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REGULAR ARTICLE

New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores

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ABSTRACT

Aim: Accurate calculations of testicular volume standard deviation (SD) scores are not currently available. We constructed LMS-smoothed age-reference charts for testicular volume in healthy boys.

Methods: The LMS method was used to calculate reference data, based on testicular volumes from ultrasonography and Prader orchidometer of 769 healthy Dutch boys aged 6 months to 19 years. We also explored the association between testicular growth and pubic hair development, and data were compared to orchidometric testicular volumes from the 1997 Dutch nationwide growth study.

Results: The LMS-smoothed reference charts showed that no revision of the definition of normal onset of male puberty – from nine to 14 years of age – was warranted. In healthy boys, the pubic hair stage SD scores corresponded with testicular volume SD scores ($r = 0.394$). However, testes were relatively small for pubic hair stage in Klinefelter's syndrome and relatively large in immunoglobulin superfamily member 1 deficiency syndrome.

Conclusion: The age-corrected SD scores for testicular volume will aid in the diagnosis and follow-up of abnormalities in the timing and progression of male puberty and in research evaluations. The SD scores can be compared with pubic hair SD scores to identify discrepancies between cell functions that result in relative microorchidism or macroorchidism.

INTRODUCTION

Measuring testicular volume is an important part of the physical examination of males. In early infancy, testicular tissue consists mainly of Sertoli cells. During puberty, under the influence of increased pulsatile secretion of follicle-stimulating hormone, Sertoli cells lining the seminiferous tubules proliferate and start supporting the production of germ cells, which increases the cord's length and width and causes the testes to enlarge (1). Meanwhile, increased pulsatile secretion of luteinising hormone stimulates the interstitial Leydig cells to produce testosterone, inducing pubic and axillary hair development, scrotal enlargement and darkening, increase in penile size and the linear growth spurt (2).

Abbreviations
IGSF1, Immunoglobulin superfamily member 1; SD, Standard deviation.

Various conditions associated with a delay or advance in the onset of puberty can be suspected based on the measurement of testicular volume. In addition, an estimation of testicular volume is warranted if microorchidism or macroorchidism is suspected.

Ultrasonography provides the most accurate estimation of testicular volume, as it only slightly overestimates the

Key notes

- Accurate calculations of testicular volume standard deviation (SD) scores are not currently available.
- We constructed LMS-smoothed age-reference charts, based on testicular volumes from 769 healthy Dutch boys aged 6 months to 19 years.
- The age-corrected SD scores for testicular volume will aid in the diagnosis and follow-up of abnormalities in the timing and progression of male puberty and can be compared with pubic hair SD scores.

true volume of the testis, in contrast to the Prader orchidometer, which also encompasses the epididymis and skin (3). Nevertheless, the orchidometer correlates well with ultrasonography (3,4) and is therefore a useful and practical tool for clinicians.

Measured testicular volumes must be compared with reference data, preferably expressed in standard deviation (SD) curves using the LMS method (5), as is routinely done for many auxological measurements. For this study, recently published data on the testicular volume of 769 Dutch boys aged 6 months to 19 years, measured with both ultrasonography and orchidometer, were used to construct smoothed reference charts and approximate standard deviation scores using the LMS method. We then compared testicular volume SD scores and pubic hair SD scores and applied this tool to testicular volume measured in the fourth Dutch growth study and patients with Klinefelter's syndrome and immunoglobulin superfamily member 1 (IGSF1) deficiency syndrome.

SUBJECTS AND METHODS

Study design

For this study, data on testicular volume measured with ultrasonography and orchidometer were fitted to construct smoothed reference charts using the LMS method, and curves were constructed for clinical practice. Furthermore, the validity and representativeness of the novel charts were assessed by applying them to the orchidometric testicular volume data from the fourth Dutch growth study (6) and patients with Klinefelter's syndrome and IGSF1 deficiency syndrome.

Subjects

Data from a recently published large cross-sectional study on testicular growth were used (4). The study included healthy boys between 6 months and 19 years of age, with two scrotal testes at birth and at the time of examination (2007–2009). Exclusion criteria were as follows: (i) history of undescended testes, hydrocele, varicocele or retractile testes, (ii) conditions that can influence testicular growth, such as various syndromes or growth disorders and (iii) previous surgery in the urogenital region. Pubic hair development was staged by the same physician (JG) according to the Marshall and Tanner scale (2). A detailed characterisation of this cohort has previously been described in Goede et al. (4).

Methods

Measurement of testicular volume

For each boy, the volume of both testes was measured by the same physician (JG) using ultrasonography and the Prader orchidometer. The orchidometer consisted of 12 solid ellipsoid models with a volume of 1 to 6, 8, 10, 12, 15, 20 or 25 mL. If the testicular volume appeared intermediate between two ellipsoids, volumes were estimated at 7, 9, 11, 13, 18 and 22 mL. If the testis appeared larger than the 25 mL model, its volume was estimated at 28 or 30 mL. The volumes of both testes were averaged.

Ultrasonography was performed using a 12-MHz linear array transducer (Falco Auto Image, Falco Software, Tomsk, Russia). Grey-scale images in the transverse and longitudinal planes were used to calculate testicular volume from the measurements of length, width and height, using the formula for an ellipsoid $\pi/6 \cdot \text{length} \cdot \text{width} \cdot \text{height}$. For each testis, the highest of three measurements was used and the volumes of both testes were averaged. A more detailed specification of the measurements has previously been described in Goede et al. (4).

Statistical method

The reference distributions were fitted by the LMS method (5) using the GAMLSS package in R. The LMS method summarises the distribution by three age-dependent smooth curves representing skewness (L curve), median (M curve) and coefficient of variation (S curve), and assumes that the data follow a standard normal distribution after a Box–Cox transformation. Prior to fitting, testicular volume was log-transformed to obtain a more even spread at the lower and upper age ranges. The transformations used were $z(a) = \log(a + 0.5)+3$ for volumes a measured by ultrasonography and $z(b) = \log(b + 10)$ for volumes b measured by the orchidometer. The smoothing family and the amount of smoothing were determined by visual inspection of the fitted curves, Q-statistics (7) and the worm plot (8). A good fit at periods of rapid growth was obtained by linearising the M curve by a monotonic age transformation (9). The model for the ultrasonographic volumes was fitted using cubic splines smoothing on age with $df(M) = 3$, $df(S) = 1.75$ and $df(L) = 1$. The orchidometer model used B-splines of degrees 5 (M), 3 (S) and 1 (L). The reference tables were exported with an age grid of 0.1 years between 6 months and 19 years of age. These values can be used to calculate SD scores from measured millilitres using the following formula: SD scores $= ((X/M)^{L}-1)/(L \cdot S)$, in which X represents the log-transformed testicular volume.

Pubic hair was expressed in SD scores using puberty plot (10), an online application that plots age-conditional reference diagrams for various Tanner stages based on the 1997 Dutch growth study (6).

RESULTS

Subjects

We obtained data from 769 healthy boys aged from 6 months to 19 years of age. The cohort characteristics have previously been described by Goede et al. (4). Pubic hair stage was I in 73.1%, II in 8.7%, III in 3.0%, IV in 5.6% and V in 9.6%.

Testicular volume

Mean testicular volumes measured by ultrasonography ranged from 0.23 to 20.23 mL and measured with orchidometer from 1 to 30 mL. In 4.5% of measurements, orchidometric testicular volume was estimated at 7, 13, 18, 22, 28 or 30 mL, as described in the methods. To investigate interobserver variation, testicular volume measured

with ultrasonography and orchidometer was investigated by a second observer in 84 boys (10.6%) and by a third observer in 44 (5.7%). Both additional investigators were blinded to the results from the first investigator. Results correlated significantly between all observers (4).

Reference charts for testicular volume measured both with orchidometer or ultrasonography were constructed. The LMS curves are displayed in Figures 1 and 2, with high-resolution versions in Supplementary Figures. The values for L, M and S are displayed in Table S1. An SD score calculation tool can be provided by the corresponding author on request.

For the onset of puberty, most clinicians use an orchidometer cut-off of 4 mL (11), although some studies showed that a volume of 3 mL can already be considered a sign of puberty (12). In our cohort, a palpated testicular volume of 3 mL was on average reached at 10.8 years of age, with the +2SD curve crossing 3 mL at 4.1 years of age. As this age limit is in clear disconcordance with the physiologic biochemical and clinical onset of puberty, a 3 mL cut-off volume is not useful in our cohort to define onset of puberty.

A palpated testicular volume of 4 mL, on the other hand, was reached on average at 11.6 years of age and at that age, the average ultrasonographic volume was 1.4 mL. The +2SD curve crosses the orchidometric 4 mL at 8.9 years of age and the ultrasonographic 1.4 mL at 9.1 years of age, and the −2SD curve crosses the orchidometric 4 mL at 14.2 years of age and the ultrasonographic 1.4 mL at 14.0 years of age. Normal onset of puberty can therefore be defined as reaching an orchidometric volume, which is the current gold standard, of 4 mL between nine and 14 years of age, corresponding with an ultrasonographic volume of 1.4 mL at those ages.

Comparison with data on orchidometric testicular volume from the fourth Dutch growth study

The new reference charts were used to calculate SD scores of orchidometric testicular volume from approximately 2000 Dutch boys aged from eight to 18 years in the 1997 Dutch growth study (6). Between eight and 13 years of age, the SD scores have an approximate normal distribution with a mean of 0.06 and an SD of 1.25. Above the age of 13 years, the SD score distribution of the Dutch boys in the growth study is below that of the new orchidometer LMS reference, with a mean of −0.88 and an SD of 1.05.

Associations between testicular volume and Tanner stages of pubertal development

Figure 3 shows the distribution of testicular volumes at different Tanner stages for pubic hair in our cohort, revealing quite a large spread of testicular size within each scored pubic hair stage, as well as an appreciable overlap between pubic hair stages. To evaluate the relationship between pubic hair development and increase in testicular volume, we calculated SD scores for pubic hair stage using the 1997 growth study as a reference population (10). As can be seen in Figure 4A, there is moderate concordance ($r = 0.394$) between these two indices of testicular function in healthy children. However, in syndromes known to exhibit disharmonious pubertal development, such as Klinefelter's syndrome (13) and IGSF1 deficiency syndrome (14), displaying this relationship reveals the disharmony (Fig. 4B).

DISCUSSION

This study provides up-to-date reference charts for testicular volume, based on the standardised measurement of 769 healthy Dutch boys aged 6 months to 19 years. It is the first study allowing clinicians and researchers to calculate SD scores from measured testicular volume either assessed with ultrasonography or the Prader orchidometer.

There are only few reports on reference values for testicular volume in childhood and adolescence, most of which were performed in non-Western or unhealthy children or with incomplete age ranges (15–19). Furthermore, only the most recent Dutch epidemiological study from 1997 by Mul et al. (6) used the LMS method to construct reference curves. However, they measured testicular volume only with the orchidometer, only included boys aged 8 years or older, and did not provide LMS charts. To compare the distribution of testicular volume of that cohort with ours, we calculated SD scores for the 1997 data, based on the LMS charts from the current study. In the preadolescent ages, the distribution of SD scores for testicular volume in the validation data had a similar mean and higher variance. The higher variance is likely to be the result of increased measurement error by multiple observers. The validation sample employed hundreds of physicians to measure testicular volume, whereas the same physician collected all the measurements in the current study. Therefore, the new LMS references do not contain inter-rater variation. Above the age of 13 years, the distribution of testicular volumes in the validation sample was considerably lower than in the current study. An explanation for this finding is the different method to estimate testicular volume at the upper end: in the validation study, all volumes of >25 mL were rounded down to 25 mL, while our study also used estimates between 25 and 30 mL. This might also explain the lower reference values from a large study in 1974 (20). We conclude that the new LMS charts depict normal orchidometric volume more accurately.

Adult testicular volume remains stable after pubertal development is complete, according to most studies (21–23). Therefore, although with caution, the current study's LMS values for 19 years of age can also be used to calculate SD scores for young- to middle-aged adults.

Normal and delayed puberty were defined in Mul et al. (6) as an orchidometric testicular volume of 4 mL at 11.5 years and 13.8 years of age, respectively. Precocious puberty was defined based on the G2 stage for boys, at 9.8 years of age. In our study, a precocious, average and delayed onset of puberty were defined as an orchidometric testicular volume of 4 mL at ≤8.9 years, 11.6 years and ≥14.2 years of age, respectively, corresponding with an

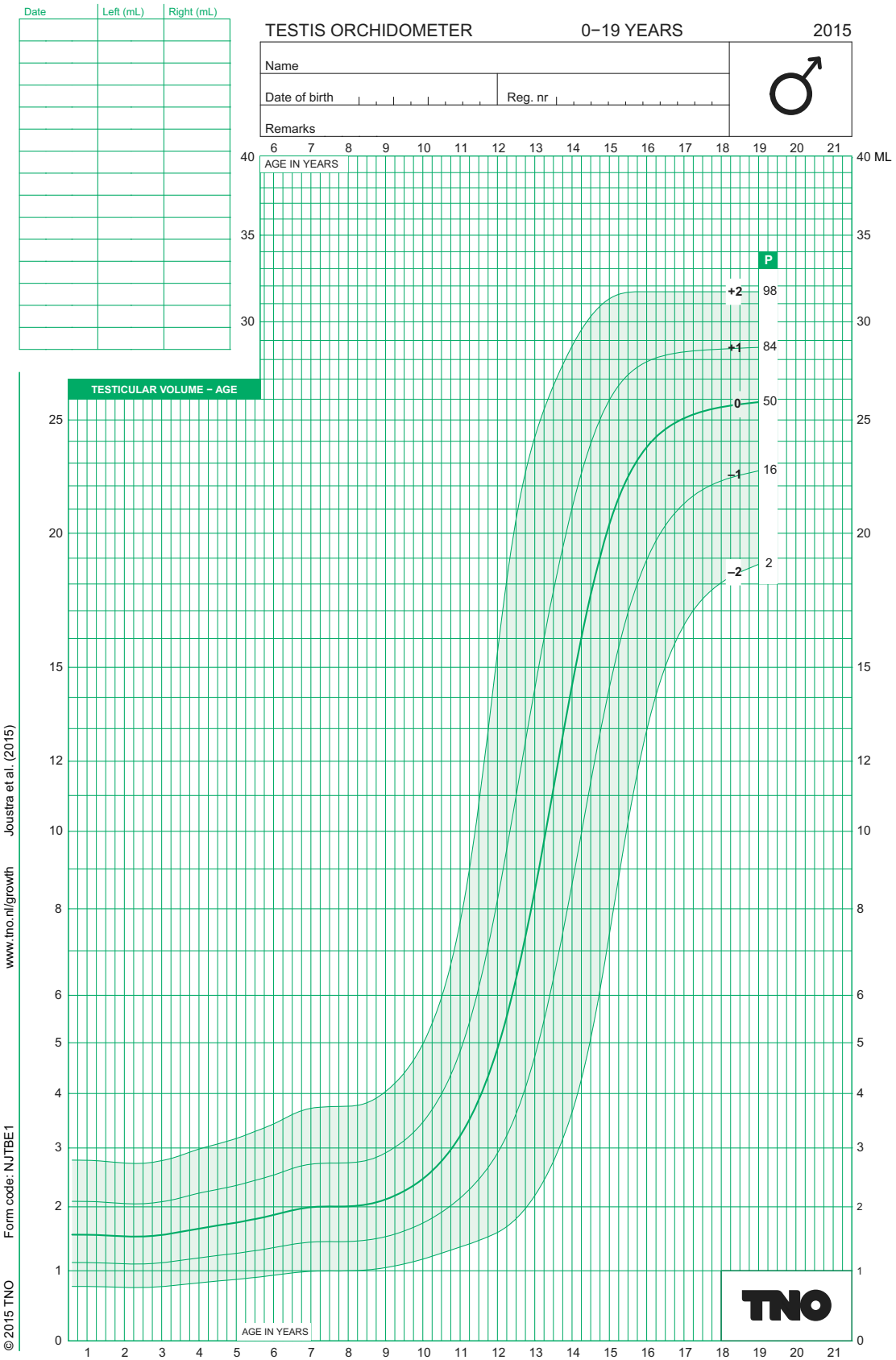

Figure 1 LMS-smoothed reference chart of testicular volume measured with orchidometer in healthy boys aged 6 months to 19 years.

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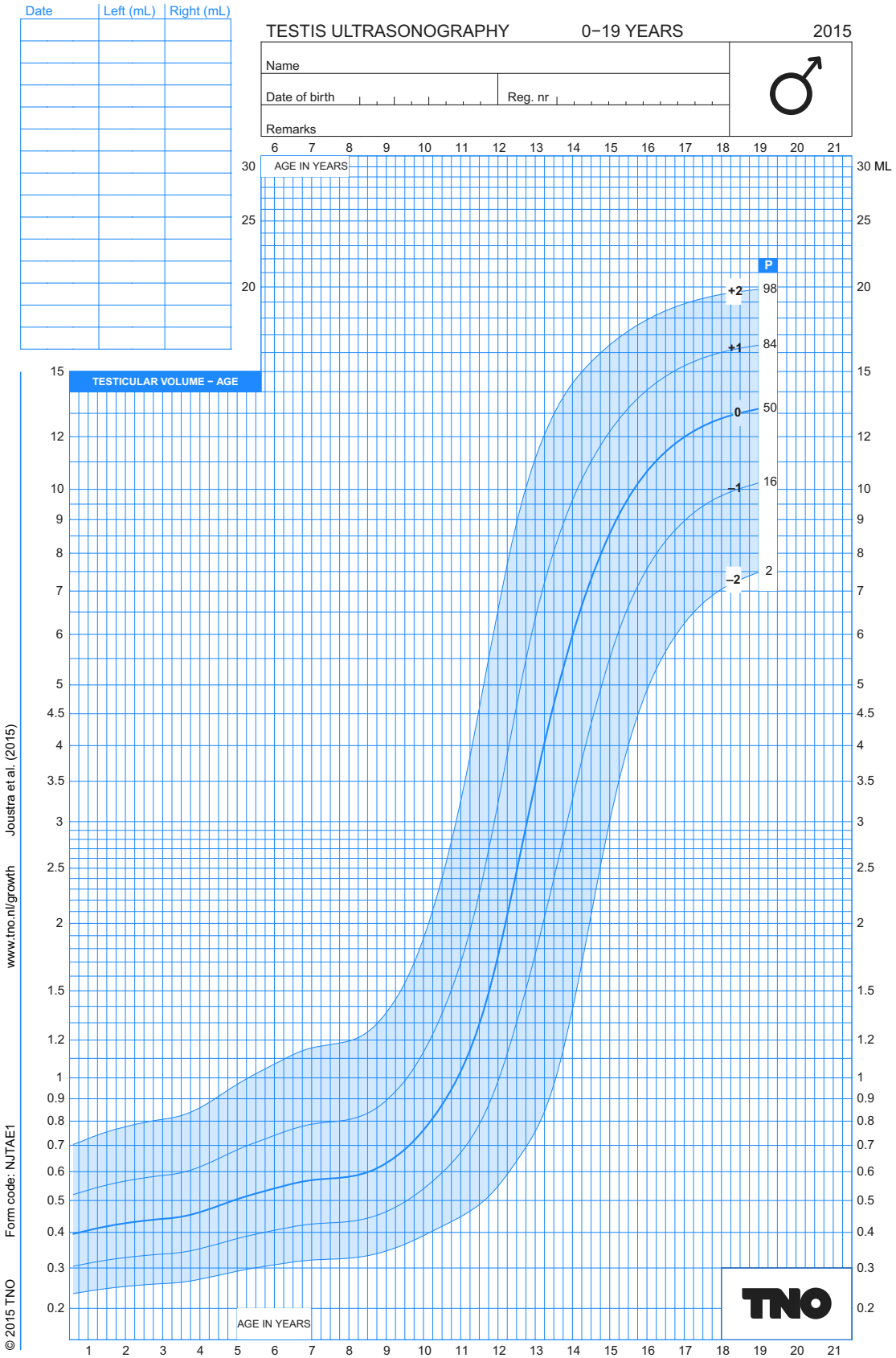

Figure 2 LMS-smoothed reference chart of testicular volume measured with ultrasonography in healthy boys aged 6 months to 19 years.

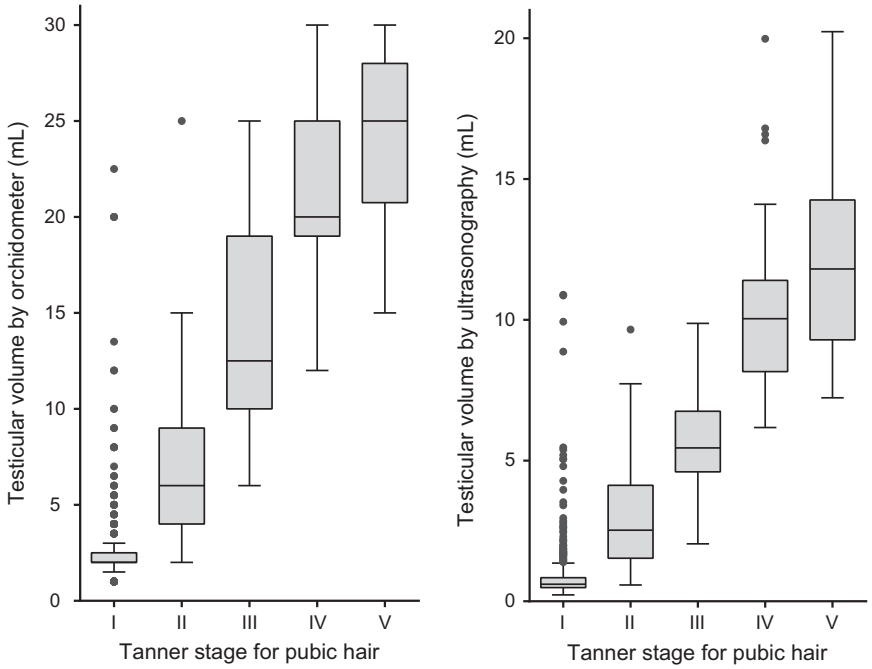

Figure 3 Distribution of testicular volumes as measured with orchidometer or ultrasonography within Tanner stages I to V of pubic hair development.

ultrasonographic testicular volume of 1.4 mL at those same ages. There is a minor discrepancy in the lower age limit of normal in the two studies, caused by Mul et al. using G2 stage and the current study using orchidometric testicular volume. Nevertheless, the definition of normal onset of puberty in boys between nine and 14 years of age can be maintained.

A limitation of the current study is that the data were collected in a cross-sectional design. Strictly speaking, the references are only valid for a one-shot comparison of a measurement with the reference group and do not allow for longitudinal interpretation. Puberty, as many of the growth and developmental parameters in children, is subject to differential timing. This increases variation of the measurement, especially during mid-puberty, and might lower the sensitivity of detecting altered testicular growth when compared to other children of the same age. Thus, similar to height, the longitudinal testicular growth curve for a given boy will be steeper than the median curve calculated for the entire group. Our references do not indicate how steep is still normal. Also, a boy with testicular growth outside the 1.0 SD curves might be just early or just late. Therefore, the interpretation of the charts should be done in conjunction with other developmental parameters that measure timing.

The reference charts can furthermore be used to diagnose conditions that affect testicular size. Testicular volumes below −2SD indicate microorchidism. The early recognition of this symptom, and its relation with age, can be used for the diagnosis and follow-up of many endocrine disorders. For instance, microorchidism in hypogonadotropic hypogonadism often presents in childhood and is associated with an absence of spontaneous puberty, while microorchidism in Klinefelter's syndrome usually becomes manifest at mid-puberty (24) and is characterised by the disharmonious pubertal development illustrated in Figure 4B. In addition, testicular pathology can be diagnosed and monitored. For instance, the inguinal thermal environment to which the congenital or acquired undescended testis is exposed affects testicular growth and spermatogenesis (25). Assessment of ultrasonographic testicular size in SD scores in this condition might serve valuable in research settings or possibly even in clinical practice and decision making.

Testicular volumes above the age-dependent +2SD curve indicate macroorchidism. For instance, patients with the recently discovered IGSF1 deficiency syndrome show macroorchidism in late adolescence and adulthood (14), as well as delayed pubertal testosterone rise (Figure 4B). In the reported cross-sectional data on IGSF1 deficiency (14), macroorchidism in either testis was present in all five adolescents aged 12.7 to 18.4 years, in contrast to only one (8.0 years) of the six children aged 3.3 to 10.5 years. In fragile X syndrome, on the other hand, patients have significantly larger testes throughout childhood, with true macroorchidism becoming manifested usually just prior to puberty at 8 years of age (13).

Of note, as macroorchidism in adults should be defined as an orchidometric testicular volume of over 31.1 mL (+2SD at 19 years of age), and most orchidometers do not contain beads >25 mL (although orchidometers with larger beads are available), most of these tools cannot be used to

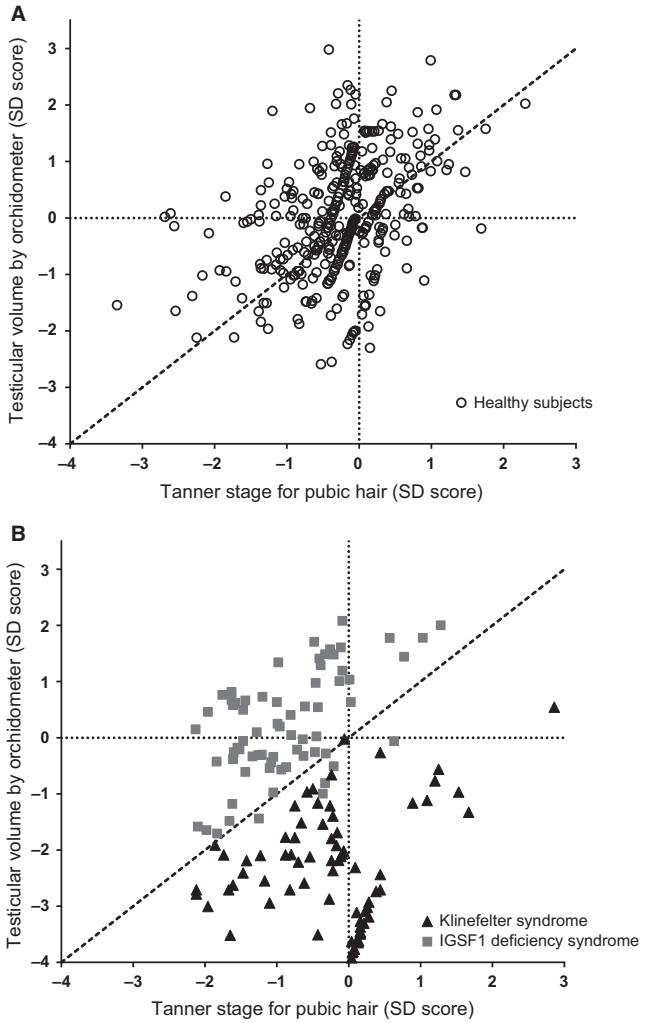

Figure 4 (A) SD scores for pubic hair stage, calculated using puberty plot (10), vs. SD scores for orchidometric testicular volume, calculated using the LMS charts, in 769 healthy boys aged 6 months to 19 years. (B) SD scores for pubic hair stages vs. SD scores for orchidometric testicular volume in 67 patients with Klinefelter's syndrome aged 9 months to 23.5 years (26–28) and 13 patients with IGSF1 deficiency syndrome aged 8.7 to 15.4 years (reference 14 and personal data). Both patient groups include longitudinal data.

diagnose adult macroorchidism. We therefore recommend ultrasonography for diagnosis and follow-up of adult macroorchidism.

For over 60 years, various stages of male pubertal development are classified based on the morphology of either pubic hair or genitalia, for which Marshall and Tanner provided the first normative age values in 1970 (2). The genitalia scores comprise a combination of testes size, scrotum size and aspect, and penis size. However, as mentioned earlier, the increase in testes size is based on follicle-stimulating hormone and Sertoli cell function, and the morphological changes in the scrotum and penis on luteinizing hormone and Leydig cell function. The genitalia score has therefore, in addition to being quite ill-defined, limited utility in discriminating between different aetiologies of disease. However, although the pubic hair score is only based on semi-quantitative categories, it has the benefit of scoring mostly the effects of testosterone production by Leydig cells, and to a lesser extent that of the adrenarche. Testicular volume, on the other hand, is mostly the result of Sertoli cell function and can be measured quantitatively. Furthermore, Figure 3 shows large spread of normal testicular volume within pubic hair stages, and Figure 4B illustrates that testicular growth and pubic hair development do not always in coincide. This underlines the relevance of separating these two processes. The combination of testicular volume and pubic hair development, as well as linear growth spurt, might therefore be considered the most useful tool to assess pubertal development in boys.

Within our cohort, data from ultrasonography and the orchidometer correlated well ($R^2 = 0.978$) (4). However, there are several disadvantages of the orchidometer. First, as mentioned in the introduction, it overestimates testicular volume, which highlights the fact that an orchidometric mL denotes quite a different testicular volume than an ultrasonographic mL. Second, the orchidometric volumes are categorical. Therefore, as there are only few categories especially at the lower ages (usually only 1 and 2 ml) and the LMS interprets these categories as continuous data, SD scores at the lowest ages are unreliable. Also, true testicular volume will often not precisely equal a bead from the orchidometer. Subsequently, in 4.5% of the measurements in the current study, testicular volume had to be estimated based on adjacent beads. However, the orchidometer remains is a practical and low-cost tool for general assessment of testicular volume. Nevertheless, we recommend using ultrasonography, especially at lower testicular volumes, if accurate SD score calculation is required, for example in complicated clinical cases or for research purposes.

In conclusion, this study provides paediatricians, endocrinologists and researchers with reliable reference charts for testicular volume measured with either ultrasonography or orchidometer, and the ability to accurately calculate SD scores. The results will aid in the diagnosis and follow-up of abnormalities in the timing and progression of male puberty, as well as of microorchidism or macroorchidism.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. LMS-smoothed reference chart of testicular volume measured with orchidometer in healthy boys aged six months to 19 years.

Figure S2. LMS-smoothed reference chart of testicular volume measured with ultrasonography in healthy boys aged six months to 19 years.

Table S1. LMS values from reference charts for testicular volume measured with orchidometer or ultrasonography.

