International Journal of Obesity (2014) 38, S26–S31

www.nature.com/ijo

OPEN

ORIGINAL ARTICLE

C-reactive protein reference percentiles among pre-adolescent children in Europe based on the IDEFICS study population

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OBJECTIVES: C-reactive protein (CRP) is involved in a wide range of diseases. It is a powerful marker for inflammatory processes used for diagnostic and monitoring purposes. We aimed to establish reference values as data on the distribution of serum CRP levels in young European children are scarce.

SUBJECTS: Reference values of high-sensitivity CRP concentrations were calculated for 9855 children aged 2.0–10.9 years, stratified by age and sex. The children were recruited during the population-based European IDEFICS study (Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and infantS) with 18 745 participants recruited from 2007 to 2010.

RESULTS: In 44.1 % of the children, CRP values were below or equal the detection limit of 0.2 mg/l. Median CRP concentrations showed a slight negative age trend in boys and girls, whereas serum CRP values were slightly higher in girls than in boys across all age groups.

CONCLUSIONS: Our population-based reference values of CRP may guide paediatric practice as elevated values may require further investigation or treatment. Therefore, the presented reference values represent a basis for clinical evaluation and for future research on risk assessment of diseases associated with increased CRP levels among children.

International Journal of Obesity (2014) **38,** S26–S31; doi:10.1038/ijo.2014.132

INTRODUCTION

C-reactive protein (CRP) is an acute-phase protein of the pentraxin family produced in the liver in response to inflammatory signals, particularly interleukin-6, which is synergistically enhanced by IL-1β. It is released during infection, systemic inflammation and tissue damage and activates the classical complement pathway. CRP binds to phosphocholine and related molecules on micro-organisms and on membranes of apoptotic and necrotic cells. Its binding to phosphocholine improves opsonisation and phagocytosis by macrophages. In addition, CRP binds to certain Fc-gamma receptors on the surface of leukocytes, which also lead to the stimulation of phagocytosis and the release of cytokines.[1]

In healthy subjects, the average serum CRP concentration is $< 1\ mg\ l^{-1}$.[2] However, the CRP concentration rises quickly up to $1000\ mg\ l^{-1}$ in the first few days following an acute-phase stimulus and decreases back to baseline with a half-life of 19 h when the stimulus has ceased.[3]

Slightly elevated levels of CRP are related to overweight and obesity in children and adults.[4] Elevation of CRP in obesity results from infiltration of the expanded adipose tissue by macrophages that release inflammatory signals and cytokines such as interleukin-6, the main stimulus for CRP production.[5]

Moreover, slightly elevated CRP is an independent predictor of coronary events and stroke and has been associated with cardiovascular mortality and all-cause mortality in adults.[6] An association between elevated CRP and markers for cardiovascular risk was reported in children as well.[7–10] In addition, increased CRP has been associated with cancer[11,12] and has shown to be a prognostic marker in various malignancies in adults,[13–15] whereas data in children are scarce. Furthermore, elevated CRP levels are associated with an increased risk of type 2 diabetes in adults[16] and are correlated with insulin resistance in children.[17] As serum CRP concentration reflects the magnitude of inflammation, it has also become a routine laboratory parameter in daily clinical practice for diagnosis and monitoring of autoimmune inflammatory disorders such as rheumatoid arthritis.[18,19] To render CRP a useful marker in children, age-specific reference values are needed.

Although CRP reference percentiles of a large US cohort of children have been reported,[20] there are only few population-based studies on paediatric CRP reference values in Europe. Most previous studies were restricted to a small number of subjects from healthy or hospital-based populations and only few nationwide or large-scale population studies reported CRP values.[21–23] Therefore, the aim of this study was to establish reference values for prepubertal children in Europe.

SUBJECTS AND METHODS

Study subjects

A cohort of 16 228 children aged 2.0–8.9 years was examined in a population-based baseline survey (T_0) in eight European countries ranging from North to South and from East to West (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium and Estonia) from autumn 2007 to spring

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2008.[24] Additional 2517 children aged 2.0–10.9 years were newly recruited during a second survey (T_1) 2 years later to add up to the study sample of the present analysis ($n = 18\,745$).

Examinations and CRP analysis

The examination programme included standard anthropometric measures such as body weight and height, a personal interview on health conditions and the collection of blood for analysis, reported in detail elsewhere.[24,25] In addition, a detailed description of blood processing, shipment, storage and quality management during the baseline survey has been published earlier.[26,27] The same procedures were applied during the second survey. The examinations were suspended during the summer holidays. As the fasting status was important for other blood marker analyses (for example, blood lipids) with regard to IDEFICS main topic on lifestyle and dietary behaviour in children, blood samples were collected mainly in the morning (83% before 10 a.m., 95% before 11 a.m.) and stored in the order of appearance at the study site. The blood samples were analysed directly after shipment from each country to a central laboratory according to a predefined shipment schedule. IDEFICS samples were randomly integrated in the normal routine samples of the lab. CRP levels were measured with latex-enhanced nephelometry (BN2-Nephelometer, Siemens, Eschborn, Germany) with a lower detection limit of 0.2 mg/l. The CRP measurements were performed under the legally regulated quality management system RiliBÄK that includes internal and external control samples on different levels. The RiliBÄK system does not allow the release of samples without valid quality control and includes a system to check for drifts. External quality control certificates are published on the laboratory website (www.labmed.de).

Inclusion and exclusion criteria for the reference population

Of the 18 745 participants aged 2.0–10.9 years, serum concentrations of CRP were analysed from all 11 641 children who provided a blood sample. Defining the reference population, our aim was to present CRP values for a large European population of children. Serum levels of CRP $\geqslant 10\ mg\,l^{-1}$ are generally believed to be attributed to infection or inflammation in adults.[28] Therefore, we used this cut-off to exclude 274 children with pathologically elevated CRP levels. Furthermore, the body mass index was determined as obesity is associated with inflammatory processes as well.[4] The parents were asked: 'Has the child taken any kind of medication within the last 24 h?' For every child who had taken medication, that is, prescription drugs or over-the-counter drugs within the last 24 h before blood collection we assumed a poor health status and excluded them all, regardless of indication. Thus, 1786 children were excluded from the analysis group because of pathologically elevated CRP levels ($n = 274$), obesity classified according to Cole and Lobstein[29] ($n = 773$) or current medication ($n = 882$), leaving 9855 children aged 2.0–10.9 years in the analysis group (Figure 1). Unfortunately, the categories on the checklist of diseases queried during the medical interview did not allow the identification of pathophysiological conditions that were related to inflammation and hence may have influenced CRP levels. But we performed sensitivity analyses to assess the impact of varying inclusion criteria on the serum levels of CRP.

Statistical analyses

To calculate percentile curves, we used the General Additive Model for Location Scale and Shape that was developed by Rigby and Stasinopoulos.[30] This method is an extension of the LMS method to model the distribution of CRP depending on multiple covariates while accounting for dispersion, skewness and particularly the kurtosis of this distribution.[30,31]

We calculated percentile curves of CRP as a function of the covariate age, stratified by sex, using the General Additive Model for Location Scale and Shape method. The General Additive Model for Location Scale and Shape method is an extension of the LMS method that models three parameters depending on one explanatory variable: M accounts for the median of the outcome variable and the coefficient of variation (S) accounts for the variation around the mean and adjusts for non-uniform dispersion, whereas the skewness (L) accounts for the deviation from a normal distribution using a Box–Cox transformation. We used the gamlss package (version 4.2–6) of the statistical software *R* (version 3.0.1).[32]

Different distributions, that is, the Box–Cox transformation, gamma and inverse Gaussian distribution were fitted to the observed distribution of CRP. Moreover, the influence of age on parameters of the considered distributions was modelled either as a constant, as a linear function or as a cubic spline of the covariate. Goodness of fit was assessed by the Bayesian Information Criterion and Q–Q plots to select the final model including the fitted distribution of CRP and the influence of the covariate on distribution parameters. The CRP serum values were measured with a detection limit of $0.2\ mg\,l^{-1}$ and a precision of $0.1\ mg\,l^{-1}$ (for example, values $\leqslant 2.4\ mg\,l^{-1}$ rounded to $0.2\ mg\,l^{-1}$). As 44.1 % of CRP blood values of the reference population were below or equal to the detection limit the distribution of CRP was strongly skewed and distributions available in the gamlss package could not accurately be fitted regarding the correct percentage of cases below the percentile curves. Hence, CRP observations equal to $0.2\ mg\,l^{-1}$ were repeatedly randomised within [0, 0.24] based on the uniform distribution to identify the best fitted model. The final model for boys and girls is based on a Box–Cox transformation distribution where the distribution parameters were modelled as follows: the location parameter μ linearly, the scale parameter $\log(\sigma)$ and the shape parameters ν and $\log(\tau)$ as constants. CRP observations equal to $0.2\ mg\,l^{-1}$ were again uniformly randomised within (0, 0.24) for 100 replications to eventually calculate mean percentile curves in the final analysis. With 44.1 % of serum CRP values below or equal the detection limit only the 50th, 75th, 90th, 95th, 97th, 97.5th and 99th CRP percentiles were calculated.[31,32]

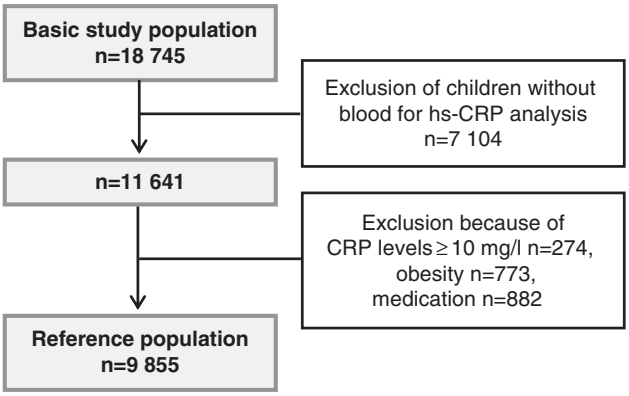

Figure 1. Selection of the reference population.

RESULTS

The characteristics of the reference population are summarised in Table 1. Our analysis group did not differ substantially from the whole study population (see Ahrens *et al.*[33] on prevalence of overweight; this issue). Median CRP levels ranged from 0.2 to $0.3\ mg\,l^{-1}$ in all countries, except for Italy where median CRP levels were higher ($0.5\ mg\,l^{-1}$).

Serum CRP concentrations ranged from 0.2 to $9.7\ mg\,l^{-1}$ in boys and from 0.2 to $9.8\ mg\,l^{-1}$ in girls (min to max). Almost half of the children (44.1 %) had CRP values lower or equal to the detection limit of $0.2\ mg\,l^{-1}$. Age- and sex-specific 50th, 75th, 90th, 97th and 99th CRP percentiles of the reference population of 2.0–10.9-year-old children are presented in Figure 2 and in Table 2 (95th and 97.5th CRP percentiles in Supplementary Figure A; Supplementary Table A). Median CRP concentrations showed a slightly negative age trend, both, in boys and girls. Upper percentiles displayed higher CRP values in younger children. CRP percentiles were generally higher in girls than in boys of the same age.

Sensitivity analyses were performed to confirm the robustness of our data. The influence of obesity on CRP levels was confirmed by excluding obese children as shown in Supplementary Figure B. Multiple definitions of obesity were applied according to the International Obesity Task Force as published by Cole and Lobstein,[29] the Centers for Disease Control and Prevention (CDC)[34] and the World Health Organisation (WHO).[35,36] This sensitivity analysis showed that the 75th, 90th and 95th CRP percentiles from non-obese children were lower, in particular for older children, and thus confirmed our decision to exclude obese

children from the analysis. Moreover, an influence of medication was apparent, especially in younger children, with higher CRP levels in children having taken medication (Supplementary Figure C). An additional sensitivity analysis revealed that there were negligible differences in CRP levels when children with any kind of reported current disease or elevated blood pressure were excluded (Supplementary Figure D).

Table 1. Basic characteristics of the reference population for boys and girls

	Girls	*Boys*
	N *(%)*	N *(%)*
Sex	4854 (49.3)	5001 (50.7)
	Mean (s.d.)	*Mean (s.d.)*
Age, years	6.53 (1.91)	6.45 (1.91)
Weight, kg	23.40 (6.52)	23.76 (6.67)
Height, cm	119.8 (13.2)	120.5 (13.2)
BMI, kg m^{-2}	16.00 (1.84)	16.05 (1.78)
BMI, *z*-score	0.15 (0.99)	0.11 (1.00)
	N *(%)*	N *(%)*
Age categories		
2–2.9-year olds	99 (2.0)	101 (2.0)
3–3.9-year olds	478 (9.9)	531 (10.6)
4–4.9-year olds	639 (13.2)	689 (13.8)
5–5.9-year olds	592 (12.2)	645 (12.9)
6–6.9-year olds	757 (15.6)	766 (15.3)
7–7.9-year olds	1067 (22.0)	1055 (21.1)
8–8.9-year olds	796 (16.4)	794 (15.9)
9–9.9-year olds	288 (5.9)	297 (5.9)
10–10.9-year olds	138 (2.8)	123 (2.5)
BMI categories		
Thinness grade I–III	592 (12.2)	617 (12.3)
Normal weight	3557 (73.3)	3766 (75.3)
Overweight	705 (14.5)	618 (12.4)
Survey		
T_0	4351 (89.6)	4546 (90.9)
T_1	503 (10.4)	455 (9.1)

Abbreviations: BMI, body mass index; %, sex-specific percentage of corresponding categories.

DISCUSSION

Age

In adults, CRP concentrations are often assumed to be independent of age. Children have lower CRP levels than adults, which are assumed to increase with age to reach adulthood levels. Therefore, we generated age-specific CRP percentiles to serve as reference values for children. Surprisingly, we observed a slight negative trend of median CRP concentrations with age in both, boys and girls. This slight negative trend is driven by the upper percentiles, which showed a marked negative trend until the age of 11. In a large German cohort study, the mean CRP level was quite stable for 2–11-year-old children, but increased in adolescent girls >13 years old.[37] In North American children from the NHANES study, the geometric mean of CRP barely increased between the age of 3 and 14 years, but was rising in females from the age of 15 years onwards.[20] Cook *et al*.[8] found a stronger rise in CRP levels by 15 % between the age of 10 and 11 years in British children calculating the proportional change in CRP for a 1 s.d. increase. Overall, available data indicate that average CRP values change only marginally in younger children, whereas they seem to rise during adolescence. It is still an open question to what extent maturational stage or increasing use of oral contraceptives or smoking behaviour might be associated with elevated CRP levels.[37]

The strong negative trend of the upper percentiles observed in our data is confirmed by the findings of Soldin *et al*.[38] In children aged 13 months to 14 years, the 97.5th percentiles were highest in the youngest groups. Young children often suffer from infections as they start to get in contact with other children in kindergartens.[39–41] Higher CRP values in the upper percentiles in younger age groups might thus represent beginning or fading subclinical infections that are more likely in these age groups.

Sex

In line with several other studies,[8,20,37,38] we found CRP to be higher in girls than in boys. On the contrary, two other studies reported no sex differences in children.[21,42] Both reported only CRP reference intervals (2.5th and 97.5th percentile) for broad age groups and did not investigate sex differences more closely in between. Surprisingly, in male participants of an adolescent study population even higher CRP concentrations were measured than in female participants.[23]

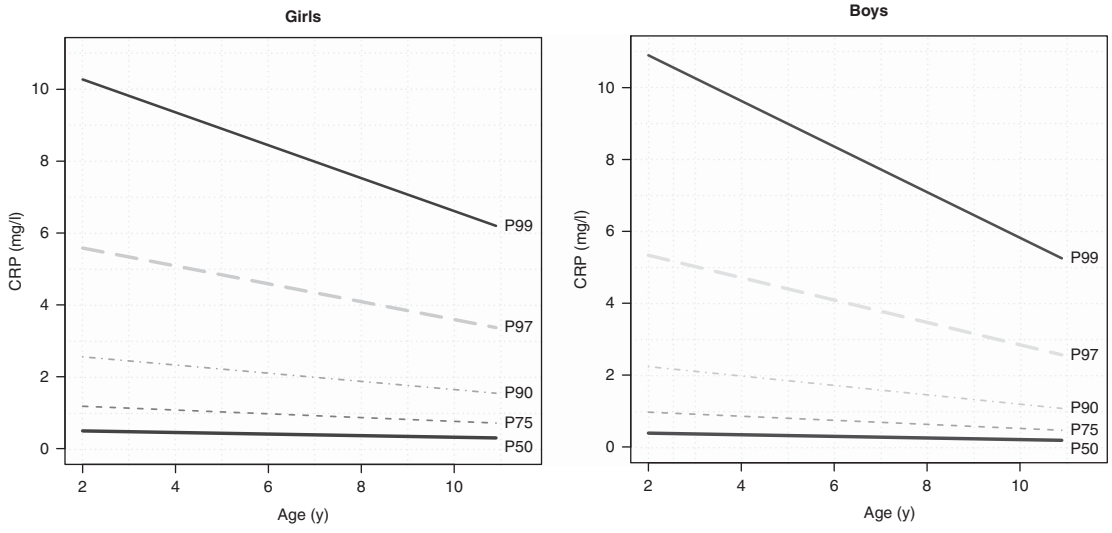

Figure 2. Age- and sex-specific CRP percentiles of the reference population. P*n* indicates the *n*th percentile.

Table 2. Percentiles of hs-CRP (mg l^{-1}) calculated with GAMLSS

Age (years)	*Percentiles for girls*					Age (years)	*Percentiles for boys*				
	50	75	90	97	99		50	75	90	97	99
2– < 2.5	0.5	1.2	2.5	5.5	10.2	2– < 2.5	0.4	1.0	2.2	5.3	10.7
2.5– < 3	0.5	1.1	2.5	5.4	9.9	2.5– < 3	0.4	0.9	2.1	5.1	10.4
3– < 3.5	0.5	1.1	2.4	5.3	9.7	3– < 3.5	0.4	0.9	2.1	4.9	10.1
3.5– < 4	0.5	1.1	2.4	5.1	9.5	3.5– < 4	0.3	0.9	2.0	4.8	9.8
4– < 4.5	0.4	1.1	2.3	5.0	9.2	4– < 4.5	0.3	0.8	1.9	4.6	9.5
4.5– < 5	0.4	1.0	2.2	4.9	9.0	4.5– < 5	0.3	0.8	1.9	4.5	9.2
5– < 5.5	0.4	1.0	2.2	4.8	8.8	5– < 5.5	0.3	0.8	1.8	4.3	8.8
5.5– < 6	0.4	1.0	2.1	4.6	8.6	5.5– < 6	0.3	0.8	1.7	4.2	8.5
6– < 6.5	0.4	1.0	2.1	4.5	8.3	6– < 6.5	0.3	0.7	1.7	4.0	8.2
6.5– < 7	0.4	0.9	2.0	4.4	8.1	6.5– < 7	0.3	0.7	1.6	3.9	7.9
7– < 7.5	0.4	0.9	2.0	4.3	7.9	7– < 7.5	0.3	0.7	1.5	3.7	7.6
7.5– < 8	0.4	0.9	1.9	4.2	7.6	7.5– < 8	0.3	0.6	1.5	3.5	7.3
8– < 8.5	0.4	0.9	1.8	4.0	7.4	8– < 8.5	0.2	0.6	1.4	3.4	6.9
8.5– < 9	0.3	0.8	1.8	3.9	7.2	8.5– < 9	0.2	0.6	1.4	3.2	6.6
9– < 9.5	0.3	0.8	1.7	3.8	7.0	9– < 9.5	0.2	0.6	1.3	3.1	6.3
9.5– < 10	0.3	0.8	1.7	3.7	6.7	9.5– < 10	0.2	0.5	1.2	2.9	6.0
10– < 10.5	0.3	0.7	1.6	3.5	6.5	10– < 10.5	0.2	0.5	1.2	2.8	5.7
10.5– < 11	0.3	0.7	1.6	3.4	6.3	10.5– < 11	0.2	0.5	1.1	2.6	5.3

Abbreviations: GAMLSS, General Additive Model for Location Scale and Shape; hs-CRP, high-sensitivity C-reactive protein.

CRP concentration

Median CRP concentrations in our 2.0–10.9-year-old children were low, close to the detection limit of high-sensitive assays. Previously reported median CRP values were similar to our data and ranged from 0.21 mg l^{-1} in 5–13-year-old boys[22] to 0.3 mg l^{-1} in 3–9-year-old children[20] and up to 0.33 and 0.54 mg l^{-1} in 11–13-year-old girls and boys, respectively.[43] A comparison of median CRP concentrations between studies is difficult as values depend on detection limits and are often reported for different age groups.

Our 95th percentiles ranged from 1.8 to 4.1 mg l^{-1} for 2.0–10.9-year-old boys and girls. This range is similar to the one reported by the large North American cohort study NHANES. In children, 3–9 years of age, the 95th percentile was 3.4 mg l^{-1} if CRP concentrations >10 mg l^{-1} were excluded.[20] In a French study, the 95th percentiles of apparently healthy children with CRP <20 mg l^{-1} were lower, with 1.45 mg l^{-1} in 5–13-year-old boys and 1.9 mg l^{-1} in 5–18-year-old girls,[22] although the inclusion criteria regarding elevated CRP levels were less strict than ours. As we measured the highest CRP levels in the youngest children, the lower 95th percentile in the French study population is probably due to the older age range.

Reporting of the 97.5th instead of the 95th percentiles probably explain the higher concentrations that were observed in 4–10-year-old children recruited regardless of their health status in a study by Soldin *et al*.[38] In those children, the 97.5th percentiles varied from 7.9 mg l^{-1} in boys to 10.0 mg l^{-1} in girls, whereas our 97.5th percentiles ranged from 2.8 to 6.3 mg l^{-1} in 2.0–10.9-year-old boys and girls. The highest 97.5th percentile of 11.3 mg l^{-1} CRP was reported in a Swedish study with children between the age of 6 months and 18 years although children with chronic diseases or infections were excluded.[21] On the contrary, the lowest 97.5th CRP percentiles of 1 mg l^{-1} for boys and girls were reported from a Canadian study.[42] We can only speculate whether the removal of outliers or the exclusion criteria had an impact on these reference values, as it was not clearly stated how many outliers had been removed and how the information regarding the health status was obtained. Taken together, differences in published upper percentiles mainly arise because studies report different percentiles and apply different exclusion criteria.

Inflammation

The increase of CRP in the pathophysiology of inflammation was the reason for the exclusion criteria in the present study in order to remove the influence of inflammatory processes in children with pathologically elevated CRP levels $\geqslant 10$ mg l^{-1}, obesity and medication. Any of the applied exclusion criteria indicating poor health led to lower CRP concentrations for each percentile in the reference population. By excluding all children who have taken any kind of medication, many children were excluded whose serum CRP concentrations have not been altered. As these children could be considered similar to those in the reference population the data were not biased by this exclusion.

The questionnaire was not sufficiently specific for the differentiation of diseases and their underlying inflammatory pathophysiologies. We did not exclude children with current diseases since a sensitivity analysis showed negligible differences in CRP levels when children reporting current diseases were excluded. We assume that the proportion of children with inflammatory conditions was only small.

Obesity

A cross-sectional and longitudinal analysis relating CRP to overweight/obesity and cardiometabolic risk factors in European children of the IDEFICS project was recently published.[7] In agreement with numerous other studies, high CRP concentrations were positively associated with adiposity in children and adolescents as recently reviewed by Choi *et al*.[4] A sensitivity analysis confirmed the effect of obesity on CRP levels in our study population as well.

It is a major strength of our data that they are based on a large heterogeneous population. Therefore, it was decided for the reference values to not discriminate between countries. Actually, CRP levels were quite comparable between countries. Only in Italian children the median CRP levels were elevated, probably caused by the extremely high prevalence of overweight and obesity in this country.[44]

As blood samples in IDEFICS were collected mainly in the morning our data are not appropriate to address a possible influence of the time of day of sampling on serum CRP levels.

Moreover, there are very few hints in the literature on a possible role of time of day of sampling as a covariate for CRP, but also few data against it. Diurnal variation of CRP is not commonly regarded as an influence and our data cannot contribute to this issue.

No seasonal variations of CRP levels were observed in our data. This confirms a previous study that was especially designed to examine seasonal changes in cardiovascular risk biomarkers and that did not report seasonal variation of CRP levels.[45]

Besides the influence of age, sex and obesity, serum CRP concentrations underlie substantial intra-individual variability.[46] Thus, the Centers for Disease Control and Prevention and the American Heart Association recommend to assess the mean of at least two measurements, taken 2 weeks apart, for evaluation of a person's true CRP status. Elevated CRP concentrations ($\geqslant$10 mg l^{-1}) without obvious signs of disease are usually due to an asymptomatic inflammatory response or subclinical infection. In this case, the measurement should be repeated.[28] Moreover, in the case of recurrent CRP elevations below 10 mg l^{-1}, the underlying cause should be further investigated.

CONCLUSION

Reference percentiles of CRP have an important role in clinical practice for diagnosis and monitoring of inflammatory diseases. The present study is the first to provide pan-European age-specific reference values of CRP for children that may guide paediatric practice and provide a basis for future clinical and epidemiological research on inflammation-related disorders in children.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was done as part of the IDEFICS Study (www.idefics.eu). We gratefully acknowledge the financial support of the European Community within the Sixth RTD Framework Programme Contract No. 016181 (FOOD) and we thank the IDEFICS children for their participation in this extensive examination. Isabelle Sioen was financially supported by the Research Foundation – Flanders (Grant no. 1.2.683.14. N00). In addition, we are grateful to the Volkswagen Foundation that financially supported the production of this supplement.

DISCLAIMER

The information in this document reflects the authors' view and is provided as it is.

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