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Serum CrossLaps: Pediatric Reference Intervals from Birth to 19 Years of Age, *Patricia M. Crofton,[1,2*] Nancy Evans,[2] Mervyn R.H. Taylor,[3] and Celia V. Holland[4]* ([1] Department of Paediatric Biochemistry, Royal Hospital for Sick Children, Edinburgh EH9 1LF, United Kingdom; [2] Section of Child Life and Health, Department of Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh EH9 1UW, United Kingdom; [3] The National Children's Hospital, Tallaght and Department of Paediatrics, Trinity College, Dublin 4, Ireland; [4] Department of Zoology, Trinity College, Dublin 4, Ireland; * address correspondence to this author at: Department of Paediatric Biochemistry, Royal Hospital for Sick Children, Sciennes Road, Edinburgh EH9 1LF, United Kingdom; fax 44-131-536-0410, e-mail croftonp@aol.com)

During childhood growth, bone undergoes extensive modeling involving separate osteoblastic and osteoclastic processes. Markers of bone formation and resorption circulate at higher concentrations in children than in adults, parallel the childhood growth curve, and correlate with height velocity *(1, 2)*. Not only do these markers provide insight into the pathophysiology of bone turnover during growth, but they also give an early surrogate measure of its response to treatment. The markers of bone formation are all measured in plasma, and their use as markers of growth and bone formation in children is well established *(1, 2)*. However, most markers of bone resorption have traditionally been measured in urine. In infants and children, the practical difficulties associated with urine collection are compounded by marked circadian variation and high within-individual biological variation in urinary markers. Results are generally expressed in relation to creatinine, itself subject to considerable biological variation and changing with age as muscle mass increases. There is therefore a need for a sensitive and specific marker of bone resorption that can be measured in plasma and directly compared with markers of bone formation measured in the same sample.

Serum CrossLaps™ is a promising new marker for bone resorption *(3)*, but its application in children has been hampered by lack of suitable reference data. Here we report age- and sex-related reference data for serum CrossLaps in children from birth to 19 years of age.

Neonates, infants, and children 0–5 years of age. Surplus plasma remaining after routine biochemical tests had been completed was retrieved from 59 neonates, infants, and children (37 males) who presented with various minor conditions that were considered not to have either a short- or long-term effect on growth. Children with systemic disease or intercurrent infections were specifically excluded. Samples were fully anonymized and stored at −70 °C until analysis.

Children 4–19 years of age. We analyzed stored plasma from 287 children (142 males, 145 females) 4–19 years of age who had participated in an earlier population-based epidemiologic study on the seroprevalence of toxocariasis in Irish schoolchildren *(4)*. Ten samples from each sex and year group were analyzed, except for girls 4 years of age and boys 18 years of age, for whom only five and two samples, respectively, were available. Nonfasting samples were collected between 0900 and 1500. All children were well enough to attend school that day. No formal pubertal staging was undertaken because it would have been ethically inappropriate in this context. Blood samples were taken for the original study with the informed consent of both children and parents and after approval by the local ethics committee. The excess plasma remaining after completion of that study was fully anonymized and stored at between −40 and −70 °C until analysis.

We measured serum CrossLaps using a sandwich ELISA assay (Osteometer Biotech) as described *(3)*. The assay has recently been restandardized on a weight basis by the manufacturer, using a synthetic, cross-linked polypeptide containing two identical residues of the sequence EKAHD-β-GGR originating from the C-telopeptide of type I collagen. The conversion from pmol/L [based on the previous reference material prepared from desalted urinary antigens *(3)*] to the new units of ng/L is: x (ng/L) = [y (pmol/L) − 138]/7.75. In addition to the manufacturer's control material, we included pooled plasma from prepubertal and pubertal children as further quality-control samples. Within- and between-run CVs in our study were 8.7% and 12% at 223 ng/L, 8.8% and 10% at 463 ng/L, and 8.2% and 14% at 786 ng/L, respectively.

The data were analyzed separately for neonates (younger than 1 month postnatal age), for infants (ages 1 month to 1 year), for each sex and year of age thereafter (e.g., the 1-year-old age band comprised children ≥1.0 to <2.0 years), and also for various combinations of ages. We compared medians, means, geometric means, and indices of skewness and kurtosis for log-transformed and untransformed data and found that log transformation gave

a better fit to a gaussian distribution and tended to equalize variances. Log-transformed data from males and females in each age band were compared with unpaired *t*-tests. Within each sex, changes with age were assessed by one-way ANOVA on the log-transformed data, followed by Fisher protected least-significant difference as a post hoc test. On the basis of the ages at which statistically significant changes occurred, results in adjacent age bands were then combined to derive appropriate age- and sex-related reference intervals. The 95% reference interval was defined as the arithmetic mean of the log-transformed data ± 2 SD, raised to the power of 10. Means (and SD) of the log-transformed data are presented to facilitate calculation of SD scores by age and sex.

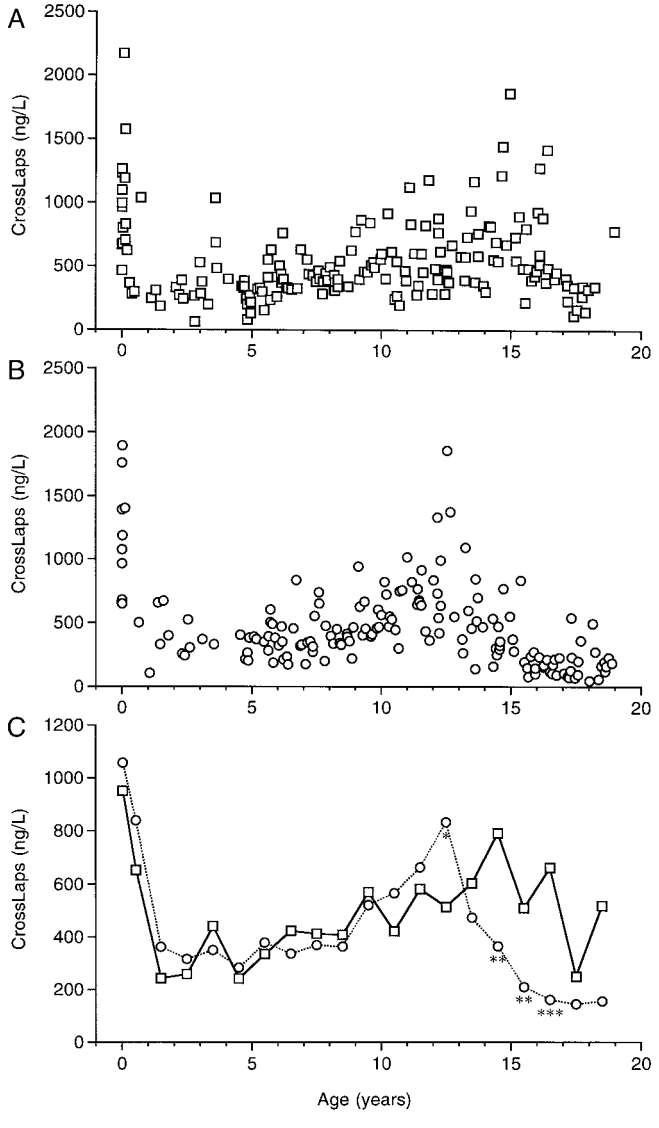

Fig. 1. Serum CrossLaps concentrations in relation to age and sex.

(*A*), individual boys; (*B*), individual girls; (*C*), geometric means (defined as the arithmetic mean of log-transformed CrossLaps concentrations, raised to the power of 10). □, boys; ○, girls. Geometric means for each age band are plotted at the midpoint of that age band. Unpaired *t*-tests between age-matched boys and girls: ∗, $P < 0.05$; ∗∗, $P < 0.01$; ∗∗∗, $P < 0.001$.

The concentrations of serum CrossLaps, plotted by age and sex in individual children, are shown in Fig. 1, A and B. The geometric means by age and sex are displayed in Fig. 1C. The highest concentrations occurred in neonates during the first month of life, with slightly lower concentrations in infants ages 1 month to 1 year ($P < 0.05$) and a further marked decrease in children older than 1 year ($P < 0.0001$). CrossLaps showed a significant variation with age in both boys and girls older than 1 year (ANOVA, $P < 0.0001$). Post hoc testing indicated that no significant change occurred in either sex between the ages of 1 and 9 years. In boys, there were then progressive increases in serum CrossLaps from 1–9 years to 9–14 years ($P < 0.0001$), peaking at 14–17 years ($P < 0.08$) before decreasing again at 17–19 years ($P < 0.0001$). In girls, serum CrossLaps increased progressively from 1–9 years to 9–11 years ($P < 0.001$), peaking at 11–13 years ($P < 0.05$) before decreasing at 13–15 years ($P < 0.001$) and further at 15–19 years ($P < 0.0001$; Fig. 1). Neonates, infants, and children 1–9 years of age showed no significant differences in serum CrossLaps between males and females ($P > 0.5$). However, girls 12–13 years of age had higher serum CrossLaps concentrations compared with age-matched boys ($P < 0.05$), whereas girls 14–15 years, 15–16 years, and 16–17 years of age all had lower concentrations than age-matched boys ($P < 0.01$).

Shown in Table 1 are the medians, logarithmic means (and SD), and derived 95% reference intervals for serum CrossLaps based on the above age groupings. Combined reference data are given for boys and girls younger than 9 years because there were no statistically significant sex differences in these age groups, but separate reference data are presented for the two sexes in older children.

Serum CrossLaps was highest in neonates, then de-

Table 1. Age- and sex-specific reference data.

Age ranges	n	Median, ng/L	Log-transformed mean[a] (SD)	95% reference interval,[b] ng/L
Males and females				
<1 month	19	994	3.0000 (0.1782)	440–2272
1 month–1 year	11	705	2.8347 (0.2645)[c]	202–2311
1–9 years	124	352	2.5383 (0.1872)[d]	146–818
Males				
9–14 years	50	539	2.7279 (0.1824)[d]	231–1238
14–17 years	30	578	2.8095 (0.2148)	240–1734
17–19 years	12	331	2.4512 (0.2235)[d]	101–791
Females				
9–11 years	20	538	2.7348 (0.1268)[e]	303–996
11–13 years	20	704	2.8714 (0.1792)[c]	326–1697
13–15 years	20	462	2.6185 (0.2308)[e]	144–1202
15–19 years	40	169	2.2204 (0.2711)[d]	48–579

[a] Mean (SD) of log-transformed serum CrossLaps concentrations (in ng/L).

[b] Arithmetic mean of log-transformed data ± 2 SD, raised to the power of 10.

[c–e] Compared with the previous age/sex group (ANOVA followed by post-hoc testing; see text): [c] $P < 0.05$; [d] $P < 0.0001$; [e] $P < 0.001$.

creased during infancy to reach a nadir between 1 and 9 years of age. The marker increased again during early adolescence in both sexes, attaining its peak earlier in girls (11–13 years) than in boys (14–17 years). It subsequently decreased more rapidly in girls than in boys in later adolescence to reach a second, lower nadir in girls after the age of 15 years. In girls in late adolescence, serum CrossLaps concentrations were comparable to those previously reported for premenopausal adult women *(3)*. The variation with age and sex shown by serum CrossLaps is similar to patterns previously observed for other markers of collagen formation and breakdown in children *(1, 2, 5, 6)* and reflects the pediatric growth curve. The timing of peak concentrations of serum CrossLaps in relation to chronological age coincided with the timing of peak height velocity in each sex on a population basis. No individual data were available for pubertal stage, height velocity, or bone mass acquisition in our study. As reported for other markers of collagen and bone turnover, at any age the range of serum CrossLaps values was wide, presumably reflecting individual variations in recent growth, pubertal progression, and bone modeling and remodeling.

In our study, nonfasting blood samples were collected between 0900 and 1500. There is no published information on circadian variation of serum CrossLaps in infants or children. In adults, it has been demonstrated that circadian variation for serum CrossLaps is minimized by 24 h of fasting *(7)*. This is not a viable option for children. In nonfasting adults, highest concentrations of serum CrossLaps occur overnight (as observed for other markers of bone collagen turnover), decreasing to relatively constant concentrations between 1000 and 1500 *(7)*. The timing of collection of the blood samples in our study almost coincided with this optimal period and is appropriate to most routine clinical samples.

In summary, we have produced age- and sex-related 95% reference intervals for serum CrossLaps from birth to 19 years of age, together with log-transformed mean (and SD) values that will allow calculation of SD scores for use in future studies.

We thank Lothian University Hospitals, National Health Service Trust, for support of this work through a small project grant. Collection of the Irish blood samples was supported by the National Children's Hospital Ladies Guild, the Garfield Weston Foundation, and the Trinity Trust.

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Release Kinetics of Cardiac Troponin T in Survivors of Confirmed Severe Pulmonary Embolism, *Margit Müller-Bardorff, Britta Weidtmann, Evangelos Giannitsis, Volkhard Kurowski, and Hugo A. Katus*[*] (Universitätsklinikum Lübeck, Medizinische Klinik II, Ratzeburger Allee 160, 23538 Lübeck, Germany; * author for correspondence: fax 49-451-5006437, e-mail katus@medinf.mu-luebeck.de)

Cardiac troponins may be increased in patients with confirmed pulmonary embolism (PE), even in the absence of significant coronary artery disease (CAD), and indicate increased risk for subsequent death *(1)*. Cardiac troponin T (cTnT) correlates with the presence and degree of right-ventricular dysfunction *(1, 2)*. In our recent study *(1)*, 18 of 56 patients (32%) with PE had significant increases in cTnT. Eight of the nine patients with fatal outcome had increased cTnT. In the present study, we investigated all consecutive survivors of angiographically confirmed acute PE who developed cTnT ≥0.1 μg/L to evaluate cTnT time release in PE and to provide a rationale for an optimal blood-sampling protocol to improve risk stratification.

The study was approved by the local ethics committee of the University of Luebeck. All patients gave informed consent.

We enrolled nine consecutive patients with confirmed PE developing cTnT concentrations ≥0.1 μg/L, who survived the acute event and sampling period until normalization of cTnT concentrations. PE was suspected in the presence of an acute onset of symptoms such as dyspnea, pleuritic chest pain, syncope, hypotension, or shock and was confirmed by pulmonary angiography. The diagnostic work-up included transthoracic echocardiography, electrocardiography, blood-gas analysis, ventilation-perfusion scan, and coronary angiography. PE was graded according to the Goldhaber classification *(3)*. PE patients were subsequently followed throughout their hospital stay. For controls, we studied six patients with confirmed acute coronary syndromes and microinfarction, defined as cTnT concentrations ≥0.1 μg/L with normal electrocardiograms and creatine kinase MB activity.

Standard therapy consisted of a parenteral bolus of 500 mg of acetylsalicylic acid and therapeutic doses of unfractionated heparin and β-blocker therapy adjusted according to activated partial thromboplastin time. Other medication was given at the discretion of the cardiologist on duty.

Serum samples were obtained on admission, every 4 h