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Misclassification of chronic hepatitis B natural history phase: Insight from new ALT, AST, AKP, and GGT reference intervals in Chinese children

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ABSTRACT

Background: The age- and sex-specific reference intervals (RIs) for liver chemistry in children are not available in China. Our study aimed to establish age and gender related RIs for ALT, AST, AKP, and GGT in China, and apply the new RI for ALT in children with chronic hepatitis B to use as a biochemical marker for disease progression.

Methods: Data were collected from the Children's Healthcare Center. The measurements of ALT, AST, AKP and GGT were performed on a Hitachi 7600 Chemistry Analyzer. Age- and sex-specific RIs were determined using a percentile (3rd-97th) method. The sensitivity and specificity were determined to test the ability of the newly proposed ALT thresholds to classify children with chronic HBV infection.

Results: The age- and sex-specific RIs of ALT, AST, AKP and GGT were established based on 4232 Chinese healthy children. Using the new median ALT threshold, the sensitivity was higher. The detection of chronic HBV infection was 31.2% in boys and 35.5% in girls, while a very slight decrease in specificity was found. Based on the newly proposed RIs of ALT, approximately 16.1% boys and 19.0% girls would be classified in the HBeAg-positive chronic hepatitis phase, but using the current ALT threshold of children's hospitals they were in HBeAg-positive chronic infection phase.

Conclusions: Based on a large healthy population, we established the sex- and age-specific RIs of ALT, AST, AKP and GGT serum activities for Chinese children. Meanwhile, newly proposed liver chemistry RIs will benefit the understanding of liver function and the natural history of chronic HBV infection in children.

1. Introduction

Since hepatitis B virus (HBV) was discovered in 1965, it has become one of the most common sources of infection worldwide [1]. In China, the major route of HBV infection is vertical transmission, which has led to chronic hepatitis B (CHB) becoming one of the most common chronic liver diseases in children [2]. Although CHB is a relatively benign process during childhood and adolescence [3], there are some children and adolescents who will develop cirrhosis or cancer of the liver [4,5].

Furthermore, infants or children with chronic HBV infection have a lifetime risk of developing hepatocellular carcinoma [6]. Therefore, special attention should be given to the monitoring and timely antiviral treatment of chronic HBV infection in children.

Clinicians rely on liver chemistry, especially alanine aminotransferase (ALT) activities, HBV markers and fibrosis markers to monitor and assess the condition of children with hepatitis B [7]. A slight-moderate increase of ALT serum activities may mark chronic liver diseases caused by a variety of etiologic factors, including HBV infection. Abnormal activities of liver chemistry are usually the first direct evidence of liver disease and can identify asymptomatic patients [8–10].

Although liver chemistry is vital for the diagnosis and treatment of chronic liver diseases such as hepatitis B in children, the reference standards from population-based liver chemistry measurements are not available in China. In many Chinese hospitals, pediatric reference intervals (RIs) of liver chemistry are replaced by reference standards for adults, which have covered all ages and sexes. However, an increasing number of recent studies have demonstrated that age- and sex-

Abbreviations: CHB, chronic hepatitis B; CHCMU, Children's Hospital of Chongqing Medical University; LLN, lower limit of normal; P3, 3rd percentiles; P50, 50th percentiles; RIs/RI, reference intervals/reference interval; ULN, upper limit of normal

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dependent thresholds of pediatric liver chemistry should be applied [11–14]. Therefore, it is essential to establish age- and sex-specific RIs for liver chemistry in Chinese children.

2. Materials and methods

2.1. Study population

This is a cross-sectional study. All consecutive participants who visited the Children's Hospital of Chongqing Medical University (CHCMU) for a routine health examination between April 2014 and November 2017 were enrolled. The inclusion criteria were as follows: ages 1–14 y; without any disease; and underwent liver function tests (ALT, AST, AKP, and GGT). The exclusion criteria included missing or incomplete data on liver function or hepatitis virus infection; any illness or use of any known or potentially hepatotoxic drugs within one month; a history of hepatobiliary surgery; a family history of inherited liver disease; exhibition of risk factors for metabolic disease such as abnormal serum lipids, glucose, ceruloplasmin and iron; underweight, overweight or obese; and implausible value for liver enzymes that were more than or < 3 standard deviations. Finally, 4232 eligible children (boys = 2415; girls = 1817) were included for the establishment of new liver chemistry thresholds in this study. The flow of inclusion and exclusion details are described in Fig. 1.

In addition, we collected a CHB group that contained 601 children from the CHCMU from 2010 to 2016. Chronic HBV infection was defined by a persistent HBsAg (+) for > 6 months [7]. The natural history phases of children with chronic HBV infection were classified according to EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection as follows: HBeAg-positive chronic HBV infection phase (HBeAg-positive and ALT activity ≤ ULN), HBeAg-positive chronic hepatitis B phase (HBeAg-positive and ALT activity > ULN), HBeAg-negative chronic HBV infection phase (HBeAg-negative and ALT activity ≤ ULN), and HBeAg-negative chronic hepatitis B phase (HBeAg-negative and ALT activity > ULN) [15]. This study was approved by the ethics committee of Chongqing Medical University.

2.2. Variables collected

The data, including the participants' personal information, laboratory tests and diagnosis, were obtained from the electronic database system, which was maintained at the Children's Hospital of Chongqing Medical University.

2.2.1. Personal information

The personal information included age, sex, height, weight, medical history (any illness, history of hepatobiliary surgery), past and current medication use (known or potentially hepatotoxic drugs), family history (inherited liver disease, metabolic disease), and appraisal of growth and development (underweight, normal, overweight or obese) [16].

2.2.2. Laboratory test

After overnight fasting for 8–12 h, the participants were asked to avoid strenuous exercise before blood collection. Then, 3 ml of blood was collected in gel separator vacuum test tubes from 8 AM to 12 AM by professional pediatric nurses. All blood samples were kept at room temperature for up to 60 min. To separate the serum, samples were centrifuged at 4000*g* for 8 min and were analyzed within 4 h of collection. Serum samples were measured for ALT (NADH method, with pyridoxal-5′-phosphate), AST (NADH method, with pyridoxal-5′-phosphate), AKP (enzymatic method), and GGT (enzymatic method) on a Hitachi 7600 Automatic Biochemistry Analyzer (Hitachi, Ltd.). Two levels of quality control were run every day. The accuracy and precision data are presented in Supplemental Table C. Serum HBV markers (HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb) were analyzed on an automatic chemiluminescence immune analysis apparatus (Abbott i2000SR) and the HBsAg positive sample concentration was ≥ 0.05 IU/ml. Serum concentrations of lipids and glucose were detected using a chemical analyzer (Vitros 4600, Ortho Clinical Diagnostics). The normal range of total cholesterol, triglycerides, high-density lipoproteins and low-density lipoproteins was 0–5.17 mmol/l, 0–1.7 mmol/l, 0.91–2.27 mmol/l, and 0–3.36 mmol/l respectively. Additionally, the normal range of glucose was 3.89–6.11 mmol/l.

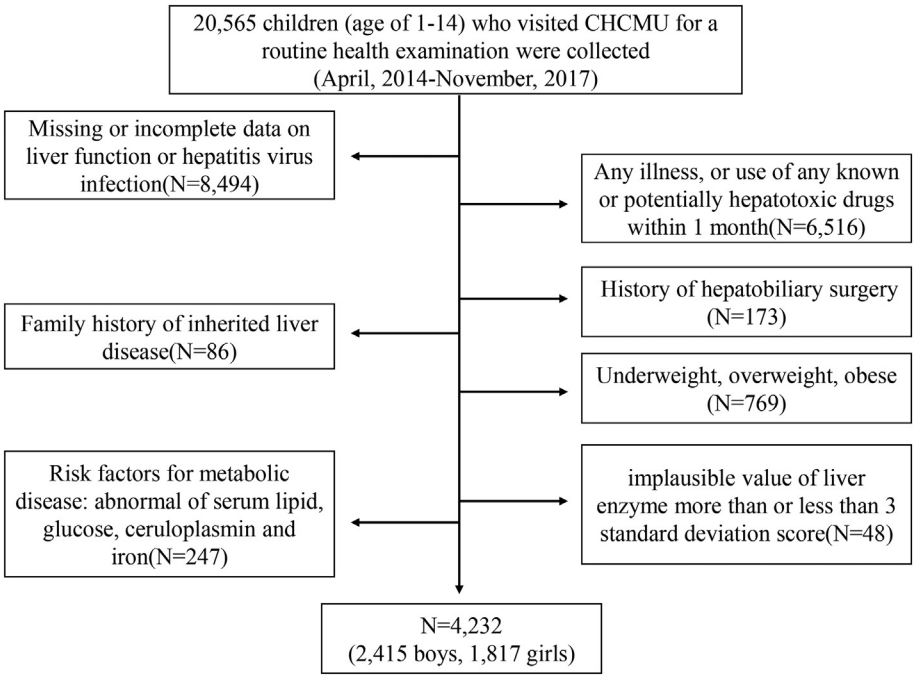

Fig. 1. Inclusion and exclusion criteria for the study population.

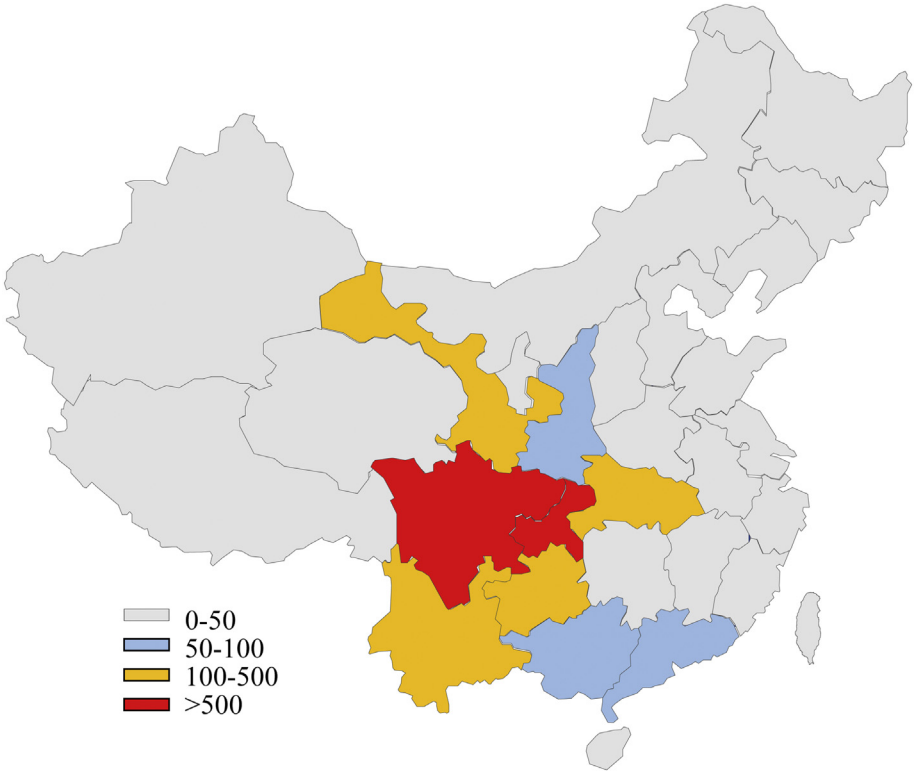

Fig. 2. Participating regions in the study.

2.3. Statistical analysis

The distribution of data was tested by SPSS 23.0. The RIs of ALT, AST, AKP and GGT for each age group and different sexes were determined by the percentile method. The lower limit of the RI was the 3rd percentile and the upper one was the 97th percentile [17]. The smoothed percentile curves of the liver chemistry were determined by JMP 13.0 software (SAS Institute). The sensitivity and specificity were performed to test the ability of the newly proposed ALT threshold for classifying children with chronic HBV infection.

3. Results

Fig. 1 describes the process of participant screening. Out of 20,565 children screened, 4232 children (2415 boys and 1817 girls) were eligible and included in the reference interval study. The participants were mainly born in and living in the southwestern region of China (Fig. 2).

3.1. Age- and sex-related RIs of ALT, AST, AKP and GGT derived from a primarily healthy cohort

The smoothed percentile curves for age- and sex-specific reference intervals of ALT, AST, AKP and GGT are provided (Fig. 3A–D). The changes of ALT with age were not substantial. A slow decline before four y old was observed, and then was followed by a slower increase from the age of five y to 10 y in both boys and girls. The median of ALT for boys was always higher than that for girls at the same age (see Fig. 3A). The lower limit of normal (LLN) range was 11.3–14.7 U/l and 12.1–16.1 U/l in boys and girls, respectively, and the upper limit of normal (ULN) range was 40.4–44.5 U/l and 38.9–45.4 U/l in boys and girls, respectively (Table 1A).

AST showed apparent age-specific decrease, and it was slightly sex-specific. The median AST activity for boys was slightly higher than that for girls (see Fig. 3B). The AST ULN ranged from 53.3 U/l to 34.9 U/l in boys and from 50.8 U/l to 34.0 U/l in girls (Table 1B). Likewise, the LLN range decreased with age from 26.6 U/l to 15.7 U/l in boys and 24.8 U/l to 14.1 U/l in girls.

AKP showed variation with age and gender paralleling times of rapid growth; AKP declined for 3 y after birth, and then peaked at 13 y for boys, and 10 y for girls (see Fig. 3C). The AKP ULN range in boys, which fluctuated more, was 278.1–437.6 U/l and it was 234.2–351.9 U/l in girls (Table 1C). In the same way, the LLN range of AKP was 101.5–131.2 U/l and 46.6–139.9 U/l in boys and girls, respectively.

There was a continuous rise in serum GGT activities in both boys and girls over the measured age span. The median serum concentrations increased more quickly in boys than it did in girls (see Fig. 3D). The LLN range of GGT spanned from 5.5 to 8.9 U/l and from 4.7 to 9.8 U/l in boys and girls, respectively. The ULN range spanned from 17.7–33.3 U/l and 17.8–35.9 U/l in boys and girls, respectively (Table 1D).

3.2. Verify the ability of the new proposal for ALT RIs for classifying children with chronic HBV infection

As the ALT ULN range changes little with age, in calculating the sensitivity and specificity, we applied the median ALT threshold values for all ages to different genders (43 U/l for boys, 41 U/l for girls) (Table 2). For the current ALT threshold, sensitivities were 22.9% (92/401) and 24.0% (48/200) for classifying chronic HBV infection in boys and girls, respectively (Supplemental Table A.). In contrast, the specificities were high in both boys and girls, which were 99.8% (2409/2415, 1813/1817). The new ALT RIs showed higher sensitivities of 31.2% (125/401) in boys and 35.5% (71/200) in girls, while specificities presented a very slight decrease (97.6% (2356/2415) in boys and 97.5% (1772/1817) in girls).

3.3. Re-evaluation of the natural history of chronic HBV infection in Chinese children

Since the judgment of HBeAg-positive chronic HBV infection phase

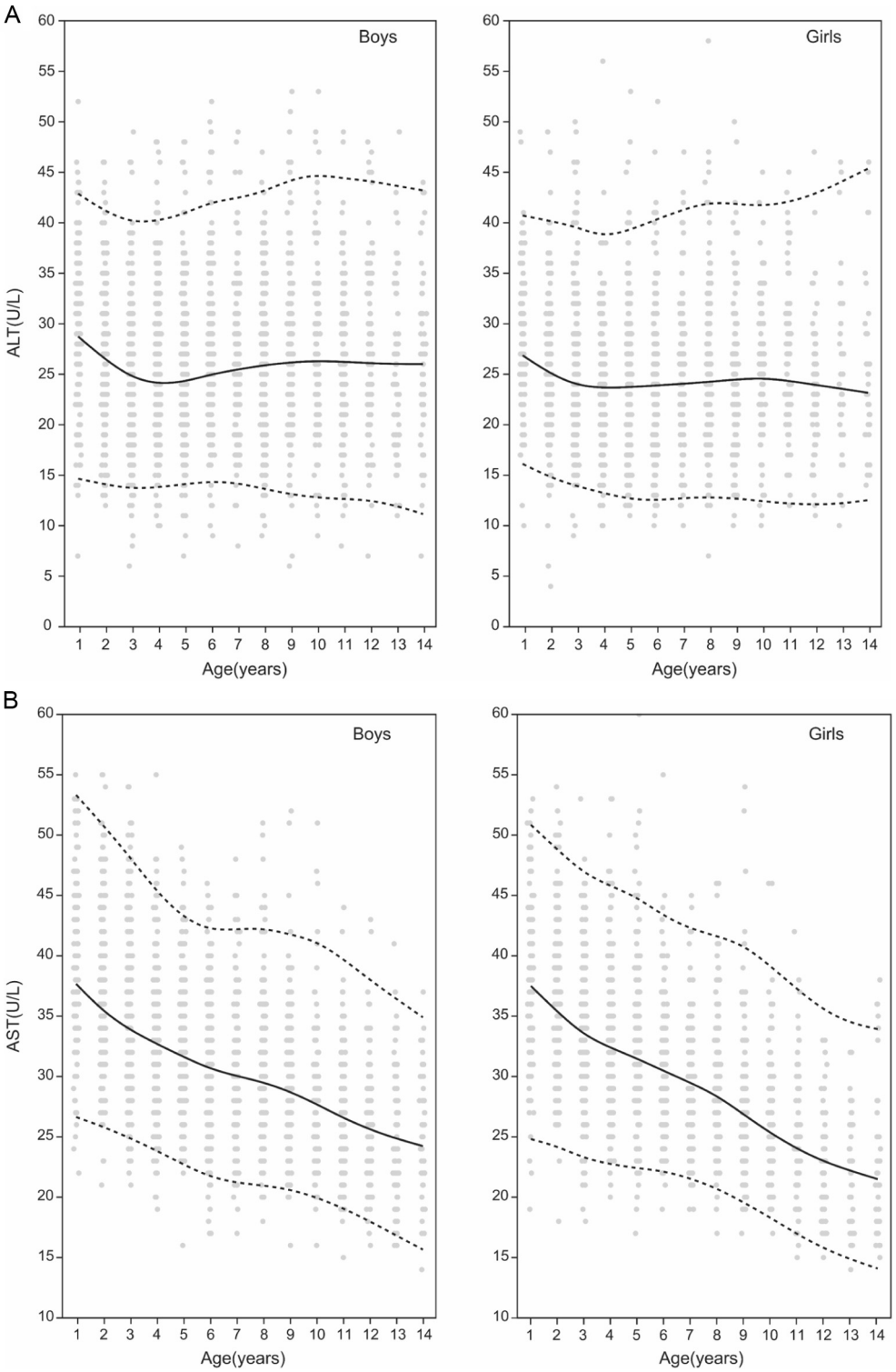

Fig. 3. Age- and sex-related smoothed percentile curves of alanine aminotransferase (ALT) (A), aspartate aminotransferase (AST) (B), alkaline phosphatase (AKP) (C) and γ-glutamyl transferase (GGT) (D) according to sex and age (1 to 14 y) based on healthy children. Upper, median, and lower curves represent the 97th, 50th, and 3rd percentiles, respectively.

and HBeAg-positive chronic hepatitis B phase is mainly based on ALT ULN, the natural history phases of children in the CHB group in the HBeAg-positive chronic infection phase (Supplemental Table B) was re-evaluated using the newly proposed cut-points for ALT. Approximately 21.1% of boys with chronic HBV infection had abnormal ALT activities in the first year. Meanwhile, approximately 15.9% of the boys with chronic HBV infection aged two to 14 y in each age partition had elevated ALT. In total, 16.1% of boys were previously classified in the HBeAg-positive chronic infection phase but had abnormal ALT activities based on the new ALT threshold. The misclassification rate was higher in girls than boys, where the rate was 19.0%. Like boys with chronic HBV infection, 22.2% of girls with chronic HBV infection at one year of age were misclassified. As well, approximately 17.0% of girls aged two to 14 y were misclassified in each age partition.

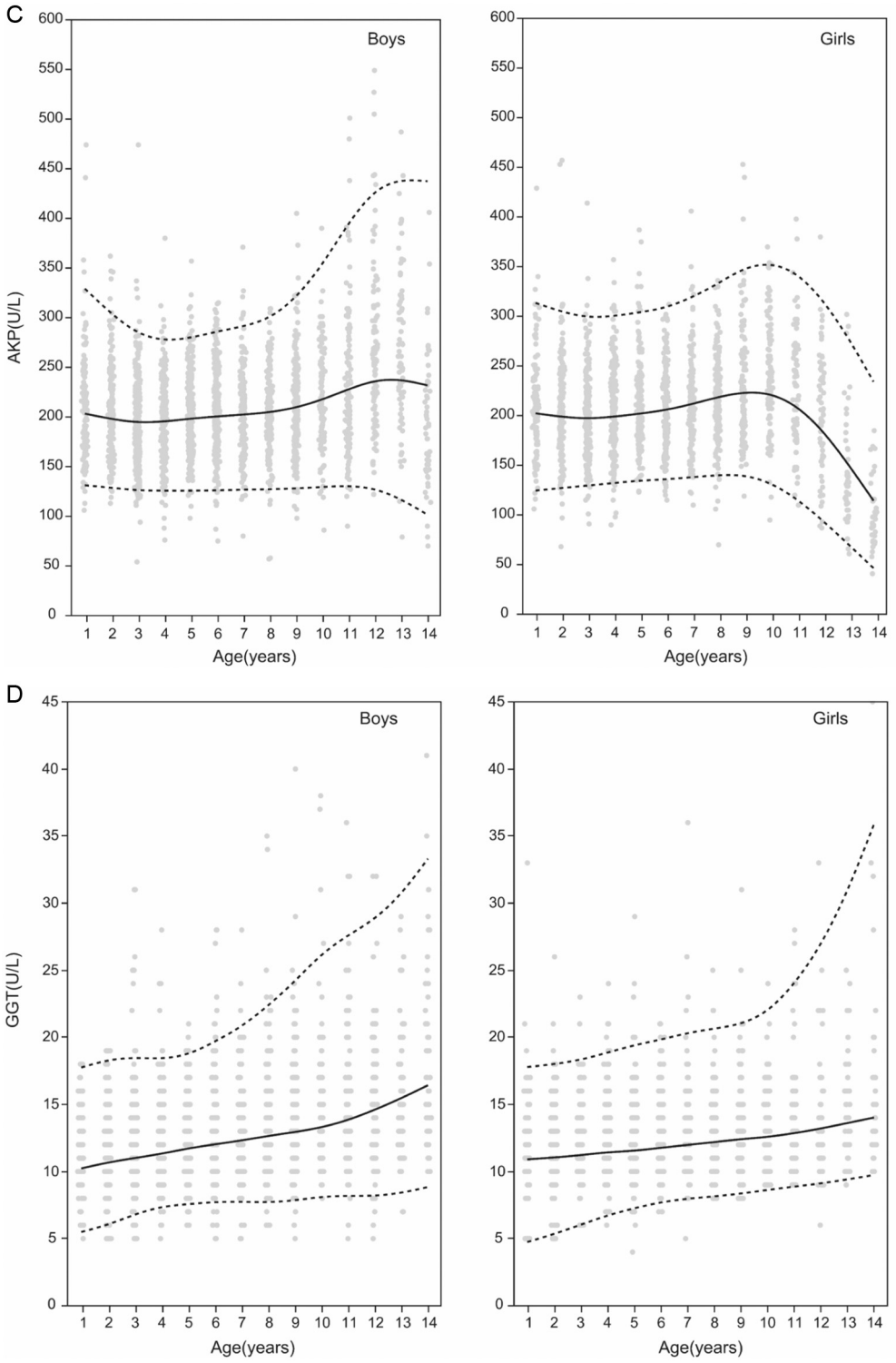

Fig. 3. (*continued*)

4. Discussion

We established the sex- and age-specific RIs for ALT, AST, AKP and GGT serum activities for Chinese children at ages 1–14 y based on a large healthy population. The new proposed ALT RIs were applied to identify children with chronic HBV infection and re-evaluate the natural history of chronic HBV infection in children.

We found that trends in serum ALT, AST, AKP and GGT concentrations over the ages observed in our study were similar to those in studies from different regions such as Germany, US and Canada [13,17–19]. The serum activities of ALT and AST were generally higher in boys than in girls. However, obvious different RIs can be found in these studies. In our study, we derived a new ALT ULN range for boys (40.4–44.5 U/l) and girls (38.9–45.4 U/l), which were both higher than ALT ULN in aforementioned studies, and they fluctuated between 20 and 40 U/l, respectively. One explanation for this is that different sources of the study population affect values of ALT ULN. For example, in the studies in adults, the ALT ULN values were 30.0 U/l for men and

Table 1A
Percentiles of alanine aminotransferase (ALT, U/l) in healthy boys and girls aged 1 to 14 y (*N* = 4232; boys = 2415; girls = 1817).

Boys	Age	N	P3*	P50*	P97*	Girls	Age	N	P3	P50	P97
	1	146	14.7	28.7	42.9		1	115	16.1	26.8	40.7
	2	189	14.1	26.5	41.2		2	172	14.9	25.2	40.2
	3	297	13.9	25.0	40.4		3	227	14.0	24.1	39.6
	4	267	13.9	24.4	40.4		4	203	13.3	23.7	38.9
	5	287	14.1	24.4	41.0		5	197	12.7	23.7	39.3
	6	243	14.2	24.9	41.8		6	180	13.6	23.9	40.3
	7	170	14.1	25.4	42.5		7	147	12.7	24.1	41.2
	8	175	13.7	25.8	43.2		8	142	12.8	24.2	41.9
	9	177	13.2	26.1	44.1		9	116	12.7	24.4	41.9
	10	110	12.9	26.2	44.5		10	104	12.4	24.6	41.8
	11	122	12.6	26.2	44.4		11	71	12.2	24.3	42.1
	12	106	12.4	26.2	44.1		12	63	12.1	24.0	42.9
	13	74	11.9	26.1	43.8		13	43	12.2	23.6	44.1
	14	52	11.3	26.0	43.4		14	37	12.5	23.2	45.4

* P3 is the 3rd percentile value, P50 is the 50th percentile value and the P97 is the 97th percentile value.

Table 1B
Percentiles of aspartate aminotransferase (AST, U/l) in healthy boys and girls aged 1 to 14 y (N = 4232; boys = 2415; girls = 1817).

Boys	Age	N	P3*	P50*	P97*	Girls	Age	N	P3	P50	P97
	1	146	26.6	37.7	53.3		1	115	24.8	37.5	50.8
	2	189	25.8	35.5	50.8		2	172	24.1	35.3	48.8
	3	297	24.9	34.0	48.1		3	227	23.3	33.5	46.9
	4	267	23.8	32.7	45.4		4	203	22.7	32.4	45.8
	5	287	22.7	31.6	43.4		5	197	22.4	31.4	44.7
	6	243	21.8	30.7	42.3		6	180	22.1	30.4	43.4
	7	170	21.3	30.1	42.2		7	147	21.5	29.4	42.3
	8	175	21.0	29.5	42.2		8	142	20.7	28.3	41.6
	9	177	20.6	28.7	41.8		9	116	19.5	26.8	40.7
	10	110	20.0	27.7	41.1		10	104	18.2	25.4	39.1
	11	122	19.1	26.6	39.7		11	71	17.0	24.0	37.2
	12	106	18.0	25.6	38.0		12	63	15.7	23.0	35.6
	13	74	16.8	24.9	36.4		13	43	14.9	22.2	34.5
	14	52	15.7	24.2	34.9		14	37	14.1	21.5	34.0

* P3 is the 3rd percentile value, P50 is the 50th percentile value and the P97 is the 97th percentile value.

19.0 U/l for women in Italy [20], compared to 54.0 U/l for men and 31.0 U/l for women in Asia [21]. A recent study on Chinese adolescents' ALT activities showed similar results to ours [22]. Bussler et al. [17] published the AST percentiles, which were generally higher than ours. The AKP peak values in the studies of western countries were higher than those of Chinese children, and the peak value of boys was higher than the peak value for girls in most studies [13,18]. GGT percentiles

Table 1D
Percentiles of γ-glutamyl transferase (GGT, U/l) in healthy boys and girls age 1 to 14 y (N = 4232; boys = 2415; girls = 1817).

Boys	Age	N	P3*	P50*	P97*	Girls	Age	N	P3	P50	P97
	1	146	5.5	10.2	17.7		1	115	4.7	10.9	17.8
	2	189	6.1	10.7	18.3		2	172	5.4	11.1	18
	3	297	6.8	11	18.4		3	227	6	11.2	18.3
	4	267	7.4	11.4	18.4		4	203	6.7	11.4	18.9
	5	287	7.6	11.7	18.8		5	197	7.3	11.6	19.4
	6	243	7.7	12	19.7		6	180	7.7	11.8	19.9
	7	170	7.8	12.3	20.8		7	147	8	12	20.3
	8	175	7.8	12.7	22.4		8	142	8.1	12.2	20.7
	9	177	7.9	13	24.2		9	116	8.4	12.4	21
	10	110	8.1	13.4	26.1		10	104	8.6	12.6	22.1
	11	122	8.2	13.8	27.6		11	71	8.9	12.9	24.1
	12	106	8.2	14.6	28.9		12	63	9.1	13.2	27
	13	74	8.4	15.5	30.8		13	43	9.4	13.6	31
	14	52	8.9	16.5	33.3		14	37	9.8	14.1	35.9

* P3 is the 3rd percentile value, P50 is the 50th percentile value and the P97 is the 97th percentile value.

Table 2
The sensitivity and specificity of ALT for boys and girls in detecting children with chronic HBV infection using the current and the newly proposed median ALT thresholds.

ALT threshold	Sensitivity		Specificity	
	Boys	Girls	Boys	Girls
Current[a]	22.9% (92/401)	24.0% (48/200)	99.8% (2409/2415)	99.8% (1813/1817)
Newly proposed[b]	31.2% (125/401)	35.5% (71/200)	97.6% (2356/2415)	97.5% (1772/1817)

[a] Current ALT ULN is 50 U/l for both boys and girls.
[b] Applied the median ALT ULN values for all ages to different genders (43 U/l for boys, 41 U/l for girls).

proposed by *Bussler* et al. [17] were generally lower than ours. However, the 3rd percentiles (P3) and 50th percentiles (P50) of GGT in our study were close to the 2.5th percentiles and P50 GGT values, which were mentioned in the Jakob Zierk et al.'s research [13]. These differences may be caused by ethnic diversity, different characteristics in children's growth and development or geographic location [11,19]. Different detection methods and test kits can also cause the differences described above [11].

ALT in liver chemistry is the most direct marker to assess liver damage in acute or chronic liver diseases [18,23]. In the diagnosis and treatment of children with chronic HBV infection, ALT activities not only determine the division of natural history but are also an important

Table 1C
Percentiles of alkaline phosphatase (AKP, U/l) in healthy boys and girls aged 1 to 14 y (N = 4232; boys = 2415; girls = 1817).

Boys	Age	N	P3*	P50*	P97*	Girls	Age	N	P3	P50	P97
	1	146	131.2	203.3	329.1		1	115	124.7	202.4	313.6
	2	189	128.7	198.1	304.2		2	172	127.2	199.1	305
	3	297	126.3	194.9	285.5		3	227	129.7	197.6	299.9
	4	267	125.8	195.6	278.1		4	203	132.3	199.2	300.7
	5	287	125.8	198.2	280.1		5	197	134.5	202.2	304.2
	6	243	126.2	200.5	285.8		6	180	136.0	206.0	309.4
	7	170	126.7	202.4	291.5		7	147	138.1	211.9	319.7
	8	175	127.3	205.0	301.1		8	142	139.9	218.5	333.1
	9	177	128.1	209.9	321.3		9	116	139.3	223.0	347.2
	10	110	129.5	217.8	354.1		10	104	131.8	221.2	351.9
	11	122	130.2	227.8	394.2		11	71	116.4	208.8	342.2
	12	106	126.8	235.9	425.7		12	63	95.0	185.0	316.6
	13	74	116.3	236.8	437.6		13	43	71.0	152.0	279.0
	14	52	101.5	231.7	437.4		14	37	46.6	114.6	234.2

* P3 is the 3rd percentile value, P50 is the 50th percentile value and the P97 is the 97th percentile value.

indicator for monitoring the condition of chronic hepatitis B [7,15]. In our study, the children with chronic HBV infection were identified using the newly proposed median cut-points for ALT. The result was that, with an apparent increase in sensitivity, there was a very slight decrease in specificity. Similarly, some related studies [24–26] mentioned that, at the new and lower ALT thresholds, the sensitivity for detection of HBV, hepatitis C virus and nonalcoholic fatty liver disease increases and is more favorable for the detection of potential liver disease or mild liver damage in both children and adults.

Wei et al. [27] reported that there were varying degrees of liver inflammatory injuries among the Chinese chronic HBV infected patients with “normal liver function”. This suggests that the current ALT threshold has limitations, and we need to reconsider the ALT threshold for classifying the natural history of chronic HBV infection. In our study, > 15.0% of the boys and girls in the “HBeAg-positive chronic infection” phase were misclassified using the newly proposed ALT RIs. It shows that new ALT cutoff values allow better classification of natural history for chronic HBV infection in children.

Our study has several limitations. First, although our research was based on a large healthy population, most of the patients reside in the southwestern region of China and are Han Chinese. Therefore, the geographical limitations and ethnic homogeneity of this study means that it is impossible for the proposed RIs to represent the liver chemistry of all Chinese children. Second, our judgment of healthy children depended on the data from the electronic database system of CHCMU. As each child's health status has not been systemically evaluated, the healthy participants may be mixed with undetected sick children. If these children suffer from diseases affecting liver function, it may affect the establishment of liver chemistry RIs.

In conclusion, based on a large healthy population, we established the sex- and age-specific RIs of ALT, AST, AKP and GGT serum activities for Chinese children aged 1–14 y. Meanwhile, the newly proposed liver chemistry RIs will benefit the understanding of liver function and the natural history of chronic HBV infection in children.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2018.11.034.

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