution is not normal, calculating the mean is misleading and statistical tests based on a normal distribution would give meaningless answers. Normalization (standardizing the data by introducing a common index) of data is required to plot the results as a normal distribution. Hence, primary IgE concentrations [IgE] were normalized according to the following formula:

$$Z_{age,i} = \frac{[IgE]_{age,i} - [IgE]_{age}}{SD_{age}} \quad [1]$$

Skewness was calculated according to the following equation:

$$Skewness = \frac{N}{(N-1)(N-2)} \sum_i Z_{age,i}^3 \quad [2]$$

Since the distribution of IgE is asymmetric, upper percentiles in non-atopic children and lower percentiles in atopic children were determined to compare values of total serum IgE between non-atopic and atopic children. Performance characteristics were calculated for the cut-off value, the optimal decision point on a receiver operating characteristic (ROC) curve. ROC analysis was used to optimize the cut-off value for total serum IgE with regard to the number of true positives against the false positive rate for different possible cut-off points. The cut-off value minimizes the sum of false positive and false negative results. ROC analysis allows assignment of the diagnostic power for determination of total serum IgE in discriminating atopic from non-atopic children. The ROC curve (which implies sensitivity and specificity) was used to compare the accuracy of total serum IgE determination: larger area under the curve (AUC) indicates greater accuracy.

Results

Subject comparison

For non-atopic children the male/female distribution was 1667 (49.7%) males and 1688 (50.3%) females. The greatest number of subjects was in the 1–3-year age groups, and the lowest in the 13–15-year age groups. Male children predominated in groups ≤ 7 years and female children in the 12–16-year groups (Table 1). The prick test was negative in 3087 out of 3355 children (92%). Of subjects with undetectable specific IgE, 8% (268/3355) had a positive skin prick to 1 or 2 allergens (mainly cow milk protein, egg white and *Dermatophagoides pteronyssinus*).

For atopic children the male/female distribution was 2931 (63.4%) males and 1689 (36.6%) females. The greatest number of subjects was in the 3–8-year age groups, and the lowest in the youngest age groups (< 2 years). Male children predominated in almost all age groups and female children in the 13-year age group (Table 1). The prick test was positive in 94% of children (4343/4620). Monosensitization was present in 67% and polysensitization in 33% of subjects. A negative prick test was found in 6% of children (277/4620).

IgE comparison

Serum total IgE increased with age in both groups, although at a variable level and rate (Table 2, Figure 1), until a plateau was reached (“saturation” behavior) at 9 and 10 years in non-atopic and atopic children, respectively. The increase in total IgE (mean and median) in non-atopic children showed a non-linear trend until the age of 9 years: a mean annual IgE index of increase of 1.30 was recorded during the first 3 years of life, whereas in the next 6 years (from the 4th to the 9th year) the increase was less pronounced (index of increase varied from 1.03 to 1.19). A statistically significant difference ($p < 0.05$) compared to the

preceding age group was confirmed in the 1st, 2nd, 3rd, 4th, 6th and 9th years. After the age of 9 years, serum concentrations of IgE showed no major changes ($p > 0.05$; Table 2).

Atopic children had higher serum total IgE compared to non-atopic children (arithmetic mean 14-fold and median 8.7-fold higher). The increase in total IgE also followed “saturation” behavior, but at a variable level and rate compared to non-atopic children (Table 2, Figure 1). The increase in total IgE (mean and median) showed a slightly non-linear trend until it reached a plateau at 10 and 8 years for the mean and median, respectively. The greatest increase in mean total IgE was observed in the 2nd (1.85 index of increase vs. 1 year, $p < 0.05$) and 4th years (1.40 index of increase vs. 3 years, $p < 0.05$). From the age of 11 years on, mean IgE no longer increased, but showed a gradual decreasing trend (Table 2). In atopic children, the lowest coefficient of variation (CV) was 105%. CV varied to a greater extent during the first 2 years of life (139–184%). In non-atopic children younger than 11 years, the CV was $\leq 60\%$, while in children ≥ 11 years it was $> 60\%$.

The index of increase in IgE concentration followed a similar trend in both groups (Table 2, Figure 2) with a stable decreasing tendency, except in the first 3–4 years and in the 15th year, when greatest fluctuations of relative IgE increases were recorded.

In both groups, the median was lower than the corresponding mean: the mean/median ratio in non-atopic and atopic children was 1.11 and 1.79, respectively (Table 2, Figure 1).

There were no sex differences for IgE concentration in non-atopic children, with the exception of children aged 6 and 11 years: in 6-year-old boys the mean serum IgE concentration was 20.7% (median 51.7% higher) higher than that measured in age-matched girls (mean 36.8 vs. 30.5 kIU/L; t-test, $p = 0.0213$; Wilcoxon test, $p = 0.005$; median 35.8 vs. 23.6 kIU/L). In 11-year-old boys mean IgE was 26.5% higher than

Table 1 Age and sex distribution of non-atopic and atopic children.

Age, years	Non-atopic, n			Atopic, n		
	Male	Female	All	Male	Female	All
< 1	85	58	143	35	20	55
1	200	177	377	99	37	136
2	194	178	372	178	73	251
3	159	144	303	246	123	369
4	125	146	271	249	118	367
5	127	117	244	250	129	379
6	117	99	216	303	151	454
7	111	95	206	239	149	388
8	81	94	175	231	136	367
9	86	84	170	175	119	294
10	78	64	142	199	106	305
11	75	72	147	172	101	273
12	54	92	146	164	101	265
13	50	57	107	101	102	203
14	44	67	111	100	91	191
15	39	63	102	89	53	142
16	42	81	123	101	80	181
Total	1667	1688	3355	2931	1689	4620

Table 2 Total serum IgE in non-atopic and atopic children.

Age, years	$\bar{x} \pm \text{SD}$, kIU/L	Index of increase for mean (1)	CV, %	Median, kIU/L	Index of increase for median (1)	Min–Max, kIU/L
Non-atopic						
<1	11.5 $\pm$ 5.0	–	43	10.2	–	1.2–37.6
1	15.3 $\pm$ 8.1 *	1.33	53	13.4	1.31	0.6–57.8
2	18.6 $\pm$ 10.4 *	1.22	56	16.8	1.25	1.8–68.6
3	25.4 $\pm$ 12.8 *	1.37	50	23.6	1.40	2.2–90.6
4	28.2 $\pm$ 15.0 *	1.11	53	25.4	1.08	1.0–95.4
5	29.8 $\pm$ 17.5	1.06	59	26.8	1.06	4.2–98.2
6	33.9 $\pm$ 20.2 *	1.14	60	30.0	1.12	1.4–97.2
7	36.6 $\pm$ 21.0	1.08	57	33.4	1.11	3.2–99.2
8	37.8 $\pm$ 21.1	1.03	56	37.7	1.13	4.8–98.4
9	44.8 $\pm$ 25.1 *	1.19	56	42.9	1.14	4.8–98.4
10	43.9 $\pm$ 24.9	0.98	57	38.8	0.90	3.6–102.6
11	43.7 $\pm$ 27.9	1.00	64	36.6	0.94	1.8–123.8
12	42.6 $\pm$ 27.8	0.97	65	34.7	0.95	1.0–174.9
13	39.5 $\pm$ 26.6	0.93	67	35.0	1.01	2.0–153.0
14	42.4 $\pm$ 26.2	1.07	62	36.4	1.04	5.4–112.8
15	48.3 $\pm$ 29.5	1.14	61	48.9	1.34	6.8–118.0
16	44.2 $\pm$ 26.7	0.92	60	39.2	0.80	5.6–110.0
Atopic						
<1	197.5 $\pm$ 362.6	–	184	73.2	–	13.2–2136.4
1	209.8 $\pm$ 292.0	1.06	139	113.6	1.55	11.0–1682.8
2	388.5 $\pm$ 558.9 *	1.85	144	170.4	1.50	12.6–> 4000
3	359.0 $\pm$ 395.6	0.92	110	211.0	1.24	16.0–> 4000
4	502.4 $\pm$ 641.3 *	1.40	128	255.2	1.21	3.8–> 4000
5	488.3 $\pm$ 611.0	0.97	125	267.6	1.05	21.0–> 4000
6	482.0 $\pm$ 581.2	0.99	121	289.4	1.08	9.2–> 4000
7	483.3 $\pm$ 549.6	1.00	114	291.0	1.00	19.4–> 4000
8	544.8 $\pm$ 590.9	1.13	108	334.0	1.15	20.8–> 4000
9	539.3 $\pm$ 565.4	0.99	105	321.3	0.96	31.8–> 4000
10	572.6 $\pm$ 619.7	1.06	108	340.0	1.06	20.8–> 4000
11	555.9 $\pm$ 629.8	0.97	113	317.0	0.93	40.2–3674.2
12	538.0 $\pm$ 607.5	0.97	113	346.6	1.09	24.8–> 4000
13	503.8 $\pm$ 547.2	0.94	109	324.6	0.94	36.4–> 4000
14	470.2 $\pm$ 593.0	0.93	126	272.2	0.84	3.0–> 4000
15	488.6 $\pm$ 546.1	1.04	112	297.2	1.09	37.1–3226.6
16	519.6 $\pm$ 546.9	1.06	105	340.2	1.14	69.4–> 4000

$\bar{x}$, arithmetic mean; SD, standard deviation; CV, coefficient of variation; Min, minimum value; Max, maximum value. *Statistically significant difference vs. preceding age group, $p < 0.05$.

in age-matched girls (48.7 vs. 38.5 kIU/L; t -test, $p = 0.0134$; Wilcoxon test, $p = 0.0119$; median 44.2 vs. 32.3 kIU/L). An inverse pattern was observed after the age of 12 years, when female children showed a tendency to higher IgE concentrations than male children; however, the differences did not reach statistical significance. There was no statistically significant sex difference for total serum IgE in atopic children.

For each of the two groups, as well as for each age subgroup, the distribution skewness was calculated using the normalized data obtained by Eq. [1]. The distribution of children according to IgE concentration was asymmetric: skewness was always positive (group I, 0.87; group II, 0.91). These findings clearly indicate systematic distributions tailing towards higher values in both groups of children. Log-normal transformation of the same data, as often presumed in immunochemistry (results not shown here) did not correct the distribution asymmetry. Owing to the asymmetrical distribution of the IgE concentrations, centiles were used. These gave a satisfactory description of data up to the age of 9 years, but not there-

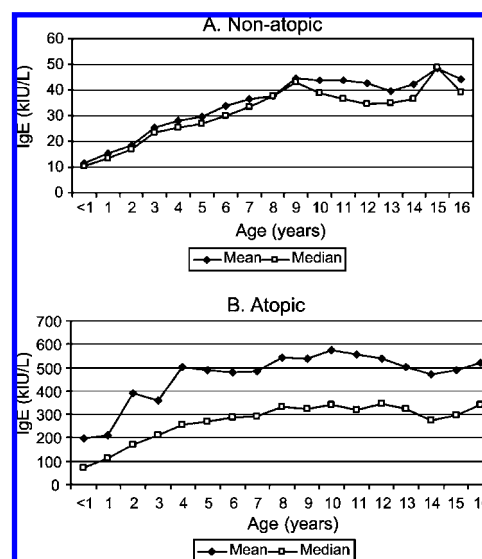


Figure 1 Concentration of serum total IgE in (A) non-atopic and (B) atopic children. Mean and median are presented, respectively.

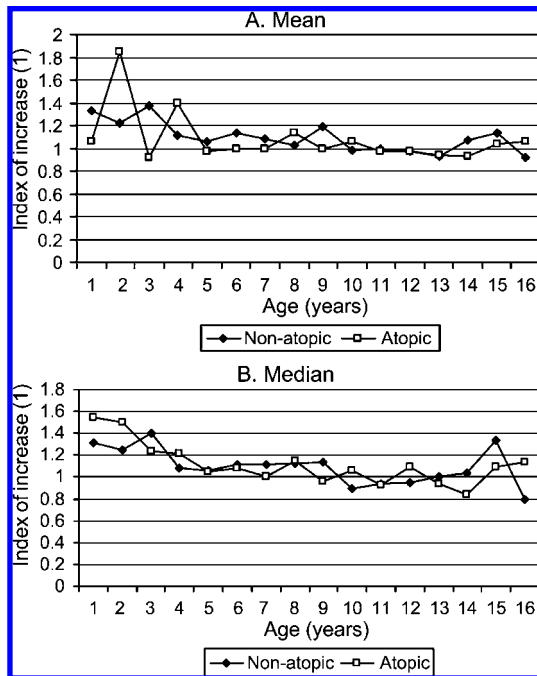


Figure 2 Relative increase of total IgE in non-atopic and atopic children: (A) mean and (B) median.

after. Therefore, for definite optimization of cut-off values, ROC analysis was implemented.

There was an overlap between the maximum IgE values in non-atopic children and the minimum IgE values in atopic children, as observed from the minimum-maximum interval (Table 2) and centile values (Table 3, Figure 3).

The 10th percentile for atopic children corresponded to the 95th percentile for non-atopic children and the 90th percentile up to the age of 8 years (except in the 2–3-year age groups), but from 9 years on the dis-

crepancy was wider. The cut-off values are closer to the 95th percentile for non-atopic children than to the 10th percentile for atopic children (Figure 3). In almost all age groups, the 95th percentile for non-atopic children corresponded to the cut-off values obtained, whereas the 10th percentile for atopic children corresponded to the respective cut-off values only up to the age of 8 years, after which greater differences between the cut-off values and the 10th percentile were recorded.

Diagnostic efficiency

The AUC of 0.950–0.997 (Table 4) shows that serum total IgE has excellent discriminatory power between non-atopic and atopic subjects. The cut-off value that best separates non-atopic and atopic children is determined on the basis of mutually maximized sensitivity and specificity. The sensitivity and specificity for particular optimal decision points (cut-offs) exceeded 80%. Cut-off values for total serum IgE in children up to 16 years were determined with average diagnostic sensitivity and specificity of 96% and 91%, respectively. In younger age groups, the selected cut-off values were associated with higher specificity, mostly >90% up to the age of 8 years. An exception is specificity of 87.6% for the cut-off value of 76.4 kIU/L in 7-year olds. In older age groups the selected cut-off values were associated with higher sensitivity, mostly >98% from the 9-year-old group on. An exception occurs in the 14-year-old group (94.2 kIU/L). These results suggest that cut-off values in children ≤8 years better define non-atopic children, whilst the same is true for atopic children in groups >8 years old.

The negative predictive value (NPV) of 77.5–100% was generally slightly better than the positive predictive value (61.3–98.2%), indicating that there may

Table 3 Upper (90–97.5th) and lower (2.5–10th) percentiles for total serum IgE in children with specific IgE <0.35 kIU_A/L (non-atopic) and ≥0.35 kIU_A/L (atopic), respectively.

Age, years	Non-atopic			Atopic		
	Percentile			Percentile		
	90th	95th	97.5th	2.5th	5th	10th
< 1	19.6	23.5	25.8	19.4	22.2	25.0
1	26.1	30.4	34.8	20.0	23.5	29.0
2	32.8	37.6	45.8	23.7	31.1	48.9
3	42.4	51.2	56.3	37.4	46.5	64.5
4	46.6	55.9	63.9	35.5	41.7	55.6
5	52.6	60.9	75.8	34.0	41.1	60.5
6	65.3	70.7	76.5	41.9	55.4	73.3
7	67.4	76.2	81.6	39.3	52.1	74.5
8	68.7	75.7	84.9	42.5	64.1	81.4
9	82.9	88.4	93.7	44.3	57.8	80.1
10	82.7	85.1	92.3	43.0	58.5	74.7
11	89.2	97.3	101.8	51.6	68.7	86.3
12	84.2	96.7	101.3	40.4	62.3	89.0
13	79.0	87.6	96.5	55.2	64.1	80.5
14	83.1	91.1	98.3	45.7	58.6	70.8
15	90.3	97.6	105.0	51.3	56.6	76.2
16	83.3	90.7	98.0	99.1	107.0	122.0

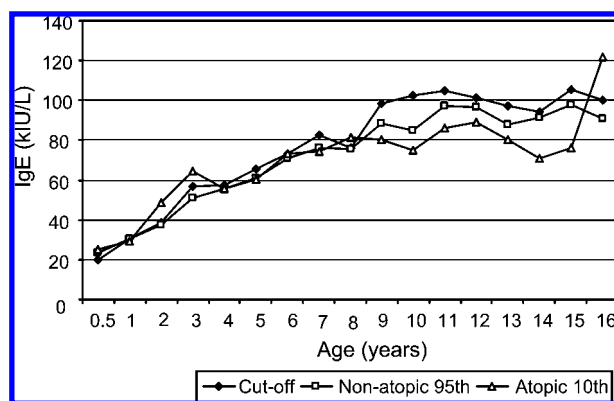


Figure 3 Comparison of total serum IgE cut-off values with the 95th and 10th percentile in non-atopic and atopic children, respectively: ◆ cut-off values with the best diagnostic power (i.e., with the highest sensitivity and specificity); □ 95th percentiles for total IgE in non-atopic children; △ 10th percentile for total IgE in atopic children.

have been more false positive than false negative findings at the respective cut-off points. The probability that atopy is not present when the test is negative is stronger than the probability that atopy is present when the test is positive. In the 9-year age group, an NPV of 100% indicated that in all children with total IgE <98.4 kIU/L atopy is not present.

It can generally be stated that 5% of non-atopic children had serum total IgE above the cut-off point, while 10% of atopic children had serum total IgE within the reference range (in children up to 8 years). The 95th percentile for total IgE in non-atopic children and the 10th percentile for total IgE in atopic children could be taken as cut-off values in children up to 8 years, after which significant percentile discrepancies between non-atopic and atopic children were recorded. Beyond that age, atopic subjects showed a much more irregular distribution of the lower centile values.

Discussion

Serum IgE concentration (influenced by genetic and environmental factors) is normally very low, accounting for only 0.001% of all immunoglobulin isotypes in healthy individuals (15). Our results support the general notion that serum concentrations of total IgE increase with age (2, 16). A progressive increase in serum total IgE during the first decade of life in both groups of subjects points to a common maturation process. Genetic background, contact with allergens and environmental factors (pre- and post-natal) contribute to the final expression of atopic disease. Since genetic factors may not have changed significantly during the last few decades (several generations are required), it is likely that environmental factors have a critical influence on increased prevalence of atopic disease over this period of time (4, 5, 8).

The extent to which this increase is due to the impact of various environmental factors on IgE synthesis depends on the reference group of subjects selected. The results of this study point to a difference in the index of IgE increase in IgE-non-sensitized compared to IgE-sensitized children. In both groups, total IgE concentrations increased continuously from 1 to 9 years of age, indicating a similar trend. The increase in total IgE (mean and median) in non-atopic children followed a slightly non-linear trend until it reached a plateau at 9 years of age ("saturation" behavior). This increase was quite pronounced during the first 3 years of life, showing a mean annual increase of 30% (index of increase 1.30), after which it gradually slowed down to a 10.4% annual increase for 4–9 years and no major changes after the age of 9 years. A statistically significant difference ($p < 0.05$) compared to the preceding age group was confirmed in the 1st, 2nd, 3rd, 4th, 6th and 9th years of life. The increase in total IgE in atopic children also showed "saturation" behavior (plateauing at 10 years of age), but at a variable level and rate compared to non-atopic chil-

Table 4 ROC analysis of total serum IgE in non-atopic and atopic children.

Age, years	AUC	Cut-off, kIU/L	Sensitivity, %	Specificity, %	PPV, %	NPV, %
< 1	0.975	20.2	95.0	91.5	61.3	99.2
1	0.974	30.4	89.6	95.2	87.0	96.2
2	0.983	38.6	95.7	94.0	95.9	93.7
3	0.987	57.0	99.0	92.7	91.8	99.1
4	0.972	57.2	89.6	97.1	97.6	87.5
5	0.974	65.4	89.2	96.7	97.7	85.2
6	0.975	73.0	95.8	90.5	82.7	97.8
7	0.971	82.6	98.5	87.6	80.7	99.1
8	0.977	76.4	96.0	92.6	86.3	98.0
9	0.962	98.4	100.0	84.4	78.5	100.0
10	0.959	102.6	99.3	84.2	75.0	99.6
11	0.974	104.8	99.3	87.5	81.2	99.6
12	0.968	101.4	98.0	86.8	80.7	98.7
13	0.979	97.4	98.1	86.2	78.6	98.9
14	0.960	94.2	83.8	97.3	98.2	77.5
15	0.950	105.2	98.1	81.7	79.7	98.3
16	0.997	100.0	98.3	97.2	95.9	98.9

AUC, area under the ROC curve. Sensitivity, probability that a test result will be positive when atopy is present (true positive rate). Specificity, probability that a test result will be negative when atopy is not present (true negative rate). PPV, probability that atopy is present when the test is positive. NPV, probability that atopy is not present when the test is negative

dren. This increase was rather discontinuous, showing sharp increments in the age groups 1–2, 3–4 and 7–8 years, with little further change after 11 years of age. A statistically significant difference ($p < 0.05$) compared to the preceding age group was confirmed in the 2nd and 4th years of life. Although absolutely greater in atopic children, total IgE showed a greater relative increase with age in the non-atopic than in the atopic group (3.9- vs. 2.7-fold increase on average). It should be noted that values $> 4000 \text{ kIU}_A/\text{L}$ were not taken into consideration.

In both groups, the median was lower than the corresponding mean and the distribution skewness was always positive. Because of the asymmetry of IgE concentration distributions, centile values were preferred for comparison purposes. This proved satisfactory up to the age of 9 years, but was unsatisfactory thereafter. Overlap between the 95th percentile for total IgE in non-atopic children and the 10th percentile for total IgE in atopic children was observed up to 8 years of age. Thereafter a significant discrepancy in centile values between non-atopic and atopic children was recorded. This was the basis for introducing ROC analysis for final optimization of cut-off values. The selected cut-off values were associated with higher specificity in younger age groups and higher sensitivity in older age groups. This means that the calculated cut-off values function more reliably in defining non-atopic subjects of ≤ 8 years of age and in defining atopic subjects > 8 years of age. The skin prick test was negative in 92% of non-atopic children and 8% were positive for 1 or 2 allergens. The skin prick test was positive in 94% and negative in 6% of atopic children.

The reference ranges reported in the literature differ from study to study, primarily owing to the different reference populations included and the different immunochemistry methods used to determine total serum IgE concentrations. These have involved multicenter studies (17), occasionally, randomly selected groups of subjects (18–20), rarely, longitudinal studies (17, 21), and most frequently, children hospitalized for various reasons (2, 22) or, like our study subjects, referred for general respiratory and allergy testing (23). In Croatia, literature data on “normal” values of total IgE or ranges provided by reagent manufacturers have been used. Since the ranges for IgE concentrations vary, they cannot help in differentiating non-atopic and atopic children in our population. Absolute comparison of our data with those from other studies is impossible because of the different pediatric populations included and the different methods of IgE determination employed. Furthermore, none of the other studies included determination of total IgE concentration in children with unmeasurable concentrations of specific IgE ($< 0.35 \text{ kIU}_A/\text{L}$). For example, using the ImmunoCAP-FEIA method in children aged ≤ 16 years, Liappis et al. recorded significantly lower ranges than those obtained in our study (3). The reference values reported by Soldin et al. (children from Washington) could be compared with our results, because the same method was used to determine IgE

concentration in both studies (2). While the results in children below the age of 1 year were consistent, children aged > 1 year and adolescents included in our study had lower values than those in the study by Soldin et al. (2). In Danish children and adolescents aged 7–16 years who were free of allergy, greater ranges were determined by the PRIST method (18) than for age-matched children in our study. In this age group (7–16 years), the geometric mean IgE showed a decreasing trend: the youngest children (7 years) had the highest IgE levels and the oldest children (16 years) had the lowest IgE levels. In children from Athens aged 1–14 years (IgE determined by EIA, Pharmacia) (22) higher IgE ranges were also determined than for the children in our study. In the Greek study, the increase in IgE concentration in the first 5 years of life was 80% per year, which is significantly higher than the annual increase recorded in our study. In Greek children, the 90th percentiles for IgE showed an increasing trend until a plateau was reached at 5–6 years of age in non-atopic children only.

IgE concentration also showed sex differences, being higher in male than in female subjects for both adults (19) and children (2, 17, 23). A longitudinal study by Johnson et al. also pointed to higher IgE concentrations in male than in female children aged < 4 years (21). Soldin et al. reported that male children aged 1–3 years had higher IgE ranges than age-matched female children (2). A study from Germany also revealed higher IgE values in male children aged ≤ 6 years; however, this sex difference was found in non-atopic, but not in atopic children (17). Sex differences in IgE concentrations were demonstrated in our study only in non-atopic children aged 6 and 11 years ($p < 0.05$). At the age of 13 years, an inverse relation was observed, as females showed a tendency towards higher IgE concentrations than males ($p = 0.0521$). No sex difference for IgE concentrations was observed in atopic children.

Mean values for total IgE in children with positive allergy tests were 10- to 20-fold (median 6- to 10-fold) higher than those found in age-matched children with negative allergy tests. Hence, the medians were consistently lower than the arithmetic means, indicating a tendency for total IgE distribution towards higher values in both groups. The frequently assumed log-normal transformation of immunoglobulin concentration (18, 24) did not resolve this problem (data not presented). These values could be higher than expected just on the basis of the means and standard deviations. The distribution asymmetry observed has to be taken into consideration in determining the upper limits for normal IgE ranges. To date, we have been using literature data on “normal” values for total IgE concentration or ranges provided by reagent manufacturers. The present study showed that cut-off values obtained in non-atopic children aged > 9 years were generally below reference values obtained by the same method (2). Thus, if a laboratory defines its own reference intervals in non-atopic children, this should facilitate differentiation of atopic children from the same geographic region and of comparable socioeconomic status.

Borderline values that differentiate atopic from non-atopic children reported in the literature vary. Hansen et al. reported that total IgE values exceeding 63 kIU/L in cord blood indicate an increased age-specific IgE concentration (25). In their study of toddler-aged children with wheezing, Guilbert et al. demonstrated that children with hypersensitivity to nutritional allergens and aeroallergens had total IgE concentrations > 100 kIU/L (26). Sinclair and Peters found that children aged < 16 years with total IgE concentration < 10 kIU/L were generally free from increased allergen-specific IgE (27). A cut-off value of 138 kIU/L to differentiate between atopic and non-atopic Turkish children was of low sensitivity (57%) and specificity (67%) (28). According to the study of Lindeberg and Arroyave, cut-off values for total IgE in children aged 1–12 years were determined with average diagnostic sensitivity and specificity of 83% and 91%, respectively (29). The results of Kulig et al. (17) reflect the moderate capacity of total IgE to discriminate between non-atopic and atopic children: sensitivity, specificity, positive predictive value and AUC were 45%, 91%, 70% and 0.750, respectively (for a cut-off value of 100 kIU/L for children of 6 years of age). For comparison purposes, the respective values in the present study for the same age (6 years, cut-off 73 kIU/L) were 95.8%, 90.5%, 82.7% and 0.975. Cut-off values for IgE in children up to 16 years had mean diagnostic sensitivity and specificity of 96% and 91%, respectively. In younger age groups (< 8 years), the selected cut-off values were associated with higher specificity (great probability that total IgE is lower than the cut-off value in non-atopic children; more true negative results). In older age groups (≥ 9 years) the selected cut-off values were associated with higher sensitivity (great probability that total IgE is higher than the cut-off value in atopic children; more true positive results).

Our study showed that the 95th percentile for total IgE in non-atopic children and the 10th percentile in atopic children could be taken as cut-off values only in children up to 8 years of age, after which significant percentile discrepancies between non-atopic and atopic children were recorded. Since atopic subjects showed a much more irregular distribution of centile values, cut-off values are best determined using ROC analysis. Cut-off values obtained using ROC analysis may help the physician to interpret total IgE values more practically, i.e., to consider the probability of an allergic disease in a child with greater reliability.

References

1. Lewkowich IP, Rempel JD, HayGlass KT. In vivo IgE levels in exogenous antigen stimulated responses: measurement of total IgE as valid, simple surrogate for Ag-specific IgE. *J Immunol Methods* 2004;286:123–32.
2. Soldin SJ, Morales A, Albalos F, Lenherr S, Rifai N. Pediatric reference ranges on the Abbott IMx for FSH, LH, prolactin, TSH, T4, T3, free T4, free T3, T-uptake, IgE and ferritin. *Clin Biochem* 1995;28:603–6.
3. Liappis N, Schlebusch H, Niesen M. Reference values for IgE concentration in serum of children, method: Pharmacia CAP system – ImmunoCAP-FEIA. *Monatsschr Kinderheilkd* 1992;140:300–2.
4. Berciano FA, Crespo M, Bao CG, Alvarez FV. Serum levels of total IgE in non-allergic children. Influence of genetic and environmental factors. *Allergy* 1987;42:276–83.
5. Herz U, Petschow B. Perinatal events affecting the onset of allergic diseases. *Curr Drug Targets Inflamm Allergy* 2005;4:523–9.
6. Nickel R, Lau S, Niggemann B, Gruber C, von Mutius E, Illi S, et al. MAS Group. Messages from the German Multicentre Allergy Study. *Pediatr Allergy Immunol* 2002;13(Suppl 15):7–10.
7. Strachan DP, Wong HJ, Spector TD. Concordance and interrelationship of atopic diseases and markers of allergic sensitization among adult female twins. *Allergy Clin Immunol* 2001;108:901–7.
8. Salvatore S, Keymolen K, Hauser B, Vandenplas Y. Intervention during pregnancy and allergic disease in the offspring. *Pediatr Allergy Immunol* 2005;16:558–66.
9. Cullinan P, MacNeill SJ, Harris JM, Moffat S, White C, Mills P, et al. Early allergen exposure, skin prick responses, and atopic wheeze at age 5 in English children: a cohort study. *Thorax* 2004;59:855–61.
10. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;56:813–24.
11. Klink M, Cline MG, Halonen M, Burrows B. Problems in defining normal limits for serum IgE. *J Allergy Clin Immunol* 1990;85:440–4.
12. Voller A, Bartlerr A, Bidwell D. *Immunoassays for '80s*. Baltimore: University Press, 1981:508–12.
13. Paganelli R, Ansotegui IJ, Sastre J, Lange C-E, Roovers MH, De Groot H, et al. Specific IgE antibodies in the diagnosis of atopic disease. Clinical evaluation of a new in vitro test system, UniCAPTM, in six European allergy clinics. *Allergy* 1998;53:763–8.
14. Zar JH. *Biostatistical analysis*, 4th ed. London: Prentice-Hall, 1999.
15. Dolen WK. The diagnostic allergy laboratory. In: Rose NR, Hamilton RG, Detrick B, editors. *Manual of clinical laboratory immunology*, 6th ed. Washington, DC: ASM Press, 2002:883–90.
16. Lindberg RE, Arroyave C. Levels of IgE in serum from normal children and allergic children as measured by an enzyme immunoassay. *J Allergy Clin Immunol* 1986;78:614–8.
17. Kulig M, Tacke U, Forster J, Edenharter G, Bergmann R, Lau S, et al. MAS Study Group. Serum IgE levels during the first 6 years of life. *J Pediatr* 1995;134:453–8.
18. Backer V, Ulrik CS, Wendelboe D, Bach-Mortensen N, Hansen KK, Laursen EM, et al. Distribution of serum IgE in children and adolescents aged 7 to 16 years in Copenhagen, in relation to factors of importance. *Allergy* 1992;47:484–9.
19. Simoni M, Biavati P, Baldacci S, Carrozzi L, Pedreschi M, Di Pede F, et al. The Po River Delta epidemiological survey: reference values of total serum IgE levels in a normal population sample of north Italy (8–78 yrs). *Eur J Epidemiol* 2001;17:231–9.
20. Chan EY, McKenzie SA. Normative data for total serum immunoglobulin E measurements in children of three ethnicities. *Clin Exp Allergy* 2003;33:752–6.
21. Johnson CC, Peterson EL, Ownby DR. Gender differences in total and allergen-specific immunoglobulin E (IgE) concentrations in a population-based cohort from birth to age four years. *Am J Epidemiol* 1998;147:1145–52.

22. Petridou E, Kanariou M, Liatsis M, Spanou K, Revinthi K, Mandalenaki-Lambrou K, et al. Factors influencing serum immunoglobulin E levels in Greek children. *Allergy* 1995;50:210–4.
23. Malinowska E, Kaczmarek M. Total IgE levels in children under three years of age. *Med Sci Monit* 2002;8:113–8.
- ▶ 24. Kerkhof M, Dubois AE, Postma DS, Schouten JP, de Monchy JG. Role and interpretation of total serum IgE measurements in the diagnosis of allergic airway disease in adults. *Allergy* 2003;58:905–11.
25. Hansen LG, Halken S, Host A, Moller K, Osterballe O. Prediction of allergy from family history and cord blood IgE levels. A follow-up at the age of 5 years. Cord blood IgE. *Pediatr Allergy Immunol* 1993;4:34–40.
- ▶ 26. Guilbert WT, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, et al. Atopic characteristic of children with recurrent wheezing at high risk for the development of childhood asthma. *J Allergy Clin Immunol* 2004;114:1282–7.
- ▶ 27. Sinclair D, Peters SA. The predictive value of total serum IgE for a positive allergen specific IgE results. *J Clin Pathol* 2004;57:956–9.
- ▶ 28. Yilmaz N, Bayraktaroglu Z, Özaslan J. Efficiency of some in vitro allergy tests for evaluating atopy in children and adults. *Clin Chem Lab Med* 1999;37:981–5.
- ▶ 29. Lindberg RE, Arroyave C. Levels of IgE in serum from normal children and allergic children as measured by an enzyme immunoassay. *J Allergy Clin Immunol* 1986;78: 614–8.

Received August 31, 2005, accepted February 6, 2006