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TESTICULAR ASYMMETRY IN HEALTHY ADOLESCENT BOYS

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Abstract

Objectives: To assess the presence of testicular asymmetry and its currently used cut-off values in varicocele management in a healthy adolescent population.

Patients and methods: We conducted an observational cross-sectional study from April 2015 until December 2016 in which we recruited 539 adolescent boys aged 11 to 16 years. A clinical examination including testicular size measurement by ultrasonography was performed. Testicular volume (TV) was calculated using Lambert's formula ($L \times W \times H \times 0.71$). The testicular atrophy index (TAI) was calculated using the formula $[(TV \text{ right} - TV \text{ left}) / \text{largest TV}] \times 100$. The data for all statistical analyses were stratified for Tanner stage for genital development (TSG) and pubic hair (TSP). Non-parametric tests were used to assess the difference between right and left TV and the prevalence of a

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smaller left testis for the entire population and between each TSG and TSP. Parametric tests were used to determine the difference in mean TAI between each TSG and TSP and to compare the mean TAI to a test value of 0.

Results: Of the 539 recruited boys, we excluded 194 due to a current or past pathology, including varicoceles, influencing normal (testicular) growth or due to incomplete data. Most boys were in the second Tanner stage, followed by the third Tanner stage. Mean age of the entire population was 13,33 years (SD 1,25 years). Of the 345 included participants the mean left TV was 7,67 mL (SD 5,63 mL) and mean right TV was 7,97 mL (SD 5,90 mL). The mean TAI was 2,85% (SD 17,00%). 203 (58,84%) boys had a smaller left testis, whereas 142 (41,16%) had a smaller right testis. 51 (14,78%) had a TAI greater than 20%, 45 (13,04%) had a TVD of 2mL or more in disadvantage of the left TV and 69 (20,00%) had a TAI greater than 20% or a TVD of 2mL or more in disadvantage of the left TV. Related-samples Wilcoxon signed rank test showed a significant difference in mean left and right TV for the entire population and more specifically for TSG3 ($p < 0,001$) and TSP3 ($p = 0,004$). One-sample T-test showed a significant difference in mean TAI versus test value 0 for the entire population ($p = 0,002$) and more specifically for TSG3 ($p < 0,001$) and TSP3 ($p = 0,003$).

Conclusion: Testicular asymmetry, in disadvantage of the left testis, was observed in a considerable number of healthy adolescents. 1 out of 5 adolescents had a smaller left testis and met one of the cut-off values currently used in varicocele management. Therefore in left-sided unilateral inguinoscrotal pathology, a smaller ipsilateral testis in combination with a TAI $> 20\%$ and/or TVD $> 2\text{mL}$ requires a careful interpretation and serial measurements of the testicular volume should always be performed. Furthermore, this study provides reference values for TV, TVD and TAI according to TSG and TSP for a healthy population.

Key words: testis, reference value, healthy adolescents, testicular volume, testicular atrophy index

Abbreviations:

TV:	Testicular volume
TVD:	Testicular volume difference
TAI:	Testicular atrophy index $[(\text{volume TV right} - \text{volume TV left}) / \text{largest TV}] \times 100$
TSG (1-5):	Tanner stage for genital development according to the Marshall and Tanner scale.
TSP (1-5):	Tanner stage for pubic hair according to the Marshall and Tanner scale.
TVdiff:	Testicular volume differential

Introduction

Male adolescence starts by definition when the testes reach an orchidometric volume of 4mL (1), which is shown to correspond with an ultrasonographic volume of 1,4mL in boys between the age of nine and fourteen.(2) At that moment the production of testosterone and the spermatogenesis will start under influence of the gonadotrophins LH and FSH, respectively. The latter is accompanied by an increase in the testicular volume due to development of the Sertoli cells. Consequently, testicular volume (TV) is an important reflection of the testicular function in puberty.(3)

In the primary health care setting, the orchidometer is most often used for estimating the TV. An alternative to the orchidometer is scrotal ultrasonography. Although there is a statistically significant correlation between volumes measured by these two methods (4), ultrasound provides greater accuracy as the orchidometer usually overestimates TV.(5,6) Therefore, when abnormal genital development is suspected, measurement and follow-up of the TV using scrotal ultrasound is recommended.

An important inguinoscrotal pathology in which testicular volumes are determined are varicoceles, defined as abnormally dilated scrotal veins. Varicoceles mostly presents at the beginning of puberty and are found in 14-20% of adolescents. They appear on the left side in 78-93% of all cases. (7,8) In approximately 20% of adolescents with a varicocele, fertility problems will arise in later life. (9) Moreover, small testicular volumes and high testicular volume differences (TVD) are associated with poor semen analysis outcome.(10–12) As a result, the guidelines of the European (EAU) and American (AUA) Association of Urology both state a varicocele in association with a small testis is one of the indication criteria for varicocelectomy in children and adolescents.(13,14) The EAU states a small testis is present when the testicular atrophy index (TAI) exceeds 20% or a TVD of 2mL or more between both testes is noted. (14)

Large volume differences are expected to be observed in patients with unilateral inguinoscrotal disorders. However, there is currently no knowledge about the volume difference between the two testes in a healthy population.

Therefore, this study aims to give a picture of the testicular volumes and testicular volume differences in a healthy population stratified according to the Tanner scale . We aim to do this by determining TV, TVD and TAI. Moreover we determine the presence of a smaller left testis, a TAI exceeding the cut-off value of 20% and a TVD greater than the cut-off value of 2mL. This is to evaluate whether a difference in testicular size is a naturally occurring phenomenon during puberty, independent of the presence of a varicocele or other inguinoscrotal pathologies.

Material and Methods

Study Design

This is a cross-sectional observational study. Data were collected between April 2015 and December 2016.

Setting

Recruitment took place at medical checkups in 15 secondary schools in Antwerp and West-Flanders and at a tertiary hospital.

Participants

Boys aged 11 to 16 years were recruited by written invitation at secondary schools and by posters and flyers, which were present at several departments (i.e. department of: neurology, radiology, abdominal surgery, oftalmology, paediatrics, physical medicine and rehabilitation, vascular surgery and orthopedic surgery) at a tertiary hospital. In the latter case, the patients visited the hospital for non-urological indications. The parent or guardian of the participant was asked to complete a questionnaire. Thereafter, a researcher performed a physical examination on the participant, which included measurement of testicular dimensions by scrotal ultrasound.

Inclusion criteria

This study included healthy boys aged 11-16 years without any evidence of a current or past pathology influencing testicular growth.

Exclusion criteria

We excluded boys with a current or past inguinoscrotal pathology with the potential to influence TV, including clinical and subclinical varicocele, testicular microlithiasis, cryptorchidism, retractile testis, testicular torsion, orchitis or epididymitis, hypospadias, inguinal hernia, hydrocele and epididymal cysts. We also excluded boys with evidence of

disorders influencing testicular growth, for example growth disorders. Boys with missing or incomplete data on Tanner stages for genital development or pubic hair were excluded as well. A more detailed description of exclusion criteria and a schematic summary of the participant selection process can be found in figure 1.

Study Protocol

Informed consent

Participants were invited to participate on a voluntary basis. An informed consent was obtained from both the participant and the parent or representing guardian. Participants were given the option to leave from the study at any time.

Questionnaire

The questionnaire included questions about demographics (i.e. date of birth and ethnicity), medical and familial precedents (e.g. inguinoscrotal disorders and congenital syndromes likely to influence testicular growth or normal development and growth during puberty).

Physical examination

The participants were screened for varicocele, in both upright and supine position, and for other scrotal pathologies. Anthropometric measurements (i.e. height, weight) were obtained. Body mass index was calculated using the formula: $\text{weight}/\text{length}^2$.

Tanner stages

Tanner stages for genital development (TSG) and pubic hair (TSP), according to the Marshall and Tanner scale, were determined.⁽¹⁵⁾

Scrotal ultrasound

In each participant, testicular dimensions were measured using scrotal ultrasonography. Boys were asked to lie down on the examination table with the legs slightly spread.

Testicular height and depth were measured bilaterally with the probe in the sagittal plane, testicular width was measured with the probe in the transversal plane. Gentle pressure was applied to the scrotum to obtain a good contact between the scrotal skin and the ultrasound probe without compressing the testes. Testicular dimensions were measured three times. Two ultrasonography devices were used: MyLabTM50 XVision, Esaote® with a linear Esaote®-probe of 5,0-10,0 MHz for the examinations in the tertiary hospital and GE Logiq® e portable Ultrasound (model 2007) with a GE® 12L-RS probe of 5,0-13,0 MHz (model 2010) for the examinations at medical checkups in secondary schools. The examinations were performed by 7 different researchers. All researchers were trained by the same coordinating supervisor and all examinations were reviewed for quality control by the supervisor.

Calculation of Testicular Volume and Testicular Atrophy Index

TVs were calculated using the formula of Lambert (length x width x height x 0.71) and were expressed in milliliters(mL).(16) The value of each testicular dimension was obtained by calculating the mean of three consecutive measurements. Due to the healthy nature of the study population and given the fact that in pathological circumstances right TV is always used as a reference, the TAI in this study was calculated using a modified formula in which the volume of the largest testis was used as a reference: $[(\text{right TV} - \text{left TV})/\text{TV of the largest testis}]$. This is a slight modification to the usual formula for calculating TAI, which divides the TVD by the right TV. A smaller left testis corresponds with a positive TAI value, while a smaller right testis corresponds with a negative TAI value.

Ethical approval

This study protocol (15/7/65) was approved by the ethical committee of the Antwerp University Hospital.

Statistical methods

The values of TV, TVD and TAI are presented as mean with SD (Standard Deviation). The prevalence of a smaller left or right testis, TAI >20% and/or TVD >2mL are presented as percentage (%).

The difference in mean left and right TV for the entire population and between each TSG and TSP were determined using related-samples Wilcoxon signed rank test.

The differences in mean TVD between each TSG and TSP was assessed using the Kruskal-Wallis test, after which post hoc testing was performed using the Bonferroni correction.

The differences in mean TAI between each TSG and TSP was tested using the Welch's ANOVA test.

The differences in mean TAI when compared to a test value of 0 was assessed for the entire population and between each TSG and TSP using the one-sample t-test.

The differences in the prevalence of a smaller left testis compared to right between each TSG and TSP was determined using the Chi-square test.

The differences in prevalence of the cut-off values for varicocele patients (TAI>20% and/or TVD>2mL) between each TSG and TSP were assessed using the Chi-square test.

The differences in the mean left, right and total TV between ethnicities was determined using the Welch's ANOVA.

All statistical analyses were done using IBM SPSS Statistics 23®. All statistical tests were two-tailed and were conducted with type I error probability of 0.05.

Results

Subjects

Of the 539 examined boys, 179 were excluded because of a current or past inguinoscrotal disorder possibly influencing TV. Another 12 boys were excluded because they had a condition influencing normal development and growth during puberty and 3 boys were excluded because of missing data. The final study population consisted of 345 boys. Study subjects were stratified according to Tanner scale. The demographic characteristics for the

study population recruited in secondary schools, in a tertiary hospital and the entire study population can be found in table 1.

Testicular volumes and testicular volume differences

The mean left TV of all study subjects was 7,67 mL (SD 5,63 mL), the mean right TV was 7,97 mL (SD 5,90 mL), the mean total TV was 15,61 mL (SD 11,42 mL) and the mean TVD was 0,32 mL (SD 1,64mL). The mean left, right, total TVs and mean TVD were further stratified according to TSG and TSP and listed in table 2. Figure 2 shows box plots (median, 25th, 75th percentiles, minimum and maximum) of left, right and total TV according to TSG (A-C), TSP (D-F) and age (G-I).

Related-samples Wilcoxon signed rank test showed that the mean left and right TV differed significantly overall ($p < 0,001$) and more specifically in TSG3 ($p < 0,001$) and TSP3 ($p = 0,004$). No significant difference between mean left and right TV was noted in the other TSG and TSP.

Kruskall-Wallis test showed that TVD differed significantly between TSG ($p = 0,009$) and TSP ($p = 0,038$) for the entire study population. Post-hoc testing using the Bonferroni correction showed that this difference was significant between TSG1 and TSG3 ($p = 0,015$) and between TSG 2 and TSG 3 ($p = 0,039$). No significant difference was withheld between the other TSG or TSP.

Mean testicular atrophy index

The mean TAI for all study subjects was 2,85% (SD 17,00%). Mean TAI for all TSG and TSP are listed in table 2. Mean TAI was the highest in TSG3 and TSP3, with a value of 5,94% (SD 14,78%) and 5,33% (SD 15,10%) respectively. Welch's ANOVA showed no significant difference in mean TAI between each TSG ($p = 0,210$) and TSP ($p = 0,469$). One-sample T-test showed a significant difference in mean TAI vs test value 0 for the entire population ($p = 0,002$) and more specifically in TSG3 ($p < 0,001$) and TSP3 ($p = 0,003$). No significant difference in mean TAI vs test value 0 was noted in the other TSG and TSP. Figure 3 shows

box plots (median, 25th, 75th percentiles, minimum and maximum) of the TAI according to TSG (A) and TSP (B).

The percentage of study subjects with a TAI greater or equal to a given value (cumulative prevalence)

In figure 4 and 5, cumulative prevalence, defined as the percentage of study subjects with a TAI greater or equal to a given value, is displayed for the total study population. Figure 4 shows the cumulative prevalence after stratification for TSG. Figure 5 shows the cumulative prevalence after stratification for TSP.

Smaller left testis vs smaller right testis

203 (58,84%) of all study subjects were found to have a smaller left testis, while only 142 (41,16%) had a smaller right testis. For each TSG and TSP, the left testicle was most frequently the smallest, excluding the first TSG, in which a smaller right testicle was more frequently observed. The numbers and percentages of study subjects with a smaller left testis and a smaller right testis are shown in table 3. Chi-square analysis showed no significant difference in the prevalence of a smaller left testis or a smaller right testis between the different TSG ($p=0,157$) and TSP ($p=0,355$).

TAI threshold: 20%

The number of study subjects with a TAI $> 20\%$, the cut-off value often used in clinical practice, was determined. This threshold is highlighted as a vertical line in figure 4 and figure 5. 51 (14,78%) boys have a TAI greater than 20%. 32 (9,28%) had a TAI less than -20%. We further stratified for TSG and TSP. For example, of all study subjects in TSG2, 26 (20,16%) had a TAI greater than 20%. All data are shown in table 4. In boys with a smaller left testis, Chi-square analysis showed that these differences in prevalence was not significant amongst TSG ($p=0,330$) and TSP ($p=0,673$).

Testicular volume thresholds: 2 mL

In total, 67 (19,42%) boys had a TVD greater than 2mL, of which 45 (13,04%) had a smaller left testis and 22 (6,38%) had a smaller right testis. All data stratified for TSG and TSP are depicted in table 4. In boys with a smaller left testis, Chi-square analysis showed that these differences in prevalence was not significant amongst TSG ($p=0,200$) and TSP ($p=0,592$).

Both thresholds: TAI >20% and TV >2 mL

In total, 40 (11,59%) boys had a TAI greater than 20% and a TVD greater than 2mL, of which 27 (7,83%) had a smaller left testis and 13 (3,77%) had a smaller right testis. All data stratified for TSG and TSP are depicted in table 4. In boys with a smaller left testis, Chi-square analysis showed that these differences in prevalence was not significant between TSG ($p=0,063$). A significant difference in prevalence was noted between TSP ($p=0,028$).

However, post-hoc analysis using the Bonferroni correction showed no TSP with a significant different prevalence of both threshold values in comparison to the another TSP.

One of both thresholds: TAI >20% or TV >2 mL

In total, 110 (31,88%) boys had a TAI greater than 20% or a TVD greater than 2mL, of which 69 (20,00%) had a smaller left testis and 41 (11,88%) had a smaller right testis. All data stratified for TSG and TSP are depicted in table 4. For example, of the 69 with a smaller left testis 51 (14,78%) had a TAI greater than 20% and 45 (13,04%) had a TVD greater than 2mL. In boys with a smaller left testis, Chi-square analysis showed that these differences in prevalence was not significant amongst TSG ($p=0,990$) and TSP ($p=0,673$).

Ethnicity and testicular volume

Welch's ANOVA showed a significant difference in mean left TV between the different ethnicities ($p = 0,037$). Post-hoc analyses using Games-Howell showed a significant larger left TV for Asian study subjects in comparison with Afro-American study subjects ($p = 0,023$). Welch's ANOVA showed no significant difference in mean right TV ($p = 0,084$) and total TV ($p = 0,050$) between the different ethnicities.

Discussion

The aim of this study was to assess the testicular asymmetry in a healthy adolescent population to evaluate whether a difference in testicular size is a naturally occurring phenomenon during puberty, independent of the presence of a varicocele or other inguinoscrotal pathologies. To our knowledge, this is the first large scale cross-sectional study to assess testicular asymmetry and its cut-off values, used in varicocele management, in a healthy adolescent population.

Testicular volume increases rapidly during puberty, making interpretation of TV difficult. (4) Therefore, formulas comparing right versus left TV were brought to life providing a relative measure of testicular asymmetry. The first formula was termed the testicular atrophy index (TAI), which compares the difference between right and left TV to right TV: $TAI = [right\ TV - left\ TV] / [right\ TV]$. (17) The second formula was termed TV differential (TVdiff) and compares the difference between right and left TV to total TV: $TVdiff = [right\ TV - left\ TV] / [total\ TV]$. (18) It was shown both formulas are completely synonymous and TAI and TVdiff can be converted by the formulas $TVdiff = [(e^{TAI}) - 1] / 1.97$ and $TAI = \ln[(1.97 * TVdiff) + 1]$, respectively. (19)

When we look at our data of the TAI, we observe the mean TAI is positive in almost all Tanner stages, meaning the left testis is smaller than the right one. Only in TSG1 we encounter a negative value for the mean TAI, meaning the left testis is larger than the right one.

Furthermore, we note the mean TAI is normally distributed across the different Tanner stages and the mean values are rather low. The highest mean TAI values are 5,94% (SD 14,78%) and 5,33% (SD 15,10%) and are seen in TSG3 and TSP3, respectively. Also, in both of the latter Tanner stages a significant difference in mean TAI is seen when compared to a test value of 0, meaning the highest relative volume differences, expressed as a percentage, of the left TV compared to the right TV are seen in these Tanner stages.

Interestingly, these Tanner stages are associated with the highest growth velocity during puberty.(20)

A smaller left testis is frequently observed in varicocele patients. (10–12) As most varicoceles are left-sided (7,8), it is assumed that the smaller left testis is due to the inhibitory effect of the varicocele on the ipsilateral TV.

However, our data shows a positive TAI for the entire population and for all Tanner stages, excluding TSG1, meaning there is a natural difference between left and right TV in a healthy adolescent population in disadvantage of the left TV. This is important information when interpreting the effect of a left-sided unilateral inguinoscrotal disorder on testicular development. The danger exists that the effect of the disorder on the ipsilateral TV will be overestimated if one is not aware of this natural asymmetry in TV in a healthy adolescent population.

The negative influence of a varicocele on testicular function and growth is reported to possibly result in infertility. (9) Therefore, the goal in adolescents is to prevent testicular injury in order to maintain testicular function and growth for future fertility. Consequently, early varicocele correction is often recommended in the literature. (17,21–26) Nonetheless, in only 20% of adolescents with a documented varicocele, fertility problems will arise in later life. (9) Several studies have postulated to achieve improvement in sperm parameters after adolescent varicocele correction. (25,27–29) However, when looking at paternity as the outcome, the only 2 studies comparing paternity rate in patients with a treated varicocele to a control group, report contradictory results. (30,31) When comparing both studies it is important to notice both study populations were different. Bogaert et al. (30) did not show any difference in paternity in a large group of treated and non-treated adolescents with a unilateral varicocele with a follow-up of 15 years. Bilateral varicoceles were excluded. Cayan et al. (31) however found a shorter time to conception and a higher conception rate in the treated group but included only varicoceles with a TAI > 20% and more than 50% of the

patients had bilateral varicoceles. Also the follow-up was significantly shorter compared to the previous study. Nevertheless, we conclude correction of a varicocele during adolescence in improving paternity remains debatable.

When considering indication criteria to decide whether a varicocele needs to be corrected semen analysis seems the ideal examination as it reflects spermatogenesis. Still, interpretation of semen analysis in adolescents remains difficult for there are currently no standard norms of semen parameters in an adolescent population. (32)

Therefore examining TV remains a very important part of the evaluation of a varicocele.

The current guidelines postulate indication criteria for varicocele correction, based on TV, are a TAI >20% or a TVD >2mL.

We investigated the prevalence of adolescents who met these cut-off values, described above, in a healthy population. As a varicocele is located most frequently on the left side our main interest were healthy adolescent with a smaller left testis.

When we look at our data, we see that 3/5 of all adolescents had a smaller left testis. In TSG3 a smaller left testis was present in more than 2/3 of adolescents.

14,78% met the cut-off value of TAI >20%, 13,04% met the cut-off value of TVD >2mL in disadvantage of the left testis, 11,59% met both cut-off values and 1 out of 5 had a smaller left testis and met one of both cut-off values.

These findings suggest there is a natural difference between left and right TV in a healthy adolescent population in disadvantage of the left TV. As many aberrant values for TAI and TVD are present in a healthy population, with 1 out of 5 healthy adolescents having a smaller left testis and exceeding one of both cut-off values of TAI or TVD, the clinician should be cautious when interpreting the effect of a left-sided unilateral inguinoscrotal disorder on the TV of the affected testis.

Also important to point is that the TAI and TVD give complementary information. A large TVD in boys with smaller TV, as seen in lower Tanner stages, will correspond with a high TAI, while in boys with higher TV, as seen in higher Tanner stages, the same TVD will have a less significant impact on the TAI. For example, when a boy has a testicular volume in the 2 to 4ml range in a Tanner 1 or 2 stage, a TDV of 2mL can represent asymmetry of 50% while the same TVD of 2mL in a boy in a Tanner 4 or 5 stage can represent less than 20% asymmetry. Therefore we believe the indication criteria of a TVD of 2mL for varicocelectomy is an ambiguous criteria, especially in earlier Tanner stages, and its removal from the guidelines seems justifiable.

As the previous paragraph again points out, it is crucial to always assess the TAI in relation to TVD and individual TV according to the scale of physical development (Tanner scale).

In this study we listed the mean TV, TVD and TAI for the entire population and according to each TSG and TSP (table 2).

When looking at mean left and right TV, we only note little difference between both TV. A statistically significant difference was calculated between both TV for TSG3 ($p < 0,001$) and TSP3 ($p = 0,004$). However, when we look at the corresponding values for TVD, we note this difference is 0,71mL (SD 1,85mL) and 0,65mL (SD 1,95mL), respectively. These values, compared to the mean TV in TSG3 and TSP3, which are >10 mL, represent only a minor difference in TVD. Therefore, one can argue whether these statistical significant results also translate to clinical relevant information.

Nevertheless, this study provides important reference values for the clinician in the assessment of the measured left, right and total TV in function of the Tanner stages and age in adolescents.

We created practical boxplots (figure 2) which show left, right and total TV (median, 25th, 75th percentiles, minimum and maximum) in function of the TSG (A-C), TSP (D-F) as well as per age group of 11 to 16 years (G-I). These boxplots can provide an important reference for the clinician in the assessment of the measured TV in adolescents. The first step is to assess

the corresponding Tanner stages for the age of the adolescent (table 1). Consequently, median, 25th, 75th percentiles, minimum and maximum can be assessed for left, right and total TV in function of Tanner stages and age to give an idea of what to expect of normal left, right and total TV (figure 2). One can estimate how the adolescent's left, right and total TV relate to the median, 25th, 75th percentiles, minimum and maximum of left, right and total testicular volume of a healthy population. Even more, the evolution of left, right and total TV in relation to the median, 25th, 75th percentiles, minimum and maximum can be followed up over time by repeating the steps, described above, in each of the following measurements.

Regarding the use of the Tanner stages, it is important to mention the onset of puberty is defined by the transition from TSG1 to TSG2, as TSG1 describes the aspect of the prepubertal male genitals. (15) It has been reported that this corresponds to a volume that exceeds 1.5mL. (1) However, our data shows that 40 (63.49%) and 41 (65.08%) of the 63 adolescents in TSG1 exceed a volume of 1.5mL for left and right TV, respectively. This is possibly explained by the fact that the formula used to calculate this 1.5mL volume is the formula of an ellipsoid ($L*B*D*0.52$). (2) It has been shown to be less accurate to the true TV compared to Lambert's formula ($L*B*D*0.71$) as used in this study. (5,6) When we convert the value of 1,5mL, calculated by the formula of an ellipsoid, to the corresponding value when calculated by the Lambert formula, this results in a volume of 2.05mL. Consequently, the transition from TSG1 to TSG2 for our data is 2.05mL.

It is important to notice all above analyses are derived from a single measurement. This means they represent TV, TVD and TAI on one single occasion in time and give no information about testicular growth. Therefore, this data presents only a picture of the situation. Testicular growth is probably more important to investigate in pubertal and adolescent boys, as they are in a very dynamic process in which their body is in full development. An idea of testicular growth can only be achieved by performing serial measurements of TV. Ideally, these serial measurements of TVs should be compared to data

of growth charts, developed by follow-up of TV in healthy patients in a prospective, longitudinal follow-up study. Although, some testicular growth charts are already available (2,33,34), these are all developed by means of a cross-sectional observational study design and therefore have limited clinical value in the follow up of TV growth. In these studies, TV was either measured by orchidometer (2,33) and/or scrotal ultrasound. (2,34) Those studies using scrotal ultrasound, calculated TV based on the formula for an ellipsoid ($L \cdot B \cdot D \cdot 0.52$). Both, measuring TV by orchidometer and measuring TV by scrotal ultrasound whereafter calculating TV based on the formula of an ellipsoid, have been shown to be less accurate to the true TV when compared to Lambert's formula ($L \cdot B \cdot D \cdot 0.71$) as used in this study.(5,6)

Ideally, in addition to growth charts, objective parameters should be made available to predict whether boys with a varicocele and asymmetry will end up having persistent or worsening asymmetry and abnormal semen parameters in the future. If reliable parameters were developed, then boys with a varicocele with such abnormal parameters could be operated upon in early Tanner stages, before abnormal semen parameters manifest, and thus avoid the need to reverse abnormal semen parameters once identified. (35) Peak retrograde flow has already shown to be a good predictor as a very strong relationship between high PRF and persistent and worsening asymmetry, and presumably increased abnormal semen parameters, has been shown by several authors. (36–38) Still, larger-scaled studies with even longer follow-up periods of TV are needed to further clarify the influence of peak retrograde flow on the growth of the left and right testis. Preferably, these data should be correlated with semen parameters.

Furthermore, it is important to perform serial measurements of TV for testicular asymmetry is often seen as a transient phenomenon in adolescents with a varicocele. Initially it was thought this catch-up growth was due to varicocele correction (22,36,38) However, the same phenomenon was seen when adolescents with a varicocele were followed-up non-surgically. (18,39,37) We also want to point out that the term “catch-up growth” might be suboptimal and

easy susceptible to misinterpretation. This term is used for a decrease in TAI, however this can be the result of a catch-up growth of the smaller testis or a decrease in growth speed of the larger testis. Yet, in varicocele patients we are likely dealing with retarded testicular growth. Not only might there be retarded growth on the left, the right testicle might also be small secondary to the left varicocele. (40,41)

The same holds true for the “testicular atrophy index”, which implies the ipsilateral testis was once bigger and subsequently shrunk. Therefore, the term “% testicular asymmetry” would perhaps be better to use as it is more direct and accurate.

When looking across the different Tanner stages in our study population, very Interestingly, we notice a pattern which resembles a “catch-up growth” of the left testis: TAI is small in the lower Tanner stages after which a peak is noticed in the third Tanner stage, thereafter testicular asymmetry decreases.

Therefore, this study suggest that catch-up growth is possibly a natural phenomenon which is also present in a healthy population, irrespective of the presence of a varicocele. It describes the course of the left testis, which is initially smaller than the right in the beginning of puberty and which differs the most from the right TV in the third Tanner stage, thereafter TVD lessens.

This is merely a hypothesis, and only growth charts, developed by performing serial measurements in a healthy population, will confirm whether this hypothesis holds any truth.

This study has some limitations.

Firstly, the presented data for TV, TVD and TAI are derived from a cross-sectional study. Therefore, this data presents only a picture of the situation. Pubertal and adolescent boys are in a very dynamic process, so determining growth development of the testes may be even more important.

Secondly, all the study participants took part in our study on a voluntary basis. This could have led to a participant bias as study participants and their parents were more likely to be

more involved with their health status. However, strict exclusion criteria were used to overcome a possible participant bias.

Thirdly, determining Tanner stages remains a subjective measurement that may lead to misclassification, even when performed by trained researchers.

Fourthly, we only included patients at the age of 11 years. However, we know that puberty can already take place at the age of 10 years. (42) This possibly explains our rather high values for TV in TSG1 and TSP1.

Fifthly, only 9 and 7 adolescents were included in TSG5 and TSP5, respectively. This is probably due to our rather low upper age inclusion limit of 16 years and a positively skewed age distribution. This low amount of study subjects makes interpretation of the data of TSG5 and TSP5 less accurate.

Sixthly, 7 different researchers performed the ultrasonographical measurements of the testicular volume. Although ultrasound is considered an operator dependent examination, we believe by using the measurements of several researchers our data reflects a realistic application of volume measurements by scrotal ultrasound in adolescents.

Seventhly, most of the study subjects (84,35%) were Caucasian which makes our findings mostly applicable to Caucasian adolescent boys. Although our analyses noted nearly no difference in mean TV between ethnicities (excluding the significant volume difference between Asian study subjects and Afro-American study subjects for left TV) several studies have shown differences in TV between ethnicities. (3,43,44)

In conclusion, this study suggests a natural difference between left and right TV in a healthy population, which peaks in the third Tanner stage after which a decrease is seen. Therefore, we believe that the use of testicular volume differential calculations, such as the TAI, should be used with great caution in the case of left-sided unilateral inguinoscrotal pathology. Serial measurements should always be performed to assess testicular growth. Furthermore, this study provides reference values for TV, TVD and TAI according to TSG and TSP.

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References

1. Wu FC, Brown DC, Butler GE, Stirling HF KC. Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. *J Clin Endocrinol Metab.* 1993;76(1)(Jan):26–31.
2. Joustra SD, Van Der Plas EM, Goede J, Oostdijk W, Delemarre-Van De Waal HA, Hack WWM, et al. New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores. *Acta Paediatr Int J Paediatr.* 2015;104(6):e271–8.
3. Takihara H, Cosentino MJ, Sakatoku J CA. Significance of testicular size measurement in andrology: II. Correlation of testicular size with testicular function. *J Urol.* 1987;137(3):416–9.
4. Goede J, Hack WWM, Sijtermans K, Van Der Voort-Doedens LM, Van Der Ploeg T, Meij-De Vries A, et al. Normative values for testicular volume measured by ultrasonography in a normal population from infancy to adolescence. *Horm Res Paediatr.* 2011;76(1):56–64.
5. Paltiel HJ, Diamond DA, Di Canzio J, Zurakowski D, Borer JG, Atala A. Testicular volume: comparison of orchidometer and US measurements in dogs. *Radiology.* 2002;222(1):114–9.
6. Sakamoto H, Saito K, Oohta M, Inoue K, Ogawa Y, Yoshida H. Testicular Volume Measurement: Comparison of Ultrasonography, Orchidometry, and Water Displacement. *Urology.* 2007;69(1):152–7.
7. Oster J. Varicocele in children and adolescents. *Scan J Urol Nephrol.* 1971;5:27–32.

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8. Akbay E, Cayan S, Doruk E, Duce MN BM. The prevalence of varicocele and varicocele-related testicular atrophy in Turkish children and adolescents. *BJU Int.* 2000;86(4):490–3.
 9. The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organization. *Fertil Steril.* 1992;57(6):1289–93.
 10. Diamond DA, Zurakowski D, Bauer SB, Borer JG, Peters CA, Cilento BG, et al. Relationship of Varicocele Grade and Testicular Hypotrophy to Semen Parameters in Adolescents. *J Urol.* 2007;178(4 SUPPLEMENT):1584–8.
 11. Kurtz MP, Zurakowski D, Rosoklija I, Bauer SB, Borer JG, Johnson KL, et al. Semen parameters in adolescents with varicocele: Association with testis volume differential and total testis volume. *J Urol.* 2015;193(5):1843–7.
 12. Keene DJB, Sajad Y, Rakoczy G, Cervellione RM. Testicular volume and semen parameters in patients aged 12 to 17 years with idiopathic varicocele. *J Pediatr Surg.* 2012;47(2):383–5.
 13. An AUA Best Practice Policy and ASRM Practice Committee Report. Report on Varicocele and Infertility. 2001.
 14. Riedmiller H, Androulakakis P, Beurton D, Kocvara R, Gerharz E. EAU guidelines on paediatric urology. Vol. 40, *European Urology.* 2001.
 15. Marshall W a., Tanner JM. Variations in the Pattern of Pubertal Changes in Boys. *Arch Dis Child.* 1970;45(239):13–23.
 16. Hsieh M-L, Huang S-T, Huang H-C, Chen Y, Hsu Y-C. The reliability of ultrasonographic measurements for testicular volume assessment: comparison of three common formulas with true testicular volume. *Asian J Androl.* 2009;11(2):261–5.
 17. Paduch DA NJ. Repair versus observation in adolescent varicocele: a prospective study. *J Urol.* 1997;158:1128–32.
 18. Kolon TF, Clement MR, Cartwright L, Bellah R, Carr MC, Canning DA, et al. Transient Asynchronous Testicular Growth in Adolescent Males With a Varicocele. *J Urol.* 2008;180(3):1111–5.
 19. Christman MS, Zderic SA, Kolon TF. Comparison of testicular volume differential calculations in adolescents with. *J Pediatr Urol.* 2014;10(2):396–8.

20. Granados A, Gebremariam A, Lee JM. and Pubertal Staging in Boys and Girls. *J Clin Res Pediatr Endocrinol*. 2015;7(3):235–7.
21. Jungwirth A, Diemer T, Dohle GR, Giwercman A, Kopa Z, Tournaye H, et al. Male Infertility. *Eur Assoc Urol*. 2013;
22. Cayan S, Akbay E, Bozlu M, Doruk E, Erdem E AD. The effect of varicocele repair on testicular volume in children and adolescents with varicocele. *J Urol*. 2002;168:731–4.
23. Cayan S, Acar D, Ulgur S AE. Adolescent varicocele repair: long-term results and comparison of surgical techniques according to optical magnification use in 100 cases at a single univeristy hospital. *J Urol*. 2005;(174):2003–6.
24. Lenzi A, gandini L, bagolan P, Nahum A DF. Sperm parameters after early left varicocele treatment. *Fertil Steril*. 1998;69:347–9.
25. Okuyama A, Nakamura M, Namiki M, Takeyama M, Utsonomiya M FH. Sugrical repair of varicocele at puberty: a preventive treatment for fertility improvement. *J Urol*. 1988;139(3):562–4.
26. Rogue M ES. A systematic review of clinical practice statements for the diagnosis and management of varicocele in children and adolescents. *Asian J Adnrol*. 2016;18:262–8.
27. Laven JS, Haans LC, Mali WP, te Velde ER, Wensing CJ EJ. Effects of varicocele treatment in adolescents: a randomized study. *Fertil Steril*. 1992;58(4):756–62.
28. Pinto KJ, Kroovand RL JJ. Varicocele related testicular atrophy and its predictive effect upon fertility. *J Urol*. 1994;152:788–90.
29. Nork JJ, Berger JH, Crain DS, Christman MS. Youth varicocele and varicocele treatment : a meta-analysis of semen outcomes. *Fertil Steril*. 102(2):381–387.
30. Bogaert G, Orye C, De Win G. Pubertal screening and treatment for varicocele do not improve chance of paternity as adult. *J Urol [Internet]*. 2013;189(6):2298–303.
31. Çayan S, Şahin S AE. Paternity Rates and Time to Conception in Adolescents with Varicocele Undergoing Microsurgical Varicocele Repair vs Observation Only: A Single Institution Experience with 408 Patients. *J Urol*. 2017;198(July):195–201.
32. Fine RG, Poppas PD. Varicocele: standard and alternative indications for repair. *Curr Opin Urol*. 2012;22(6):513–6.

33. Matsuo N, Anzo M, Sato S, Ogata T, Kamimaki T. Testicular volume in Japanese boys up to the age of 15 years. *Eur J Pediatr.* 2000;159(11):843–5.
34. Van Der Plas EM, Zijp GW, Froeling FMJA, Van Der Voort-Doedens LM, Meij-De Vries A, Goede J, et al. Long-term testicular volume after orchiopexy at diagnosis of acquired undescended testis. *J Urol.* 2013;190(1):257–62.
35. Glassberg KI. My indications for treatment of the adolescent varicocele (and why ?). 2014;3(1):402–12.
36. Kozakowski KA, Gjertson CK, Decastro GJ, Poon S, Gasalberti A, Glassberg KI. Peak Retrograde Flow: A Novel Predictor of Persistent, Progressive and New Onset Asymmetry in Adolescent Varicocele. *J Urol.* 2009;181(6):2717–23.
37. Poon SA, Gjertson CK, Mercado MA, Raimondi PM, Kozakowski KA, Glassberg KI. Testicular Asymmetry and Adolescent Varicoceles Managed Expectantly. *J Urol.* 2010;183(2):731–4.
38. Van Batavia JP, Badalato G, Fast A, Glassberg KI. Adolescent varicocele - Is the 20/38 harbinger a durable predictor of testicular asymmetry? *J Urol.* 2013;189(5):1897–901.
39. Spinelli C, Di Giacomo M, Lo Piccolo R, Martin A, Messineo A. The role of testicular volume in adolescents with varicocele: The better way and time of surgical treatment. *J Urol.* 2010;184(4 SUPPL.):1722–6.
40. Kass EJ, Stork BR SBB ya. Varicocele in adolescence induces left and right testicular volume loss. *BJU Int.* 2001;87(6):499–501.
41. Chen JJ, Ahn HJ, Junewick J, Posey ZQ, Rambhatla A, Steinhardt GF. Is the comparison of a left varicocele testis to its contralateral normal testis sufficient in determining its well-being? *Urology.* 2011;78(5):1167–72.
42. Tinggaard J, Mieritz MG, Sørensen K, Mouritsen A, Hagen CP, Aksglaede L, et al. The physiology and timing of male puberty. 2012;197–203.
43. Aribarg A, Kenkeerati W, Vorapaiboonsak V, Leepipatpaiboon S FT. Testicular volume, semen profile and serum hormone levels in fertile Thai males. *Int J Androl.* 1986;9(3):170–80.
44. Ku JH, Kim ME, Jeon YS, Lee NK PY. Factors influencing testicular volume in young men: results of a community-based survey. *BJU Int.* 2002;90(4):446–50.

Legends to illustrations

Figure 1. Schematic summary of the patient selection process.

Figure 2. Boxplot (median, 25th, 75th percentiles, minimum and maximum) of left, right and total testicular volume by Tanner stage for genital development (TSG) (A-C), pubic hair (D-F) and age (G-I).

Figure 3. Boxplot (median, 25th, 75th percentiles, minimum and maximum) of testicular atrophy index (TAI) by Tanner stage for genital development (A) and pubic hair (B).

Figure 4. On the horizontal axis, the TAI values are shown. Boys with a smaller left testis, therefore having a positive TAI, are represented in the positive part of the horizontal axis. A smaller right testis results in a negative TAI, this is represented in the negative part of the horizontal axis. The vertical axis represents the percentage of study subjects with a TAI equal to or greater than a given TAI. The cut-off value for a TAI of 20% for adolescent boys with a smaller testis is denoted as a vertical line on the right side of the figure. Similarly, a TAI of -20% is denoted as a vertical line on the left side of the figure. The data is presented for the entire population as well as for each Tanner stage for genital development (TSG1-TSG5).

For example, in the entire population, a smaller left testis with a TAI of $\geq 20\%$ is present in about 14,78% of adolescent boys.

Figure 5. cfr. Figure 4 but a distinction was made between Tanner stages for pubic hair (TSP1-TSP5) instead of Tanner stages for genital development.

	Medical check-ups at secondary schools	Tertiary hospital	Overall
N	256 (74,20%)	89 (25,80%)	345
All (n=345)			
Mean age (y)	13,22 (SD 1,81)	12,89 (SD 2,54)	13,33 (SD 1,25)
Mean length (m)	1,61 (SD 0,11)	1,60 (SD 0,13)	1,61 (SD 0,11)
Mean weight (kg)	49,17 (SD 12,13)	51,40 (SD 17,54)	49,73 (SD 13,71)
Mean BMI (kg/m ²)	18,78 (SD 3,22)	19,78 (SD 4,22)	19,02 (SD 3,52)
Ethnicity	C: 220 (63,8%), NA: 14 (4,1%), AA: 6 (1,7%), A: 9 (2,6%), O: 3 (0,9%), M: 4 (1,2%)	C: 71 (20,6%), NA: 7 (2,0%), AA: 1 (0,3%), A: 3 (0,9%), O: 3 (0,9%), M: 4 (1,2%)	C: 291 (84,3%), NA: 21 (6,1%), AA: 7 (2,0%), A: 12 (3,5%), O: 6 (1,7%), M: 8 (2,3%)
TSG1 TSP1 (n= 63 81)			
Mean age (y)	11,94 (SD 0,55) 12,00 (SD 0,62)	11,42 (SD 0,67) 11,5 (SD 0,71)	11,84 (SD 0,61) 11,85 (SD 0,68)
Mean length (m)	1,51 (SD 0,049) 1,51 (SD 0,056)	1,49 (SD 0,098) 1,50 (SD 0,095)	1,51 (SD 0,061) 1,51 (SD 0,071)
Mean weight (kg)	40,97 (SD 9,75) 40,99 (SD 9,30)	37,13 (SD 11,26) 40,06 (SD 11,28)	40,23 (SD 10,07) 40,68 (SD 9,95)
Mean BMI (kg/m ²)	17,84 (SD 3,57) 17,81 (SD 3,36)	18,22 (SD 2,17) 18,38 (SD 2,69)	17,91 (SD 3,35) 18,00 (SD 3,15)
Ethnicity	C: 47 (92,2%), NA: 3 (5,9%), M: 1 (2,0%) C: 51 (94,4%), NA: 3 (5,6%)	C: 23 (91,7%), NA: 1 (8,3%) C: 23 (85,3%), NA: 3 (11,1%), M: 1 (3,7%)	C: 58 (92,1%), NA: 4 (6,3%), M: 1 (1,6%) C: 74 (91,4%), NA: 6 (7,4%), M: 1 (1,2%)
TSG2 TSP2 (n= 129 121)			
Mean age (y)	12,45 (SD 0,77) 12,45 (SD 0,77)	11,95 (SD 1,03) 11,95 (SD 1,03)	12,30 (SD 0,89) 12,38 (SD 0,94)
Mean length (m)	1,57 (SD 0,072) 1,57 (SD 0,071)	1,55 (SD 0,086) 1,58 (SD 0,067)	1,56 (SD 0,077) 1,57 (SD 0,069)
Mean weight (kg)	44,53 (SD 8,73) 44,92 (SD 9,23)	46,48 (SD 11,51) 47,98 (SD 11,02)	45,14 (SD 9,68) 45,68 (SD 9,75)
Mean BMI (kg/m ²)	18,16 (SD 2,99) 18,23 (SD 3,05)	19,20 (SD 3,68) 19,24 (SD 3,82)	18,49 (SD 3,24) 18,48 (SD 3,27)

Ethnicity	C: 79 (88,8%), NA: 4 (4,5%), AA: 4 (4,5%), A: 1 (1,1%), O: 1 (1,1%) 1C: 80 (87,9%), NA: 3 (3,3%), AA: 4 (4,0%), A: 1 (1,1%), O: 1 (1,1%),	C: 34 (85,0%), NA: 3 (7,5%), A: 2 (5,0%), M: 1 (2,5%) C: 27 (90,0%), NA: 1 (3,3%), A: 1 (3,3%), M: 1 (3,3%)	C: 113 (87,6%), NA: 7 (5,4%), AA: 4 (3,1%), A: 3 (2,3%), O: 1 (0,8%), M: 1 (0,8%) C: 107 (88,4%), NA: 4 (3,3%), AA: 4 (3,3%), A: 2
TSG3 TSP3 (n =83 78)			
Mean age (y)	13,35 (SD 0,99) 13,38 (SD 0,96)	13,55 (SD 1,70) 13,71 (SD 1,64)	13,40 (SD 1,20) 13,44 (SD 1,11)
Mean length (m)	1,65 (SD 0,091) 1,66 (SD 0,094)	1,70 (SD 0,11) 1,74 (SD 0,10)	1,66 (SD 0,090) 1,67 (SD 0,099)
Mean weight (kg)	52,37 (SD 9,80) 52,31 (SD 9,77)	61,31 (SD 24,35) 67,83 (SD 25,87)	54,52 (SD 14,99) 55,10 (SD 15,07)
Mean BMI (kg/m ²)	19,10 (SD 2,72) 18,92 (SD 2,85)	20,82 (SD 6,10) 22,12 (SD 6,82)	19,52 (SD 3,84) 19,50 (SD 4,00)
Ethnicity	C: 53 (84,1%), NA: 3 (4,8%), A: 3 (4,8%), O: 1 (1,6%), M: 3 (4,8%) C: 54 (84,4%), NA: 4 (6,3%), A: 3 (4,7%), O: 1 (1,6%), M: 2 (3,1%)	C: 15 (75,0%), NA: 2 (10,0%), AA: 1 (5,0%) , O: 2 (10%) C: 10 (71,4%), NA: 2 (7,1%), AA: 1 (7,1%), O: 2 (14,3%)	C: 68 (81,9%), NA: 5 (6,0%), AA: 1 (1,2%), A: 3 (3,6%), O: 3 (3,6%), M: 3 (3,6%) C: 64 (82,1%), NA: 5 (6,4%), AA: 1 (1,3%), A: 3 (3,8%),
TSG4 TSP4 (n= 61 58)			
Mean age (y)	13,96 (SD 0,99) 14,14 (SD 0,81)	14,31 (SD 1,03) 14,40 (SD 1,06)	14,03 (SD 1,00) 14,21 (SD 0,88)
Mean length (m)	1,73 (SD 0,077) 1,73 (SD 0,068)	1,72 (SD 0,051) 1,72 (SD 0,049)	1,73 (SD 0,072) 1,73 (SD 0,063)
Mean weight (kg)	60,83 (SD 11,91) 62,08 (SD 11,04)	63,91 (SD 8,77) 63,63 (SD 8,46)	61,45 (SD 11,35) 62,44 (SD 10,43)
Mean BMI (kg/m ²)	20,29 (SD 3,36) 20,72 (SD 3,21)	21,60 (SD 3,17) 21,50 (SD 3,05)	20,55 (SD 3,34) 20,90 (SD 3,16)
Ethnicity	C: 38 (79,2%), NA: 4 (8,3%), AA: 1 (2,1%), A: 4 (8,3%), O: 1 (2,1%) C: 32 (76,2%), NA: 4 (9,5%), AA: 1 (2,4%), A: 4 (9,5%), O: 1 (2,4%)	C: 8 (61,5%), NA: 1 (7,7%), A: 4 (8,3%), O: 1 (2,1%) C: 9 (56,3%), NA: 2 (12,5%), A: 1 (6,3%), O: 1 (6,3%), M: 3 (18,8%)	C: 46 (75,4%), NA: 5 (8,2%), AA 1 (1,6%), A: 5 (8,2%), O: 2 (3,3%), M: 2 (3,3%) C: 41 (70,7%), NA: 5 (10,3%), AA: 1 (1,7%), A: 5 (8,6%), O:
TSG5 TSP5 (n= 9 7)			
Mean age (y)	14,20 (SD 0,45) 14,40 (SD 0,55)	15,67 (SD 0,58) 15,50 (SD 0,71)	14,75 (SD 0,89) 14,71 (SD 0,76)
Mean length (m)	1,73 (SD 0,051) 1,76 (SD 0,083)	1,83 (SD 0,49) 1,83 (SD 0,049)	1,76 (SD 0,064) 1,78 (SD 0,077)
Mean weight (kg)	60,56 (SD 8,05) 63,66 (SD 8,44)	61,33 (SD 4,84) 61,33 (SD 4,84)	60,78 (SD 6,87) 62,99 (SD 7,26)
Mean BMI (kg/m ²)	20,12 (SD 2,30) 20,51 (SD 2,03)	18,39 (SD 0,46) 18,39 (SD 0,46)	19,63 (SD 2,07) 19,90 (SD 1,96)

Ethnicity	C: 3 (60,0%), AA: 1 (20,0%), A: 1 (20,0%) C: 3 (60,0%), AA: 1 (20,0%), A: 1 (20,0%), M: 1 (20,0%)	C: 3 (75,0%), M: 1 (25,0%) C: 2 (100,0%)	C: 6 (66,7%), AA: 1 (11,1%), A: 1 (11,1%), M: 1 (11,1%) C: 5 (71,4%), AA: 1 (14,3%), A: 1 (14,3%)
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Table 1. Schematic summarization of the characteristics of our study population. A distinction is made between the study population recruited at secondary schools, the study population recruited in a tertiary hospital and the entire study population. Furthermore the data is stratified according to the Tanner scale for genital development (TSG1-TSG5) and pubic hair (TSP1-TSP5). Abbreviations for ethnicity: C: Caucasian, NA: North-African, AA: African-American, A: Asian, O: other, M: missing.

Table 2.**Testicular volume, testicular volume difference and testicular atrophy index**

TSG	TSG1	TSG2	TSG3	TSG4	TSG5	Overall
n	63	129	83	61	9	345
Mean left TV (mL)	2,17 (SD 1,21)	4,65 (SD 2,79)	10,83 (SD 3,90)	14,15 (SD 4,66)	16,19 (SD 4,16)	7,65 (SD 5,63)
Mean right TV (mL)	2,13 (SD 1,23)	4,78 (SD 2,94)	11,54 (SD 4,19)	14,74 (SD 4,59)	16,33 (SD 4,19)	7,98 (SD 5,89)
<i>P-value*</i>	<i>0,533</i>	<i>0,188</i>	<i><0,001</i>	<i>0,095</i>	<i>0,767</i>	<i><0,001</i>
Mean total TV (mL)	4,38 (SD 2,37)	9,37 (SD 5,58)	22,34 (SD 7,84)	28,89 (SD 8,89)	32,52 (SD 8,18)	15,63 (SD 11,40)
Mean TVD (mL)	-0,053 (SD 1,68)	0,12 (SD 1,18)	0,71 (SD 1,84)	0,58 (SD 2,58)	0,14 (SD 1,69)	0,32 (SD 1,64)
Mean TAI (%)	-0,56 (SD 17,89)	2,21 (SD 18,47)	5,94 (SD 14,78)	3,86 (SD 16,09)	0,81 (SD 10,10)	2,85 (SD 17,00)
TSP	TSP1	TSP2	TSP3	TSP4	TSP5	Overall
n	81	121	78	58	7	345
Mean left TV (mL)	2,54 (SD 1,50)	5,29 (SD 3,39)	10,07 (SD 4,20)	14,10 (SD 4,72)	16,15 (SD 4,02)	7,65 (SD 5,63)
Mean right TV (mL)	2,56 (SD 1,44)	5,49 (SD 3,62)	11,72 (SD 4,45)	14,67 (SD 4,58)	16,63 (SD 4,63)	7,98 (SD 5,89)

<i>P-value*</i>	<i>0,365</i>	<i>0,075</i>	<i>0,004</i>	<i>0,100</i>	<i>0,499</i>	<i><0,001</i>
Mean total TV (mL)	5,10 (SD 2,84)	10,78 (SD 6,91)	22,78 (SD 8,43)	28,77 (SD 8,93)	32,78 (SD 8,49)	15,63 (SD 11,40)
Mean TVD (mL)	0,016 (SD 0,73)	0,19 (SD 1,19)	0,65 (SD 1,95)	0,57 (SD 2,59)	0,48 (SD 1,74)	0,32 (SD 1,64)
Mean TAI (%)	0,40 (SD 18,47)	2,45 (SD 17,78)	5,33 (SD 15,10)	3,89 (SD 16,18)	2,15 (SD 10,92)	2,85 (SD 17,00)

Table 2. Mean Testicular volume (TV), testicular volume difference (TVD) and testicular atrophy index (TAI) of our study population. Data are presented of the total study population and stratified according to the Tanner scale for genital development (TSG1-TSG5) and pubic hair (TSP1-TSP5).

*P-values of related-samples Wilcoxon signed rank, comparing mean left and right TV per TSG and TSP and for the entire population (overall), are noted.

Table 3.**Smaller left testis versus smaller right testis**

	Smaller left	Smaller right
TSG1 TSP1 (n= 63 81)	30 (47,62%) 42 (51,85%)	33 (52,38%) 39 (48,15%)
TSG2 TSP2 (n= 129 121)	73 (56,59%) 68 (56,20%)	56 (43,41%) 53 (43,80%)
TSG3 TSP3 (n =83 78)	56 (67,47%) 50 (64,10%)	27 (32,53%) (35,90%)
TSG4 TSP4 (n= 61 58)	38 (62,30%) 38 (65,52%)	23 (37,70%) (34,48%)
TSG5 TSP5 (n= 9 7)	6 (66,67%) 5 (71,43%)	3 (33,33%) 2 (28,57%)
Overall (n=345)	203 (58,84%)	142 (41,16%)

Table 3. The number of participants with a smaller left testis and smaller right testis for each Tanner scale of genital development (TSG1-TSG5) and pubic hair (TSP1-TSP5) and for the total study population are shown.

Table 4.						
Number of participants exceeding cut-off values of testicular volume difference > 2mL and/or testicular atrophy index >20%						
TSG	TSG1 TSP1	TSG2 TSP2	TSG3 TSP3	TSG4 TSP4	TSG5 TSP5	Overall
<i>n</i>	63 81	129 121	83 78	61 58	9 7	345
>20%	13 (20,63%) 23 (28,4%)	42 (32,56%) 32 (26,4%)	16 (19,28%) 17 (21,8%)	12 (19,67%) 11 (19,0%)	0 (0%) 0 (0%)	83 (24,06%)
smaller left (>20%)	4 (6,35%) 11 (13,6%)	26 (20,16%) 19 (15,7%)	12 (14,46%) 13 (16,7%)	9 (14,75%) 8 (13,8%)	0 (0%) 0 (0%)	51 (14,78%)
smaller right (<-20%)	9 (14,28%) 12 (14,8%)	16 (12,40%) 13 (10,7%)	4 (4,82%) 4 (5,1%)	3 (4,92%) 3 (5,2%)	0 (0%) 0 (0%)	32 (9,28%)
>2mL	10 (15,9%) 16 (19,8%)	16 (12,40%) 26 (21,5%)	26 (31,33%) 16 (20,5%)	21 (34,43%) 9 (15,5%)	2 (22,22%) 0 (0%)	67 (19,42%)
smaller left	4 (6,3%) 8 (9,9%)	9 (6,98%) 18 (14,9%)	20 (24,09%) 12 (15,4%)	14 (22,95%) 7 (12,1%)	1 (11,11%) 0 (0%)	45 (13,04%)
smaller right	6 (9,5%) 8 (9,9%)	7 (5,43%) 8 (6,6)	6 (7,23%) 4 (5,1%)	7 (11,48%) 2 (3,4%)	1 (11,11%) 0 (0%)	22 (6,38%)
>20% and >2ml	2 (3,17%) 1 (1,2%)	13 (10,08%) 7 (5,8%)	13 (15,67%) 11 (14,1%)	12 (19,67%) 8 (13,8%)	0 (0%) 0 (0%)	40 (11,59%)
smaller left	1 (1,59%) 1 (1,2%)	7 (5,43%) 7 (5,8%)	10 (12,05%) 11 (14,1%)	9 (14,75%) 8 (13,8%)	0 (0%) 0 (0%)	27 (7,83%)
smaller right	1 (1,59%) 0 (0%)	6 (4,65%) 0 (0%)	3 (3,61%) 0 (0%)	3 (4,92%) 0 (0%)	0 (0%) 0 (0%)	13 (3,77%)
>20% or >2ml	13 (20,63%) 23 (28,4%)	45 (34,88%) 32 (26,4%)	29 (34,94%) 17 (21,8%)	21 (34,43%) 11 (19%)	2 (22,22%) 0 (0%)	110 (31,88%)
smaller left	9 (14,28%) 11 (13,6%)	28 (21,71%) 19 (15,7%)	22 (26,51%) 13 (16,7%)	4 (22,95%) 8 (13,8%)	1 (0%) 0 (0%)	69 (20,00%)
smaller right	4 (6,35%) 12 (14,8%)	17 (13,18%) 13 (10,7%)	7 (8,43%) 4 (5,1%)	7 (11,48%) 3 (5,2%)	1 (0%) 0 (0%)	41 (11,88%)

Table 4. Number and percentages of study subjects who exceed the threshold for TAI (>20%), TVD (>2mL), both (TAI>20% and TVD>2mL) or one of both (TAI>20% or TVD>2mL). Data of the entire study population and for each Tanner stage of genital development (TSG1-TSG5) and pubic hair (TSP1-TSP5) are presented (columns). Furthermore, a distinction was made between boys with a smaller left testis and boys with a smaller right testis (rows). For TAI, a smaller left testis corresponds with a positive TAI while a smaller right testis corresponds with a negative TAI.