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ORIGINAL ARTICLE

Paediatric reference intervals are heterogeneous and differ considerably in the classification of healthy paediatric blood samples

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Abstract

The aim was to elude differences in published paediatric reference intervals (RIs) and the implementations hereof in terms of classification of samples. Predicaments associated with transferring RIs published elsewhere are addressed. A local paediatric (aged 0 days to < 18 years) population of platelet count, haemoglobin level and white blood cell count, based on first draw samples from general practitioners was established. PubMed was used to identify studies with transferable RIs. The classification of local samples by the individual RIs was evaluated. Transference was done in accordance with the Clinical and Laboratory Standards Institute EP28-A3C guideline. Validation of transference was done using a quality demand based on biological variance. Twelve studies with a combined 28 RIs were transferred onto the local population, which was derived from 20,597 children. Studies varied considerably in methodology and results. In terms of classification, up to 63% of the samples would change classification from normal to diseased, depending on which RI was applied. When validating the transferred RIs, one RI was implementable in the local population. *Conclusion*: Published paediatric RIs are heterogeneous, making assessment of transferability problematic and resulting in marked differences in classification of paediatric samples, thereby potentially affecting diagnosis and treatment of children.

What is Known:
- *Reference intervals (RIs) are fundamental for the interpretation of paediatric samples and thus correct diagnosis and treatment of the individual child.*
- *Guidelines for the establishment of adult RIs exist, but there are no specific recommendations for establishing paediatric RIs, which is problematic, and laboratories often implement RIs published elsewhere as a consequence.*

What is New:
- *Paediatric RIs published in peer-reviewed scientific journals differ considerably in methodology applied for the establishment of the RI.*
- *The RIs show marked divergence in the classification of local samples from healthy children.*

Keywords Classification · Paediatric · Reference interval · Transference

Abbreviations

RI	Reference interval
plt	Platelet count
hgb	Haemoglobin level
WBC	White blood cell
GP	General practitioner
CLSI	Clinical and Laboratory Standards Institute
QD	Quality demand
IFCC	Federation of Clinical Chemistry and Laboratory Medicine
CBC	Complete blood count
LH	Lillebaelt Hospital
OUH	Odense University Hospital
SD	Standard deviation
IQR	Interquartile range
ER	Emergency room

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Introduction

Reference intervals (RIs) are essential for the interpretation of paediatric biochemical results, enabling distinction between healthy and diseased children. It is thus fundamental that the RIs are representative of the paediatric population, and providing age- and gender-specific RIs is considered an important responsibility of clinical laboratories [20].

The Clinical Laboratory Standards Institute (CLSI) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have created a guideline for the establishment of RIs (EP28-A3C), in which a direct sampling approach using representative, healthy individuals is recommended [5]. However, this approach is demanding, costly and time-consuming; particularly in a paediatric population, requiring large populations to create multiple age and gender partitions. Sampling from healthy children, especially very young children, is furthermore technically difficult and has considerable ethical implications [20, 25].

According to the CLSI guideline, RIs might also be transferred from other populations, e.g. from kit inserts or the literature [5]. Subsequently, transference of the RI has to be validated. Validation of a transferred RI often relies on subjective assessment, as the recommended method of validation also requires direct sampling. Assessment of RI applicability is however crucial, as misclassification of samples as either healthy or diseases can result in erroneous diagnosis and treatment. Choosing which RI to implement can therefore be precarious.

The aim of this study is to evaluate differences in published paediatric RIs for haemoglobin (hgb), platelet count (plt) and white blood cell count (WBC) by assessing how the RIs classify local laboratory results stemming from healthy children. The significance of implementing different RIs will thus be highlighted. Means of validating transferred RIs by using locally generated laboratory data will further be illustrated.

Materials and methods

Selection of RIs

A search on PubMed (17th of March, 2018) on existing paediatric plt count, hgb and WBC count RIs was conducted. We included studies which made assessment of the RI according to the topics listed in Table 1 possible. Studies were excluded if they were conducted on a study population substantially different from Southern Denmark in terms of ethnicity (i.e., Caucasian) and overall children health as this could influence the complete blood count (CBC). Physician density [32] and under-five mortality rate [31] were used to assess conformity of reference populations. Thus, we only included studies conducted on European and North American populations. Studies on neonates were also excluded.

Assessment of RIs

Studies were evaluated according to the criteria listed in Table 1.

To standardise for comparison, we converted hgb results in g/dL and g/L to mmol/L by multiplying by 0.6206 and 0.06206, respectively [30]. Plt and WBC counts were declared in $\times 10^9$/L.

The paediatric population

We defined a dataset of paediatric (age 0 days to < 18 years) haematological parameters from general practitioners (GPs) analysed at Lillebaelt Hospital (LH) and Odense University Hospital (OUH) over a 5-year period (13 September 2012–4 August 2017), which constituted our LH/OUH population. The local population was used to assess classification of samples and validate transference of RIs. We exclusively used samples originating from GPs for our paediatric population, as both hospitals manage specialised paediatric functions (i.e. paediatric emergency wards, neonatological wards and paediatric oncology wards). To ensure a healthy population, children with > 3 repeat measurements, or samples taken at hospital, were excluded, and only the first samples of each child was included in the final dataset. Both LH and OUH are situated in the region of Southern Denmark. Plt, hgb and WBC counts were analysed using Sysmex XN-9000 (Sysmex, Kobe, Japan).

Statistical validation of the transferred RIs

The LH/OUH dataset was transformed to a Gaussian distribution using Box-Cox transformation. Subsequently, the dataset was partitioned into 1-year age stratifications and subdivided according to gender. After the stratification, outliers were removed using the method by Tukey [28]. We then examined the classification of samples by determining the percentage of samples from our dataset that would be classified as above or below the RI for each transferred RI based on 1-year age intervals. We furthermore calculated the overall percentage of local samples above and below each RI. A quality demand (QD) based on desirable performance in relation to biological variance was applied [11]. With this, we determined whether RIs were transferable (i.e. validated the transference of the RI). According to the QD, RIs were deemed suitable for application in the local population if no more than 6% of local samples were outside of the RI in total, with a maximum of 4.5% outside one RI limit [17].

Frequencies are presented as numbers and percentages. Normally distributed data were presented as mean and

Table 1 Assessment criteria of published RIs based on the CLSI guideline [5]

Criteria	Specification
Sampling collection	Direct (reference individuals selected based on specific criteria) or indirect method (data compiled in a hospital laboratory over a given time period)
Preanalytical considerations	Age range of participants, sample collection method, time from sample collection to analysis, fasting of reference subjects
Health assessment of participants	Subjective health evaluation, use of questionnaires, physical examination
Number of participants	Total number of participants, number of participants in gender and age subclasses
Analytical equipment used	Manufacturer and model
Statistical methods applied	Statistical approach for partitioning, handling of outlying observations, and presentation of RI

standard deviation (SD) and median and interquartile ranges (IQRs) for non-normally distributed data. Data were analysed with Stata software package (Stata 15.1; StataCorp, College Station, TX, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

Published paediatric RIs for transference

We found 12 studies on paediatric RIs with a combined 28 RIs. Eleven RIs were found for plt count, ten for hgb and seven for WBC count. Descriptive data of the studies are presented in Table 2.

The designs of the selected published studies on RI are shown in Table 2.

The majority of studies use a direct sampling approach, as only Soldin et al. [23] and Zierk et al. [33, 34] based their RIs on laboratory data. Health assessment of reference individuals in the studies using a direct sampling approach varied considerably, and two studies used different means of health assessment depending on the children's age [2, 21]. Range and number of age partitions furthermore differed, as well as the use of gender subclasses. Two studies [1, 2] used the CLSI recommended method for partitioning, whilst four studies [8, 23, 33, 34] did not disclose the method used, five relied on subjective evaluation [12–14, 21, 27] and one used other means of partitioning [3]. Similarly, four studies adhered to the recommendations for handling outlying observations, using either Tukey's method [1, 33, 34] or Dixon's rule [2]. Venepuncture was used to draw samples in all studies using a direct approach, whereas this was not addressed in the studies using the indirect sampling method. Time from sampling until analysis varied across studies, ranging from 'immediately' [1], within 1–6 h [3, 12, 13, 21, 27] to a maximum of 3 days post sampling [8]. Four studies did not disclose time from sampling till analysis [14, 23, 33, 34]. Different analytical platforms were used across studies. RIs were mainly reported as the 2.5th–97.5th percentile [1–3, 21, 23, 33, 34].

The LH/OUH population

Selection and characteristics of individuals in the local population is shown in Fig. 1. First sampling from 20,597 individuals < 18 years with combined 17,704 hgb, 13,849 plt and 18,658 WBC analyses constituted the final local population.

The majority of samples were taken from girls (55%). Distributions of data were non-Gaussian. Median plt count was 280×10^9/L (IQR 240 - 327×10^9/L), median hgb level was 13.37 g/dL (IQR 12.73 g/dL–14.18 g/dL) (8.3 mmol/L (IQR 7.9–8.8 mmol/L), and median WBC count was 6.8×10^9/L (IQR 5.6–8.4×10^9/L). A total of 831 outlying observations (227 for plt, 243 for hgb and 361 for WBC) were removed using Tukey's method [28].

RI classification of samples

The classification of the LH/OUH samples by the individual RIs was evaluated and the percentage of samples classified as normal or abnormal (i.e. above or below the upper and lower RI limit) calculated. Details can be seen in Fig. 2. Overall, 0.6% of children had hgb results that were above the upper limits of all RIs, and 0.4% had results below all lower RI limits. In relation hereto, 36% of children had hgb results within all RI limits, meaning 63% of children in the LH/OUH population had hgb results that would change classification from normal to abnormal, depending on which RI was applied. Thus, 4.088 (42%) children had results that would change classification from normal to anaemic, whilst 2.020 (21%) children would change from normal to polycythaemic. For plt, 66% of children were within the RI limits, with 33% of children changing classification depending on RI, as 938 (12%) children would change classification form normal to thrombocytopenic and 1.570 (21%) from normal to thrombocytotic. For WBC, 22% of children would change classification, as 1.154 (12%) children would change from normal to leucopoenia, and 968 (10%) would change from normal to leukocytosis, depending on which RI was

Table 2 Detailed description of the included studies

Study (reference)	Analyte	Age range	Age partition (*n*)	Gender subclass	Sampling method	Health assessment	Method of partitioning	Handling of outlying observations	Analytical equipment	Reference range
Soldin et al. [23]	Plt ($n = 11,825$) Hgb ($n = 12,859$) WBC ($n = 10,010$)	0 ≤ 18years	9	+	Indirect; based on laboratory data	None, exclusion criteria applied[a,b]	Not disclosed	Chauvenet's Criterion, Hoffmann truncation	Sysmex XE-2100	2.5th - 97.5th pctl
Adeli et al. [1]	Plt ($n = 3790$) Hgb ($n = 6590$) WBC ($n = 6700$)	3–26 years	4 (plt) 6 (hgb) 2 (WBC)	+[c]	Direct; population representative cross-sectional study	Questionnaire and physical examination	Harris and Boyd	Tukey's method	Beckman Coulter HmX	2.5th–97.5th pctl
Hinchliffe et al. [13]	Plt ($n = 254$) Hgb ($n = 254$)	2–13 months	3	–	Direct; sampling of healthy infants participating in vaccination trial	Physical examination	Subjective evaluation	Not disclosed	Siemens-Bayer H1	95% range
Zierk et al. [33]	Plt (n = 58,040) Hgb ($n = 58,065$)	0–16 years	Continuous RI	+	Indirect; based on laboratory data	None	Not disclosed	Tukey's method	Sysmex XE-2100	2.5th–97.5th pctl
Zierk et al. [34]	Plt ($n = 32,271$) Hgb ($n = 32,276$) WBC ($n = 32,274$)	0 < 19 years	Continuous RI	+	Indirect; based on laboratory data	None	Not disclosed	Tukey's method	Sysmex XE-2100	2.5th–97.5th pctl
Biino et al. [3]	Plt ($n = 31,849$)	> 15–64 years	2	+	Direct; population representative cross-sectional study	Questionnaire and physical examination	T-test and ANOVA	Distribution evaluation	Sysmex-Beckman Coulter HmXr	2.5th–97.5th pctl
Giacomini et al. [12]	Plt ($n = 200$)	1–18 years	2	–	Direct; children participating in thalassemia open screening	Method not disclosed	Subjective evaluation	Not disclosed	Siemens-Bayer Advia 120	95% range
Aldrimer et al. [2]	Plt ($n = 436$) Hgb ($n = 436$) WBC ($n = 432–436$)[d]	6 months–18 years	2 (plt) 3 (hgb) 1–3 (WBC)[d]	Only for hgb	Direct; population representative cross-sectional study	Questionnaire and physical examination	Lahti et al.	Dixon's rule	Siemens- Bayer Advia 2120	2.5th–97.5th pctl
KiGGs [8]	Hgb ($n = 14,075$)	1.5–17.5 years	33	+	Direct; population representative cross-sectional study	Questionnaire	Not disclosed	Winsorization	Abbott Cell-Dyn 3500	3rd–97th pctl
Romeo et al. [21]	Plt ($n = 571$) Hgb ($n = 584$) WBC ($n = 574$)	13–18.5 years	5	–	Direct; school children	Questionnaire	Subjective evaluation	Not disclosed	Beckman Coulter GenST	2.5th–97.2nd pctl
Taylor et al. [27]	Plt ($n = 2107$) Hgb ($n = 2120$) WBC ($n = 2122$)	4–19 years[e]	13	+[f]	Direct; school children	Questionnaire	Subjective evaluation	Not disclosed	Beckman Coulter GenST	3rd–97th pctl
Hollowell et al. [14]	Plt ($n = 2720$) Hgb ($n = 2722$) WBC ($n = 2722$)	1–19 years	6	+	Direct; population representative cross-sectional study	Interview and physical examination	Subjective evaluation	Not disclosed	Coulter S + Jr	5th–95th pctl[g] 10th–90th pctl[h]

Hgb haemoglobin, *plt* platelet count, *WBC* white blood cell count

[a] Haematology oncology clinic patients were excluded

[b] WBC specimens from emergency room excluded for some male partitions

[c] Gender subclasses for ages 14–26 years for plt, and 11–79 years for hgb

[d] Age partitions depending on specific WBC population

[e] Ages 4–19 years for girls, 4–18 for boys

[f] Depending on WBC subset

[g] 5th–95th percentile for boys and girls aged 1–5 years and girls aged 15–19 years

[h] 10th–90th percentile for boys aged 6–19 years and girls aged 6–14 years

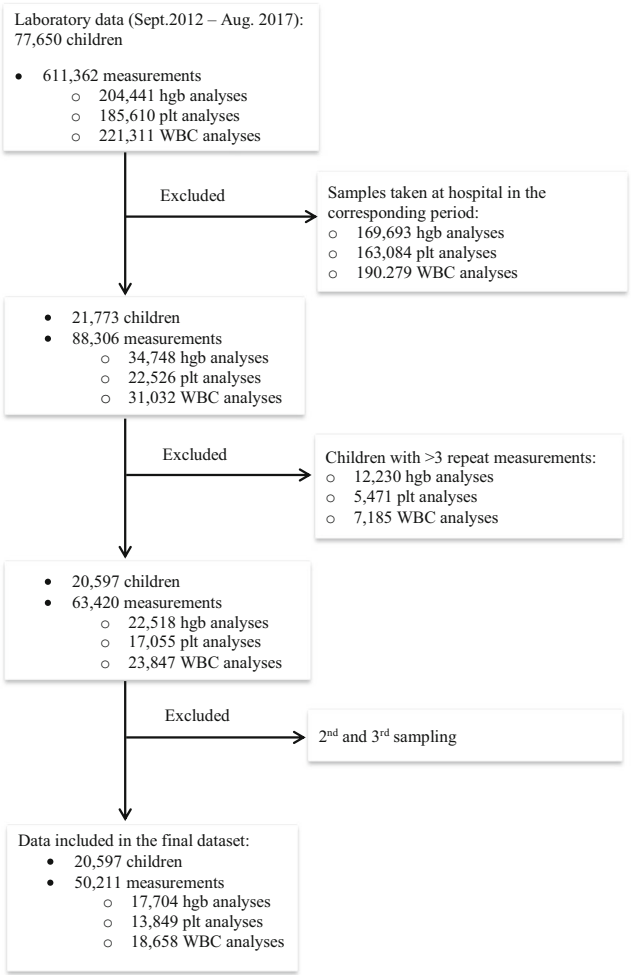

Fig. 1 Selection of the local paediatric population. Flowchart illustrating the data mining process of including and excluding local paediatric LH/OUH samples

applied. Details are shown in Fig. 2, which furthermore show the distribution of LH/OUH samples in relation to the published RI limits for hgb, plt and WBC, respectively.

Validation of transferred RIs

In accordance with the QD, RIs were suitable for implementation in the LH/OUH population if ≤ 6% of local samples were outside the RI limits in total, with a maximum of 4.5% of samples outside either the upper or lower limit. The total percentage of samples outside an RI varied from 3.7% (hgb RI, Romeo et al. [21]) to 39.3% (hgb RI, Hinchliffe et al. [13]). The hgb RI from Romeo et al. [21] was applicable in the LH/OUH paediatric population, but the remainder were not. Table 3 shows the percentage of samples from our LH/OUH population that were above or below each published RI.

The mean percentage of local samples outside the RI limits differed according to the analytical equipment used, with 14% (SD 9%) for the Sysmex analytical platform (i.e. the same platform as used in the local LH/OUH population), 17% (SD 11%) for the Beckman Coulter and 18% (SD 14%) for the Siemens/Bayer Advia equipment. On average, the mean percentage of local samples outside the RI limits furthermore differed according to the method applied for handling outlying observations, as 33% (SD 13%) of local samples were outside the RI limits of studies using the recommended methods (i.e. Dixon's rule or Tukey's method), whilst 43% (SD 29%) of local samples were outside the RI limits for studies using alternative methods for handling outlying observations (i.e. Chauvenet's Criterion, Hoffmann truncation, distribution evaluation, Winsorization, or not disclosed). Percentage of samples outside the RI for studies using the indirect method was 39% (SD 21%) and 39% (SD 27%) for studies using a direct sampling approach.

Discussion

Twenty-eight paediatric RIs for plt, hgb and WBC from populations comparable to our local population were identified. The RIs differed considerably, as nearly two thirds of the local population would change classification from normal to abnormal, depending on which RI was chosen. When applying a QD based on desirable biological variation, 1 of the 28 RIs was transferable onto the LH/OUH population.

Comparison of studies was difficult, as there was considerable variation in methodology, including preanalytical variables, where congruency between studies was limited, making it problematic to determine whether they could apply for our local population. Preanalytical considerations are critical in the establishment of RIs and interpretation of blood sample results in general, as several preanalytical factors may influence results [4, 29]. In young children, capillary sampling is commonly used in daily practice [16]. Several CBC analytes yield different values in capillary compared to venous blood in children [7, 15, 19], especially plt count, as significant underestimations have been reported in capillary blood [10, 26]. Even so, Hollowell et al. [14] was the only study addressing capillary sampling. It is essential for laboratories to be aware of the sampling method and to take it into account when conveying results to the clinicians, as interpretation of capillary samples based on venous RIs should be done with caution.

Discrepancy in means of health assessment was also prominent, with two studies even applying different approaches for health evaluation depending on the children's age [2, 12]. In large reference populations, data mining of hospital samples supersedes health, as the majority of hospital samples will be within the normal range [33,

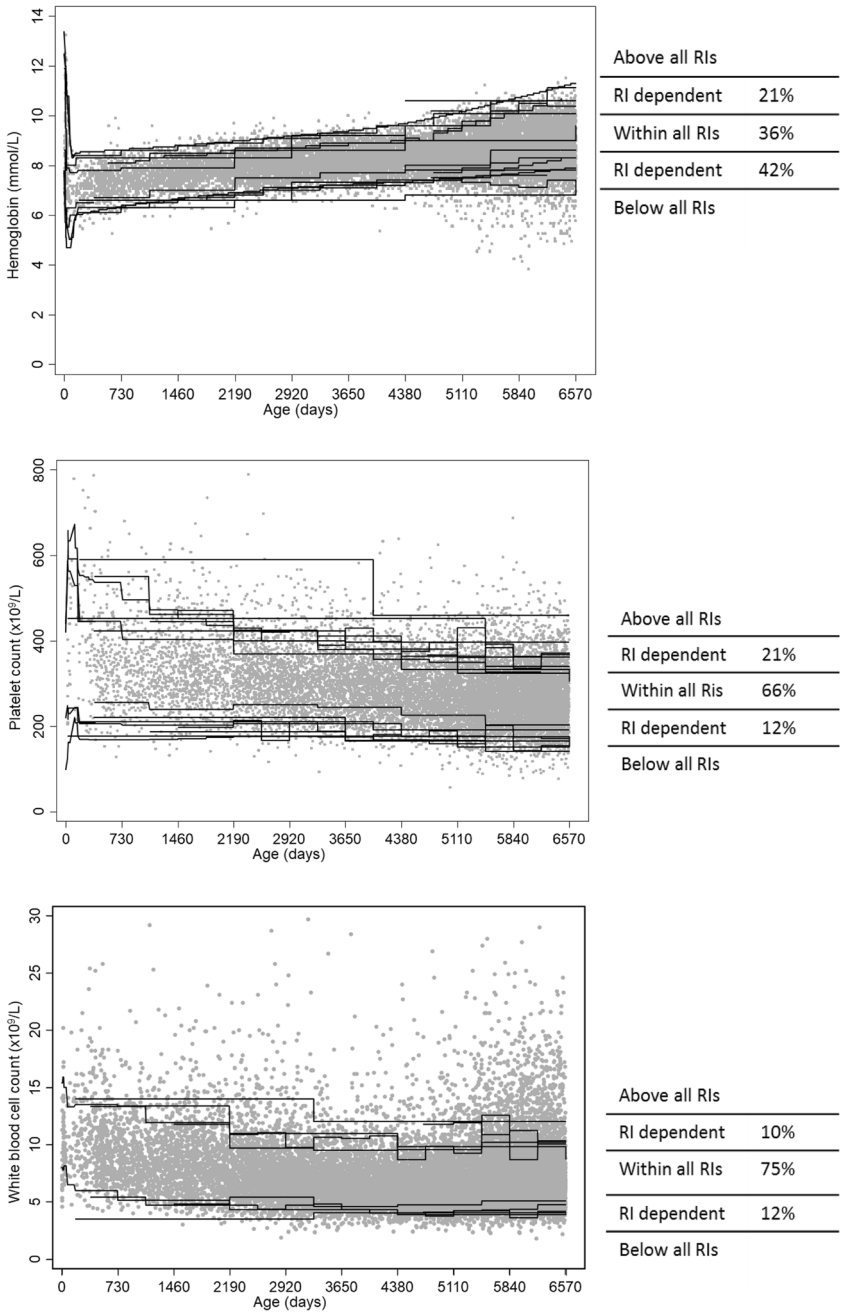

Fig. 2 Local LH/OUH samples in relation to the age-specific lower and upper RI limits set by the included studies. Black lines reflect published reference limits. Grey dots are measurements from the local dataset. Percentage of samples changing classification depending on which RI was applied on the local population is shown for each parameter. Hgb haemoglobin, plt platelet count, WBC white blood cell count

34]. Interestingly, we observed no difference in the RIs based on direct and indirect sampling in terms of the percentage of samples outside the RI. For transference of RIs in general, a data mining process is also more easily standardised and thus easier to evaluate. Another advantage of laboratory date is the size of reference population attainable. Especially in paediatrics, it is difficult to establish sufficiently large populations with the direct sampling method, which is highlighted by only two of the studies evaluated here [1, 3] having sufficient individuals for each partition (i.e. minimum 120 individuals) for all their RI parameters. Insufficient number of reference individuals diminishes the statistical validity of a RI, thus limiting its use.

Methods applied for partitioning and the ensuing age stratifications were exceedingly different among studies. Partitions are only justified in situations where they depict clinically relevant differences. As many analytes are influenced by the physiological changes during childhood [6, 9, 18, 20, 22, 24], incorrect partitioning can contribute to misclassification and hence misinterpretation of results. To amend this, Zierk et al. [33, 34] argued that continuous RIs reflect the physiological changes better than conventional RIs. In a paediatric population, this approach

Table 3 LH/OUH samples below and above the reported RIs. The dataset was evaluated in 1-year intervals, and the percentage of sample outside the RI was calculated for each 1-year interval. Range denotes the minimum and maximum percentage of samples outside the RI. RIs transferable according to the 6% QD are marked in bold. *Hgb* haemoglobin, *plt* platelet count, *WBC* white blood cell count

Study (Reference)	Plt		Hgb		WBC	
	% of samples below RI (range)	% of samples above RI (range)	% of samples below RI (range)	% of samples above RI (range)	% of samples below RI (range)	% of samples above RI (range)
Soldin [23]	4.7 (0.6–9.2)	14.3 (7.7–24.5)	0.5 (0.0–6.3)	36.4 (15.5–44.7)	3.4 (0.9–15.2)	9.7 (1.8–18.1)
Adeli [1]	1.1 (0.0–1.9)	11.2 (5.2–15.0)	9.4 (1.0–18.3)	23.8 (0.2–42.4)	1.1 (0.1–2.1)	8.6 (4.0–16.8)
Hinchliffe [13]	7.2 (6.7–12.5)	26.8 (0.0–29.2)	7.2 (7.1–10.0)	32.1 (0.0–33.3)	–	–
Zierk [33]	0.7 (0.0–2.0)	11.1 (2.0–15.2)	3.2 (0.0–7.6)	3.9 (0.1–10.4)	–	–
Zierk [34]	0.3 (0.0–1.2)	11.4 (4.2–19.1)	5.4 (0.0–13.2)	5.1 (0.0–12.4)	1.7 (0.0–2.9)	6.7 (1.2–10.0)
Biino [3]	0.6 (0.0–1.0)	6.0 (5.7–13.0)	–	–	–	–
Giacomini [12]	2.8 (0.0–8.2)	4.8 (2.7–22.6)	–	–	–	–
Aldrimer [2]	5.6 (2.2–7.9)	0.3 (0.0–9.5)	9.4 (0.0–15.4)	6.6 (0.0–25.0)	1.9 (0.0–4.0)	4.3(1.3–18.0)
KiGGs [8]	–	–	8.0 (0.0–23.7)	15.0 (6.4–25.3)	–	–
Romeo [21]	1.5 (0.0–3.7)	5.2 (1.2–7.0)	**3.2 (1.7–5.1)**	**0.5 (0.0–1.9)**	5.2 (3.0–8.1)	7.8 (0.0–12.0)
Taylor [27]	3.80.2–7.1)	6.5 (2.9–12.5)	16.2 (1.0–35.1)	12.8 (0.0–29.2)	5.7 (0.0–11.0)	9.7 (0.0–18.4)
Hollowell [14]	14.0 (8.1–20.7)	7.5 (1.9–11.3)	24.6 (2.4–47.9)	15.4 (3.4–26.5)	14.3 (1.2–20.0)	9.9 (6.6–18.9)

however only seems feasible with an indirect sampling method.

The number of samples classified as healthy in our dataset (i.e. within the RI) differed substantially according to which RI was applied. Of clinical relevance, 63% of the LH/OUH children had hgb values that would change classification from normal to diseased, depending on which RI was applied. For WBC and plt, percentages were 22% and 33%, respectively. As several precautions were taken in the present study to ensure a healthy local population, the variation is not likely to be due to underlying disease in the local population. Our study included all paediatric RIs available that were conducted on a population comparable to the local paediatric population in terms of ethnicity and health status. The difference in classification of samples is thus more likely due to the heterogeneity in study designs, rather than physiological differences between the populations and emphasises the difficulties in choosing an appropriate RI from the literature.

The troubles associated with accepting a transferred RI is supported by other parts of the present study. Ideally, when implementing a published RI to a local population, the QD should approximate the definition of an RI, thus having a maximum of 2.5% of samples outside either RI [17]. In reality, this is however not feasible. The CLSI guideline suggests accepting a transferred RI if ≤ 10% of local samples are outside the RI. This validation approach is based on direct sampling of 20 healthy representative individuals, which is often unsuitable in paediatrics, especially in young children. The alternative, subjective validation is not well described. Given how vastly different the RI classified samples from healthy children are, it is important to bear in mind that seemingly suitable RIs with comparable reference populations is not enough for this subjective evaluation. We therefore used a QD based on biological variation which also accounts for bias [11]. As demonstrated in this article, it albeit leads to very few RIs deemed acceptable for transference.

The present study clearly shows the heterogeneity in published reference intervals and emphasises the challenges associated with transference of RIs and highlights the necessity for standardisation of the methodology applied for the establishment of paediatric reference intervals in order to minimise potential misinterpretation and misclassification of paediatric samples.

In conclusion, published paediatric RIs constitute a very heterogeneous group in terms of results and methodology. As a result, classification of samples by different RIs exhibit marked divergence. Assessment of RI transferability is problematic with seemingly few transferable RIs. Laboratories seeking to transfer RIs onto their local paediatric population therefore face a wide range of pitfalls in the process, which ultimately influence the interpretation of paediatric samples, thus possibly affecting the diagnosis and treatment of children.

Authors' contributions Both authors contributed equally to the present study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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