Statistical models for pediatric endocrinology

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Data Note

Keywords: hormones, references, statistics, LMS

Posted Date: November 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1109851/v1>

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Abbreviations: E1, estrone; E2, estradiol; CI, confidence interval; CV, coefficient of variation; FSH, follicle-stimulating hormone; IGF1, insulin-like growth factor 1; LC-MS/MS, liquid chromatography tandem-mass spectrometry; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.

Funding: The current study was funded by the Western Norwegian Health Authority (grant no. 912221) and the author was not instructed or incentivized to produce any specified/predetermined outcome.

Disclosure: CEO, Anylite AS [Norway VAT no. 927 529 068]

ABSTRACT

Background: Modelling references for biomarkers as reference curves enables calculation of patient z-scores that are adjusted for both gender and covariance with age during childhood.

Aim: To establish biomarker reference curves using the 'LMS' method previously employed for conventional growth charts.

Design, Setting and Participants: Cross-sectional study of healthy Norwegian girls (n=647) and boys (n=465) in ages 6 to 16 years were recruited in the Bergen Growth Study (2016). Blood samples were analyzed using state-of-the-art instruments.

Results: Reference curves for several biomarkers were established in the 'LMS' framework and provided here for clinical implementation.

Conclusion: The 'LMS' reference framework is already used to create conventional growth charts and may also be applied in clinical biochemistry.

INTRODUCTION

Normal range references for clinical biomarkers in the pediatric population are critically lacking and crudely presented as age-partitioned nonparametric 95% confidence intervals. By this standard proposed by the clinical laboratory standards institute (CLSI C28-A3c), clinically applicable reference intervals must include at least 120 observations. Such sample sizes are not feasible in pediatric endocrinology due to ethical constraints for sampling hundreds of healthy children in any given partition. Arguably and in itself, partitioning reference intervals is a suboptimal approach due to consistent covariance of biomarkers with age, gender and developmental status. A new framework for biochemical references and patient benchmarking is required to enable systemic precision medicine in pediatric practice.

The LMS method was published by Tim Cole (PMID: 2354692) in 1990 and has since become the major statistical framework for establishing normalized growth standards. The current work proposes that application of the LMS algorithm may provide value in the clinical interpretation of hormone/biomarker levels in pediatric endocrinology.

METHODS | Cohort

The “Bergen Growth Study 2” (BGS2) was ethically approved by the regional Western Norway Ethics Authority (reference REK2015/128) prior to data collection and the cohort was described previously (PMID: 31697832; PMID: 32961560; PMID: 32140730; PMID: 31899821). Written parental consent and child’s consent plus assent was mandatorily obtained before and evaluations were performed. The study followed the standards of the Helsinki Declaration. Evaluations of children included blood sampling, puberty benchmarking by Tanner stages and testicular/breast ultrasound, bioelectric impedance analysis (BIA) and clinical examination with anthropometric measurements. Children with any known chronic disease, scrotal pathology or regular corticosteroid use were excluded from the final dataset. The cross-sectional BGS2 cohort included hormone data from n=645 girls and n=465 boys, representing a healthy population sample and was representative of the Norwegian demographic composition (approximately 90% Caucasian) at the time of data collection in 2016.

METHODS | Blood samples

Blood samples were analyzed as reported previously (PMID: 31697832; PMID: 32961560) at the Department of medical biochemistry and pharmacology, Haukeland University Hospital, in accordance with NS-EN ISO 15189:2012. Briefly, steroid hormones were quantified by in-house LC-MS/MS while lipids and peptide hormones were assayed by Siemens Immulite 2000Xpi or appropriate ELISA kits as specified. The LC-MS/MS methods used to quantify androgens and estrogens, respectively, were described previously (PMID: 23825158; PMID: 32500111).

Biomarker	Analyte	Method	LOD	Inter-assay CV
Androgens	11-Deoxycortisol	LC-MS/MS	<0.03 nmol/L	7% @ 5.8 nmol/L
	17-OH progesterone	LC-MS/MS	<0.06 nmol/L	6% @ 8.9 nmol/L
	4-Androstendione	LC-MS/MS	<0.02 nmol/L	7% @ 9.1 nmol/L
	Cortisol	LC-MS/MS	<0.1 nmol/L	7% @ 44 nmol/L
	Progesterone	LC-MS/MS	<0.06 nmol/L	6% @ 25 nmol/L
	Testosterone	LC-MS/MS	<0.01 nmol/L	4% (1.5 - 37 nmol/L)
Estrogens	Estrone (E1)	Ultrasensitive LC-MS/MS	0.15 pmol/L	8% (1.7 - 153 pmol/L)
	Estradiol (E2)	Ultrasensitive LC-MS/MS	0.28 pmol/L	9% (1.7 - 153 pmol/L)
Gonadotropins	LH	IMMULITE 2000 XPi	0.05 IU/L	7% @ 10 IU/L
	FSH	IMMULITE 2000 XPi	0.1 IU/L	5% @ 17 IU/L
Other	SHBG	IMMULITE 2000 XPi	0.02 nmol/L	6% @ 60 nmol/L
Adipokines	Leptin	Mediagnost ELISA E07	1 ug/L	5% @ 7 ug/L
	Adiponectin	Mediagnost ELISA E09	1 ug/mL	8% @ 13ug/mL
	IGF1	IMMULITE 2000 XPi	1 nmol/L	7% @ 18 nmol/L
Lipids	TC; HDL; LDL; Triglycerides	Cobas 8000	0.1 nmol/L	3% @ 1.5 nmol/L

Table 1. Analytes and assays used in the “Bergen Growth Study 2”.

Statistical approach

Continuous reference curves were modelled using the LMS algorithm provided in the 'gamlss' CRAN package in R. The following formulas were used to calculate clinically relevant biomarker ratios:

Free androgen index = $100 \cdot T / SHBG$

Vermeulen free T = $(1000 \cdot T) / (1 + (((1000000000 \cdot ((SHBG \cdot 43779) / 1000000000) / 43779) + ((36000 \cdot 43) / 69000))))$

Bioavailable T = $T / (1 + (((1000000000 \cdot ((SHBG \cdot 43779) / 1000000000) / 43779) + ((36000 \cdot 43) / 69000)))) \cdot 23.43478$

Vermeulen free E2 = $E2 / (1 + (((500000000 \cdot ((SHBG \cdot 43779) / 1000000000) / 43779) + ((45500 \cdot 43) / 69000))))$

OUTPUT MODELS

LMS parameters for the resulting reference models are provided as supplemental data for $\log(x \cdot 1e6)$ transformed data.

Analyte	Abbreviation
Sex hormone-binding globulin	SHBGF
Sex hormone-binding globulin	SHBGM
Estradiol	E2F
Estradiol	E2M
Estrone	E1F
Estrone	E1M
Luteinizing hormone	LHF
Luteinizing hormone	LHM
Follicle-stimulating hormone	FSHF
Follicle-stimulating hormone	FSHM
Cortisol	CORTISOLF
Cortisol	CORTISOLM
Androstenedione	ANF
Androstenedione	ANM
17-hydroxyprogesterone	OHPF
17-hydroxyprogesterone	OHPM
11-deoxycortisol	DOCF
11-deoxycortisol	DOCM
Progesterone	PGF
Progesterone	PGM
Testosterone	TESF
Testosterone	TESM
LH/FSH ratio	LHFSHRATIOF
LH/FSH ratio	LHFSHRATIOM
FSH/E2 ratio	FSHE2RATIOF
LH/Testosterone ratio	LHTESRATIOF
E2/E1 ratio	E2E1RATIOF
E2/E1 ratio	E2E1RATIOM
Free Testosterone (Vermeulen)	VERMEULENF
Free Testosterone (Vermeulen)	VERMEULENM
Free Androgen Index	FAIF
Free Testosterone Index	FAIM
E2/Testosterone ratio	E2TESRATIOF
E2/Testosterone ratio	E2TESRATIOM
Bioavailable Testosterone	BIOTF
Bioavailable Testosterone	BIOTM
Insulin-like growth factor 1	IGF1F
Insulin-like growth factor 1	IGF1M
Leptin	LEPTINF
Leptin	LEPTINM
Adiponectin	ADIF
Adiponectin	ADIM
Total cholesterol	TOTALCF
Total cholesterol	TOTALCM
LDL cholesterol	LDLCF
LDL cholesterol	LDLCM
HDL cholesterol	HDLCF
HDL cholesterol	HDLCM
Triglycerides	TRIGLYCF
Triglycerides	TRIGLYCM

Table 2. Index of provided LMS reference curves provided as supplemental information. References are provided for M=male, F=female genders separately.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementalcharts.zip](#)
- [Supplementaljson.zip](#)