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Clinical and Diagnostic Features of Patients With Suspected Klinefelter Syndrome

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ABSTRACT: Klinefelter syndrome, with an incidence of 1:600 male newborns, is the most frequent form of male hypogonadism. However, despite its relatively high frequency, the syndrome is often overlooked. To prevent such oversights, the clinical features should be better characterized, and simple screening tests should be used more frequently. In a cohort of 309 patients suspected of having Klinefelter syndrome, we evaluated the clinical symptoms as well as the diagnostic value of the Barr body test for screening procedures. On the basis of chromosome analysis, 85 patients (group I) were diagnosed as having Klinefelter syndrome, and 224 patients had a 46,XY karyotype (group II). Barr body analysis revealed a specificity of 95% and a sensitivity of 82% for the diagnosis of Klinefelter syndrome. General features (eg, reason for admission, age, age of the parents, body weight, and frequency of maldescended testes) were not different between the groups, except that group I had a higher proportion of patients with a lower educational background. Compared to group II, patients with Klinefelter syndrome were taller ($P < .001$); had smaller testis volumes ($P < .0001$), higher follicle-stimulating hormone (FSH) and luteinizing hormone (LH) values; and carried a tendency for less androgenic phenotype and secondary hair distribution. Testosterone, estradiol, sex hormone–binding globulin (SHBG), and prostate-specific antigen (PSA) serum levels as well as prostate volume were not significantly different between the groups. In patients who provided an ejaculate, azoospermia was found in 54% of the patients in group II and in 93% of the patients with Klinefelter syndrome. Although not exclusively characteristic for Klinefelter syndrome, the combination of low testicular volume and azoospermia, together with elevated gonadotropins, is highly indicative for a Klinefelter syndrome and should stimulate further clinical investigations. Barr body analysis provides a quick and reliable screening test, which, however, must be confirmed by karyotyping.

Key words: Male hypogonadism, testosterone substitution, 47,XXY, Barr body analysis.

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With a prevalence of 1:600 in the male population, Klinefelter syndrome, first described in 1942, is the most frequent form of male hypogonadism (Klinefelter et al, 1942). About 80% of patients with 47,XXY bear a congenital numerical chromosome aberration. The other 20% are represented either by 47,XXY/46,XY mosaics or higher-grade sex chromosomal aneuploidy or structurally abnormal X chromosomes (Nieschlag et al, 2000).

Clinically, the syndrome is characterized in adolescents and adults by the constellation of small, firm testes and symptoms of androgen deficiency. Other often-associated clinical features are azoospermia, tall stature, and bilateral painless gynecomastia. Diagnosis is confirmed by chromosome analysis performed in lymphocytes (Jacobs and Strong, 1959). In clinical routine, the occurrence of Barr bodies in a buccal smear (Moore and Barr, 1955) has been used as a rapid and simple diagnostic method in suspected Klinefelter syndrome. However, the diagnostic accuracy of screening for Barr bodies has never been evaluated systematically, and this simple test has been neglected in recent years. Since the symptoms of the Klinefelter syndrome are not exclusive and the syndrome may be overlooked during clinical diagnosis, we evaluated the sensitivity and specificity of this simple laboratory technique in the diagnostic workup of patients suspected to suffer from Klinefelter syndrome.

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Subjects and Methods

Subjects and Study Design

From October 1984 to October 1999, 10 134 new male patients (including 150 patients with Klinefelter syndrome) attended the Institute of Reproductive Medicine (IRM) of the University (Münster, Germany) mainly for infertility or hypogonadism. In 65 of the Klinefelter patients, no screening for Barr bodies was performed, as the definite diagnosis was known on the basis of karyotype analysis before initial examination in the IRM. These 65 patients were not analyzed in the present study. In addition to the 65 patients with confirmed Klinefelter syndrome for whom no Barr body analysis was performed, on the basis of clinical symptoms, general physical and genital examination, and previous external examinations in 311 patients, a Klinefelter syndrome was suspected and a Barr body screening was performed, followed by a karyotype analysis in lymphocytes. Two patients

with a 47,XYY or 45,X/46,XY karyotype were excluded from the analysis. In addition to screening for Klinefelter syndrome, a general and genital examination was performed in all patients. Body hair distribution in our institute is routinely classified as feminine, scant virile, normal virile, and strong virile. In addition, we routinely classify body composition as athletic (muscular), pyknic (obese), or leptosomal (lean). To limit interobserver variation, tick boxes in which these options are the only ones available were filled out during the clinical examination by an andrologically experienced clinician. Gynecomastia was defined as a palpable enlargement of the mammary gland in the male that is distinguishable from lipomastia (Behre et al, 2000). Further examinations (not performed in all patients) included reproductive hormone analysis, semen analysis and sonographic evaluation of the scrotal content, and transrectal ultrasonography of the prostate. Common reasons why some examinations were not performed include noncompliance of the patient (ejaculate analysis and prostate examination), previous hormone treatments shortly before examination (hormone analysis), and nonexistence of the analytical method at the time of examination (ultrasound and sex hormone–binding globulin [SHBG] analysis).

Measurements

Blood Samples and Hormone Assays—Venous blood was sampled between 0800 and 1200 hours at every visit. Blood samples for endocrine determinations were separated at 800 g and stored at −20°C until evaluation. Serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, testosterone, estradiol, SHBG, and prostate-specific antigen (PSA) were determined by highly specific routine immunoassays as published previously (Schürmeyer et al, 1984; Bals-Pratsch et al, 1986, 1988; Jockenhövel et al, 1990; Behre et al, 1992, 1994; Lemcke et al, 1996; Kamischke et al, 1998; von Eckardstein et al, 2000). The normal range in our laboratory for LH is 2 to 10 IU/L; for FSH, it is 1 to 7 U/L; for prolactin, it is less than 500 mU/L; for testosterone, it is more than 12 nmol/L; for estradiol, it is less than 250 pmol/L; for SHBG, it is 11 to 71 nmol/L; and for PSA, it is less than 4 μg/L. The hormone laboratory of the IRM participates in the national quality control program and has successfully passed all quality control exams since 1983. Since computerized quality control was introduced in 1996, mean interassay variances of all assays during this time were 5.1% for LH, 4.3% for FSH, 5.4% for prolactin, 9.8% for SHBG, 8.3% for testosterone, 6.0% for estradiol, and 8.0% for PSA.

Barr Body Analysis—After the patient rinsed his mouth with water, microscope slides were scraped along the buccal mucosa. The cells were spread on the slide and air dried at room temperature. After 10 minutes in a 1:2 ether-alcohol solution, microscope slides are left in a cresyl violet acetate solution for 15 to 20 minutes. Afterward, the slides are briefly dipped in increasing alcohol solutions (60%, 80%, and 96%). If possible, at least 200 epithelial cells at 100× magnification are microscopically analyzed for the presence of stained Barr bodies. However, often only 100 cells could be analyzed. A positive Barr body test was defined on the basis of 1 positive Barr body. The technicians performing the Barr body analysis were blinded to the clinical status of the patients, and, in general, Barr body analysis was performed long before results of karyotyping were available.

Chromosome Analysis—Definite karyotype analyses were performed after Giemsa staining on guanosine triphosphate–banded metaphase peripheral blood lymphocytes according to standard methods as reported elsewhere (Therman et al, 1980).

Semen Analysis

Semen samples were analyzed according to the current *WHO Laboratory Manual* (World Health Organization, 1999) and subjected to rigid internal (Cooper et al, 1992) and external quality control (Cooper et al, 1999). Only the first semen analysis was evaluated for this study. In cases of extremely low sperm counts or azoospermia, the ejaculates were centrifuged, and analysis was performed on the sediment. Azoospermia was defined as no sperm found after the centrifugation and analysis of the pellet. The patients were requested to abstain from sexual activity for 48 hours to 7 days before investigation.

Ultrasonography

Sonographic evaluations of scrotal content were performed with a 7.5-MHz sector scanner (Sonoline Versa Pro, Siemens, Erlangen, Germany) as published previously (Behre et al, 2000).

All measurements of prostate volume were performed by transrectal ultrasonography with a mechanical biplanar 7.5-MHz sector scanner. Prostate volume was calculated using the ellipsoid method (Behre et al, 2000).

Statistics

All variables were checked for normal distribution in the Kolmogorov-Smirnov one-sample goodness-of-fit test. Variations between study groups were evaluated by unpaired *t* tests. If data were not normally distributed, Mann-Whitney *U* tests were used instead. Proportions were analyzed using the chi-square test. Regression analysis was performed by nonlinear regression. Two-sided *P* values of .05 were considered significant. All analyses were performed using the statistical software GraphPadPrism for Windows version 2.01 (GraphPad Software Inc, San Diego, Calif). In general, results are given as mean plus or minus standard error of the mean.

Results

Chromosome and Barr Body Analysis

Chromosome analysis revealed a 46,XY karyotype in 224 patients (group II). In the remaining 85 patients (group I), 80 patients showed a 47,XXY, 3 patients showed a 47,XXY/46,XY mosaic, 1 patient showed a 48,XXXY, and 1 patient showed a 48,XXXY/47,XXY mosaic.

In 283 cases (92%), Barr body analyses were consistent with karyotype results. In 11 of 224 patients with a 46,XY karyotype, false-positive Barr bodies (mean, 2.6 false-positive Barr bodies per patient; range, 1 to 10 Barr bodies; mean, 400 cells counted) were counted, leading to a specificity of the analysis of 95%. In 15 of 85 patients

Table 1. *General features of our patients with Klinefelter syndrome*

	Klinefelter Patients			Patients With Normal Karyotype	
	Total n		*P* Value	Total n	
Age (mean ± SEM)	85	29.1 ± 1.1	.95	224	29.1 ± 0.6
Age of mother at patient's birth (mean ± SEM)	71	27.0 ± 1.0	.30	201	26.7 ± 1.1
Age of father at patient's birth (mean ± SEM)	67	31.0 ± 1.0	.22	194	28.9 ± 0.6
Married, n (%)	84	37 (44)	.379	223	123 (55)
History of maldescended testes, n (%)	84	14 (17)	.874	222	33 (15)
University graduate/student, n (%)	81	4 (4.9)	.071	218	31 (14)
Technician/master craftsman, n (%)	81	2 (2.5)	.453	218	12 (5.5)
Commercial employee, n (%)	81	6 (7.4)	.111	218	36 (16.5)
Craftsman, n (%)	81	19 (23.5)	.017	218	22 (10.1)
Industrial worker, n (%)	81	25 (30.9)	.019	218	32 (14.7)
Apprentice/schoolboy, n (%)	81	10 (12.3)	.158	218	48 (22)

with a Klinefelter karyotype, false-negative results were obtained (mean, 168 cells counted), leading to a sensitivity of the analysis of 82%. One of the false-negative patients had a 47,XXY/46,XY mosaic, while all others had a 47,XXY karyotype. In the patients with Klinefelter syndrome, 3.9 plus or minus 0.5 Barr bodies per patient were counted out of 213 plus or minus 19 buccal epithelial cells. In non-Klinefelter men, 214 plus or minus 12 cells were counted.

General Features

The majority of patients (n = 145) with a normal karyotype consulted our clinic for infertility, followed by suspected hypogonadism (n = 35), delayed puberty (n = 19), gynecomastia (n = 13), and suspected Klinefelter syndrome (n = 12). Diagnoses in this group consisted of hypogonadism of unknown origin (n = 85), present or previous maldescended testes (n = 43), varicocele (n = 38), hypogonadotropic hypogonadism (n = 25), primary nontesticular illness (n = 9), idiopathic gynecomastia (n = 7), anorchia (n = 7), constitutionally delayed puberty (n = 6), history of orchitis (n = 2), and Y chromosome microdeletion (n = 2). In the Klinefelter group, the main reasons for admission were also fertility problems (n = 37), followed by suspected Klinefelter syndrome (n = 34) or hypogonadism (n = 11), delayed puberty (n = 1), and gynecomastia (n = 2). The age of the patients and the age of the parents at the birth of the patients, as well as the frequency of maldescended testes, were not different between the groups (Table 1). Significant differences could be observed in the professions of the patients. In the patients with a normal karyotype, the proportion with an academic or higher educational background was significantly higher than in the Klinefelter group, in which more than 50% of the patients worked as craftsmen or industrial workers (Table 1).

Clinical Features

Patients with Klinefelter syndrome were significantly taller than patients with normal karyotypes (Table 2). Body weight and body mass index did not differ significantly between the groups (Table 2). In the 216 patients analyzed in group II, 37 (17%) patients had a feminine body hair distribution, 80 (37%) patients had a scant body hair distribution, 76 (35%) patients had a normal body hair distribution, and 23 (11%) patients had a strong male secondary body hair distribution. In the 83 patients with Klinefelter syndrome, a less virile male secondary body hair distribution was evident, although not significant, and 12 (15%) patients showed a feminine body hair distribution, 45 (54%) patients showed a scant body hair distribution, 18 (22%) patients showed a normal body hair distribution, and 8 (10%) patients showed a strong male body hair distribution. The body composition of patients with a normal karyotype was significantly more often judged athletic (30%) than was the body composition of patients with Klinefelter syndrome (9%). However, the proportions of patients with pyknic or leptosomal body composition did not differ significantly between both groups. No significant differences in proportions of patients with present or past gynecomastia were observed (Table 2).

Testes and Prostates

Palpated and sonographic total testicular volumes were lower ($P < .0001$) in the Klinefelter patients than in the non-Klinefelter patients (Table 2). The largest combined bitesticular volume in a Klinefelter patient was 14.5 mL. None of the Klinefelter patients had normal testicular volumes, at least on one side, while in the other group, 28 patients showed normal testicular volumes, at least unilaterally.

In the patients for whom prostate volumes were measured, no differences could be detected between the volumes of the 35 patients with Klinefelter syndrome and the volumes of the 30 patients of group II (Table 2).

Hormones, SHBG, and PSA

Previous treatments with hormones were conducted in 6 Klinefelter patients (4 injectable testosterone enanthate, 1

Table 2. *Clinical features of our patients with Klinefelter syndrome*†*

	Klinefelter Patients			Patients With Normal Karyotype	
	Total n		*P* Value	Total n	
Body height (m)	85	1.83 ± 0.01	.0004	222	1.77 ± 0.01
Body weight (kg)	84	80.8 ± 1.8	.3155	220	79.7 ± 1.2
Body mass index (kg/m2)	84	24.1 ± 0.4	.047	220	25.2 ± 0.3
Past or present gynecomastia, n (%)	84	26 (31)	.257	233	51 (22)
Sonographic bitesticular volume (mL)	79	4.7 ± 0.3	.0001	202	13.7 ± 0.7
LH (U/L)	78	27.0 ± 1.0	.0001	220	7.9 ± 0.5
FSH (U/L)	78	33.8 ± 1.7	.0001	221	20.2 ± 1.6
Prolactin (U/L)	77	180 ± 10	.1324	218	212 ± 10
Testosterone (nmol/L)	78	11.8 ± 0.8	.371	222	12.9 ± 0.6
Free testosterone (pmol/L)	44	255 ± 23	.873	115	279 ± 21
Estradiol (pmol/L)	68	79 ± 4	.889	185	80 ± 3
SHBG (nmol/L)	44	40 ± 3	.469	115	38 ± 2
Androgen sensitivity index (U × nmol/L2)	78	225 ± 18	.0001	217	101 ± 6
PSA (pg/mL)	31	0.7 ± 0.1	.5075	44	0.6 ± 0.1
Prostate volume (mL)	35	15.9 ± 0.1	.608	30	15.2 ± 1.1
Ejaculate volume (mL)	57	2.3 ± 0.2	.0005	164	3.5 ± 0.2
Patients with azoospermia, n (%)	57	53 (93)	.001	164	88 (54)

* FSH indicates follicle-stimulating hormone; LH, luteinizing hormone; PSA, prostate-specific antigen; and SHBG, sex hormone–binding globulin.
† Data are given as total n (mean ± SEM) except where noted.

human chorionic gonadotropin, and 1 unknown preparation) and in 4 patients with normal karyotypes (2 injectable testosterone enanthate, 1 oral mesterolone, and 1 tamoxifen) directly before and up to 14 days before the examination. These hormone values were excluded from the analysis. Both groups showed elevated FSH and LH values, which were higher in the Klinefelter group ($P < .001$) than in group II (Table 2). In both groups, neither FSH values (group I: $r = 0.10$, $P = .38$; group II: $r = 0.05$, $P = .42$) nor LH values (group I: $r = 0.14$, $P = .22$; group II: $r = 0.06$, $P = .38$) were significantly correlated with age. Whereas in group II FSH values were significantly correlated with total testicular volume, no significant correlation was apparent in group I (Figure 1). Highly significant differences were found in the FSH/LH ratio between the groups. None of the Klinefelter patients (except for 1 prepubertal 9-year-old boy) had an FSH/LH ratio greater than 3.5, while 48 patients in group II had a ratio exceeding 3.5 (22%). Prolactin serum levels in both groups were not significantly different (Table 2). The androgen sensitivity index (LH × testosterone; Hiort et al, 2000) was significantly higher in the Klinefelter group than in group II (Table 2). Neither testosterone nor estradiol serum levels between both groups were significantly different (Table 2). Within the groups, neither testosterone (group I: $r = 0.08$, $P = .49$; group II: $r = 0.10$, $P = .14$) nor estradiol serum levels (group I: $r = 0.01$, $P = .91$; group II: $r = 0.05$, $P = .53$) were significantly correlated to the patient's age. In both groups, testosterone values were significantly correlated with the total testicular volume (Figure 2). No significant correlations were found between testosterone and LH (group I: $r = 0.04$, $P = .72$; group II: $r = 0.03$, $P = .68$) or FSH (group I: $r = 0.08$, $P = .22$; group II: $r = 0.07$, $P = .53$) for either group, respectively. SHBG and PSA levels between the groups were not significantly different (Table 2).

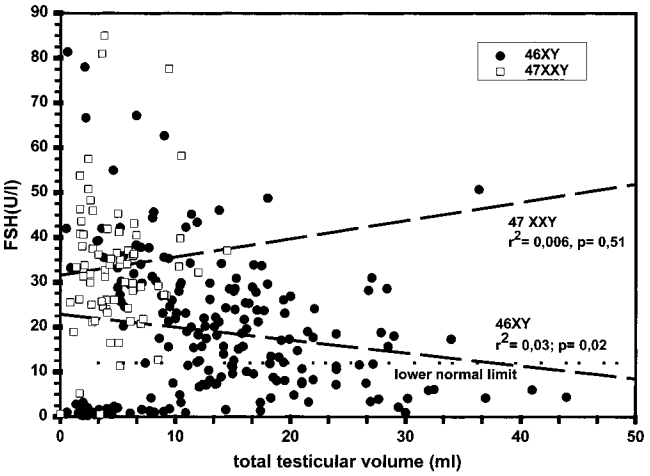

Figure 1. Total testicular volume vs follicle-stimulating hormone (FSH) in 74 patients with Klinefelter syndrome (black circles) and in 197 patients with suspected Klinefelter syndrome but normal karyotypes (white squares).

Semen Parameters

Of the 57 patients with Klinefelter syndrome who provided an ejaculate, 4 had sperm (7%). Baseline characteristics of these 4 patients are given in Table 3. Of the 164 patients in group II for whom an ejaculate analysis was performed, 88 had azoospermia, 43 had severe oligozoospermia (<1 million/mL), and only 5 were normozoospermic. For the patients in group II in whom

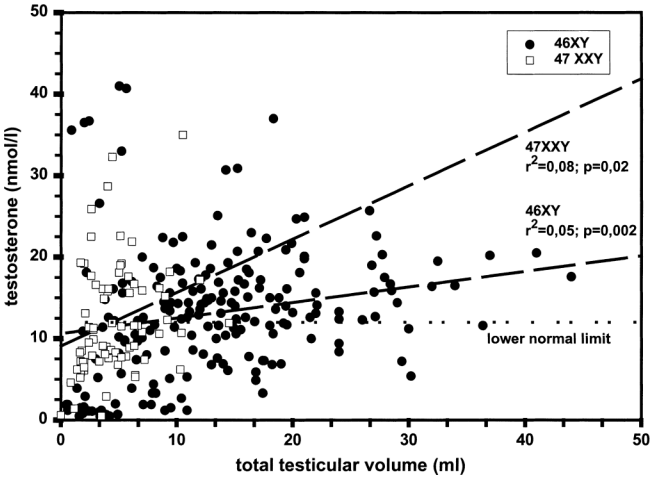

Figure 2. Total testicular volume vs testosterone in 74 patients with Klinefelter syndrome (black circles) and in 197 patients with suspected Klinefelter syndrome but normal karyotypes (white squares).

sperm could be detected (mean ± SEM), sperm concentration (6.0 ± 1.7 million/mL), sperm motility (31% ± 3% WHO grade a + b), and sperm morphology (21% ± 2%) were decreased. Ejaculate volumes (Table 2) and sperm concentrations (Table 3) were significantly lower in the Klinefelter patients.

Discussion

Klinefelter syndrome is the most common cause of gonosomal aberration in all ethnic groups, and in about 3% of our patients, a Klinefelter syndrome was suspected. A definite diagnosis of Klinefelter syndrome was established in 38% of these 309 patients on the basis of karyotype analysis in lymphocytes, which is the diagnostic gold standard (Zang, 1984). Klinefelter syndrome was diagnosed in 1.5% of our patients, which is in agreement with other infertility clinics (Chiang et al, 2000). The higher incidence of patients with Klinefelter syndrome in infertility clinics (Nielsen and Wohlert, 1991) can be explained by the severe impairment of spermatogenesis and the high incidence of signs of androgen deficiency, which accumulate in a tertiary andrology center.

We and others (Filippi, 1986) used Barr body analysis as a quick screening test, which is performed together with blood donation for karyotyping and other laboratory tests. With simple staining methods that are available in every andrology laboratory, Barr body analysis can be processed within 1 hour. In contrast, at our university, as in most other settings, karyotype analysis is performed by a genetics department. At our university, the mean time required for the results of karyotype analysis is 5 to 10 days, depending on the clinical indication. In not-urgent situations like those in this study, it took 17 days (±3 days SEM), whereas Barr body results were available within 1 hour. Barr body analysis was not only quick, but it also showed a very good specificity of 95% and a satisfying sensitivity of 82%. This diagnostic accuracy was also reported by other groups, which, however, found a higher sensitivity of Barr body analysis (97%) in a preselected collective of 64 patients with known Klinefelter syndrome (Grabski et al, 1979). However, in any case, a karyotype analysis has to be performed to obtain definite results, although karyotype analysis may also miss tissue-specific mosaic Klinefelter syndrome. In our 11 patients with false-positive results, it is notable that they also presented with a reduced mean testicular volume of 10 mL and that 7 of 10 patients also were azoospermic. In the patients with false-negative results, no obviously different characteristics compared to the patients correctly diagnosed could be observed. However, it cannot be excluded that a false-positive Barr body test may occur in a mosaic individual with a Klinefelter karyotype in buccal epithelia cells but a normal karyotype in peripheral lymphocytes, and a false-negative Barr body test may occur in a patient

Table 3. *Clinical features of 4 patients with Klinefelter syndrome and spermatozoa in the ejaculate**

Parameter	Patient 6544	Patient 7179	Patient 9847	patient 10 146
Karyotype	47, XXY	47, XXY	47, XXY	47, XXY
Age (y)	18.6	23.8	19.7	20.2
Virilization	Scant	Virile	Scant	Scant
Bitesticular volume (mL)	2.8	2.8	5.1	5.1
LH (U/L)	19.2	15.4	8.9	28.7
FSH (U/L)	37.5	21.1	16.5	41.2
Testosterone (nmol/L)	9.9	11.1	22.6	19.0
Estradiol (pmol/L)	94	88	69	34
Sexual abstinence time (d)	4	4	4	4
Ejaculate volume (mL)	1.1	1.7	2.7	3.0
Motility (WHO grade a + b)	40	0	40	0
Sperm concentration (million/mL)	0.6	<0.1	0.1	<0.1
Normal sperm morphology (%)	10	0	25	0

* FSH indicates follicle-stimulating hormone; LH, luteinizing hormone; and WHO, World Health Organization.

with mosaic Klinefelter syndrome having a normal karyotype in a buccal smear but a Klinefelter karyotype in lymphocytes.

In general clinical features, no obvious differences between the patients with confirmed Klinefelter syndrome and the group with normal karyotypes existed. In contrast to Carothers and Filippi (1988), we found no increased risk of procreating a child with Klinefelter syndrome at an advanced paternal or maternal age. This is in agreement with our finding that older men have no increase in sperm aneuploidies over younger men (Bernardini et al, 1998; Luetjens et al, 2002). The intelligence of some but not all Klinefelter patients may be slightly reduced, and deficits are observed in verbal and cognitive abilities (Rovet et al, 1995; Nieschlag et al, 2000). Moreover, in our population, the proportion of men with professions involving higher educational levels was significantly reduced in the Klinefelter patients compared to group II (Table 1).

Characteristic clinical features of adults with Klinefelter syndrome are small, firm testes, hypergonadotropic azoospermia, gynecomastia, and tall eunuchoid stature (Klinefelter et al, 1942; Nieschlag et al, 2000). However, diagnosis is often delayed because of the substantial variation in clinical presentation in adults (Smyth and Bremner, 1998; Amory et al, 2000; Wilkes, 2000) and the relatively discrete symptoms, especially prior to midpuberty (Salbenblatt et al, 1985). Moreover, according to our study, 60% of the patients with Klinefelter syndrome were not suspected of having Klinefelter syndrome in the referring secondary or primary center, despite previous external clinical investigations. In addition, 5% of the patients with a normal karyotype were referred for suspected Klinefelter syndrome, although the initial diagnosis could not be confirmed on the basis of karyotype analysis.

Compared to our group with normal karyotypes, our patients with Klinefelter syndrome were significantly taller, had azoospermia more often, and had higher gonadotropin concentrations. However, body height, presence of azoospermia, gonadotropin levels, and secondary body hair distribution, as well as the comparable incidence of gynecomastia, showed a surprising overlap between both groups in whom Klinefelter syndrome was suspected and were of low specificity. However, the group with normal karyotypes represents a heterogeneous group with various diagnoses, which might explain this missing difference. They were considered eligible for the Barr body test and karyotype analysis if they showed at least 1 clinical feature (eg, decreased testicular volume, gynecomastia, or azoospermia) of Klinefelter syndrome, while in most of these patients, hormone values were not known at the time of the Barr body test and karyotype analysis. On the other hand, not all patients with Klinefelter syndrome show all or at least some of the classical signs of Klinefelter syndrome, and the quick, cheap, and simple Barr body test in this situation can increase the diagnostic accuracy. It can be used for screening a larger proportion of men at the first visit, of whom those with a positive Barr body test and those with characteristic hormone profiles and clinical features (regardless of the result of the Barr body test) will be karyotyped at the second visit in the clinic. In the meantime, results of the Barr body test, together with hormone and ejaculate values and the results of the physical examination, would allow more precise handling of the patient.

Of the general and clinical features, bitesticular volume appears to be the most sensitive parameter (Table 2), showing the smallest overlap between the groups, and all Klinefelter patients had subnormal sonographic bitesticular volumes, with a mean of 4.7 mL. Among the patients with suspected Klinefelter syndrome but normal karyotypes, many also had reduced testicular volumes, despite a mean sonographic testicular volume (13.7 mL) in the normal range. However, the magnitude of the reduction in testicular volume was mostly not comparable to the patients with Klinefelter syndrome. Testosterone levels between the groups were not significantly different (Table 2). However, the proportion of adult men with hypogonadal testosterone values ($<$12 nmol/L) was nearly double (61%) in the Klinefelter patients compared to group II (36%). The androgen sensitivity index (Table 2) was significantly increased in the patients with Klinefelter syndrome, which was due to the markedly increased LH serum levels. However, in contrast to patients with androgen receptor mutations and in view of the similar testosterone serum levels, this probably does not reflect different androgen sensitivities compared to patients with a normal karyotype.

FSH is generally inversely correlated with total testicular volume and Sertoli cell–only tubuli (Pierik et al, 1998; von Eckardstein et al, 1999). Whereas in group II FSH was weakly correlated with total testicular volume, this correlation was absent in the patients with Klinefelter syndrome (Figure 1), reflecting the severe exocrine testicular failure. Interestingly, in both groups, a weak correlation between testosterone and total testicular volume was evident (Figure 2). Such a correlation cannot be found in normal men. In the patients with Klinefelter syndrome, this correlation might be explained by the low testicular volume, which, to a large extent, is occupied by the hyperplastic Leydig cells.

In 4 (ie, 7%) of the investigated Klinefelter patients, spermatozoa were found in the ejaculate (Table 3) that could be used for intracytoplasmic sperm injection. However, it may not be excluded that, in addition, individual Klinefelter patients with sperm could have been identified if repeated semen analysis had been performed in all patients. In all patients with repeated semen samples, the

diagnosis of azoospermia was subsequently confirmed. Testicular sperm retrieved from patients with Klinefelter syndrome demonstrate a normal pattern of sex chromosome segregation comparable with the rates found in other severe male factor infertility patients (Levron et al, 2000), and successful pregnancies from patients with Klinefelter syndrome have been reported (Palermo et al, 1998). Westlander et al (2001) have found no exclusive clinical features of patients with Klinefelter syndrome, azoospermia, and sperm in the testis. Nor was any clinical feature predictive for sperm in the ejaculate in our Klinefelter patients. However, they were younger compared to the cohort's mean age. Therefore, it might well be that, in some patients with Klinefelter syndrome, the depletion of germ cells appears later and that therefore the chance of finding sperm in younger Klinefelter patients is greater than in older ones. Early diagnosis would offer these patients the possibility of cryopreserving their spermatozoa for later use in assisted reproduction. However, to confirm this hypothesis, longitudinal examinations including semen analysis in pubertal boys with Klinefelter syndrome would be required.

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