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Pediatric reference intervals for alkaline phosphatase

Abstract

Background: Interpretation of alkaline phosphatase activity in children is challenging due to extensive changes with growth and puberty leading to distinct sex- and age-specific dynamics. Continuous percentile charts from birth to adulthood allow accurate consideration of these dynamics and seem reasonable for an analyte as closely linked to growth as alkaline phosphatase. However, the ethical and practical challenges unique to pediatric reference intervals have restricted the creation of such percentile charts, resulting in limitations when clinical decisions are based on alkaline phosphatase activity.
Methods: We applied an indirect method to generate percentile charts for alkaline phosphatase activity using clinical laboratory data collected during the clinical care of patients. A total of 361,405 samples from 124,440 patients from six German tertiary care centers and one German laboratory service provider measured between January 2004 and June 2015 were analyzed. Measurement of alkaline phosphatase activity was performed on Roche Cobas analyzers using the IFCC's photometric method.
Results: We created percentile charts for alkaline phosphatase activity in girls and boys from birth to 18 years which can be used as reference intervals. Additionally, data tables of age- and sex-specific percentile values allow the incorporation of these results into laboratory information systems.
Conclusions: The percentile charts provided enable the appropriate differential diagnosis of changes in alkaline phosphatase activity due to disease and changes due to physiological development. After local validation, integration of the provided percentile charts into result reporting facilitates precise assessment of alkaline phosphatase dynamics in pediatrics.

Keywords: alkaline phosphatase; pediatric; percentile charts; reference ranges.

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Introduction

Alkaline phosphatase is a ubiquitous enzyme in humans and an important biomarker for skeletal and hepatobiliary diseases [1–3]. Clinical interpretation is enabled with reference intervals, which allow classification of samples in the context of intra- and inter-individual variation. However, growth, changes in bone metabolism, and changes in the hepatobiliary system result in a pronounced age- and sex-dependent variability of alkaline phosphatase activity during childhood, especially in infancy and puberty.

These dynamics must be considered when defining and using reference intervals for alkaline phosphatase activity to distinguish between change due to physiological development and change due to disease.

An overview of published reference intervals for alkaline phosphatase established using current analytical methods is shown in Table 1. Age-dependent changes are represented either with separate reference intervals for different age groups or using reference intervals which change continuously with age. While separation into age groups is well-established in clinical practice and allows easy integration of reference intervals into current laboratory information systems, this approach can lead to misclassification of samples, especially at the margins of age groups (i.e. when upper and lower reference limits change abruptly due to changes in age). Continuous reference intervals reflect the continuous change of reference intervals with age and therefore allow a more accurate representation of the physiological dynamics of alkaline phosphatase activity. The most sophisticated approach to alkaline phosphatase activity interpretation are percentile charts analogous to other developmental quantities (e.g. weight- and height-for-age charts), which enable a more precise consideration of intra- and inter-individual differences than a categorization relative only to the reference interval boundaries. However, large numbers of samples are needed to create continuous representations of reference intervals and percentile charts. As access to blood samples from healthy children is restricted by ethical and practical objections, availability of percentile charts and continuous reference intervals for alkaline phosphatase from birth to adulthood is limited, leading to limitations when interpreting alkaline phosphatase activity in childhood [24].

In the studies listed in Table 1, upper and lower reference intervals for alkaline phosphatase activity are provided. Less recent studies, including surveys referred to by assay manufacturers [25, 26], only report upper reference limits because of a focus on diseases characterized by elevated alkaline phosphatase activity. However, this approach results in misclassification of abnormally low values and may result in missing medical conditions associated with reduced alkaline phosphatase activity. The introduction of therapeutic agents for hypophosphatasia and the accompanying marketing have emphasized the importance of lower reference limits for alkaline phosphatase activity as a screening marker for this congenital condition. Continuous classification of test results with percentile charts from birth to adulthood can improve the usefulness of alkaline phosphatase activity as a screening marker for hypophosphatasia and related conditions.

In a recent study – based on a data-mining approach using a single center's laboratory data – we demonstrated the complex pattern of change in alkaline phosphatase activity over time, with a high accuracy of age- and sex-dependent changes in infancy and puberty [6]. However, representation of the extensive dynamics after birth in neonates (0–28 days) with a high age-specific accuracy, and the creation of percentile charts require analysis of a more comprehensive data set.

The aim of this report is the creation of continuous reference intervals and percentile charts for alkaline phosphatase activity from birth to adulthood. To overcome restrictions due to sample size limitations, we employ a data-mining approach using multi-center laboratory data. This allows special consideration of age-specific accuracy in the first days of life and the creation of percentile charts to enable precise interpretation of patient test results over time.

Materials and methods

An established indirect method was used to generate continuous percentile charts [6].

Study population and selection of samples

We analyzed measurements of alkaline phosphatase activity performed during clinical care of in- and outpatients in six German tertiary care centers (denoted centers A, B, C, D, F, G) and measurements from outpatients sent to a German laboratory service provider (denoted center E). Supplemental Table 1 shows an overview of the participating centers and laboratories.

Alkaline phosphatase measurements performed for inpatients and outpatients aged ≤18 years, including patients from intensive care units and specialty units, were retrieved from the participating laboratories' databases. All measurements were retrieved, irrespectively of whether they were performed as part of a general panel or due to a specific indication. The time period examined covered January 2004 to June 2015 in order to provide a maximum number of samples measured with identical analytical methods. The population in Germany – and the patient population examined – is composed predominantly of Caucasian individuals; stratification according to ethnicity was not performed.

Analytical procedures

Measurements of alkaline phosphatase activity were performed on a Roche Cobas Integra 800, Roche Cobas 6000, and Roche Cobas 8000 (Supplemental Table 1). The analytical stability over time during the study period is demonstrated by stable quarterly median values (Supplemental Table 2). Statistical testing for significant trends in the quarterly median values during the study period was performed with

Table 1: Reference intervals for alkaline phosphatase.

Year	Age range	Data base/method	Analytical platform	n	Sampling period	Representation of age-dependent dynamics	Percentile charts	References
2015	1 month to 18 years	Healthy Tanzanian children	Cobas Integra 400 plus	619 children/samples	2009–2011	4 separate age groups	No	Buchanan et al. [4]
2015	Birth to 18 years	Data mining of Australian primary care data[a]	Roche analyzers	56,712 children/samples	2012–2013	Continuous	Yes	Loh and Metz [5]
2015	Birth to 18 years	Data mining of a German tertiary care center	Cobas Integra 800	14,904 children/63,270 samples	2004–2013	Continuous	No	Zierk et al. [6]
2014	½ year to 18 years	Healthy Swedish children	Abbott Architect ci8200	693 children/samples	Not specified	4 separate age groups	No	Ridefelt et al. [1]
2014	1 month to 12 months	Healthy Canadian children	Beckman Coulter UniCel DxC600	132 children, 651 samples	2007–2011	Continuous	Yes	Gallo et al. [7]
2014	2 years to 14 years (♀) 2 years to 16 years (♂)	Korean children who underwent a short stature workup but were diagnosed as normal	Hitachi 7600	2474 children/samples	2010–2012	3 separate age groups	No	Cho et al. [8]
2013	5 years to 18 years	Healthy Danish children	Roche Modular Analytics P	1429 children	2006–2008	6 separate age groups	No	Hilsted et al. [9]
2012	Birth, infants	Healthy Ethiopian newborns (cord-blood) and infants	HumaStar 300	60 newborns, 57 infants	2010–2011	separate reference intervals for newborns and infants	No	Melkie et al. [10]
2012	Birth to 18 years	Healthy Canadian children	Abbott Architect c8000	1213 children/samples	Not specified	7 separate age groups	No	Colantonio et al. [11]
2011	6 years to 18 years	Healthy Canadian children	Roche P-modular automated analyzer	205 children/samples	Not specified	4 separate age groups	No	Huang et al. [12]
2011	Birth to 18 years	Outpatient children considered healthy	Roche/Hitachi 917/MOD	1741 children/samples	2003–2006	11 separate age groups	No	Turan et al. [2]
2011	Birth	Preterm and term infants (cord-blood)	Hitachi 917	506 preterm infants, 56 term infants	2003–2005	5 separate reference intervals according to gestational age	No	Fenton et al. [13]
2010	8 years to 12 years	Healthy Australian children	Abbott Architect ci8200	852 children, 1545 samples	2005–2009	3 separate age groups	No	Southcott et al. [14]
2010	6 years to 17 years	Healthy Indian children	Hitachi 902	3327 children/samples	Not specified	Year-specific reference intervals	Yes	Marwaha et al. [15]
2009	Birth to 18 years	In- and outpatients in a German tertiary hospital considered healthy	Roche Modular Analytics	533 children/samples	2004–2006	5 separate age groups	No	Heiduk et al. [16, 17]
2009	1 year to 17 years	Healthy German children	Hitachi 917	14,255 children/samples	2003–2006	Continuous	Yes	Dortschy et al. [18]

Table 1 (continued)

Year	Age range	Data base/method	Analytical platform	n	Sampling period	Representation of age-dependent dynamics	Percentile charts	References
2008	Birth to 18 years	Data mining of British primary, secondary and tertiary care data, exclusion of outliers	Abbott Aeroset	1883 samples	Not specified	4 separate age groups[b]	No[b]	Shine [19]
2004	1 year to 14 years	Data mining of an Australian hospital laboratory[c]	Beckman Synchron CX-5 CE	187 samples	1999	single reference interval	No	Brinkworth et al. [20]
2003	Birth to 18 years	Transference of existing reference intervals				12 separate age groups	No	Ghoshal and Soldin [21]
1998	8 years to 18 years	Healthy Croatian children	Not specified	985 children/samples	1990–1995	4 separate age groups	No	Jagarinec et al. [22]
1993	Birth	Healthy Canadian newborns	Hitachi 737	390 children/samples	Not specified	single reference interval	No	Perkins et al. [23]

[a]Children were considered healthy if they were tested only once. [b]Continuous percentile charts have been created but are not provided. [c]Outpatients, patients tested more than once, and patients with annotated test results are excluded.

data from children aged 4–8 years (i.e. an age period with only minor physiological dynamics) to isolate analytical trends from trends due to changes in hospital age-composition over time. This analysis revealed no significant trend in the quarterly median values during the study period (Cox and Stuart trend test: p=0.27 for trend; Mann-Kendall test: p=0.23 for trend; 95% CI for Sen's slope: −0.27 to 0.06).

Calculation of percentile charts

Percentile charts were calculated with an indirect algorithm which we have described and validated previously [6, 27]. The method extracts samples of healthy individuals from an input data set, containing both non-pathologic and pathologic samples. The distribution of non-pathologic samples is modeled with a parametric distribution, whereas pathologic samples are assumed to be scattered randomly. The parameters of the distribution of healthy samples are estimated at different age points and used to construct reference intervals. (For each of these estimations, samples were selected from different patients.) To allow an exact representation of alkaline phosphatase dynamics from birth to adulthood the following method settings were changed: samples are partitioned into higher-resolution age groups (n=1500) in the first 30 days of life than in the remaining time period (31 days of life to 18 years, n=5000). This allows a more precise consideration of the rapid changes in alkaline phosphatase activity in the neonatal period than identically sized age groups from birth to adulthood. Secondly, we now report percentile charts (i.e. the 2.5th, 10th, 25th, 50th, 75th, 90th and 97.5th percentiles) instead of reference limits.

Differences between centers

To exclude differences in alkaline phosphatase activity between centers due to pre-analytical, analytical, and population differences we analyzed each participating center's data individually. Each center's data was split into age groups (n=1500) and the corresponding age- and sex-specific 50th percentiles are shown in Supplemental Figure 1. As center-specific medians range from 75–325 U/L, this comparison also shows cross-center analytical stability in this range. (A uniform lower age group size [n=1500 from birth to 18 years] was selected for the individual center analysis than for the combined analysis as each center's individual data set was substantially smaller than the combined data set.)

Additionally, we investigated whether a transformation of each center's measurements would benefit the combined analysis. To test this, a center-specific transformation $T(m)=a\times m+b$ was applied to each measurement m. Parameters a and b were selected for centers B, C, D, E, F, and G so that application of the transformation function T to the age- and sex-specific 2.5th, 50th, and 97.5th percentiles from each center would result in minimal differences to the corresponding percentiles from center A. The transformation $T(m)$ that minimizes the sum of the absolute differences between the 2.5th, 50th, and 97.5th percentiles of each center and center A was selected. For center A, a uniform transformation was selected ($a=1$ and $b=0$). The resulting 50th percentiles of alkaline phosphatase activity with and without this transformation are shown in Supplemental Figure 2 and further confirm that a transformation of measurements between centers is not necessary. The analysis of the

combined data set was therefore performed without transformation of measurements.

Comparison to existing reference intervals

Reference intervals from the Canadian CALIPER study [11] were selected for comparison to our results. These reference intervals were created using CLSI C28-A3 statistical guidelines from a representative cohort of 1213 healthy Canadian children. The analysis included children from birth to adulthood and the data set underlying the published reference intervals is freely accessible, enabling a detailed comparison. Measurement of alkaline phosphatase activity was performed on the Abbott Architect c8000 analyzer.

Results

We analyzed 361,405 samples from 124,440 different patients to create percentile charts for alkaline phosphatase activity from birth to 18 years. 192,972 samples from 64,670 boys and 168,433 samples from 59,770 girls were examined. Table 2 shows the age- and sex-specific distribution of samples with a substantial proportion of samples in the newborn period and infancy. Graphical representations of the percentile charts created are available in Figure 1 (2.5th, 10th, 25th, 50th, 75th, 90th, and 97.5th percentiles) and Supplemental Figure 3 (1st, 2.5th,

10th, 25th, 50th, 75th, 90th, 97.5th, and 99th percentiles). Data tables with age-specific percentile values are available in the online Supplemental Database and allow incorporation of our results into laboratory information systems.

Our results demonstrate the complex age- and sex-related dynamics in alkaline phosphatase activity: Levels surge after birth and peak at approximately 20 days of life. After this, activity decreases and reaches a local minimum at about 4 years of age, succeeded by a sex-specific rise and subsequent decline. Activity in girls peaks at 10–12 years with a median and maximum activity of 240 and 400 U/L (50th and 97.5th percentile, respectively). Males' activity peaks at 13–15 years with similar median activity (50th percentile, 250 U/L) and higher maximum activity (97.5th percentile, 450 U/L).

Comparison of our 2.5th and 97.5th percentile results with findings from the CALIPER study [11] demonstrates similar reference limits and age-dependent dynamics (Figure 2). In the first 6 months of life, the upper reference limits of the two studies intersect, but those provided by us are higher than those from CALIPER for a substantial period of time. However, direct comparison of results from these studies is complicated by the different strategies for age-stratification and different analytical devices (Roche Cobas and Abbott Architect). Comparison of the different approaches to represent age-dependent dynamics

Table 2: Number of samples and individuals analyzed.

Age group	Total		Boys		Girls	
	Samples	**Individuals**	**Samples**	**Individuals**	**Samples**	**Individuals**
0–28 days	11,513	7823	6723	4449	4790	3374
29 days to 1 year	30,780	10,924	17,422	6148	13,358	4776
1 year	18,076	7393	9668	3959	8408	3434
2 years	15,817	6605	8857	3566	6960	3039
3 years	15,456	6899	8572	3811	6884	3088
4 years	15,246	7389	8452	4111	6794	3278
5 years	15,396	7844	8502	4324	6894	3520
6 years	15,579	7646	8796	4159	6783	3487
7 years	15,617	8022	7999	4237	7618	3785
8 years	16,251	8624	8518	4475	7733	4149
9 years	16,679	8942	8999	4690	7680	4252
10 years	17,468	9526	9386	5055	8082	4471
11 years	18,212	9892	9678	5314	8534	4578
12 years	19,677	10,833	10,744	5777	8933	5056
13 years	22,361	12,279	11,486	6355	10,875	5924
14 years	23,580	12,304	11,950	6223	11,630	6081
15 years	23,191	11,884	11,397	5705	11,794	6179
16 years	25,196	12,336	13,093	5731	12,103	6605
17 years	25,237	12,312	12,699	5577	12,538	6735
Total	36,1405	12,4440	19,2972	64,670	16,8433	59,770
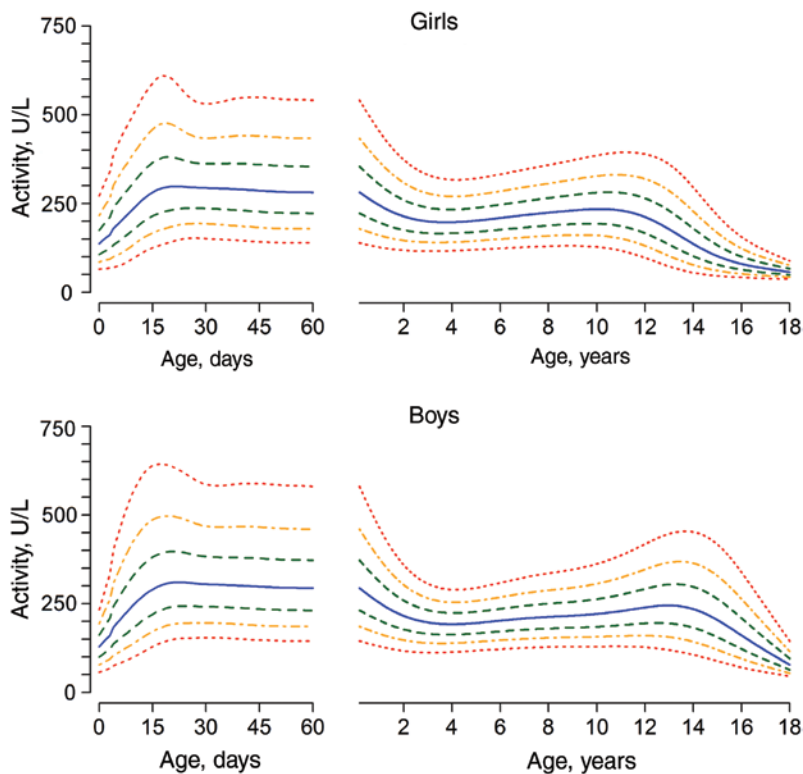
Figure 1: Age- and sex-dependent percentile charts for alkaline phosphatase activity, showing the 50th percentile (solid lines, blue), 25th and 75th percentiles (dashed lines, green), 10th and 90th percentiles (dashdotted lines, orange), and 2.5th and 97.5th percentiles (dotted lines, red); the 1st and 99th percentiles are shown in Supplemental Figure 3.
The x-axes are scaled differently on the left- and right-hand sides of the figure to account for the dynamics in young infants; the x-axis on the right-hand side starts at 61 days.

demonstrated in Figure 2 illustrates the limitations of discrete age groups to represent the dynamics of an analyte changing continuously with age.

Discussion

We report percentile charts for alkaline phosphatase activity from birth to adulthood generated with a multi-center data-mining strategy (Figure 1). This approach has provided a large number of samples for evaluation (n=361,405), even for the challenging subgroups of neo-nates (birth to 28 days, n=11,513) and infants (29 days to 1 year, n=30,780). Reference intervals for these age groups are often restricted by limited access to samples due to ethical and practical objections, although the rapid age-dependent changes in alkaline phosphatase activity after birth require a disproportionate number of samples for the construction of accurate reference intervals. Consequently, our results show a steady rise in alkaline phos-phatase activity immediately after birth – a finding not

demonstrated in other reference intervals due to parti-tioning into large-scale age groups or reduced sample availability.

The percentile charts provided allow a continuous representation of change in alkaline phosphatase activ-ity with age. This distinguishes them from the major-ity of current reference intervals, which use discrete age groups to represent age-dependent dynamics (Table 1). Partitioning into discrete age groups with significant dif-ferences, however, can only approximate the physiologi-cal dynamics leading to change in alkaline phosphatase activity. This results in difficulties in interpretation of patient test results at age group margins, as the clas-sification of identical measurements can change from pathological to normal or vice-versa due to a biologically negligible change in patient age. Continuous implemen-tations of reference intervals as percentile charts, analo-gous to other quantities typically specified in relation to age (e.g. weight- and height-for-age charts), eliminate this problem. Similarly, classification of test results accord-ing to percentiles allows a more precise consideration of
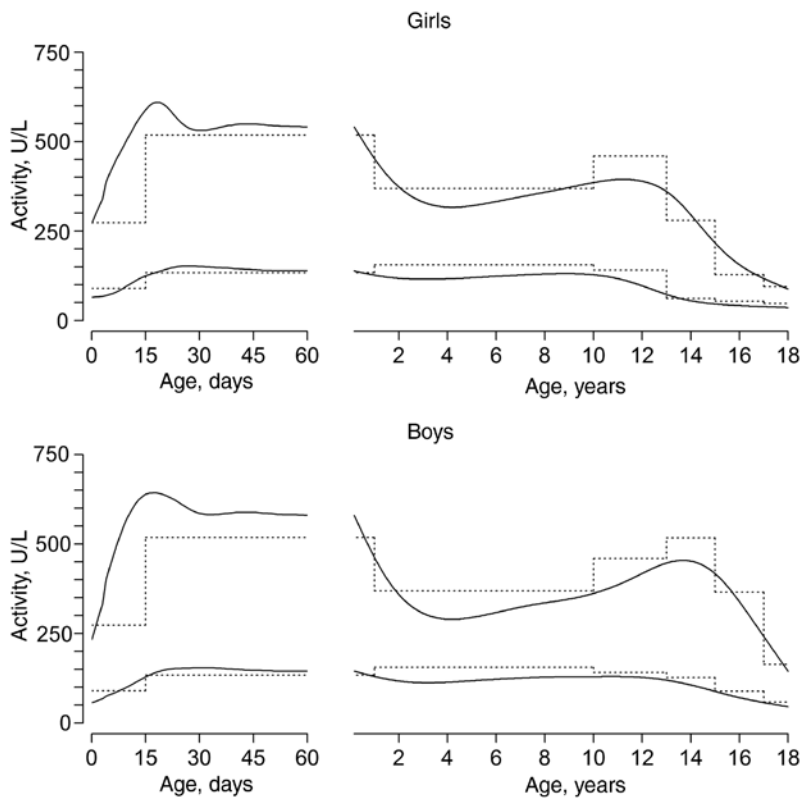
Figure 2: Comparison of 2.5th and 97.5th percentiles for alkaline phosphatase activity (solid lines) to reference intervals from the CALIPER study (dotted lines) [11].
The x-axes are scaled differently on the left- and right-hand side of the figure to account for the dynamics in young infants; the x-axis on the right-hand side starts at 61 days.

intra- and inter-individual differences than a categorization relative only to reference interval boundaries (e.g. "inside" the reference interval or multiples of the "upper limit of normal"). Use of percentiles also reflects the fact that healthy children may have test results outside the reference interval (i.e. below the 2.5th percentile or above the 97.5th percentile) and vice-versa (i.e. not all values between the 2.5th and 97.5th percentiles reflect physiological alkaline phosphatase metabolism). Interpretation of test results with percentile charts therefore seems especially appropriate for an analyte as closely linked to growth as alkaline phosphatase.

We selected a data-mining method (indirect method) for the determination of reference intervals. Most available reference intervals were created using population-based approaches (direct methods), in which blood samples are drawn from healthy children. Population-based methods are generally regarded as the best method available for the determination of reference intervals [24] because they allow a precise control of the reference population and preanalytical factors, and are not subject to the uncertainties intrinsic to data-mining approaches. The use of an

indirect method might therefore be regarded as a severe limitation of our study and might oppose application of the published reference intervals.

However, the ethical and practical challenges associated with blood sampling in healthy children required for population-based methods cause restrictions in the number of samples available for analysis, which reduces the age-specific precision in these reference intervals and often restricts analysis to certain age groups. The calculation of continuous reference intervals from birth to adulthood for alkaline phosphatase activity using population-based methods has therefore been prevented by the excessive requirement of samples for such an approach. Meanwhile, data-mining approaches give access to a large number of measurements from clinical laboratories and have therefore fewer restrictions regarding age-specific dynamics. To ensure valid reference intervals when using indirect procedures, great care must be taken in the selection of samples and statistical algorithms. Previous evaluations of the applied algorithm [6, 27] and comparison of our results to reference intervals from CALIPER, created using a direct method

(Figure 2), demonstrate the selected approach's ability to generate correct reference intervals. In the context of an analyte subject to such extensive age-dependent changes as alkaline phosphatase, the benefits of an increase in sample numbers make data-mining approaches especially appropriate.

Although comparison of our results to reference intervals from CALIPER shows consistent upper and lower reference limits, there are noteworthy differences in the first months of life. The 97.5th percentiles provided by us exceed the upper reference limits reported by CALIPER for a substantial period of time (Figure 2), which may raise doubts about the validity of our approach. However, interpretation of these differences requires inspection of the distribution in CALIPER's age groups: Reference intervals for children from birth to 14 days are taken from 46% samples of children ≤4 days old and 96% samples from children ≤7 days. Similarly, in the CALIPER data set for children aged 15 days to 1 year only 7% of samples were from children ≤30 days. In total, 16 samples from children aged 8–30 days are included in the CALIPER database, precluding a comparison of reference limits in this age group. As available reference intervals – including those from CALIPER – show a significantly lower alkaline phosphatase activity immediately after birth than in the following months, there is a strong indication of a rapid rise in alkaline phosphatase activity after birth. This surge is presumably masked by the low number of children aged 8–30 days (the age group showing most disagreement) and the selection of age groups (0–14 days, and 15 days to 1 year) in CALIPER's and other's reference intervals.

We established percentile charts for alkaline phosphatase activity in a German population of mainly Caucasian origin on the Roche Cobas platform. The reported reference intervals are therefore directly applicable only for this population and analytical platform and require transference according to guidelines before usage outside this context [28, 29]. However, the physiological dynamics in alkaline phosphatase activity with age are independent from analytical procedures; our results can therefore be used to extrapolate existing reference intervals, even if our results cannot be transferred directly.

A variety of reference intervals and percentile charts for alkaline phosphatase established with current analytical methods have been published (Table 1). While these studies provide important insights into physiological dynamics and support clinical decision making, only one investigation by Loh and Metz reports continuous reference intervals from birth to adulthood [5]. Their results close an important gap in pediatric reference intervals

using an indirect data-mining approach – laboratory data from primary care providers were analyzed and all test results from children tested only once were considered to be normal and used to construct percentile charts, allowing the accurate representation of the age-dependent dynamics for the majority of children. However, an unknown number of children will have visited their doctor due to an underlying disease affecting alkaline phosphatase activity and not undergone repeated testing, resulting in uncertainty in the 2.5th and 97.5th percentiles. On the other hand, this approach to sample selection may lead to a bias towards the existing reference intervals, as physicians might tend to retest children with results "outside the reference range". To address these issues, we performed an independent analysis of an increased number of children from another population using a different indirect approach.

Our multi-center analysis of laboratory data from six pediatric tertiary centers and one laboratory service provider has resulted in the most comprehensive data set of alkaline phosphatase activity available, containing 361,405 samples from 124,440 different children. Furthermore, the employment of identical analytical methods at distributed locations has allowed us to create reference intervals for different laboratories using the Roche Cobas platform. Considering the lack of continuous reference intervals from birth to adulthood, our results are an important addition to our understanding of the changes in alkaline phosphatase activity with age and allow a more precise consideration of these dynamics when guiding clinical decisions.

Conclusions

We provide sex- and age-dependent percentile charts for alkaline phosphatase activity from birth to adulthood. These charts allow an accurate appreciation of the changes in analyte activity with age and can therefore improve clinical decision making.

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