BMJ Open

Age-specific and sex-specific reference intervals for non-fasting lipids and apolipoproteins in 7260 healthy Chinese children and adolescents measured with an Olympus AU5400 analyser: a cross-sectional study

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To cite: Liu J, Dai Y, Yuan E, *et al*. Age-specific and sex-specific reference intervals for non-fasting lipids and apolipoproteins in 7260 healthy Chinese children and adolescents measured with an Olympus AU5400 analyser: a cross-sectional study. *BMJ Open* 2019;**9**:e030201. doi:10.1136/bmjopen-2019-030201

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2019-030201).

Received 04 March 2019
Accepted 18 July 2019

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ABSTRACT

Aims Ethnic, demographic, lifestyle, genetic and environmental factors influence lipids and apolipoproteins. The aim of this study was to establish age-specific and gender-specific reference intervals for non-fasting lipids and apolipoproteins in healthy Chinese children and adolescents.

Methods This study followed the Clinical and Laboratory Standards Institute EP28-A3c guidelines. Non-fasting samples were collected from 7260 healthy Chinese children and adolescents, and they were analysed using the Olympus AU5400 analyser for: triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 and apolipoprotein B (ApoB). The age-related and gender-related reference intervals were partitioned using the Harris-Boyd method. The non-parametric method was used to establish the lower limit (2.5th percentile) and the upper limit (97.5th percentile) for the reference intervals. The 90% CIs for the lower and upper limits were also calculated.

Results Based on the Harris-Boyd method, gender partitions were required for TC, LDL-C and ApoB. Age differences were observed for all analytes. Paediatric reference intervals were established for non-fasting lipids and apolipoproteins based on a large population of healthy children and adolescents.

Conclusions Previously used reference intervals did not take age and gender into account. These age-specific and gender-specific reference intervals established in this study may contribute to improved management and assessment of paediatric diseases.

Strengths and limitations of this study

- This study was based on 7260 participants representing one of the largest samples in the field.
- Study population, statistical analysis and sample size were defined according to the guidelines of the Clinical and Laboratory Standards Institute.
- The age-related and gender-related reference intervals were partitioned using the Harris-Boyd method.
- This study was a monocentric study.
- This study lacked data on pubertal stage of the participants.

INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death in the global adult population.[1] Childhood dyslipidaemia has become an important health concern due to its association with an increased risk for CVD and the metabolic syndrome with consequent occurrence of cardiovascular mortality and type 2 diabetes mellitus later in life, respectively. The worldwide epidemic of child and adolescent obesity in is now well appreciated, and obesity in children is commonly associated with dyslipidaemia.[2 3] Serum lipid biomarkers have an indispensable role in the assessment of CVD and dyslipidaemia, which are conditions that have become increasingly prevalent in the paediatric population.[4–6]

The criteria for normal lipid levels, however, vary among populations. The mortality rate of coronary heart disease (CHD) is quite different among countries, even with the same total cholesterol (TC) level.[7 8] Each country is encouraged to have its own criteria. Great changes have occurred in terms of dietary and lifestyle habits with the development of the economy, suggesting that the criteria should be changed even in the same area.[9 10] The prevalence and mortality rates of diseases associated with dyslipidaemia such as CHD have also increased.[10] However, few studies have been performed to establish reference intervals of serum lipids in Chinese children.

Clinicians rely on the availability of reliable and appropriate reference intervals

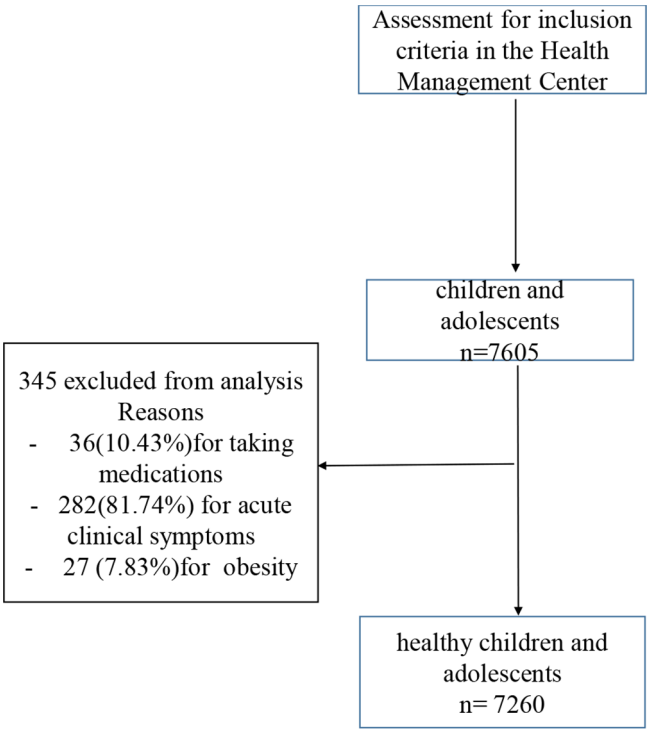

Figure 1 Flow diagram of study.

to correctly interpret laboratory test results combined with data collected through physical examinations and medical histories. Unfortunately, critical gaps currently exist in up-to-date and accurate reference intervals for clinical interpretation of laboratory test results in the paediatric population.[11] Ideally, clinical laboratories should establish reference intervals based on their own population.[12] Most clinical laboratories adopt the reference intervals reported by the medical literature or the diagnostic test manufacturer,[13 14] which may lead to misinterpretation because serum lipid levels in healthy individuals are affected by many factors such as gender, age, dietary, life style, racial differences and geographic conditions. Different methods also may play a role in variation which cannot be ignored. Thus, it is critical and urgent to establish age-specific and gender-specific reference intervals of lipids and lipoproteins for the local paediatric population.

The aim of this study was to establish age-specific and gender-specific reference intervals of lipids and lipoproteins in healthy Chinese children and adolescents.

SUBJECTS AND METHODS

Study population

This cross-sectional study was performed in Zhengzhou, Henan Province, China. From June 2016 to January 2019, 7605 children and adolescents (4125 boys and 3480 girls aged 0–13 years) were randomly recruited from the Health Management Centre of the Third Affiliated Hospital of Zhengzhou University (also known as Henan Maternal and Children Health Hospital). The study individuals were those who visited the hospital for a routine health check-up. Only individuals who resided in Zhengzhou, Henan Province, for at least 6 months were enrolled into this study. The residences of subjects covered all regions, including the Erqi District, Jinshui District, Guancheng Hui District, Zhongyuan District, Shangjie District, Huiji District and Zhengdong New District). We carefully investigated the history of the individuals enrolled in the present study. Only individuals without a history of hypertension, diabetes mellitus, CHD, renal disease and inherited metabolic diseases were included in this study based on the published article.[15] Individuals were excluded from this study for the following reasons: taking medications, acute clinical symptoms such as fever and sore throat and obesity. Weight and height were measured without shoes and in lightweight clothes using a clinical weight/height scale (Detecto, Webb City, Missouri, USA), and these measurements were used to calculated body mass index (kg/m^2). Obesity and overweight were defined according to the WHO standards.[16 17]

Of the 7605 individuals who participated in this study, 345 were excluded for the regular use of medication (n=36, 33 children and 3 adolescents), acute clinical symptoms present at the time of blood collection (n=282, 273 children and 9 adolescents), obesity (n=27, 20 children and 7 adolescents) (figure 1). Children and adolescents were included in this study after their parents or guardians signed informed consent.

SAMPLE COLLECTION

Blood samples were collected in the non-fasting state between 08:00 and 12:30 hours. Samples were collected in vacuum tubes, labelled, transported, lifted to be clotted for 30 min and then centrifuged for 10 min at 1509×*g*. Serum samples were analysed on the Olympus AU5400 system for: triglycerides (TG), TC, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A1 (ApoA1) and apolipoprotein B (ApoB). The full names, abbreviations and analytical methods were listed in table 1. The internal controls were run daily and used to calculate the precision (measured by CV%). The CV values for all analytes ranged from 1.53% to 2.51%. Accuracy (measured by Bias%) was calculated from external quality assessment schemes organised by the Chinese National Center for Clinical Laboratories.

STATISTICAL ANALYSIS

Outliers were removed using the Dixon's test.[12 18] Non-parametric method is a rank-based method. This method is reasonable for sample sizes of at least 120 observations, especially if the analyst wishes to make no assumptions regarding the underlying distribution of the data.[12] Paediatric reference intervals (2.5 and 97.5 percentiles) with their 90% CIs were calculated for each measurand using the non-parametric method performed with RefVal 4.11 program (Refval, Rykkinn, Norway) according to the Clinical and Laboratory Standards Institute (CLSI) C28-A3

Table 1 Analytical methods used to measure biochemical analytes on the Olympus AU5400 Automated Chemistry analyser

Analyte	Method	Bias (%)	CV (%)
TG	GPO-PAP	0.78	1.95
TC	CHOD-PAP	−0.66	1.87
HDL-C	Direct clearance	−4.76	2.51
LDL-C	Direct clearance	4.44	2.21
ApoA1	Immunoturbidimetric	0.06	1.53
ApoB	Immunoturbidimetric	−2.98	2.14

The total analytical imprecision for the experimental method used to calculate the reference intervals is given for each test as an average coefficient of variation (CV%) of two levels of internal controls through 1 year.
ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; CHOD-PAP, cholesterol oxidase-peroxidase-phenol-4-aminoantipyrine; CV, coefficient of variation; GPO-PAP, glycerol-3-phosphate oxidase-peroxidase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

guideline.[12] Statistical analyses were completed using SPSS 17.0 software (SPSS, Chicago, Illinois, USA). The Shapiro-Wilk (S-W) test was used to evaluate the distribution of data. In the event of a non-Gaussian, the data are first transformed to an approximate Gaussian distribution using Box-Cox transformation method.[19] And then age and gender partitioning of reference intervals were evaluated using Harris-Boyd method currently recommended by the CLSI.[12] A value of $p<0.05$ was considered significant.

Patient and public involvement

No patients or the public were involved in the design or conduct of this study.

RESULTS

The final study population consisted of 7260 healthy children and adolescents. Figure 2 illustrates a histogram for age and gender distribution of the reference population while descriptions are presented in table 2. As shown in table 3, three outliers were detected in TG and ApoA1. Four outliers were detected in TC, HDL-C, LDL-C and ApoB. These outliers were excluded from further analysis. Paediatric reference intervals for lipids and apolipoproteins are summarised in table 3, partitioned by age and/or gender, as per the Harris-Boyd method. The profiles and trends of paediatric reference intervals for lipids and apolipoproteins are demonstrated in figure 3. TG, TC, HDL-C and ApoA1 level increased gradually with increasing ages. They required 2–3 age partitions. Gender partitions were required for TC in the 0 to 12 month, 1 to 2 year and 3 to 13 year partitions. TG, HDL-C and ApoA1 required no gender partitioning. The concentrations of LDL-C and ApoB gradually increased before 5 years and then decreased. Gender partitions were required for LDL-C in the 0 to 12 month, 1 to 5 year and 6-to 13 year partitions. And gender partitions were required for ApoB in the 1 to 5 year partitions.

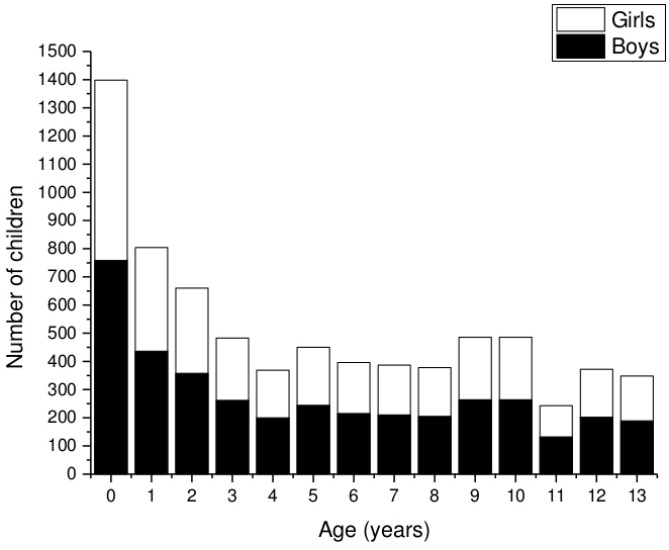

Figure 2 Histogram for age and gender distribution of the reference population.

DISCUSSION

CVD usually occurs in the fourth decade of life, but atherosclerosis begins during the first few years of life.[20] A previous study has clearly shown the relationship between dyslipidaemia and CVD.[21] Lipoproteins are the CVD risk

Table 2 Descriptive data of the study population

	Boys (n=3938)	Girls (n=3322)
Age (years)	5.08 (4.18)	4.98 (4.15)
BMI (kg/m^2)	16.88 (1.62)	16.56 (1.53)
TG (mmol/L)	1.51 (0.17)	1.55 (0.18)
TC (mmol/L)	4.17 (0.52)	4.58 (0.54)
HDL-C (mmol/L)	1.66 (0.16)	1.88 (0.16)
LDL-C (mmol/L)	3.24 (0.55)	3.81 (0.52)
ApoA1 (g/L)	1.35 (0.11)	1.36 (0.11)
ApoB (g/L)	1.12 (0.24)	1.21 (0.30)

Data are mean (SD).
ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Table 3 Age-specific and gender-specific paediatric reference intervals for lipids and apolipoproteins

Analyte (unit)	Age	Gender	N	Outliers	Medians	Lower limit (CI)	Upper limit (CI)
TG (mmol/L)	0 to <1 year	Any	1397	1	1.73	0.43 (0.39 to 0.44)	3.17 (2.85 to 3.28)
	≥1 year to ≤6 years	Any	3160	2	1.35	0.41 (0.37 to 0.43)	2.67 (2.53 to 2.72)
	>6 years to ≤13 years	Any	2700	0	1.67	0.42 (0.39 to 0.43)	2.91 (2.83 to 3.05)
TC (mmol/L)	0 to <1 year	Boys	840	0	2.81	1.60 (1.40 to 1.90)	4.08 (3.46 to 4.30)
	0 to <1 year	Girls	558	0	3.36	1.80 (1.40 to 2.00)	4.98 (4.02 to 5.60)
	≥1 year to ≤2 years	Boys	874	1	3.51	1.90 (1.50 to 2.00)	5.18 (4.84 to 5.60)
	≥1 year to ≤2 years	Girls	588	0	3.72	1.91 (1.50 to 2.10)	5.57 (5.44 to 5.98)
	>2 years to ≤13 years	Boys	2636	2	4.43	2.51 (2.30 to 2.90)	6.42 (5.93 to 6.82)
	>2 years to ≤13 years	Girls	1760	1	4.86	3.00 (2.98 to 3.20)	6.79 (5.92 to 7.14)
HDL-C (mmol/L)	0 to ≤6 years	Any	4557	3	1.50	0.47 (0.45 to 0.54)	2.57 (2.47 to 2.84)
	>6 years to ≤13 years	Any	2699	1	1.82	0.63 (0.59 to 0.69)	3.08 (3.01 to 3.52)
LDL-C (mmol/L)	0 to <1 year	Boys	840	0	2.57	0.92 (0.74 to 1.00)	4.25 (3.87 to 4.78)
	0 to <1 year	Girls	558	0	3.12	1.00 (0.56 to 1.08)	5.28 (4.23 to 6.12)
	≥1 year to ≤5 years	Boys	1611	1	3.89	1.31 (1.08 to 1.40)	6.54 (5.64 to 7.52)
	≥1 year to ≤5 years	Girls	1153	1	4.47	1.30 (1.24 to 1.40)	7.66 (6.63 to 8.75)
	>5 years to ≤13 years	Boys	1762	1	2.86	0.96 (0.83 to 1.30)	4.81 (4.60 to 5.19)
	>5 years to ≤13 years	Girls	1332	1	3.43	1.28 (1.26 to 1.39)	5.58 (5.30 to 6.50)
ApoA1 (g/L)	0 to ≤2 years	Any	2860	1	1.16	0.29 (0.20 to 0.36)	2.09 (2.02 to 2.19)
	>2 years to ≤13 years	Any	4397	2	1.41	0.52 (0.50 to 0.60)	2.36 (2.23 to 2.40)
ApoB (g/L)	0 to <1 year	Any	1397	1	0.93	0.30 (0.27 to 0.32)	1.63 (1.58 to 2.31)
	≥1 year to ≤5 years	Boys	1612	0	1.44	0.46 (0.34 to 0.51)	2.32 (1.68 to 2.62)
	≥1 year to ≤5 years	Girls	1153	1	1.57	0.49 (0.40 to 0.50)	2.68 (2.16 to 2.77)
	>5 years to ≤13 years	Any	3094	2	0.94	0.40 (0.40 to 0.41)	1.50 (1.39 to 1.66)

ApoA1, apolipoproteinA1; ApoB, apolipoproteinB; HDL-C, high-densitylipoprotein cholesterol; LDL-C, low-densitylipoprotein cholesterol; TC, totalcholesterol; TG, triglycerides.

factors showing the strongest tracking properties.[22] These findings emphasise the need to measure lipid levels in the paediatric population and identify individuals with elevated cardiovascular risk to enable early intervention.

Lipid profiles are usually measured in the fasting state. However, non-fasting TG levels are better at predicting future cardiovascular events than fasting TG levels.[23–25] Furthermore, non-fasting lipids, lipoproteins and apolipoproteins levels differ only minimally from levels in the fasting state.[25] Many countries are currently changing their guidelines, moving towards a consensus on measuring serum lipid profile for cardiovascular risk prediction in the non-fasting state.[26–30]

A previous study has shown that lipid levels are dependent on age and sexual maturation.[31] Differences in lipid levels are linked to gender, especially after the beginning of puberty.[32] Our finding of higher TC, LDL-C and ApoB levels in girls compared with boys is consistent with other studies.[33 34] In contrast, Lopez *et al* did not report any gender differences.[35] And age-related differences were

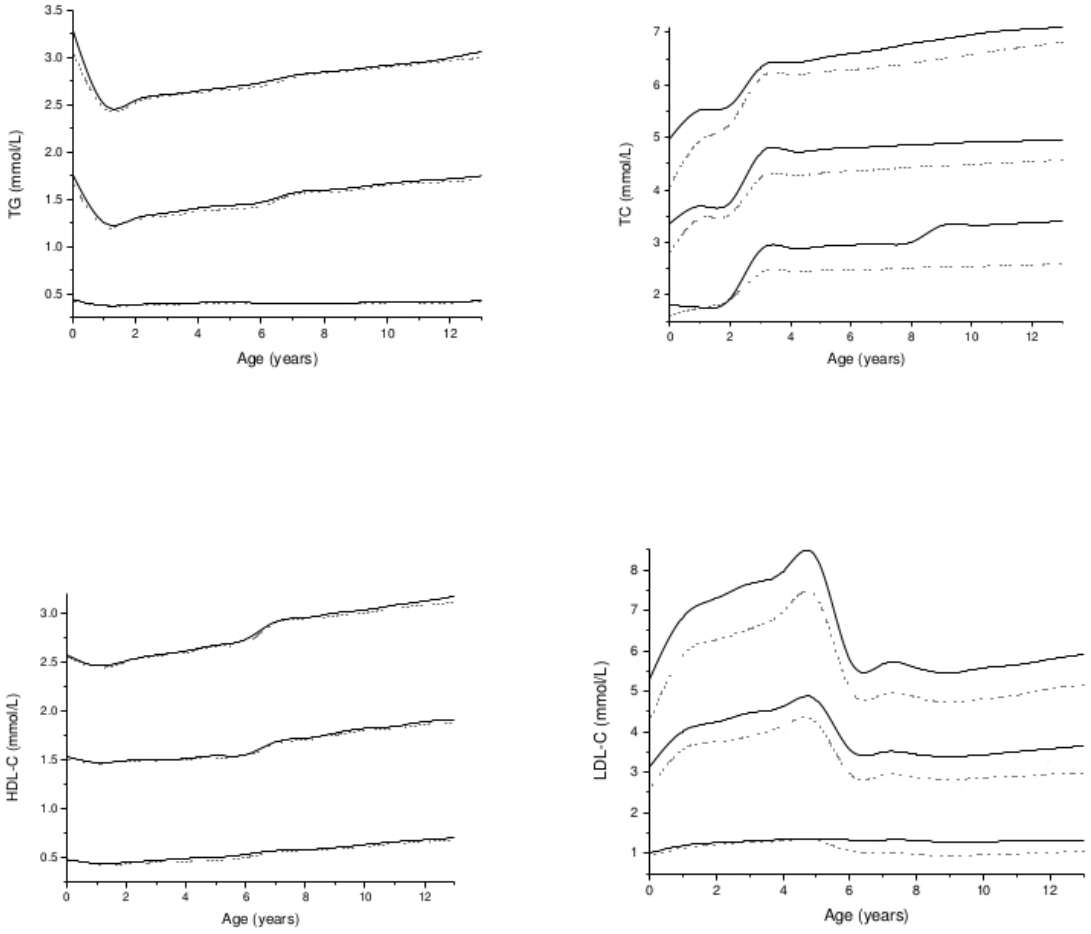

Figure 3 Percentile curves for concentrations of non-fasting serum lipids and apolipoproteins. Lower, median and upper curves denote the 2.5th, 50th and 97.5th percentiles, respectively. Solid lines denote female values and dashed lines denote male values. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

also observed for all analytes. Yip *et al* reported similar findings.[36] The lipid levels of children differ from area to area. In comparison to data from other studies,[34 36 37] Chinese children showed lower levels of TG, TC and LDL. This may be explained by the difference of diet, lifestyle, economic development and environment in various ethnicities.[32 38 39] TG, TC, HDL, LDL, ApoA1 and ApoB levels in children of Han ethnicity in our area are higher than those of children with the same age in Beijing area.[40] This may be explained by the different analytical methods, reagents and overweight epidemic. It is therefore essential to establish age-specific and gender-specific reference intervals using a local population.

In earlier studies, age intervals were arbitrarily set. In this study, comprehensive age-specific and sex-specific analyses were performed and therefore we can determine actual age-groups and sex-groups reflecting age-related and gender-related changes and cut-off ages for the reference intervals.

A major strength of this study is the recruitment of a large study population. There are two possible limitations to this study. The first limitation is the lack of data on pubertal stage of the participants. We could not assess the relationship between pubertal stage and lipid levels. A second limitation is the monocentric nature of the cohort. Due to dietary and geographical diversity in China, we cannot state that our study population is representative of the Chinese paediatric population in general.

Further study needs to be performed to present the prevalence of dyslipidaemia in China. A multicentre study needs to be performed to present the reference intervals for the general paediatric population in China.

To be the best of our knowledge, this is the first report from China on paediatric reference intervals for non-fasting lipids and apolipoproteins. These reference intervals may contribute to improved management and assessment of paediatric diseases. Those established reference intervals could be adopted in other clinical laboratories after appropriate validation.

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Acknowledgements We would like to express our sincere thanks to all the children and adolescents who participated in this study. And we also thank our coworkers at the Department of Clinical Laboratory.

Contributors Study concept and design: JL, YD. Acquisition of data: YL, EY. Interpretation of data/results: EY, YL, QW. Data analysis: LW, YS. Drafting of the manuscript: JL, YD. Critical revision of the manuscript: EY, YL. All authors approved the final manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Parental/guardian consent obtained.

Ethics approval This study was approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University on 1-1-2015, number 2015/011. All the subjects gave informed written consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available.

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