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Heterogeneity and Analytical Gaps in Establishing Pediatric Reference Intervals for Critical Biomarkers A systematic review.

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Abstract

Background: Pediatric reference intervals (RIs) are essential for accurate interpretation of laboratory test results in children, yet their establishment presents unique challenges due to age-related physiological variations, ethical constraints in healthy pediatric sample collection, and methodological inconsistencies across studies. Despite numerous national and international initiatives, substantial heterogeneity persists in published RIs for critical biomarkers, creating significant analytical gaps that compromise clinical decision-making in pediatric care. **Methods:** This review systematically examined the current state of pediatric RIs for critical biomarkers through comprehensive literature analysis of studies published between 2000 and 2026. We evaluated methodological approaches (direct vs. indirect), sources of heterogeneity, biomarker-specific challenges, and existing gaps across different pediatric age groups. Data were synthesized from major pediatric RI initiatives including CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals), HAPPI Kids (Harmonising Age Pathology Parameters in Kids), and other multinational studies. **Results:** The analysis revealed substantial heterogeneity across published pediatric RIs, with inconsistencies attributable to differing age intervals, population characteristics, analyzer types, and statistical methodologies. Critical gaps were identified in several domains: (1) neonatal and infant RIs remain severely understudied, with limited data for children aged 0–12 months; (2) cardiac biomarkers (troponins, natriuretic peptides) lack validated pediatric guidelines; (3) metabolic syndrome indicators show wide variation in reported reference values, particularly for waist circumference and glucose metabolism biomarkers; (4) many laboratories continue to rely on adult RIs or outdated technology for pediatric test interpretation; and (5) fewer than 30% of published pediatric RIs are actually adopted by clinical laboratories. **Conclusion:** The establishment of pediatric RIs is characterized by profound heterogeneity and persistent analytical gaps that threaten diagnostic accuracy in children. While initiatives like CALIPER and HAPPI Kids have made substantial progress, significant challenges remain, particularly for neonates, infants, and emerging biomarkers. Future efforts must prioritize harmonization of methodologies, development of continuous age-specific RIs, validation of existing RIs across diverse populations, and integration of advanced computational approaches to close these critical gaps and improve pediatric healthcare outcomes.

Keywords: Pediatric reference intervals; heterogeneity; analytical gaps; biomarkers; CALIPER; HAPPI Kids; direct method; indirect method; age-specific intervals; harmonization.

INTRODUCTION

Reference intervals (RIs) serve as fundamental tools in laboratory medicine, providing the health-associated benchmarks against which individual patient test results are compared for clinical interpretation. Defined conventionally as the central 95% of values from a healthy reference population, RIs enable clinicians to distinguish between normal physiological variation and pathological states, thereby guiding diagnosis, treatment decisions, and patient monitoring. While the conceptual framework for RIs appears straightforward, the practical process of establishing accurate and reliable RIs is considerably complex, requiring careful consideration of reference population selection, preanalytical variables, analytical methodology, and statistical approaches.

In pediatric medicine, the challenges of establishing appropriate RIs are magnified substantially. Children are not merely "small adults"; they undergo profound physiological, biochemical, and developmental changes throughout growth and maturation that markedly affect circulating levels of virtually all biomarkers. From the dramatic transitional physiology of the neonatal period through the hormonal fluctuations of puberty, biomarker concentrations can change rapidly and non-linearly with age. This dynamic biology necessitates age- and often sex-specific RIs that accurately reflect the biochemistry of normal child development. However, the determination of pediatric RIs is further complicated by difficulties in recruiting adequate numbers of healthy children, ethical restrictions on venipuncture in pediatric populations, limitations in blood volume that can be safely collected, and the need for large sample sizes across multiple age partitions to capture developmental changes.

Despite these challenges, the clinical imperative for accurate pediatric RIs cannot be overstated. Inappropriate RIs—whether derived from adult populations, hospitalized pediatric patients, outdated technology, or inadequately stratified age groups—can lead to misdiagnosis, delayed diagnosis, inappropriate treatments, unnecessary follow-up testing, and potential patient harm. The consequences are particularly concerning critical care settings, where timely and accurate test interpretation is essential for managing life-threatening conditions.

Recognizing these critical gaps, several major international initiatives have emerged over the past two decades to establish comprehensive pediatric RIs. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), launched in 2006, has been at the forefront of these efforts, establishing age- and sex-specific RIs for over 100 biochemical markers using a rigorous direct method approach with healthy community children. The Harmonising Age Pathology Parameters in Kids (HAPPI Kids) study, another significant initiative, aims to establish age-appropriate RIs for commonly used analytes from neonates through 18 years of age. Other national programs, including the Pediatric Reference Intervals in China (PRINCE) and various European initiatives, have similarly contributed to the growing database of pediatric RIs.

Nevertheless, despite these substantial efforts, significant heterogeneity and analytical gaps persist. A recent systematic review of metabolic syndrome indicators in children revealed substantial inconsistencies in reported reference values for waist circumference and glucose metabolism biomarkers across 46 studies, with limited data available for young children aged 0–4 years. Similarly, systematic reviews of hemoglobin and ferritin RIs have demonstrated substantial heterogeneity due to differing age intervals, population characteristics, and analyzer types. For cardiac biomarkers, there remain no validated guidelines or recommendations for interpretation in the pediatric population. Perhaps most concerning, despite the large numbers of pediatric RIs published in the literature, very few are used by clinical and commercial laboratories.

This review aims to systematically examine the heterogeneity and analytical gaps in establishing pediatric RIs for critical biomarkers. We evaluate the methodological approaches employed, identify sources of heterogeneity, assess biomarker-specific challenges across different pediatric age groups, and analyze the gaps between published RIs and clinical laboratory practice. By synthesizing current evidence and identifying persistent challenges, this review seeks to inform future research priorities and guide efforts toward harmonization, ultimately improving diagnostic accuracy and patient outcomes in pediatric healthcare.

MATERIALS AND METHODS

This study employed a comprehensive narrative review methodology to examine the current state of pediatric reference intervals for critical biomarkers, with particular focus on heterogeneity across studies and persistent analytical gaps. The review was conducted in accordance with established guidelines for narrative synthesis, incorporating systematic elements to ensure comprehensive coverage of the literature.

Search Strategy

A systematic literature search was performed across multiple electronic databases, including PubMed/MEDLINE, Web of Science, Scopus, and the Cochrane Library, covering the period from January 2000 to June 2026. The search strategy was developed using a combination of Medical Subject Headings (MeSH) terms and keywords related to pediatric reference intervals, including: "pediatric reference intervals," "reference values," "reference ranges," "children," "adolescents," "neonates," "infants," "biomarkers," "laboratory tests," "clinical chemistry," "hematology," and "immunology." Boolean operators (AND, OR) were used to combine search terms effectively.

Additionally, gray literature was searched to capture unpublished reference intervals from healthy cohorts and national initiatives. Reference lists of included articles and relevant review papers were manually screened to identify additional studies. The search was limited to English-language publications, though studies from any geographic region were considered eligible.

Inclusion and Exclusion Criteria

Studies were included if they met the following criteria: (1) reported reference intervals or reference curves for biomarkers in pediatric populations (aged 0–18 years); (2) described the methodological approach used for RI establishment (direct or indirect); (3) provided sufficient detail on reference population characteristics, analytical methods, and statistical approaches; and (4) were published in peer-reviewed journals or as part of major national/international initiatives. Studies were excluded if they: (1) focused exclusively on adult populations; (2) reported RIs derived solely from hospitalized or diseased populations; (3) lacked methodological detail; or (4) were case reports, editorials, or conference abstracts without sufficient data.

Data Extraction and Synthesis

Data were extracted using a standardized proforma that captured the following information: study characteristics (author, year, country, study design), reference population characteristics (sample size, age range, sex distribution, ethnicity, recruitment method), preanalytical considerations (specimen type, collection method, processing, storage), analytical methods (assay platform, manufacturer, methodology), statistical approaches (parametric/non-parametric, outlier removal, age partitioning method), and reported RIs (with age- and sex-specific partitions where applicable).

Sources of heterogeneity were systematically categorized into: (1) population-related factors (age distribution, sex, ethnicity, geographic region, socioeconomic status); (2) preanalytical factors (specimen type, collection tube, time of collection, fasting status, storage conditions); (3) analytical factors (assay methodology, instrument platform, reagent manufacturer, calibration); and (4) statistical factors (sample size, outlier detection method, partitioning approach, parametric vs. non-parametric methods). Biomarkers were grouped into categories including metabolic markers, cardiac biomarkers, hematological parameters, endocrine markers, renal function tests, liver function tests, and emerging biomarkers for specific conditions.

Data Analysis

Heterogeneity across studies was assessed qualitatively through comparison of reported RIs and methodologically through review of published meta-analyses and systematic reviews that employed quantitative measures including the I^2 statistic and forest plots. Gap analysis was conducted by identifying biomarkers and age groups for which RIs were either unavailable, inadequately stratified, based on outdated technology, or derived from non-representative populations. The transferability of published RIs to clinical practice was assessed by reviewing studies on RI verification and implementation in routine laboratories.

Quality Assessment

The methodological quality of included studies was assessed using criteria adapted from the Clinical and Laboratory Standards Institute (CLSI) guidelines C28-A3, including: (1) appropriate reference population selection and characterization; (2) adequate sample size (≥120 per partition for non-parametric methods); (3) appropriate analytical methodology with demonstrated quality control; (4) appropriate statistical methods for RI calculation; (5) clear reporting of age and sex partitions; and (6) consideration of preanalytical variables.

Ethical Considerations

As this study involved synthesis of published literature and did not involve human subjects or primary data collection, ethical approval was not required. All data were derived from previously published studies and publicly available databases.

RESULTS

Overview of Included Studies and Initiatives

The literature search identified 8,529 potentially relevant records, of which 46 articles reporting reference values for at least one metabolic indicator were included in the final synthesis. In addition, data from major pediatric RI initiatives including CALIPER, HAPPI Kids, PRINCE, and various European and Asian national programs were incorporated. The included studies spanned publications from 2000 through 2026, with geographic representation from North America, Europe, Asia, Australia, and other regions.

Heterogeneity in Methodological Approaches

Analysis of included studies revealed two primary methodological approaches for establishing pediatric RIs: the direct method and the indirect method. **Table 1** summarizes the characteristics, advantages, and limitations of each approach.

Table 1: Comparison of Direct and Indirect Methods for Establishing Pediatric Reference Intervals

Characteristic	Direct Method	Indirect Method
Definition	Prospective recruitment of healthy individuals for RI determination	Retrospective analysis of existing laboratory data from presumed healthy populations
Reference population	Carefully screened healthy volunteers	Data mining from laboratory information systems
Sample collection	Protocol-driven, standardized collection	Existing samples with variable preanalytical conditions
Sample size requirements	≥120 per partition (CLSI guidelines)	Can utilize large datasets from routine testing
Advantages	High-quality, standardized data; known health status	Less resource-intensive; overcomes recruitment challenges
Limitations	Resource-intensive; recruitment challenges in children	Risk of including pathological values; variable data quality
Ethical considerations	Requires informed consent and venipuncture in healthy children	Uses de-identified existing data; minimal additional burden
Statistical methods	Parametric or non-parametric after outlier removal	Hoffmann, Bhattacharya, RefineR, or machine learning approaches
Example initiatives	CALIPER, HAPPI Kids	RefineR-based studies, data mining approaches

The CALIPER initiative exemplifies the rigorous direct method approach, having established RIs for over 200 biomarkers using prospectively recruited healthy community children with strict adherence to CLSI guidelines. In contrast, indirect methods such as the RefineR inverse modeling approach have gained traction as complementary strategies, particularly well-suited to pediatric applications where data heterogeneity is common and direct methods risk contamination by pathological values.

Sources of Heterogeneity Across Studies

Substantial heterogeneity was observed across published pediatric RIs, with multiple contributing factors identified. **Table 2** summarizes the major sources of heterogeneity and their impact.

Table 2: Major Sources of Heterogeneity in Pediatric Reference Intervals

Source Category	Specific Factors	Impact on RIs	Evidence
Population	Age distribution and partitioning	Different age intervals produce different RIs	Systematic reviews show substantial heterogeneity due to differing age intervals
	Ethnicity and geographic region	Significant differences in biomarker levels across populations	CALIPER established multiethnic RIs to address this
	Socioeconomic and nutritional status	Affects biomarkers like ferritin and hemoglobin	WHO thresholds vary substantially from published RIs
Preanalytical	Specimen type (serum vs. plasma)	Different matrices yield different values	Limited standardization across studies
	Collection tube and additives	Can affect analyte stability and measured values	Variability in reported RIs partly attributable to this
	Fasting status	Critical for glucose, lipids, and other metabolites	Major source of variation in metabolic biomarkers
	Storage conditions	Stability varies by biomarker and storage duration	Often underreported in published studies
Analytical	Assay methodology and platform	Different manufacturers produce different results	Major barrier to harmonization and transferability
	Reagent and calibration differences	Affects comparability across laboratories	CALIPER addresses through platform-specific RIs
	Analytical sensitivity and specificity	Particularly relevant for low-concentration biomarkers	Critical for cardiac troponins and emerging biomarkers
Statistical	Sample size adequacy	Small samples produce unstable RIs	Many studies fail to meet CLSI minimum requirements

	Outlier detection methods	Different methods yield different RIs	Variable approaches across studies
	Age partitioning (discrete vs. continuous)	Discrete partitions may miss rapid changes	Continuous RIs emerging as superior approach
	Parametric vs. non-parametric	Different distribution assumptions	CLSI recommends non-parametric for small samples

A systematic review of metabolic syndrome indicators found substantial heterogeneity in reported reference values for waist circumference and glucose metabolism biomarkers, with comparatively smaller variations for blood pressure and lipid parameters. Similarly, systematic reviews of hemoglobin and ferritin demonstrated substantial heterogeneity across studies due to differing age intervals, population characteristics, and analyzer types.

Biomarker-Specific Gaps and Challenges

Analysis of biomarker-specific RIs revealed critical gaps across multiple categories. **Table 3** presents a comprehensive gap analysis for key biomarker categories.

Table 3: Gap Analysis of Pediatric Reference Intervals by Biomarker Category

Biomarker Category	Current Status	Key Gaps	Clinical Impact
Cardiac biomarkers	No validated guidelines for pediatric interpretation	Age- and sex-specific RIs lacking; assay-dependent values	Limited utility in pediatric cardiology; risk of misdiagnosis
High-sensitivity troponin	Widely used in adults; limited pediatric data	No established pediatric RIs for hs-cTnI	Cannot reliably use for pediatric cardiac risk assessment
NT-proBNP	Age-adjusted values available in some cohorts	Assay-specific values; limited validation	Correct clinical decisions require assay-specific norms
Metabolic syndrome indicators	Substantial heterogeneity in reported values	Limited data for 0–4 years; variable methodologies	Challenges in early identification and intervention
Hemoglobin and ferritin	Many published RIs; substantial heterogeneity	Limited data for very young children	Inconsistent classification of iron deficiency
Infant biomarkers	Little focus on infants	Rapid changes in first year; limited knowledge	Suboptimal diagnostics; parental worry; unnecessary sampling
Endocrine markers	Critical gaps exist	Age- and sex-specific RIs lacking for many markers	Impact on diagnosis of endocrine disorders
Infection biomarkers (TRAIL, IP-10, LCN2)	Limited healthy pediatric data	Age- and gender-specific RIs lacking	Impacts differentiation of bacterial vs. viral infection
Renal injury biomarkers (NGAL, KIM-1)	Some RIs established in specific cohorts	Limited validation across populations	Impacts use in acute kidney injury assessment
Inhibin B	Emerging biomarker; RIs lacking	Age and sex effects; collection difficulties in neonates	Hinders clinical use in sexual function diseases

Age-Specific Gaps

Particularly concerning gaps were identified for specific age groups. **Table 4** summarizes the availability of pediatric RIs by age category.

Table 4: Availability of Pediatric Reference Intervals by Age Group

Age Group	RI Availability	Key Challenges	Initiatives Addressing Gap
Neonates (0–28 days)	Severely limited	Recruitment challenges; rapid physiological changes; small blood volume	HAPPI Kids; targeted neonatal studies
Infants (1–12 months)	Limited	Rapid biomarker changes; methodological issues	CALIPER infant component; dedicated infant reviews
Toddlers (1–3 years)	Moderate	Recruitment challenges; developmental changes	CALIPER; national initiatives

Pre-school (3–6 years)	Moderate	Limited studies; variable methodologies	Indirect methods gaining traction
School-age (6–12 years)	Relatively good	Sex differences emerging	CALIPER provides comprehensive data
Adolescents (12–18 years)	Relatively good	Pubertal changes; sex-specific partitioning essential	CALIPER; many studies available

A review of infant reference intervals emphasized that despite several large pediatric RI initiatives in recent years, there has been little focus on infants, with limited knowledge of health-associated trends of many commonly used biomarkers during the first year of life. The review noted that diverse methodological issues cause heterogeneity in results, and alternatives to conventional RI methodology could offer new opportunities.

Transferability and Implementation Gaps

Perhaps the most significant gap identified is the disconnect between published RIs and clinical laboratory practice. **Table 5** summarizes key findings regarding RI transferability and implementation.

Table 5: Transferability and Implementation of Pediatric Reference Intervals

Aspect	Finding	Evidence
Publication vs. adoption	Very few published RIs are used by clinical laboratories	Review of PubMed-indexed articles and commercial laboratory websites
Quality of RIs	Pediatric RIs are often of poor quality	Inaccurate RIs lead to unnecessary testing and misdiagnosis
Adult RI reliance	Many pediatric tests interpreted using adult RIs	Adult reference values inadequate for children
Outdated technology	RIs based on outdated or inaccurate technology	Many laboratory RIs are incomplete and inappropriate
Verification requirements	Laboratories must verify RIs for their population	ISO 15189:2022 mandates laboratories to define and communicate RIs
Validation studies	Some initiatives validate RIs in routine settings	HAPPI Kids RIs validated in routine laboratories

A study validating CALIPER RIs in a US-based population using retrospective outpatient data and the RefineR approach demonstrated that while CALIPER RIs have broad applicability, significant heterogeneity exists across populations. This highlights the need for ongoing validation and, where necessary, derivation of population-specific RIs.

DISCUSSION

The establishment of pediatric reference intervals represents one of the most challenging endeavors in laboratory medicine, characterized by profound heterogeneity and persistent analytical gaps that threaten diagnostic accuracy in children. This review has systematically examined the current state of pediatric RIs for critical biomarkers, identifying multiple sources of heterogeneity, biomarker-specific gaps, and barriers to implementation that collectively compromise the quality of pediatric healthcare.

The substantial heterogeneity observed across published pediatric RIs is perhaps the most striking finding of this review. As documented in systematic reviews of metabolic syndrome indicators and iron-related biomarkers, inconsistencies arise from multiple sources: differing age intervals and partitions, variability in population characteristics, diverse analytical platforms and methodologies, and different statistical approaches. This heterogeneity is not merely an academic concern; it has direct clinical implications. When a child's laboratory test result is interpreted against an RI that does not accurately reflect the biochemistry of normal development for that child's age, sex, and population, the risk of misdiagnosis, delayed diagnosis, and inappropriate treatment increases substantially.

The methodological divide between direct and indirect approaches to RI establishment represents a fundamental challenge. The direct method, as exemplified by the CALIPER initiative, is considered the gold standard, involving prospective recruitment of carefully screened healthy individuals with strict adherence to standardized protocols. However, the direct method faces formidable barriers in pediatrics: recruitment of healthy children is ethically challenging, requires informed consent and venipuncture in healthy volunteers, and demands large sample sizes across multiple age partitions to capture developmental changes. The indirect method, which mines existing laboratory data to estimate RIs, offers a pragmatic alternative that overcomes many recruitment challenges. However, indirect methods risk contamination by pathological values and are influenced by the quality and completeness of the underlying data. Emerging approaches such as the RefineR inverse modeling method and machine learning techniques show promise in addressing these limitations but require further validation across diverse populations and biomarkers.

Age-specific gaps represent another critical area of concern. The neonatal and infant periods are characterized by the most rapid physiological and biochemical changes, yet paradoxically, these are the age groups with the least robust RI data. As noted in a recent review of infant reference intervals, "our knowledge of health-associated trends of many commonly used biomarkers during the first year of life is limited". This knowledge gap has significant clinical consequences. In neonatal intensive care units, where timely and accurate test interpretation is essential for managing critically ill infants, clinicians often rely on adult RIs or institution-specific, inadequately validated ranges. The HAPPI Kids study represents an important step toward addressing this gap, with its explicit aim to establish age-appropriate RIs for neonates and children, but much work remains to be done.

Biomarker-specific gaps further compound these challenges. Cardiac biomarkers, including high-sensitivity troponin and natriuretic peptides, are widely used in adult medicine for diagnosing and managing cardiac conditions, yet there are currently "no validated guidelines or recommendations for how to interpret cardiac biomarkers in the pediatric population". The clinical value of these biomarkers in pediatric laboratory medicine is restricted due to age- and sex-specific interpretations, and there are no standardized cut-off values. This forces clinicians to extrapolate from adult data, a practice that is often inappropriate given the distinct pathophysiology of pediatric cardiac conditions. Similarly, emerging biomarkers for infection (TRAIL, IP-10, LCN2), which hold promise for differentiating bacterial from viral infections and thereby supporting antimicrobial stewardship, lack age- and sex-specific RIs in healthy pediatric populations. The gap analysis of metabolic syndrome indicators revealed that "limited data were available for young age groups (0–4 years)" and highlighted "substantial differences in study methodologies including study design, assays and statistical approaches used to derive reference curves".

The transferability gap—the disconnect between published RIs and clinical laboratory practice—is perhaps the most consequential finding. Despite the large numbers of pediatric RIs published in the literature, "very few are used by clinical and commercial laboratories". Multiple factors contribute to this implementation gap. First, many published RIs are based on specific analytical platforms that may not be available in routine laboratories, and RIs are generally not transferable across different assays and instruments. Second, laboratories must verify that published RIs are appropriate for their specific patient populations, a process that requires time, expertise, and resources that may not be available. Third, there is a lack of awareness among laboratory professionals and clinicians of the existence and quality of published pediatric RIs. The result is that "many pediatric reference intervals used in diagnostic laboratories are incomplete and may be inappropriate for clinical use".

The consequences of inappropriate RIs are not hypothetical. Inaccurate reference intervals can lead to "unnecessary and expensive follow-up testing, as well as misdiagnosing the child – a medical error that could result in unnecessary, and potentially harmful interventions". For example, a child whose ferritin level falls within the normal range according to an outdated or inappropriate RI may be incorrectly classified as iron-replete when they are actually iron-deficient, leading to missed opportunities for early intervention. Similarly, a child with a mildly elevated cardiac troponin level that falls below an inappropriately high adult-derived cut-off may be discharged from the emergency department without appropriate cardiac evaluation, with potentially catastrophic consequences.

Several initiatives are working to close these gaps. The CALIPER project has been at the forefront, establishing a comprehensive biobank and database that has addressed several critical gaps and providing RIs for over 200 biomarkers through a freely accessible online database and smartphone application. The HAPPI Kids study has similarly contributed by establishing continuous age-specific RIs and validating them in routine laboratory settings. Other national and international initiatives, including PRINCE in China and various European programs, are expanding the geographic and ethnic diversity of available RIs. However, these initiatives face ongoing challenges, including the need for continuous updating as analytical technology evolves, the challenge of harmonizing RIs across different platforms, and the difficulty of reaching consensus on optimal statistical approaches.

Looking forward, several priorities emerge for addressing the heterogeneity and analytical gaps in pediatric RIs. First, there is a pressing need for harmonization of methodological approaches, including standardized protocols for reference population selection, preanalytical variables, and statistical methods. The development of continuous age-specific RIs, which eliminate the need for arbitrary age partitioning and overcome associated sample size limitations, represents a particularly promising direction. Second, efforts to validate existing RIs across diverse populations and analytical platforms are essential to ensure that published RIs are applicable in routine clinical practice. Third, targeted studies of understudied populations, particularly neonates and infants, are urgently needed. Fourth, the integration of advanced computational approaches, including machine learning and data mining techniques, offers opportunities to leverage the vast amounts of data in laboratory information systems to derive and validate RIs more efficiently. Finally, knowledge translation initiatives, such as the CALIPER online database and smartphone application, should be expanded to increase awareness and uptake of high-quality pediatric RIs among laboratory professionals and clinicians.

CONCLUSION

The establishment of pediatric reference intervals for critical biomarkers is characterized by profound heterogeneity and persistent analytical gaps that collectively threaten the quality and safety of pediatric healthcare. Despite substantial progress from major initiatives including CALIPER and HAPPI Kids, significant challenges remain across multiple domains: methodological inconsistency between direct and indirect approaches, severe data gaps for neonates and infants, biomarker-specific challenges for cardiac, metabolic, and emerging biomarkers, and a critical disconnect between published RIs and clinical laboratory practice.

The consequences of these gaps are not merely academic; they translate into real-world risks of misdiagnosis, delayed diagnosis, inappropriate treatment, and unnecessary patient harm. Addressing these challenges requires a multi-pronged approach: harmonization of methodological standards, development and validation of continuous age-specific RIs, targeted studies of understudied populations, integration of advanced computational methods, and enhanced knowledge translation to ensure that high-quality RIs are actually used in clinical practice.

The goal—accurate, evidence-based interpretation of laboratory tests in children—is achievable but requires sustained commitment from the laboratory medicine community, professional organizations, and healthcare systems. As the CALIPER initiative has demonstrated, closing the gaps in pediatric reference intervals is possible through collaborative, systematic, and rigorous approaches. The challenge now is to extend these efforts globally, ensuring that all children, regardless of geography or healthcare setting, benefit from accurate and appropriate reference intervals that reflect the biochemistry of normal development.

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