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Serum eosinophil cationic protein (ECP): reference values in healthy nonatopic children

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Background: Although serum ECP concentrations have been reported in normal children, there are currently no published upper cutoff reference limits for serum ECP in normal, nonatopic, nonasthmatic children aged 1–15 years.

Methods: We recruited 123 nonatopic, nonasthmatic normal children attending the Royal Belfast Hospital for Sick Children for elective surgery and measured serum ECP concentrations. The effects of age and exposure to environmental tobacco smoke (ETS) on the upper reference limits were studied by multiple regression and fractional polynomials.

Results: The median serum ECP concentration was 6.5 µg/l and the 95th and 97.5th percentiles were 18.8 and 19.9 µg/l. The median and 95th percentile did not vary with age. Exposure to ETS was not associated with altered serum ECP concentrations ($P=0.14$).

Conclusions: The 95th and 97.5th percentiles for serum ECP for normal, nonatopic, nonasthmatic children (aged 1–15 years) were 19 and 20 µg/l, respectively. Age and exposure to parental ETS did not significantly alter serum ECP concentrations or the normal upper reference limits. Our data provide cutoff upper reference limits for normal children for use of serum ECP in a clinical or research setting.

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Activated eosinophils release granular proteins such as eosinophil cationic protein (ECP), which can be measured in the serum (1). Increased levels of ECP seem to be related to the presence and activity of asthma and other atopic disorders (2–5). Serum ECP has been suggested as a useful marker for airway inflammation as it is known to correlate with both percentage of eosinophils and ECP in broncho-

alveolar lavage in asthmatic adults (6) and children (7). However, normal reference ranges for serum ECP in a large group of children without atopic disease over all ages up to 15 years are lacking. Reference levels have been reported from children under the age of 4 years, but these included atopic infants (8). Reference intervals have been reported in 68 nonatopic children aged 7–12 years (9). In addition, it is unclear what effect age has on serum ECP concentrations. Carlsen et al. reported that serum ECP was higher in infants less than 2 years old (8). However, another study with a small number of control children reported lower serum ECP values in children less than 2 years of age (10). Remes et al. found that elevated serum ECP concentrations became more common with increasing age at 7–12 years (9). In adulthood, age does not appear to influence serum ECP concentration (11). It has also been suggested that exposure to environmental tobacco smoke (ETS) be taken into account when applying serum ECP levels (8). In this paper, we report our age-related normal reference range for serum ECP concentrations in nonatopic, nonasthmatic children.

Material and methods

As part of an ongoing study of airways inflammation in childhood asthma, we recruited 123 control subjects, who were nonatopic, nonasthmatic children attending the Royal Belfast Hospital for Sick Children for an elective surgical procedure, for a noninflammatory condition. The majority of these surgical procedures comprised plastic surgery such as correction of prominent ears or ophthalmic surgery such as correction of squint. Venous blood was transferred on ice to the laboratory, where it was allowed to stand at room temperature (21°C) for 90 min before being centrifuged at 1500 g for 15 min. The serum was removed and stored at –70°C until analysis. Serum ECP was measured by a commercially available radioimmunoassay (Pharmacia Upjohn, Diagnostics, Milton Keynes, UK) (1). The manufacturer's detection limit for the assay is 2 µg/l. Values below this detection limit were recorded as 1.99 µg/l.

A physician recorded a respiratory and allergy history as previously described (11). A detailed history was taken so that children with symptoms in keeping with asthma or other respiratory disorders were excluded. In addition, children with evidence of other allergic disease (atopic eczema, hay fever, allergic conjunctivitis, or food allergy) or an elevated total serum IgE compared to age normal values (> 2 SD from age normal) were excluded. Children with first-degree relatives with definite atopic disease (eczema,

asthma, and rhinitis) were excluded. The children were regarded as being free from infection, with parents reporting no recent infection such as that of the upper respiratory tract within the previous 4 weeks. In each case, the anaesthetist regarded the child as being suitable for the elective surgical procedure. The parents were carefully questioned about their smoking habits and about any other persons who lived and smoked at the home. Exposure to cigarette smoke was recorded as Yes/No to any reported parental ETS and according to whether the mother, father, both parents, or neither smoked.

Statistical analysis

Descriptive statistics were used to calculate the median, interquartile range (IQR), upper 95th and 97.5th percentiles (with 95 % confidence intervals [CI]) for the group as a whole and for patients grouped by exposure to parental ETS. As serum ECP is not normally distributed but positively skewed and values below the detection limit were coded as 1.99 µg/l, we used the Mann–Whitney U test for comparisons of serum ECP concentrations grouped according to sex and exposure to parental ETS (Yes/No). The Kruskal–Wallis test was used for comparisons when cases were grouped according to exposure to ETS (maternal/paternal/both/neither).

Only four of the 123 samples were found to be below the detection limit and coded as having a serum ECP concentration of 1.99 µg/l; therefore, we logarithm (log) transformed the serum ECP concentrations and tested for normality by inspecting the normal plot and using the Shapiro–Wilk W test. The log serum ECP fulfilled the criteria for and conformed to normality (Shapiro–Wilk $W=0.99$, $P=0.1$). As there is no consensus for reporting reference intervals and for comparison with other publications, we also quote the antilogarithm of both the logarithmic mean serum ECP (geometric mean [GM]) and the reference limits produced by back-transforming the upper and lower limits of the mean ± 1.96 SD of the logarithm serum ECP (12). Simple linear regression and Pearson correlation were used to determine how log serum ECP was associated with and varied with age. A multiple linear regression model was used to determine whether age, sex (male/female), or reported exposure to parental ETS had statistically significant and independent effects on log serum ECP concentrations.

The limits of an age-specific reference interval are defined by curves. The gap between the upper and lower percentiles may change as the standard deviation changes with age.

Median and upper 95th percentiles were calculated as conditional on age with maximum likelihood and accounting for nonnormal skewness in the data. We used the software package Stata 5 to model each as a function of age with fractional polynomials and plotted the median and 95th percentiles against age (13).

Results

A total of 123 nonatopic children (70 male), with mean age of 6.9 years (range: 1.25–14.5 years), were studied. Twenty children were aged 1–3 years; 32, 3–6 years; 31, 6–9 years; and 34, 9–12 years; and six were older than 12 years. Neither parent smoked in 63 cases, both parents smoked in 22, mother only in 15, and father only in 16 (data available on 116 subjects only).

Table 1 summarizes the values for serum ECP and for those reportedly exposed and not exposed to parental ETS. No statistically significant differences were observed in the serum ECP between those exposed and not exposed to passive cigarette smoke ($P=0.14$). The 95th and 97.5th percentiles were similar (Table 1).

Table 1. Summary of statistics and calculated reference intervals for serum ECP concentrations

	All patients	Exposure to parental ETS†	
		No	Yes
Number	123	63	73
Age (years)			
Mean (SD)	6.9 (3.2)	6.7 (3.2)	7.1 (3.3)
Range	1.25–14.5	1.6–13.0	1.25–14.5
Sex (male/female)	70/53	38/25	29/24
Serum ECP (μg/l)			
Median	6.5	7.1	6.0
IQR	4.5–9.2	5.0–9.2	3.6–9.1
Range	1.99–21.1	1.99–20.0	1.99–21.1
95th percentile	18.8	18.9	18.3
(95% CI)	16.0–20.0	14.2–20.0*	11.3–21.1*
97.5th percentile	19.9	20.0	20.2
(95% CI)	18.1–21.1*	17.7–20.0*	16.2–21.1*
GM	6.4	6.8	5.8
(GM ± 1.96 SD)	2.1–20.0	2.3–20.2	1.8–18.9
Geometric mean (GM) ± 1.96 SD was obtained from mean ± 1.96 SD of logarithm serum eosinophil cationic protein (ECP) and back-transforming mean and each of upper and lower limits.			
*Upper 95% confidence intervals for 95th and 97.5th percentiles are held at maximum of sample. Interquartile range (IQR).			
†Data available on only 116 cases.			

In addition, there were no statistically significant differences in serum ECP ($P=0.5$) when cases were classified according to exposure to parental ETS (maternal/paternal/both/neither). Sex had no statistically significant influence on the log serum ECP ($P=0.8$, data not shown).

With simple linear regression, log serum ECP was not found to vary significantly with age (log serum ECP = $0.0001 \times \text{age [years]} + 1.8$, 95% confidence interval of the beta coefficient -0.32 to 0.032 , $P=0.99$, $r=0.0001$). Table 2 summarizes the regression coefficients when age, sex, and exposure to parental ETS were included simultaneously in the regression equation. Fig. 1 plots the median and upper 95th percentiles against age and shows that the two percentiles run parallel and horizontal and do not vary with age.

Discussion

Although the children that we studied were attending hospital, we believe that they are representative of the healthy non-hospital population in that they were attending hospital for a noninflammatory, non-neoplastic, elective operation, which was usually plastic or ophthalmic surgery. Therefore, we conclude that serum ECP concentrations of 19 and 20 μg/l could be employed as the 95th and 97.5th percentiles for normal, nonatopic children. This is slightly lower than the 95th percentile for nonasthmatic children aged 24–41 months quoted by Carlsen et al. of 24.1 μg/l, but their study included some atopic children (8). A further study with 68 healthy nonatopic children reported a value of 24.7 μg/l for their 97.5th percentile (9). The determination of serum ECP is fraught with methodological problems, in that the assay measures the ability of blood eosinophils to release ECP. Thus, the clotting time and temperature must be carefully controlled. In our study, these were absolutely standardized with samples standing at 21°C for 90 min

Table 2. Multiple regression analysis to examine effects of age, sex, and exposure to parental environmental tobacco smoke (ETS) on serum ECP concentrations

Log serum ECP	Beta coefficient	P value	95% CI
Age (years)	0.002	0.9	−0.03 to 0.035
Sex	−0.06	0.6	−0.27 to 0.16
Parental ETS	−0.15	0.16	−0.37 to 0.06
Constant	2.15	0.00	1.61 to 2.7
Multiple linear regression table of beta coefficients for logarithm (log) serum ECP against age (years) adjusted for simultaneous inclusion of sex (male/female) and exposure to parental ETS (yes/no).			

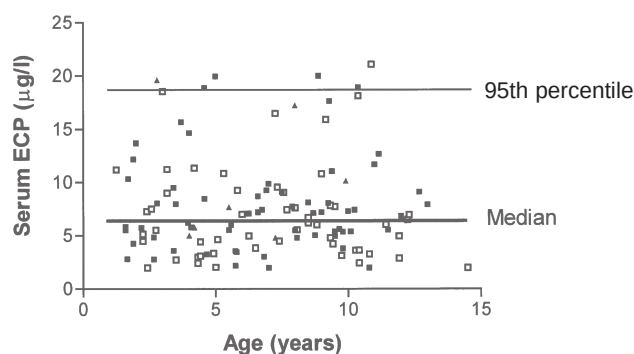


Figure 1. Serum ECP concentration vs age (untransformed data) showing median and 95th percentiles. □ Exposed to environmental tobacco smoke (ETS), ■ not exposed to ETS, ▲ unknown ETS exposure.

before centrifugation. Although other papers state that room temperature was used, the exact temperatures have not been given (8–10). A temperature even slightly above 21°C would also give rise to higher ECP concentrations. Allowing blood to clot for 90 min, as opposed to the 60 min used in some other studies, would potentially produce higher values of serum ECP concentrations (8–10). However, we found the opposite, and it is more likely that the slightly higher upper limits of serum ECP concentrations noted in the other studies were due to the inclusion of some atopic children as controls.

The effects of age, sex, and passive exposure to cigarette smoke on serum ECP concentrations have been investigated. From our data, age does not significantly affect the serum ECP concentrations for children aged 1–15 years. For children aged less than 4 years, Carlsen et al. (8) showed that there was a slight decline in serum ECP with increasing age. However, this study is not directly comparable with ours because they included atopic children and children under 1 year. By contrast, in the study of Ingram et al., serum ECP tended to increase with age in 59 nonasthmatic children (10). However, in their study, the control patients were attending for clinical conditions (rashes, gastrointestinal symptoms) that theoretically could elevate the serum ECP concentration, and the study also included some children who may have been atopic (elevated total IgE and positive RAST tests). The lack of variation in serum ECP with age in our study is in agreement with findings in adults that ECP concentrations do not vary with age (1). We found no influence of sex on serum ECP in children. In our study, we did not find an association between parental smoking (maternal, paternal, or both) and serum ECP. The study of Carlsen et al. reported a weak positive association with maternal, but not paternal, smoking and suggested that this factor should be taken into account (8). In their study, the

mean number of maternally smoked cigarettes was higher in the children with higher serum ECP levels. However, it is difficult to tell from their data whether maternal smoking was associated with a higher upper cutoff value. In our study, we did not record the numbers of cigarettes smoked daily by parents and have not validated the reported parental ETS by cotinine analysis. The group of children reportedly exposed to parental ETS had upper 95th and 97.5th percentiles that were similar to those of subjects not exposed. Our data revealed no evidence that this factor should be taken into account when considering what is the upper 95th or 97.5th percentile for “cutoff” values in normal children.

It has been suggested that a minimum sample size of 120 is required when reference intervals are calculated, that this number should apply to each subgroup, and that confidence limits be quoted for the reference limits (14). A limitation is that the upper 95% confidence intervals for the upper 95th and 97.5th percentiles are difficult to calculate accurately when smaller sample sizes are used in subgroup analysis (e.g., exposure to parental ETS). Ethically, it is difficult to justify taking blood samples from such large numbers of normal children. In our study, we analysed a minimum of 20 and usually over 30 cases in each of the age groups 1–3 years, 3–6 years, 6–9 years, and 9–12 years, and we consider this a reasonable age spread. We used fractional polynomials to compute the percentile curves rather than analysing the data in small age categories, as it is likely that any age-related changes will occur smoothly.

Reference intervals can be reported using either parametric or nonparametric analysis (12, 14). As our data were positively skewed, undetectable values coded as 1.99 µg/l, and we were primarily interested in the upper cutoff values, we have reported the median and the upper 95th and 97.5th percentiles. Since logarithm transforming serum ECP was successful in normalizing the data, we also quote the parametrically obtained upper reference intervals so that comparisons can be made with other published reports.

Serum ECP measurements may be of use in clinical practice. Although several studies have shown serum ECP to be significantly increased in atopic asthmatics compared to nonasthmatic subjects, the value of serum ECP as an aid to diagnosis is doubtful (9). This is because of its low specificity and sensitivity for diagnosing asthma (15). However, measuring serum ECP may be more useful for monitoring disease activity in asthma. Currently, we have no reliable means of assessing airway inflammation in the clinical setting, although many agree that this would be a useful adjunct for clinicians managing patients with asthma

(16). Serum ECP correlates with markers of airways inflammation in asthma and with other markers of disease activity such as symptom severity and blood eosinophil count. Normal upper reference limits are important if serum ECP is to be used as a “noninvasive” clinical test for monitoring disease activity in atopic conditions.

In conclusion, in this study, the 95th and 97.5th percentiles for serum ECP for normal nonatopic children (aged 1–15 years) were 19 and 20 µg/l, respectively. Age, sex, and exposure to parental ETS did not significantly affect

serum ECP concentrations or alter the upper normal reference limits.

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