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Anticardiolipin and anti-β₂ glycoprotein I antibodies in sera of 61 apparently healthy children at regular preventive visits

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Abstract

Objectives. To determine anticardiolipin (aCL) and anti-β₂ glycoprotein I antibodies (anti-β₂-GPI) in apparently healthy children and express the cut-off levels in concentrations of monoclonal antibodies, and to compare the mean values and frequencies of aCL and anti-β₂-GPI in children with those in blood donors.

Methods. Blood samples were collected from 29 preschool children and 32 adolescents during their routine preventive follow-up visits. The control group consisted of 52 blood donors. aCL and anti-β₂-GPI were assayed by an ELISA method. Two monoclonal β₂-GPI-dependent aCL (HCAL and EY2C9) were used as calibrators.

Results. The estimated cut-off values for immunoglobulin G (IgG) and immunoglobulin M (IgM) aCL, expressed in concentrations of monoclonal antibodies and standardized international units (GPL/MPL units), were 13.9 ng/ml (7.6 GPL) and 33.1 ng/ml (3.3 MPL) for preschool children, 13.5 ng/ml (7.2 GPL) and 36.9 ng/ml (4.0 MPL) for adolescents, and 14.4 ng/ml (8.0 GPL) and 42.6 ng/ml (5.1 MPL) for blood donors. No statistically significant differences in the mean values for IgG and IgM aCL were found between the age groups. The mean value of IgA aCL was significantly higher in blood donors than in preschool children and adolescents (P < 0.037 and P < 0.025 respectively). Seven (11.4%) of 61 apparently healthy children had low positive values for aCL (IgG for all seven). The estimated cut-off values for IgG and IgM anti-β₂-GPI were 4.2 and 13.1 ng/ml respectively for preschool children, 3.2 and 13.1 ng/ml for adolescents, and 2.9 and 20.5 ng/ml for blood donors. The mean value for IgG anti-β₂-GPI was found to be higher in preschool children than in adolescents and blood donors (P < 0.0001 and P < 0.0001). The mean values for IgM and IgA anti-β₂-GPI were higher in blood donors than in preschool children (IgM, P < 0.007; IgA, P < 0.0001) and adolescents (IgM, P < 0.01; IgA, P < 0.0001). Four (6.6%) of 61 apparently healthy children had positive values for anti-β₂-GPI (two for IgG and two for IgA).

Conclusions. This is the first report in which the cut-off values for aCL and anti-β₂-GPI in children are expressed in concentrations of monoclonal antibodies. Low titres of aCL, which were identified frequently in apparently healthy children, were hypothesized to be the result of previous infections. The high mean value of IgG anti-β₂-GPI observed in preschool children was an unexpected result of the study and might indicate a default response to nutritional exposure to β₂-GPI in this age group.

Key words: Antiphospholipid antibodies, Anticardiolipin antibodies, Anti-β₂ glycoprotein I antibodies, Healthy children.

Antiphospholipid antibodies (aPL) have been well recognized in patients with autoimmune diseases [1–3] as well as in association with various infections [4]. They have also been described, but less commonly, in individuals without any underlying disorders [5–7]. As with most other autoantibodies, an increased frequency of aPL has been reported in the healthy elderly population compared with healthy adults [5, 8]. As high levels of aPL in the elderly population are not usually associated with any clinical disease, it has been generally...
assumed that this autoimmune phenomenon might be a manifestation of age-related senescence of the immune system [5, 8].

In recent years, the clinical features of aPL have been recognized increasingly in children, and a growing number of reports have provided information on the frequency of aPL in childhood disorders [9, 10]. However, there are few data available on the normal values and the frequency of aPL in healthy children.

Among the best studied and clinically most widely accepted aPL are anticardiolipin (aCL) antibodies. Several studies have investigated aCL in childhood disorders, but in most cases the control group for the normal population was poorly defined. Some studies have used healthy adult individuals as the control group [11–13] while others have used apparently healthy children [14–20]. In most cases, the source of apparently healthy children was not stated clearly [15, 18], while sometimes sera from children hospitalized for minor surgical or psychosomatic problems were used [14, 16, 17, 19]. There were also substantial differences in the methods used for enzyme-linked immunosorbent assay (ELISA) and in the methods of statistical analysis used for defining cut-off points for immunoglobulin G (IgG) and immunoglobulin M (IgM) aCL. Consequently, no accurate information has been produced on normal ranges of IgG and IgM aCL in children and comparisons between studies have not been possible.

Recently, it has become clear that most autoimmune aPL are directed against β2 glycoprotein I (β2GPI) [21–23], and anti-β2GPI antibodies (anti-β2GPI) have been associated with the clinical features of the anti-phospholipid syndrome [24, 25]. Because aPL induced by infections do not require any plasma protein for binding to negatively charged phospholipids, the detection of anti-β2GPI might also be a useful marker for discriminating between presumably pathogenic autoimmune and post-infectious aPL [26, 27]. Furthermore, human and chimaeric monoclonal antibodies against β2GPI have been developed which could serve as the standard for aCL and anti-β2GPI ELISA [28, 29]. To our knowledge, no study has addressed the presence of anti-β2GPI in healthy children or has used concentrations of monoclonal antibodies to express aCL and anti-β2GPI values in children.

In our study, the values of aCL and anti-β2GPI isotypes in apparently healthy children were examined and the cut-off levels were expressed in standardized international units (GPL/MPL units) and concentrations of monoclonal antibodies. In addition, the mean values and frequencies of aCL and anti-β2GPI in children were compared with those in blood donors.

Patients and methods

Participants

Blood samples were collected from 61 apparently healthy children during their routine preventive follow-up visits in community health centres between March and July 1999. The children included in the study did not have clinical infections at the time of examination or any documented serious medical problems. On the basis of their age they were divided into two groups: group I consisted of preschool children (15 boys and 14 girls, mean age 5.0 yr, range 4.9–5.3) and group II consisted of adolescents (10 boys and 22 girls, mean age 13.5 yr, range 13.0–14.2).

Blood was drawn by clean venipuncture and collected into glass tubes. Informed consent for drawing extra blood at the time of routine venipuncture was obtained from the parents of all participating children. As the control, we used sera from 52 healthy blood donors (31 men and 21 women, mean age 34.0 yr, range 18.0–65.0). All sera were separated within 2 h after collection, aliquoted and stored at −30°C until assayed. The study was approved by the Ethics Committee of the Slovenian Ministry of Health.

Detection of aCL antibodies

aCL antibodies were detected by a slightly modified aCL ELISA, described previously [30–32]. Microtitre plates (Costar Medium Binding EIA/RIA plates; Costar, Cambridge, MA, USA) were coated with 40 μl/well of cardiolipin (Sigma, St Louis, MO, USA) in ethanol and allowed to evaporate at 4°C overnight. After incubation with 120 μl/well of 10% fetal bovine serum (Sigma) in phosphate-buffered saline, pH 7.4 (FBS–PBS) for 1 h at room temperature (22–26°C) the plates were washed once with 300 μl/well of PBS. Then 100 μl/well of standards and serum samples diluted 1:100 in 10% FBS–PBS were added in duplicate and the plates were incubated for 2.5 h at room temperature. The plates were washed four times with PBS, and 100 μl/well of alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA (ACSC, Westbury, NY, USA) diluted in 10% FBS–PBS was added. After 1 h of incubation at room temperature, the plates were washed four times with PBS and 100 μl/well of p-nitrophenyl phosphate (Sigma) dissolved at 1 mg/ml in 1 M diethanolamine buffer (pH 9.8) was added. Optical density at 405 nm (OD) was measured first after 10 min and then every 3 min by a microtitre plate reader Rainbow Spectra Thermo® (Tecan Austria GmbH, Grödig/Salzburg, Austria) vs a reagent blank until optimal fitting to predicted OD of standards was obtained. To prevent the influence of interassay variation, sera from all three groups were selected randomly for each run. The plate positioning of sera was also changed for each run to prevent possible edge effects affecting the final results.

Detection of anti-β2GPI antibodies

β2GPI was purified by the perchloric acid precipitation method using the same principles as described previously [33]. A modified anti-β2GPI ELISA was developed on the basis of a method described previously [34–36] and our preliminary results. Microtitre plates (Costar High Binding EIA/RIA plates) were coated with 50 μl/well of β2GPI dissolved at 10 μg/ml in PBS for 2 h at room temperature. After one wash with
200 μl of PBS containing 0.05% Tween 20 (PBS–Tween), 50 μl/well of standards and serum samples diluted 1:100 in PBS–Tween were applied in duplicate and incubated for 30 min at room temperature. After four washes, 50 μl/well of alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA (ACSC) diluted in PBS was added. After 30 min of incubation at room temperature and four washes, 100 μl of substrate was added. OD was measured as described for the aCL ELISA.

Monoclonal antibodies
Two monoclonal β2GPI-dependent aCL were used as calibrators: (i) the chimaeric IgG monoclonal antibody HCAL, consisting of human κ and γ1 constant regions and variable regions from the mouse monoclonal β2GPI-dependent aCL WBCAL-1 [29, 37]; and (ii) the IgM monoclonal antibody EY2C9, derived from a patient with antiphospholipid syndrome [28]. Monoclonal antibodies were aliquoted in amounts sufficient for one assay and frozen at −20°C. Monoclonal antibodies in appropriate concentrations were used to create reference curves. Calibrations according to original KAPS (Kingston Anti-phospholipid Antibody Study) standards [38, 39] and calibrations according to monoclonal antibodies were performed simultaneously in the same plates as the tested sera, to allow more accurate comparisons.

Statistical analysis
The distributions of OD and log OD were assessed by the use of histograms. Because of the skewed frequency distribution of OD in the aCL and anti-β2GPI ELISAs, log-transformed data were used in the statistical test procedures. Student’s t-test was used to compare the mean values and Fischer’s exact test was used where appropriate. The statistical significance of intergroup frequency rate was determined by χ2 analysis. All statistical analyses were performed using the program MS Excel 6.0 for Windows. Differences were considered statistically significant whenever \( P < 0.05 \).

Results

Distributions of aCL
The cumulative results of aCL assays in all of the participants studied are shown in Fig. 1. The frequency distributions of OD for IgG, IgM and IgA aCL isotypes in the three age groups demonstrated a non-symmetrical, positively skewed main peak with some outliers. For IgG aCL, the data were slightly more dispersed than for the other two isotypes. Additionally, in group II there was a trend towards a bimodal distribution of the OD for IgG aCL. To reduce the skewness of the main peaks, OD measurements were transformed to the log scale. The frequency distributions of log OD for aCL were nearly symmetrical, showing less skewness than the distributions for the untransformed measurements (Fig. 2).
Table 1. Estimated cut-off points for aCL antibodies in groups I, II and III

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
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<td>II</td>
<td>III</td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>95th percentile (OD)</td>
<td>0.114</td>
<td>0.100</td>
<td>0.133</td>
<td>0.064</td>
<td>0.071</td>
<td>0.084</td>
</tr>
<tr>
<td>Mean ± 2 S.D. (OD)</td>
<td>0.156</td>
<td>0.151</td>
<td>0.158</td>
<td>0.083</td>
<td>0.095</td>
<td>0.112</td>
</tr>
<tr>
<td>GPL/MPL units</td>
<td>7.6</td>
<td>7.2</td>
<td>8.0</td>
<td>3.3</td>
<td>4.0</td>
<td>5.1</td>
</tr>
<tr>
<td>mAb (ng/ml)</td>
<td>13.9</td>
<td>13.5</td>
<td>14.4</td>
<td>33.1</td>
<td>36.9</td>
<td>42.6</td>
</tr>
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</table>

mAb, concentration of monoclonal antibodies HCAL/EY2C9.

Fig. 3. (A) Relationships between mOD and concentrations of HCAL/EY2C9 in the aCL antibody assay. (B) Relationships between GPL/MPL units and concentrations of HCAL/EY2C9 in aCL antibody assay. The concentration of HCAL can be converted to GPL units with the formula GPL units = 0.978 × [concentration of HCAL (ng/ml)] − 5.542 (R² = 0.999), and the concentration of EY2C9 to MPL units with the formula MPL units = 0.012 × [concentration of EY2C9 (ng/ml)]^0.12 (R = 0.999).

Estimation of cut-off points and comparison of mean values for aCL between age groups

Because the frequency distributions for the log OD were nearly symmetrical, the cut-off points were defined as the mean value plus two standard deviations for the log OD values. Corresponding values for the OD were then computed by taking the anti-logarithm of the values for the log OD. The estimated cut-off points for aCL expressed as OD were transformed into GPL/MPL KAPS units and concentrations of monoclonal antibodies (Table 1). The relationships between the mOD (10⁻³ OD), GPL/MPL units and appropriate concentrations of HCAL/EY2C9 are presented in Fig. 3, the legend of which gives equations for converting the concentration of HCAL into GPL units and the concentration of EY2C9 into MPL units.

Mean aCL values obtained from the log-transformed data were also compared between different age groups. For IgG and IgM aCL there were no statistically significant differences in the mean values of antibodies between age groups. However, the mean value of IgA aCL was significantly higher in group III than in groups I and II (P < 0.037 and P < 0.025 respectively).

Frequency of elevated aCL

According to our cut-off points, a total of 7/61 (11.4%) apparently healthy children (groups I and II combined) had low positive values for aCL. They were all positive for only IgG aCL, and no child had elevated IgM or IgA aCL. In the blood donors, the overall frequency of aCL positivity was 9.6% (5/52); three (5.8%) aCL-positive donors had a low titre of IgG and two (3.8%) had a low titre of IgA aCL antibodies. The differences in frequency between apparently healthy children and blood donors were not statistically significant for either aCL isotype.

Distributions of anti-β₂GPI

The cumulative results of the anti-β₂GPI assays in all participants studied are shown in Fig. 4. The frequency distributions of OD for anti-β₂GPI were more uniform than for aCL. Each of the three isotypes of anti-β₂GPI demonstrated one asymmetrical, positively skewed main peak with some outliers, and the same pattern was observed in all three age groups. As with aCL, the frequency distributions of log OD for anti-β₂GPI were nearly symmetrical, showing less skewness than the distributions for untransformed measurements (Fig. 5).

Estimation of cut-off points and comparison of mean values for anti-β₂GPI between age groups

The cut-off points for anti-β₂GPI isotypes were determined by the same principle as for aCL. The estimated values were expressed as OD and transformed into concentrations of monoclonal antibodies (Table 2). The relationships between OD and concentrations of HCAL and EY2C9 are presented in Fig. 6.

Mean values for anti-β₂GPI were also compared between age groups. The mean value for IgG anti-β₂GPI
Table 2. Estimated cut-off points for anti-β2 GPI antibodies in groups I, II and III

<table>
<thead>
<tr>
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<th>IgG</th>
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<td>II</td>
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<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>95th percentile</td>
<td>0.050</td>
<td>0.041</td>
<td>0.037</td>
<td>0.059</td>
<td>0.057</td>
<td>0.071</td>
</tr>
<tr>
<td>Mean + 2 s.d. (OD)</td>
<td>0.068</td>
<td>0.052</td>
<td>0.044</td>
<td>0.059</td>
<td>0.061</td>
<td>0.092</td>
</tr>
<tr>
<td>mAb (ng/ml)</td>
<td>4.2</td>
<td>3.2</td>
<td>2.9</td>
<td>13.1</td>
<td>13.1</td>
<td>20.5</td>
</tr>
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</table>

mAb, concentration of monoclonal antibodies HCAL/EY2C9.

Fig. 4. Comparison of IgG, IgM and IgA anti-β2 GPI mOD values in preschool children (group I), adolescents (group II) and blood donors (group III); the data are means of results of two consecutive tests for each participant. The horizontal lines indicate means of pooled data for each age group.

was higher in group I than in groups II and III ($P < 0.0001$ and $P < 0.0001$ respectively). In contrast, the mean values for IgM and IgA anti-β2 GPI were higher in group III than in group I (IgM, $P < 0.007$; IgA, $P < 0.0001$) and group II (IgM, $P < 0.01$; IgA, $P < 0.0001$).

**Frequency of elevated anti-β2GPI**

A total of 4/61 (6.6%) apparently healthy children (groups I and II combined) had positive values for anti-β2 GPI. They were positive for either IgG (3.3%; 2/61) or IgA (3.3%; 2/61) anti-β2 GPI. The overall frequency of anti-β2 GPI-positive blood donors was 7.7% (4/52); positive donors exhibited either IgG (1.9%; 1/52) or IgA (5.8%; 3/52) anti-β2 GPI. As with aCL, the intergroup differences in frequency for all three anti-β2 GPI isotypes were not statistically significant.

**Comparison between aCL and anti-β2GPI assays**

Among seven apparently healthy children who were positive in the aCL assay, only one was also positive in the anti-β2 GPI assay; similarly, among five aCL-positive blood donors only one was also anti-β2 GPI-positive. We also found that, among four apparently healthy children who were positive in the anti-β2 GPI assay, only one was also positive for aCL. Similarly, among four anti-β2 GPI-positive blood donors only one was also positive in the aCL assay.

**Discussion**

In the present study, all serum samples were taken from apparently healthy children during their regular routine preventive visits in community health centres. Their health was assessed as part of a preventive visit before
blood was drawn, and those failing to pass this health test were excluded from the study. Our results therefore demonstrated unequivocally the presence of low levels of aCL and anti-β2GPI in sera of apparently healthy children.

To quantitate the risk associated with aCL in childhood disorders, it is essential to establish the normal range in healthy children. There has been considerable controversy over the most appropriate way of defining ‘normal’ and ‘abnormal’ levels of aCL.Gattorno et al. [18] considered an aCL test to be positive when the values were at least two standard deviations above the mean value for 25 healthy children. Siamopoulos-Mavridou et al. [15] considered the cut-off value to be the mean plus three standard deviations of the 66 normal sera obtained in children without rheumatic disease. In three studies performed in the same laboratory [14, 16, 17], the upper limits for aCL were set as the mean plus five standard deviations of 42 apparently healthy children who were hospitalized for minor surgical procedures or for bone marrow donation.

Our results demonstrate that, for aCL, the frequency distributions of OD in apparently healthy children were not normal but had a pronounced positive skew. Similar asymmetrical distributions of OD for aCL and for most other autoantibodies have been observed in the healthy adult population [6, 40]. For this reason, non-parametric tests were preferred, and these gave better estimates of normal ranges. Serra et al. [20] therefore used the percentile principle and set the cut-off point as the 95th percentile for 52 healthy children. However, to obtain a more accurate estimate of the 95th percentile requires a larger sample size than that used by Serra et al.

In this study, we used the method proposed by Verrier Jones et al. [40], in which OD measurements were transformed to the log scale, thus substantially reducing the skewness. Although not Gaussian, the frequency distribution of log-transformed data was nearly symmetrical and hence the cut-off point of the mean value plus two standard deviations could be used as the estimate of the 95th percentile. Our sample size (29 preschool children and 32 adolescents) seems a reasonable compromise to estimate the mean values and standard deviations accurately. Furthermore, such analysis of log-transformed data appears to be more appropriate than some of the non-parametric tests performed on a small number of untransformed data. However, in spite of the methodological differences discussed above, our results were consistent with the values obtained by Serra et al. [20], who defined normal aCL values in healthy children as being equal to or higher than 9 GPL and 4 MPL. To our knowledge, the present study was the first in which the cut-off values for IgG and IgM aCL in children were expressed in concentrations of monoclonal antibodies, which could easily be compared between different laboratories using the same detection method. Monoclonal antibodies for IgA aCL were not available; therefore the cut-off values for IgA aCL were expressed only in OD. Mean values of IgG and IgM aCL were comparable between different age groups and only the mean value of IgA aCL was found to be significantly higher in blood donors than in preschool children ($P < 0.037$) and adolescents ($P < 0.025$).

The frequency of low titres of aCL was high in apparently healthy children, and was similar to values obtained in healthy adults. Because aCL are also expressed frequently in various viral and bacterial infections [4] that are commonly acquired in childhood, we speculate that some positive aCL values in apparently healthy children could be the result of previous infections. This hypothesis is supported by the observation that six out of seven children positive in the aCL assay were negative in the anti-β2GPI assay.

We also established the cut-off values for IgG, IgM and IgA anti-β2GPI isotypes. Like those of aCL, the distributions of OD for anti-β2GPI isotypes were positively skewed, and transforming the results to the log scale substantially reduced the skewness. Therefore, the same statistical test procedures as those used for aCL were used to determine the cut-off values. The estimated cut-off values for IgG and IgM anti-β2GPI were expressed as HCAL and EY2C9 concentrations while the cut-off values for IgA anti-β2GPI were expressed only in OD for the same reasons as for aCL.

One of the most remarkable findings of the present study was that the mean value of IgG anti-β2GPI was highest in preschool children and in this group it was significantly higher than in adolescents ($P < 0.0001$) and blood donors ($P < 0.0001$). It appears, therefore, that the mean value of IgG anti-β2GPI decreases after the preschool period, in spite of the fact that the total IgG level increases gradually and progressively [41]. The relatively high mean value of IgG anti-β2GPI in preschool children might be related to several factors. First, it might be influenced by absorption of nutritional β2GPI at gastrointestinal surfaces and might therefore represent a possible default response to mucosal challenge in infants. It is well known that β2GPI has

![Figure 6](image-url)  
**Fig. 6.** Relationships between mOD and concentrations of HCAL and EY2C9 in the anti-β2GPI antibody assay. The concentration of HCAL can be converted to mOD with the formula mOD = 16.941 \times [\text{concentration of HCAL (ng/ml)}]^{0.955} (R^2 = 0.999)$, and the concentration of EY2C9 to mOD with the formula mOD = 5.592 \times [\text{concentration of EY2C9 (ng/ml)}]^{0.920} (R^2 = 0.999).
been remarkably conserved during the evolution of animal species [42], and thus it is possible that ingestion of bovine or some other type of β2-GPI found in different milk or meat products could act as a peroral immunization agent and induce transitory production of anti-β2-GPI in infants, in whom the intestinal mucosa is more permissive for large molecules [43, 44]. Secondly, some previous exposure to viral infection, together with nutritional exposure to β2-GPI, might have an adjuvant effect on antibody production. An opposite trend to that for IgG anti-β2-GPI was observed for IgM and IgA isotypes of anti-β2-GPI, for which significantly higher mean values were obtained in blood donors than in preschool children (IgM, \( P < 0.007; \) IgA, \( P < 0.0001 \)) and adolescents (IgM, \( P < 0.01; \) IgA, \( P < 0.0001 \)). These findings were in accordance with the increasing levels of total IgM and IgA after birth [41].

The frequency of anti-β2-GPI positivity in apparently healthy children was lower than that of aCL. However, among four anti-β2-GPI-positive children only one was simultaneously positive for both anti-β2-GPI and aCL. Three children clearly exhibited the presence of anti-β2-GPI without aCL, which was surprising and could indicate that anti-β2-GPI might be present without aCL in children. The explanation of this phenomenon could be that the subset of sera positive only for anti-β2-GPI contain antibodies directed to epitopes expressed on β2-GPI when attached to a high-binding microtitre plate but not when bound to cardiolipin [45]. In our study, the ELISA for anti-β2-GPI was performed without a blocking step because of the relatively frequent occurrence of antibodies recognizing the most commonly used blocking agents (bovine serum albumin, gelatin), giving high background readings. On the other hand, sample blank values obtained by our modification of the anti-β2-GPI ELISA were negligible (data not shown). In our opinion, no additional interference effects were introduced into the assay system by using only PBS and Tween.

To provide an additional illustration of the message of this article, we report preliminary data on aCL and anti-β2-GPI titres in children with juvenile idiopathic arthritis (unpublished data). According to our cut-off points, slightly or moderately elevated aCL antibody concentrations were found in 46.4% of 28 children with juvenile idiopathic arthritis, and the level was usually below 37 ng/ml HCAL (30 GPL) and 85 ng/ml EY2C9 (15 MPL). The frequency of anti-β2-GPI in our children with juvenile idiopathic arthritis was 10.7% and the quantitative expression of anti-β2-GPI was usually below 10 ng/ml HCAL and 20 ng/ml EY2C9.

In conclusion, in the present study the cut-off values for aCL and anti-β2-GPI were determined in apparently healthy children and were expressed as concentrations of HCAL and EY2C9 monoclonal antibodies. This could facilitate clinical comparisons in childhood disorders and enables comparison of data derived from different laboratories. Low titres of aCL were common in apparently healthy children; this might have been the result of previous infections. Therefore, the clinical significance of low titres of aCL in childhood disorders has to be judged critically. The higher mean value of IgG anti-β2-GPI observed in preschool children than in adolescents and adults is an unexpected result of the study. It has not been demonstrated previously and might indicate a default response to nutritional exposure to β2-GPI in this age group.

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References


42. Matsuura E, Igarashi M, Igarashi Y, Nagae H, Ichikawa K, Yasuda T et al. Molecular definition of human β2-glycoprotein I (β2-GPI) by cDNA

