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BRIEF COMMUNICATION

Serum and CSF soluble CD26 and CD30 concentrations in healthy pediatric surgical outpatients

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Key words
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Abstract

Activated T-helper type 1 (Th1) lymphocytes induce a cellular type immune response, and Th2 lymphocytes, a humoral or antibody-mediated type immune response. Soluble CD26 (sCD26) and soluble CD30 (sCD30) are regarded as markers of Th1 and Th2 lymphocyte activation, respectively. Serum from 112 generally healthy pediatric surgical patients and cerebrospinal fluid (CSF) from 39, aged 1–17 years were measured for sCD26 and sCD30 using an enzyme-linked immunosorbent assay method. The detection limit for sCD26 was 6.8 ng/ml and for sCD30, 1.9 IU/ml. For serum sCD26 and sCD30, 2.5% and 97.5% percentiles constituted the reference limits, and the 95% credible intervals for the percentiles were calculated using regression models with a Bayesian approach. A significant between-gender difference was observed ($P = 0.015$) in serum sCD26 concentration, of which the lower limits ranged between 273 and 716 ng/ml for girls and 235 and 797 ng/ml for boys. The upper limits ranged between 1456 and 1898 ng/ml for girls and between 1419 and 1981 ng/ml for boys. Moreover, the concentrations of sCD26 increased in infants and children up to 10 years in girls and 12 years in boys. After this however, the values decreased. The serum sCD30 concentration was highest among the youngest infants aged 1 year (80–193 IU/ml), after which a consistent age-related decrease was found. The lowest values were found at the age of 17 years (10–89 IU/ml). A significant between-gender difference in sCD30 concentration was observed ($P = 0.019$). sCD26 and sCD30 concentrations were low in the CSF samples analyzed: 13.3 ng/ml (median); range 8.3–51.5 ng/ml and 7.6 IU/ml; 2.1–18.5 IU/ml, respectively. Reference limits for serum sCD26 in children aged 1–17 years were established as being 235–1800 ng/ml in toddlers and 400–1800 ng/ml in female adolescents and 700–2000 ng/ml in male adolescents. For sCD30; reference limits of 80–190 IU/ml were established in the youngest age group and 10–90 IU/ml in adolescents.

Introduction

There has recently been wide clinical interest in the functional roles of T-helper lymphocytes in infectious diseases (1–3), allergy and atopic diseases (4, 5), rheumatoid arthritis (6, 7) and other autoimmune diseases (8, 9), and in kidney transplantation (10, 11), among other cases. Activated T-helper type 1 (Th1) lymphocytes are responsible for the induction of the cellular type immune response, since they activate cytotoxic T lymphocytes to kill infected cells. Th2 lymphocytes are responsible for the humoral or antibody-mediated type immune response, because activated Th2 lymphocytes are needed to help B lymphocytes secrete protective antibodies (12).

Lymphocyte activation leads to the expression of activation molecules on activated cells, e.g. the membrane form of CD30 is expressed on the surface of activated T lymphocytes (13). Soluble CD30 (sCD30) is an 85 kDa molecule (14) and is released upon T-cell activation by proteolytic cleavage to circulating plasma (14). It has been regarded as a measure

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of the activity of Th2 lymphocytes (13, 15); however, the CD30 antigen has recently been suggested not to reflect Th2 activation but rather to act as an important regulatory molecule in the balance between Th1 and Th2 cells (16).

CD26 is a 110 kDa cell surface glycoprotein with a dipeptyl peptidase IV activity (17). It is expressed on activated T lymphocytes and on the epithelial cells of the liver, kidney and intestine (18, 19). CD26 may help activated T lymphocytes in localizing to inflammatory regions (20), and has been regarded as a marker of Th1 activation (2, 3).

The measurement of soluble CD26 (sCD26) and sCD30 concentrations in a serum sample or in other body fluids might be useful for clinical use, since changes in the concentrations could reflect the activation state of lymphocytes. There are commercial enzyme-linked immunosorbent assays (ELISA) for analyzing sCD26 and sCD30 that could be used for routine clinical analysis; however, little data concerning serum and cerebrospinal fluid (CSF) sCD26 and sCD30 in generally healthy pediatric patients is available.

If the laboratory analytes of interest are known to depend on characteristics such as age and sex, the guideline for calculating reference limits suggests partitioning the reference values into homogeneous subgroups (21). However, if there is not enough data in each subgroup, the reliable statistical estimation of these reference limits may be problematic. Therefore, regression models have been proposed for the estimation of reference limits in children (22, 23).

The aim of this study was to measure the sCD26 and sCD30 concentrations in serum in generally healthy surgical pediatric outpatients, in order to calculate the reference limits for these two new immunological lymphocyte activation markers. We used a regression model with a Bayesian approach to calculate age-specific reference limits for serum sCD26 and sCD30 in children aged 1–17 years. CSF concentrations of sCD26 and sCD30 were also measured from samples when available.

Materials and methods

Patients

We enrolled a consecutive group of 112 generally healthy children (51 girls and 61 boys) undergoing elective surgery in a pediatric outpatient unit of the Department of Pediatric Surgery at Kuopio University Hospital, Kuopio, Finland. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio. The parents (and children if old enough to understand) were asked whether the children had any symptoms of autoimmune diseases such as asthma, allergic rash, chronic bowel disease, chronic arthritis, diabetes or renal failure, malignant diseases, liver inflammation disease or acute respiratory infection. All patients with a previous medical history or signs or symptoms of these diseases in a preoperative medical evaluation were excluded from the study. Four cohorts of pediatric patients of both genders, aged between 1 and 3 ($n = 28$), 4 and 7 ($n = 28$), 8 and 11 ($n = 28$) and 12 and 17 ($n = 28$) years were enrolled, to obtain an adequate number of equally distributed samples for the calculation of reference intervals. Parental written informed consent and the children's assent were obtained before obtaining the blood and CSF samples.

Samples

Blood samples of 3 ml were obtained by venipuncture with the routine preoperative laboratory tests or in the operating theater from an in-dwelling venous catheter inserted for perioperative fluid administration and pharmacotherapy. Blood was collected into tubes without anticoagulants, and after a 10 min centrifuge of the clotted samples at 20°C and 2084 ***g***, the serum was separated and aliquoted.

In 39 children undergoing spinal anesthesia for surgery, a CSF sample of 0.5 ml was obtained before intrathecal local anesthetic injection. The CSF was centrifuged at 20°C and 3100 rpm for 10 min. Both serum and CSF aliquots were stored in plastic tubes at −70°C until analysis.

Measurements

Serum sCD26 and sCD30 measurements were performed using the ELISA method (Bender Med Systems, Vienna, Austria), according to the manufacturer's instructions. The detection limit for sCD26 was estimated to be 6.8 ng/ml and for sCD30, 1.9 IU/ml. Pooled serum samples were run in every assay. The intra-assay precision of serum sCD26 concentrations of 627 ng/ml ($n = 10$) and 1395 ng/ml ($n = 10$), were 8.1% and 7.6% respectively, whereas the inter-assay precision of a serum sCD26 concentration of 840 ng/ml ($n = 9$) was 15.1%. The intra-assay precisions of serum sCD30 concentrations of 16.9 IU/ml ($n = 11$), 27.5 IU/ml ($n = 9$) and 44.7 IU/ml ($n = 5$) were 20.2%, 5.9% and 6.8%, respectively. The inter-assay precision of a serum sCD30 concentration of 34.9 IU/ml ($n = 13$) was 13.5%.

Statistical methods

Regression models

We modeled the observed values of serum sCD26 and sCD30 using Bayesian hierarchical linear regression models (i.e. linear in parameters) (24). For all parameters we used standard non-informative prior distributions (24, 25). As reference limits, we reported the 2.5th and 97.5th percentiles of the fitted Gaussian distributions (21). The 95% credible intervals of the percentiles were estimated using a Markov chain Monte Carlo (MCMC) simulation algorithm, implemented in the WinBUGS (26) software package. Details of the regression models and MCMC analysis are given in Appendix.

Investigating the difference between sexes

In order to investigate the differences in the distributions of the observed values between sexes, we estimated the mean

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difference and its 95% credible interval between boys and girls at different ages using model 1 (see Appendix).

As a further investigation of the statistical significance of the difference in the observed values between boys and girls, we fitted a regression model which considers both sexes jointly. Using this model, we simulated replicate data sets under the hypothesis that the values for boys and girls would follow the same distribution. We then compared the simulated replicates to the observed data. As a result of these comparisons, we obtained posterior *P*-values (24) which reflect the probability of observing a certain characteristic at least as extreme as that seen in the observed data, under the null-hypothesis of no difference between boys and girls (see Appendix).

Finally, in order to estimate the fit of the different models, we compared models 1 and 3 with the deviance information criterion (DIC) criterion (27). This provided an estimate of the prediction accuracy of the models employed.

Results

From the 112 serum samples collected from healthy children aged 1–17 years, we established age-dependent reference limits for serum sCD26 and sCD30 using a Bayesian approach in a regression-based estimation of reference limits (Tables 1 and 2).

The lower limits of serum sCD26 ranged between 273 and 716 ng/ml for girls, and between 235 and 797 ng/ml for boys. The upper limits ranged between 1456 and 1898 ng/ml and 1419 and 1981 ng/ml for girls and boys, respectively. The serum sCD26 concentration increased in infants and children up to 10 years in girls and up to 12 years in boys, after which the values were found to decrease (Figure 1, Table 1). A significant between-gender difference ($P = 0.015$) was observed, and separate reference intervals for girls and boys were created. The differentiation between boys and girls was seen to be largest in the oldest age groups with a 95% credible interval, slightly excluding the value zero (Figure 2).

The serum sCD30 concentration was found to be highest among the youngest infants aged 1 year (80–193 IU/ml), after which came a consistent age-related decrease. The lowest values were found at the age of 17 years (10–89 IU/ml; Figure 3, Table 2). Overall, a significant between-gender difference in sCD30 concentration was observed ($P = 0.019$), with the largest difference seen in the oldest age groups, where the whole 95% credible interval lied above zero after the age of 14 (Figure 4).

Of 38 measured sCD26 concentrations in CSF, 22 were above the detection limit. The mean concentration of sCD26 in CSF samples was 17.2 ng/ml (median 13.3 ng/ml, range 8.3–51.5 ng/ml). sCD30 concentrations in all 39 CSF samples were above the detection limit, the mean concentration being 8.0 IU/ml (7.6 IU/ml, 2.1–18.5 IU/ml). No correlation between age and the concentration of sCD26 and sCD30 in CSF was found.

Discussion

A curved association could be found between age and serum sCD26 in both boys and girls. Interestingly, the peak concentration in serum sCD26 in girls occurred at the age of 10 years, two years earlier than noted in boys. T-cell production by the thymus is most active in young children, decreasing after the onset of puberty due to the effect of

Table 1 Reference limits (2.5% and 97.5%) for girls and boys, and their 95% credible intervals at different age points for serum sCD26 concentrations (ng/ml)

	Girls ($n = 51$)		Boys ($n = 61$)	
Age (years)	Lower limit	Upper limit	Lower limit	Upper limit
1	273 (88–447)	1456 (1292–1648)	235 (51–394)	1419 (1253–1601)
2	371 (210–515)	1555 (1417–1720)	334 (176–464)	1517 (1377–1671)
3	455 (311–588)	1639 (1519–1790)	422 (281–537)	1605 (1481–1742)
4	529 (387–653)	1713 (1597–1858)	498 (366–612)	1683 (1568–1817)
5	591 (446–714)	1775 (1657–1921)	568 (438–682)	1753 (1637–1886)
6	640 (492–763)	1823 (1701–1973)	629 (494–746)	1813 (1695–1947)
7	676 (526–805)	1859 (1735–2014)	680 (541–800)	1865 (1742–2003)
8	701 (550–833)	1884 (1757–2040)	722 (578–844)	1907 (1780–2050)
9	715 (562–845)	1897 (1770–2055)	754 (609–879)	1939 (1813–2084)
10	716 (567–841)	1898 (1773–2050)	778 (635–900)	1962 (1839–2108)
11	705 (557–828)	1887 (1765–2036)	792 (647–916)	1976 (1854–2120)
12	681 (531–803)	1863 (1743–2011)	797 (646–923)	1981 (1856–2128)
13	645 (491–773)	1828 (1701–1980)	794 (635–924)	1976 (1846–2128)
14	599 (428–741)	1780 (1640–1949)	779 (605–926)	1963 (1820–2127)
15	538 (336–706)	1722 (1552–1915)	755 (560–924)	1941 (1776–2126)
16	466 (226–668)	1650 (1441–1877)	724 (489–934)	1908 (1705–2130)
17	382 (94–636)	1566 (1306–1843)	682 (408–941)	1867 (1624–2137)

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Table 2 Reference limits (2.5% and 97.5%) for girls and boys and their 95% credible intervals at different age points for serum sCD30 concentrations (IU/ml).

	Girls ($n = 51$)		Boys ($n = 61$)	
Age (years)	Lower limit	Upper limit	Lower limit	Upper limit
1	85 (70–101)	193 (170–220)	80 (66–93)	185 (165–207)
2	78 (65–89)	181 (163–203)	71 (60–81)	171 (156–189)
3	70 (60–80)	170 (156–187)	64 (55–71)	160 (147–174)
4	63 (54–72)	159 (147–174)	57 (49–64)	149 (138–162)
5	57 (49–65)	149 (137–164)	51 (44–58)	139 (129–152)
6	51 (43–59)	140 (127–154)	46 (39–53)	131 (120–144)
7	46 (38–54)	131 (118–145)	41 (34–48)	123 (112–136)
8	41 (33–48)	122 (110–136)	37 (31–44)	116 (106–129)
9	36 (29–43)	114 (102–127)	34 (27–41)	111 (100–123)
10	32 (25–38)	106 (95–118)	31 (25–38)	105 (95–118)
11	28 (22–33)	98 (88–110)	29 (23–35)	101 (91–113)
12	24 (19–29)	91 (82–103)	27 (22–33)	97 (88–109)
13	21 (16–25)	85 (75–96)	26 (20–31)	95 (85–107)
14	18 (13–22)	78 (68–90)	24 (18–31)	92 (82–105)
15	15 (10–20)	72 (61–85)	24 (17–31)	91 (78–105)
16	12 (7–19)	67 (53–83)	23 (15–31)	90 (75–108)
17	10 (4–18)	61 (46–80)	23 (14–34)	89 (71–111)

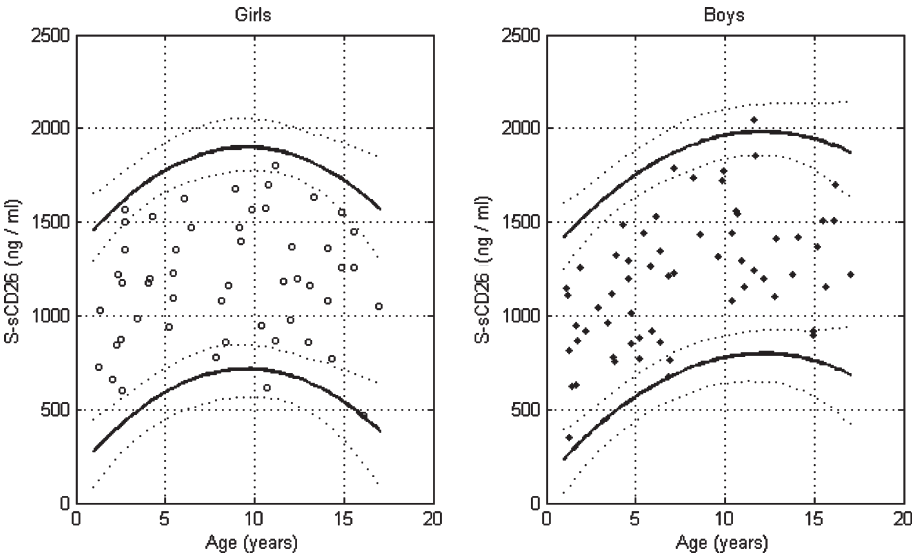

Figure 1 Calculated reference limits for serum sCD26. Both plots show the measured values of serum sCD26. The solid dots represent boys and open circles girls. The plot on the left shows the calculated reference limits for girls as a function of age. The solid lines represent the 97.5th and 2.5th percentiles of the distribution of sCD26. The dotted lines indicate the 95% credible intervals for the percentiles. The plot on the right shows the same percentiles as applied to boys.

sex hormones (28, 29). Relatedly, we wonder if the peak concentration of serum sCD26 at the age of 10 years in girls could reflect the earlier onset of puberty in girls.

Our findings concerning the between-gender difference of serum sCD26 are in accordance with our previous study (30), in which serum sCD26 was observed to be higher in boys than girls at school age. This was, however, in another patient population including patients having clinical or possible asthma and controls. In contrast to that study (30), we can now show a relationship between age and the level of serum sCD26. The reason for this may be the rather narrow age range of children at school age. The mean age of the children was 10.4 years and the range was 7.6–13.5 years, in our previous study (30), compared to this study in which the age ranged from 1 to 17 years. The difference in patient populations between our studies may also partly explain differences in results, as a delay in Treg cell maturation has been reported in atopic children, when compared to age-matched non-atopic children (31).

Our findings concerning sCD30 agree with those of two earlier studies, in which an inverse relationship was found between plasma sCD30 and age, in pediatric patients with tuberculosis (age range from 5 months to 14 years, $n = 46$) (32) and in atopic children and normal controls (61 patients, mean age ± SD, 6.3 ± 2.7 years, and 27 controls, 5.6 ± 3.0 years) (33). Recently, a negative correlation between the age and the concentration of serum sCD30 was reported in healthy children aged 0–18 years (34); however, they could

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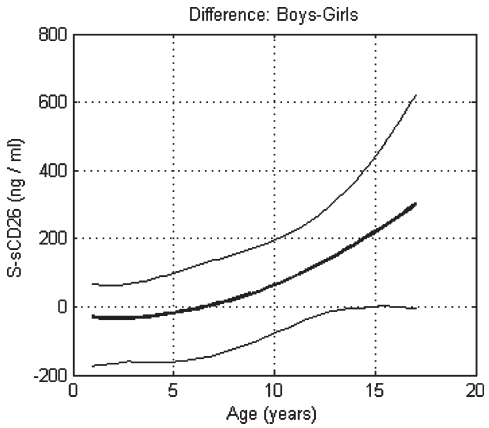

Figure 2 Mean difference between boys and girls for serum sCD26. The thick solid line shows the estimated mean difference between the values of boys and girls as a function of age. The thin lines show the corresponding 95% credible interval.

not find a sex difference of serum sCD30 between boys and girls (34). This may be due to a smaller patient population, when compared to our study. In contrast, there is a study in which serum sCD30 was not found to correlate with age in 18 atopic dermatitis patients (6 months to 8 years), in 22 bronchial asthma patients (6–14 years), or in 20 controls (6 months to 14 years) (4). The number of patients studied, their age range and the differences in patient populations may have had an effect on these results.

During the preschool period (<5-year-old infants and children), an association among increased serum sCD30 concentration, detectable circulating CD30 expressing cells and reduced Th1-type immune response has been reported (35). Our finding of the highest serum sCD30 concentrations among the youngest children could reflect an increased Th2-type activation in this age group. Th2-type activation has been connected to the down regulation of Th1 activation (2), which could explain the reduced Th1-type immune response in early infancy, seen in our study. Our findings show that serum sCD26 concentrations increased, while serum sCD30 concentrations decreased after early infancy, which might reflect an increasing Th1-type response in children up to adolescence (35). The levels of serum sCD30 were lower in boys compared to girls aged less than 10 years; however, those boys older than 10 years had higher levels of serum sCD30 than girls of the same age.

Our findings indicate that the sCD26 and sCD30 concentrations in CSF in healthy children are very low. We expected to find low levels of CSF sCD26 and sCD30, because there are few normal mononuclear cells such as lymphocytes, present in CSF. The number of lymphocytes in general is small and with sensitive ELISA methods, we could show measurable but low immunoactivity in our CSF samples.

We used the Bayesian statistical framework (24, 25) to fit the statistical models specified earlier in the *Materials and methods* section. Unlike the classical Frequentist approach which assumes that the parameters of a model have a fixed but unknown value, the Bayesian approach assigns probabilities to the possible values of unknown quantities. We adopted the Bayesian approach because it allows an intuitive interpretation, in terms of probabilities and flexible calculations within the hierarchical model (36).

The hierarchical model can be interpreted as if boys and girls are modeled separately, but takes into account the fact that the regression curves for both sexes are expected to be similar. Thus, the hierarchical model is an intermediate between a model which considers both sexes jointly and one which has independent parameters for boys and girls. The benefit of using such a model is that it allows some variation in the results for boys and girls. By utilizing the information that the results for the different sexes are expected to be similar, it avoids the introduction of additional independent parameters, which would lead to increased uncertainty during estimation. Initially, both models were used for both laboratory analytes to calculate reference intervals, and following this, the most suitable model was selected (see Appendix).

Large numbers of samples are needed if the partitioning of data into subgroups is performed, which poses difficulties in the case of pediatric reference samples. In our study, partitioning data into subgroups could have led to moderate changes in the reference limits between subgroups, but this was avoided with the regression method used (data not shown). Additionally, the sample sizes in partitioned age groups would have proved to be too small for valid reference limit estimation. As such, both serum sCD26 and sCD30 concentrations were age dependently distributed in the pediatric samples tested.

One limitation of this study was that only one sample from each of the subjects was obtained. Thus, we were unable to detect whether there would have been any fluctuation or variation over a time in the levels of sCD26 and sCD30 for a single patient. Moreover, it is open to discussion, as to whether the stressful situation or preoperative blood withdrawal, would have effects on the levels of these two activation molecules. Further studies are needed to establish whether there is variation in sCD26 and sCD30 levels prior to and after surgery. Moreover, in future the reference values obtained here should be compared with those seen in patients with altered activation of T cells.

In conclusion, continuous reference limits with 95% credible intervals were constructed for serum sCD26 and sCD30 concentrations in children aged 1–17 years. A Bayesian regression model-based approach was able to estimate the reference limits on a continuous age scale, and the partitioning of data was unnecessary. The CSF sCD26 and sCD30 concentrations were seen to be very low. Now that reference limits for sCD26 and sCD30 for children have been established, the measurement of sCD26 and sCD30 concentrations in a serum sample or in CSF could be useful clinically, since changes in

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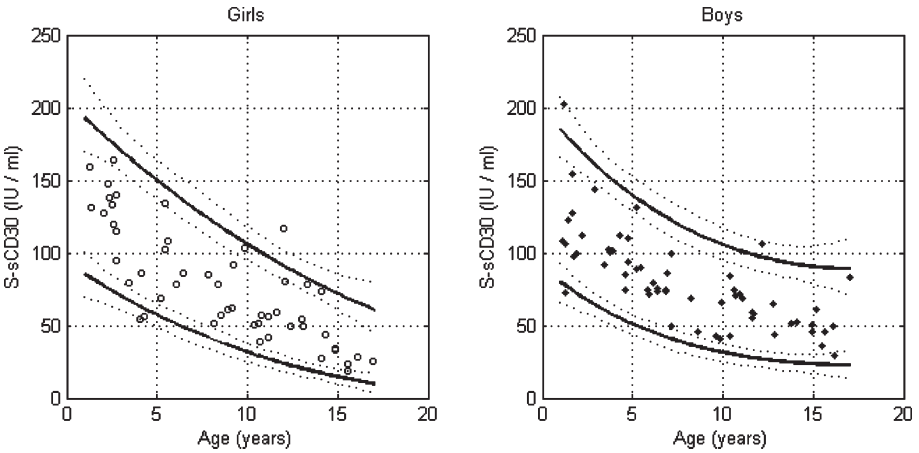

Figure 3 Calculated reference limits for serum sCD30. Both plots show the measured values of sCD30. The solid dots represent boys and open circles girls. The plot on the left shows the calculated reference limits for girls as a function of age. The solid lines represent the 97.5th and 2.5th percentiles of the distribution of sCD26. The dotted lines indicate the 95% credible intervals for the percentiles. The plot on the right shows the same percentiles as applied to boys.

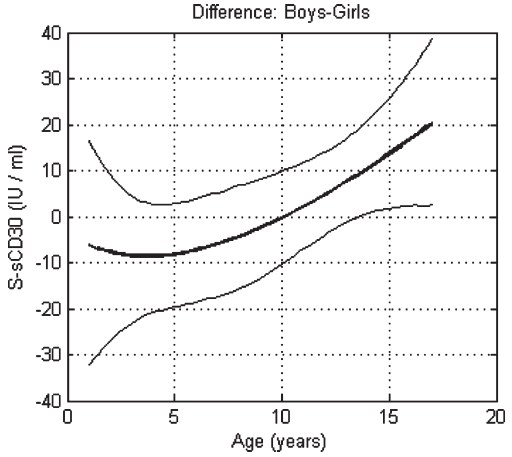

Figure 4 Mean difference between boys and girls for serum sCD30. The thick solid line shows the estimated mean difference between the sCD30 values of boys and girls as a function of age and the thin lines show the corresponding 95% credible interval.

the concentrations of sCD26 and sCD30 can reflect the activation state of lymphocytes, which differs in various types of disease.

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Conflict of interest

The authors have declared no conflicting interests.

Appendix

Bayesian hierarchical linear regression models

For statistical analysis, we used Bayesian hierarchical linear regression models (24):

$$y_i = \beta_0^{s(i)} + \beta_1^{s(i)} x_i + \beta_2^{s(i)} x_i^2 + \epsilon_i. \qquad (1)$$

In this, x_i denotes the age of the ith sampled individual, y_i denotes the measured level of either sCD26 or sCD30–the square root transformation was applied to sCD30 concentrations (see below).$\varepsilon_i \sim N(0, \sigma^2)$, and $s(i) \in \{\text{boy, girl}\}$ specifies the gender of the ith individual. The similarity in values of boys and girls was taken into account by specifying the gender-specific regression parameters to be drawn from common distributions:

$$\beta_j^{\text{boy}}, \beta_j^{\text{girl}} \sim N\left(\mu_j, \sigma_j^2\right), \qquad \text{for} \qquad j = 0, 1, 2. \qquad (2)$$

As reference limits, we reported the 2.5th and 97.5th percentiles of the fitted Gaussian distributions (21) given by

$$\text{Percentile}_{97.5} = \mu + 1.96\sigma$$

and

$$\text{Percentile}_{2.5} = \mu - 1.96\sigma.$$

In this, μ and σ are the mean and standard deviation estimated using the model defined in Equation [1].

To further investigate the statistical significance of the differences in observed values between boys and girls, we fitted a regression model which considered both sexes jointly:

$$y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2 + \epsilon_i. \qquad (3)$$

Using this model, we simulated K replicate data sets from the estimated posterior predictive distribution. For each replicate data set we calculated a test score: $T_k, k = 1, \ldots, K$. The posterior P-values (24) were then obtained as the proportion of test scores for those resampled data sets whose

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values were greater than or equal to the value of the test score (T) for the original data, i.e.

$$P\text{-value} = \frac{\#\{T_k : T_k \geq T\}}{K}.$$

In this, # denotes the set cardinality operator.

As test scores, we used

$$T_1 = \sum_{i \in \text{boys}} \Delta_i - \sum_{i \in \text{girls}} \Delta_i$$

and

$$T_2 = k_{\text{boys}} - k_{\text{girls}}.$$

Here, Δ_i denotes the residual of the ith sampled individual, and the terms k_{boys} and k_{girls} are slopes of the linear regression curves fitted to the residuals of boys and girls respectively.

Prior distributions for the parameters of the joint model defined in Equation [3] were set as follows:

$$\begin{aligned} \tau &\sim \text{Gamma}\,(0.001, 0.001)\,, \text{ where } \tau = 1/\sigma^2, \\ \beta_j &\sim N\left(\mu_j, \sigma_j^2\right), \text{ for } j = 0, 1, 2, \\ \mu_j &\sim N\left(0, 10^6\right), \text{ for } j = 0, 1, 2, \\ \sigma_j &\sim \text{Unif}\,(0, 100)\,, \text{ for } j = 0, 1, 2. \end{aligned}$$

Prior distributions for the hierarchical model defined in Equation [1] were set as above, except for the prior specifications given in Equation [2].

We used two chains in the WinBUGS MCMC analysis, and the convergence was detected by inspecting the trace plots for sampled parameter values. We used a burn-in equal to 20,000 and a thinning value equal to 100. After the convergence was reached, we simulated yet another 2000 iterations and saved the sampled parameter values from both chains (in total, 4000 samples). The presented distributions are based on these saved values.

Preliminary model checking

Before selecting the models which were used in the final analyses, we considered a number of alternative models for the two data sets. Regression models were tested with first, second and third order terms for age, and also models in which the two sexes were considered either jointly or separately. For sCD30, we also considered different transformations to make the variance of observations approximately equal over the age groups. We tested ln, log2 and square root transformations, of which the square root transformation provided the best fit.

In the preliminary investigations, the models were assessed by comparing the estimated posterior predictive distributions with the observations, both visually and using the numerical P-values (e.g. 24), and also by using the DIC criterion (27) obtained from the WinBUGS software for different models. For both sera, the lowest DIC score (i.e. the highest estimated accuracy) was obtained using the hierarchical model with second order terms, although some alternative models showed almost equal DIC values (exact results not shown).

References

1. Kemp K, Kurtzhals JAL, Akanmori BD et al. Increased levels of soluble CD30 in plasma of patients with *Plasmodium falciparum* malaria. *Clin Diagn Lab Immunol* 2002: **9**: 720–2.
2. Liao YR, Po-Yen C, Lin CC, Fu LS, Chiu CC, Chi CS. Soluble CD26/30 levels before and after treatment with interferon–alpha and ribavirin combination therapy in a pediatric hepatitis C patient. *J Microbiol Immunol Infect* 2004: **37**: 67–70.
3. Jafari-Shakib R, Shokrgozar MA, Nassiri-Kashani M, Malakafzali B, Nikbin B, Khamesipour A. Plasma sCD26 and sCD30 levels in cutaneous leishmaniasis. *Acta Trop* 2009: **109**: 61–3.
4. Heshmat NM, El-Haddidi ES. Soluble CD30 serum levels in atopic dermatitis and bronchial asthma and its relationship with disease severity in pediatric age. *Pediatr Allergy Immunol* 2006: **17**: 297–303.
5. Lun SWM, Wong CK, Ko FWS, Hui DSC, Lam CWK. Increased expression of plasma and CD4+ T lymphocyte costimulatory molecule CD26 in adult patients with allergic asthma. *J Clin Immunol* 2007: **27**: 430–7.
6. Gerli R, Lunardi C, Bocci EB et al. Anti-tumor necrosis factor-α response in rheumatoid arthritis is associated with an increase in serum soluble CD30. *J Rheumatol* 2008: **35**: 14–9.
7. Savolainen E, Matinlauri I, Kautiainen H, Luosujärvi R, Kaipiainen-Seppänen O. Serum soluble CD30 in early arthritis: a sign of inflammation but not a predictor of outcome. *Clin Exp Rheumatol* 2008: **26**: 922–5.
8. Krams SM, Cao S, Hayashi M, Villanueva JC, Martinez OM. Elevations in IFN-γ, IL-5, and IL-10 in patients with autoimmune disease primary biliary cirrhosis: association with autoantibodies and soluble CD30. *Clin Immunol Immunopathol* 1996: **80**: 311–20.
9. Pellegrini P, Totaro R, Contasta I, Berghella AM, Carolei A, Adorno D. CD30 antigen and multiple sclerosis: CD30, an important costimulatory molecule and marker of a regulatory subpopulation of dendritic cells, is involved in the maintenance of the physiological balance between TH1/TH2 immune responses and tolerance. *Neuroimmunomodulation* 2005: **12**: 220–34.
10. Pelzl S, Opelz G, Wiesel M et al. Soluble CD30 as a predictor of kidney graft outcome. *Transplantation* 2002: **73**: 3–6.
11. Matinlauri IH, Kyllönen LEJ, Salmela KT, Helin H, Pelzl S, Süsal C. Serum sCD30 in monitoring of alloresponse in well HLA-matched cadaveric kidney transplantations. *Transplantation* 2005: **80**: 1809–12.
12. Delves PJ, Roitt IM. The immune system. Second of two parts. *N Engl J Med* 2000: **343**: 108–17.
13. Del Prete G, De Carli M, Almerigogna F et al. Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. *FASEB J* 1995: **9**: 81–6.
14. Falini B, Pileri S, Pizzolo G et al. CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. *Blood* 1995: **85**: 1–14.

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15. Romagnani S, Del Prete G, Maggi E, Chilosi M, Caligaris-Cappio F, Pizzolo G. CD30 and type 2 T helper (Th2) responses. *J Leukoc Biol* 1995: **57**: 726–30.
16. Pellegrini P, Berghella AM, Contasta I, Adorno D. CD30 antigen: not a physiological marker for TH2 cells but an important costimulator molecule in the regulation of the balance between Th1/Th2 response. *Transpl Immunol* 2003: **12**: 49–61.
17. Morimoto C, Schlossman SF. The structure and function of CD26 in the T-cell immune response. *Immunol Rev* 1998: **161**: 55–70.
18. Dong RP, Tachibana K, Hegen M et al. Determination of adenosine deaminase binding domain on CD26 and its immunoregulatory effect on T cell activation. *J Immunol* 1997: **159**: 6070–6.
19. Hegen M, Niedobitek G, Klein CE, Stein H, Fleischer B. The T cell triggering molecule Tp103is associated with dipeptidyl aminopeptidase IV activity. *J Immunol* 1990: **144**: 2908–14.
20. Masuyama J, Berman JS, Cruikshank WW, Morimoto C, Center DM. Evidence for recent as well as long-term activation of T cells migrating through endothelial cell monolayers in vitro. *J Immunol* 1992: **148**: 1367–74.
21. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline – third edition CLSI document C28-A3; 2008.
22. Virtanen A, Kairisto V, Irjala K, Rajamäki A, Uusipaikka E. Regression-based reference limits and their reliability: example on hemoglobin during the first year of life. *Clin Chem* 1998: **44**: 327–35.
23. Virtanen A, Uusipaikka E. Computation of profile likelihood-based confidence intervals for reference limits with covariates. *Stat Med* 2008: **27**: 1121–32.
24. Gelman A, Carlin JB, Stern HS, Rubin DB. *Bayesian Data Analysis*, *2nd edn. Boca Raton, Florida*: *Chapman & Hall/CRC Press*, *2003*.
25. Bernardo JM, Smith AFM. *Bayesian Theory*. *Chichester*: *John Wiley & Sons Ltd*, *1994*.
26. Lunn DJ, Thomas A, Best N, Spiegelhalter D. WinBUGS – a Bayesian modelling framework: concepts, structure, and extensibility. *Stat Comp* 2000: **10**: 325–37.
27. Spiegelhalter DJ, Best NG, Carlin BP, van der Linde A. Bayesian measures of model complexity and fit. *J Roy Stat Soc Ser B Stat Met* 2002: **64**: 583–639.
28. Shames RS. Gender differences in the development and function of the immune system. *J Adolesc Health* 2002: **30S**: 59–70.
29. Calder AE, Hince MN, Dudakov JA, Chidgey AP, Boyd RL. Thymic involution: Where endocrinology meets immunology. *Neuroimmunomodulation* 2011: **18**: 281–9.
30. Remes ST, Delezuch W, Pulkki K, Pekkanan J, Korppi M, Matinlauri IH. Association of serum – soluble CD26 and CD30 levels with asthma, lung function and bronchial hyper-responsiveness at school age. *Acta Pediatrica* 2011: **100**: e106–11.
31. Tulic MK, Andrews D, Crook ML et al. Changes in thymic regulatory T-cell maturation from birth to puberty: differences in atopic children. *J Allergy Clin Immunol* 2012: **129**: 199–206.
32. Hanekom WA, Hussey GD, Hughes EJ, Potgieter S, Yogev R, Check IJ. Plasma-soluble CD30 in childhood tuberculosis: Effects of disease severity, nutritional status and vitamin A therapy. *Clin Diagn Lab Immunol* 1999: **6**: 204–8.
33. Chen JY, Fu LS, Chu JJ, Chen HC, Chi CS. Plasma soluble CD30 level correlates negatively with age in children. *J Microbiol Immunol Infect* 2007: **40**: 168–72.
34. Chrul S, Polakowska E. Age-dependent changes of serum soluble CD30 concentration in children. *Pediatr Transplant* 2011: **15**: 515–8.
35. Krampera M, Vinante F, Tavecchia L et al. Progressive polarization towards a T helper/cytotoxic type-1 cytokine pattern during age-dependent maturation of the immune response inversely correlates with CD30 cell expression and serum concentration. *Clin Exp Immunol* 1999: **117**: 291–7.
36. Hibbert DB, Armstrong N. An introduction for analyzing chemistry data Part II: a review of applications of Bayesian methods in chemistry. *Chemometr Intell Lab Syst* 2009: **97**: 211–20.

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