Closing the Gaps in Pediatric Reference Intervals for Biomarkers of Pediatric Disease

The CALIPER Program

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Central Role of Laboratory Medicine In Healthcare Delivery

Laboratory Medicine is part of the multi-disciplinary team at the centre of healthcare.

The quality of the Clinical Laboratory Service is critically dependent on:

- Accurate/Precise Testing Process (validated methods/systems)
- Accurate interpretation of lab results based on appropriate reference intervals or decision limits
Definition of Reference Interval

• Limiting values within which a specified percentage (usually 95%) of apparently healthy individuals’ results would fall

• Usually the 0.025 and 0.975 fractiles of the test result distribution in the reference population

• Newer consensus approaches define reference interval endpoints for some analytes based upon
  – epidemiology of disease
  – outcomes analysis
  – economics

James C. Boyd, M.D.
University of Virginia Health System
Charlottesville, VA USA
Why Reference Intervals?

• Reference Intervals serve as health-associated benchmarks to accurately assess laboratory test results

• Patient Care (adult and pediatric) is highly dependent on the use and interpretation of medical lab tests

• Reference Intervals can be influenced by many covariates such as Age, Sex, Ethnicity, BMI

• Until recently, appropriately partitioned/up-to-date Reference Intervals have not been clearly defined in pediatric (and even adult) populations
Reference Intervals vs Decision Limits
(Common Source of Error in Result Interpretation)

• Reference Intervals appropriate for most assays but not all

• Decision Limits must be used for some analytes (e.g. HbA1c, lipids, Vitamin D) based on clinical guidelines

• A common source of error is application of reference intervals in result interpretation when decision limits are more appropriate
Test Result Report/Interpretation

Current Challenges

• Major Gaps in Adult and Pediatric Reference Intervals and Appropriate Decision Limits

  >>>> Leading to inaccurate/erroneous interpretation

• Lack of Standardization or Harmonization of Assay Methodology

  >>>> Considerable variation in established reference intervals for both adults and children

Should be regarded as a major source of laboratory error affecting patient care and patient safety
Lack of Harmonization of Test Methodology in Medical Labs Globally

Multiple Assays for Every Test

Lack of Harmonized Reference Intervals Reported by Labs Across Canada/Globally
Children are not small adults:

- **Body weight:**
  - Doubles by 6 months of age
  - Triples by the first birthday

- **Body length** increases by 50% during the first year

- **Major organ systems** grow and mature

- Important changes take place during puberty
  - Accelerated growth and sexual maturation occur

*Pediatric Reference Intervals Need to reflect differences in:*

- Development & physiologic function at different ages
- Key covariates including gender, age, BMI, sexual development (Tanner stage), and ethnicity

Distribution of Alkaline Phosphatase In Relation To Age In Young Females

Age Dependence of Serum Enzymatic Activities (Alkaline Phosphatase, Aspartate Aminotransferase, and Creatine Kinase) in Healthy Children and Adolescents
Pediatric vs Adult Reference Ranges for ALP
(Canadian Health Measures Survey [CHMS] 2015)

*Canada-wide survey at 16 sites across Canada

National Survey 2016

Reference Intervals in use across Canada: >30 Clinical Laboratories surveyed

Clinical Biochemistry (2017)
Reference Intervals Used in Clinical Practice in Healthcare Centres across Canada - May 2016

Alkaline Phosphatase

ALP Reference Intervals
2 Year Old Male
## Major National Initiatives

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Age Range (years)</th>
<th>Sex</th>
<th>Statistical Method</th>
<th>Examples of Groups of Biomarkers Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>AACB</td>
<td>Australia and New Zealand</td>
<td>All age groups</td>
<td>Both</td>
<td>Central 95%</td>
<td>Common blood analytes (mostly ions and enzymes)</td>
</tr>
<tr>
<td>CALIPER</td>
<td>Canada</td>
<td>0-18</td>
<td>Both</td>
<td>Central 95%</td>
<td>Common biochemical markers Endocrine markers Tumor markers Vitamins Metabolic disease biomarkers Testosterone indices</td>
</tr>
<tr>
<td>CHILDx</td>
<td>United States</td>
<td>0.5-17</td>
<td>Both</td>
<td>Median, mean and central 95%</td>
<td>Enzymes Coagulation tests Hormones Vitamins Bone markers</td>
</tr>
<tr>
<td>COPENHAGEN</td>
<td>Denmark</td>
<td>5-20</td>
<td>Both</td>
<td>Central 95%</td>
<td>Common blood analytes</td>
</tr>
<tr>
<td>KiGGS</td>
<td>Germany</td>
<td>0-18</td>
<td>Both</td>
<td>Median and central 90%</td>
<td>Nutrient deficiency markers Non-communicable diseases and lipids Immunology markers Thyroid hormones</td>
</tr>
<tr>
<td>LOOK</td>
<td>Australia</td>
<td>8, 10 and 12</td>
<td>Both</td>
<td>Median and central 95%</td>
<td>Cardiac Biomarker Common blood analytes</td>
</tr>
</tbody>
</table>

Adapted from Tahmasebi et al. (2017)
PRINCE Study in China
CALIPER Initiative in Canada
CALIPER Initiative

• To determine the effects of these key covariates on biochemical parameters on reference intervals in healthy children

• To develop a comprehensive database of covariate stratified reference intervals

• To disseminate study results to pediatric healthcare community worldwide using novel knowledge translation strategies
**CALIPER in Toronto/Hamilton**

1. **Promotional Campaign**
   - Community Sample Collection Clinics
   - Onsite Sample Collection at SickKids

2. **Sample Processing & Storage**
   - Sample Processing & Storage
   - Statistical Analysis (CLSI C28-A3)
   - Calculation of Reference Interval

3. **Laboratory Analysis**

4. **Online CALIPER Database**
   - www.caliperproject.ca
CALIPER Participants
CALIPER Participants
CALIPER Promotional Events

CALIPER Participants
CALIPER Biobank – Toronto

• Serum Biobank: ~ 10,300 samples (males/females)

• Age Range: Birth to 18 years

• Health Information: Family History, Health Status, BMI, Waist Circumference, Tanner Stage (9-15yrs)

www.caliperproject.ca
Age and Sex Distribution of CALIPER Participants
As of November 2018 (>10300 children recruited)

www.caliperproject.ca
CALIPER Biobank – Toronto

- **Serum Biobank:** ~ 9835 samples (males/females)
- **Age Range:** Birth to 18 years
- **Health Information:** Family History, Health Status, BMI, Waist Circumference, *Tanner Stage* (9-15yrs)

“Confidential. For Internal Use Only.”
I. Biochemical Markers (Chemistry, Proteins, Enzymes)

II. Endocrine and Fertility Markers

III. Vitamins, Cancer Markers

IV. Metabolic & Specialized Biochemical Markers
   Teodoro-Morrison Clin Biochem 2015

Highlighted Publications


Biochemical Markers


“Confidential. For Internal Use Only.”
Endocrine and Metabolic Markers

**Testosterone**

Concentration (nmol/L) vs. Age (years)

- Male
- Female

**Estradiol**

Concentration (pmol/L) vs. Age (years)

- Male
- Female

**LH**

Concentration (IU/L) vs. Age (years)

- Male
- Female

**FSH**

Concentration (IU/L) vs. Age (years)

- Male
- Female

*Colantonio D. et al. Clin Chem 2012*

Biochemical Markers in the CALIPER Cohort – Alkaline Phosphatase

Age/Gender Adjusted Reference Intervals are Critical to Diagnosis

CALIPER Database

www.caliperdatabase.ca

Online Database

Old Apps: >70 Biomarkers
New Apps: >170 Biomarkers

Full Tables of Age- and Gender-Specific Reference Intervals

CALIPER Mobile App
(Available on Apple Store & Google Play)

Targeted to Community Pediatrician and Healthcare Workers
To Facilitate Easy Access to CALIPER Database

New Mobile & Web Apps Available as of September 2018
CALIPER Web & Mobile Apps
(New 2018 Update)

Developed for paediatricians to facilitate accurate interpretation of medical test results in children and adolescents!

CALIPER
Reference Mobile & Web App now available!

How does the App work?
Access comprehensive and up-to-date paediatric reference intervals in 3 easy steps!
1. Download the app from the Apple or Google Play store for free!
2. Input patient information and test result
3. View whether patient’s result is normal or abnormal

Why is it useful?
- Based on healthy Canadian population (10,000+)
- Contains over 170 laboratory tests!
- Prevents misdiagnosis through easier and more accurate test interpretation

Questions?
Please contact us at:
416-813-7654 Ex. 202673
caliper.project@sickkids.ca

SickKids
THE HOSPITAL FOR SICK CHILDREN
CALIPER Phase II Studies 2017- 2020

• Canada-Wide Dissemination and Harmonization
  • Harmonization across labs and across instruments
    *(In Progress)*

• Influence of Ethnicity on Pediatric Reference Intervals
  • Caucasians, South Asians, East Asians, & Blacks
    *(REB Approved; Study Completed)*

• Postprandial Biomarkers
  • Non-fasting/postprandial markers in healthy and obese adolescents
    *(REB Approved; Study completed)*

• CALIPER Mother & Child Prospective Study
  • Comprehensive database of pregnancy, neonatal, and infantile reference intervals
    *(Initial planning and discussion phase)*
Closing the Gaps in Pregnancy, Neonatal & Infantile Reference Intervals:

The CALIPER Mother & Child Study (2017-2020)

**Pregnancy Reference Intervals**
*Trimester Specific:*
12-14, 24-26, 34-36 weeks

**Preterm Reference Intervals**
*Sex, Gestational Age & Birth Weight Partitions*

**Neonatal Reference Intervals**
*Sex & Age Specific:*
1-3 days, 4-7 days, 8-15 days, 16-30 days; Gestational Age and Birth Weight Partitions

**Infantile Reference Intervals**
*Sex & Age Specific:*
1-3 months, 4-6 months, 7-12 months

Study Milestones
## Analytes with Ethnic Partitions

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Ethnicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase (M+F)</td>
<td>CA</td>
</tr>
<tr>
<td>IgA (M+F)</td>
<td>CA + BK</td>
</tr>
<tr>
<td>IgG (M+F)</td>
<td>CA</td>
</tr>
<tr>
<td>IgM (F)</td>
<td>CA + BK</td>
</tr>
<tr>
<td>IgM (M)</td>
<td>CA + BK + SA</td>
</tr>
<tr>
<td>Ferritin (M)</td>
<td>CA + BK + SA</td>
</tr>
<tr>
<td>FSH (M)</td>
<td>CA + BK + SA</td>
</tr>
<tr>
<td>Vitamin D (M+F)</td>
<td>CA</td>
</tr>
</tbody>
</table>
Unpublished Data (to be submitted for publication)
25(OH) Vitamin D

Unpublished Data (to be submitted for publication)
Influence of Ethnicity on ALP Reference Ranges

Unpublished Data (to be submitted for publication)
Influence of Ethnicity on ALP Reference Ranges

Unpublished Data (to be submitted for publication)
SUMMARY: CALIPER Database

• CALIPER Database
  • Comprehensive Pediatric Reference Intervals from Birth to 18 years of age; most robust from 1 to 18 yrs
  Partitioned by Age, Sex, Ethnicity, BMI, Tanner Stage
  • New Mother & Child study initiated to obtain more robust neonatal and infantile reference values

• Key Resources:
  • CALIPER Web Database (www.caliperproject.ca)
  • CALIPER Mobile App (iTunes & Google Play)
  • CALIPER White Paper (Critical Reviews in Clinical Laboratory Sciences 2017)
  • CALIPER Review (BMJ 2018)
Acknowledgements - Team CALIPER

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