REVIEW ARTICLE

Consensus statement on diagnosis and clinical management of Klinefelter syndrome

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ABSTRACT. Nearly 70 years after its description, Klinefelter syndrome (KS) remains a largely undiagnosed condition. As its clinical presentation may be subtle, many of those affected may be unaware or diagnosed only during evaluation for hypogonadism and/or infertility. In February 2010 an interdisciplinary panel of specialists met in Abano Terme (Padua, Italy) in a workshop on "Klinefelter Syndrome: diagnosis and clinical management". The main aim of this meeting was to discuss several aspects related to the epidemiology, pathogenesis, and evaluation of KS and to develop a consensus defining its early diagnosis and treatment. In the present consensus we have highlighted the features that may prompt the physicians to look after patients with KS both for the syndrome and correlated diseases. We have provided evidences that, during the different phases of life, there might be some advantages in establishing the diagnosis and starting proper follow-up and treatment. The workshop was carried out under the auspices of the Italian Society of andrology and Sexual Medicine (SIAMS).

(J. Endocrinol. Invest. 33: 839-850, 2010)

BACKGROUND

Although first described about 70 years ago, Klinefelter Syndrome (KS) has remained a largely undiagnosed condition. Its clinical presentation may be subtle, meaning that affected subjects often never consult any doctor, nor are physicians prompted to screen the population adequately for the syndrome. In consequence, many individuals carrying an extra X in the karyotype remain unaware throughout their life or are diagnosed only during evaluation for hypogonadism and/or infertility in adulthood.

In February 2010 an interdisciplinary panel of specialists met in Abano Terme (Padua, Italy) in a workshop on "Klinefelter Syndrome: diagnosis and clinical management". The main aim of this meeting was to discuss several aspects related to the pathogenesis and evaluation of KS and to develop a consensus defining its early diagnosis and treatment.

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This review paper summarises the main points of the consensus statement.

EPIDEMIOLOGY AND PATHOGENESIS

KS was first described in 1942 (1) as a "not infrequent" syndrome, but the actual prevalence remained unknown until recently, when karyotype analysis was confirmed as the best method for its diagnosis. Its cause was unknown until 1959, when Jacobs and Strong demonstrated the presence of an extra X chromosome in the karyotype of patients with KS (2). The term KS describes a group of chromosomal disorders defined by the presence of at least one supernumerary X chromosome added to a normal 46,XY male karyotype. Epidemiological studies in different countries and populations have estimated its prevalence as 152 cases per 100,000 males, corresponding to 1 in 660 live births (3-6). KS is thus the most common numerical chromosomal aberration among infertile males, affecting 10-15% of azoospermic men and about 5% of severely oligozoospermic men (7), and is the most common form of congenital male hypogonadism.

There is a discrepancy between the pre- and post-natal prevalence of KS, probably due to under-diagnosis. In fact, only 25% of estimated cases are diagnosed postnatally and <10% are detected at or before birth (4).

The most frequent karyotype is 47,XXY (80-90% of cases), with other cases presenting supernumerary X chromosomes (48,XXXY, 49,XXXXY) or mosaicism with a mixture of normal and 47,XXY cells (or mixtures of 47,XXY and other karyotypes, such as 47,XXY/48,XXXY). Recent studies have reported cytogenetic variants with structural anomalies rather than aneuploidies (8).

The severity of the phenotype in KS is directly correlated with the number of extra X chromosomes, suggesting an effect related to the gene dosage. It has been estimated that each extra X is associated with a decrease in intelligence quotient (IQ) of approximately 15-16 points, with language, and particularly expressive skills, being most affected (9). Mosaicism generally results in a milder phenotype. The true prevalence of mosaic forms might be

Key-words: Aneuploidy, epidemiology, hypogonadism, rare diseases, testosterone.

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Accepted September 1, 2010.

underestimated, as the phenotype might not be severe enough to induce diagnosis and chromosomal mosaicism could be present in the testes alone, with the karyotype of peripheral leucocytes being normal.
The genetic cause of 47,XXY KS is meiotic non-disjunction during the first or second meiosis, either during oogenesis or spermatogenesis (abnormal partitioning of chromosomes or chromatids during paternal or maternal meiosis, respectively, leading to gametes with an extra X chromosome) or, less often (about 3%), during early divisions of the fertilised egg. Maternal and paternal meiotic non-disjunction each account for approximately 50% of cases of KS (10-13). Advanced maternal – and possibly paternal – age has been linked to an increased risk of KS. Post-fertilisation non-disjunction is responsible for mosaicism, which is seen in approximately 10% of KS patients.
In KS, apart from the aneuploidy *per se*, the dosage effect of X chromosomal genes, the presence of mosaicism and the number of supernumerary X chromosome/s, other possible modulators of clinical manifestations are the paternal or maternal derivation of the extra X chromosome/s, the timing of androgen deficiency, hypothalamic-pituitary or androgen receptor (AR) activity, the expression and inactivation status of X-chromosome genes and the activity of the genes located on the pseudo-autosomal regions of the sex chromosomes.

CLINICAL AND HORMONAL MANAGEMENT

Traditionally, men with KS have been described as tall, with small testes, gynecomastia, broad hips, sparse body hair, and androgen deficiency. However, an alternative phenotype has also been recognised, in which patients present with fewer clinical features. Early detection of the syndrome is important because it allows identification of speech problems and scholastic difficulties requiring speech therapy and educational support. It also facilitates prevention or recovery of the long-term consequences of gonadal insufficiency.
Pre-natal diagnosis by routine amniocentesis is quite rare, as the association with advanced maternal age is weak (14-17). During fetal life there are no specific features to distinguish KS from XY fetuses and the hormonal pattern is the same (18). At birth, most 47,XXY babies appear normal. Genital abnormalities such as small penis, scrotum bifidus, cryptorchidism, hypospadias, and inguinal hernia and variable degree of hypotonia are only occasionally described (19, 20).
Hormonal evaluations have produced conflicting data: serum T was lower in 3-months-old KS infants than in controls (21), but, more recently, in the same period of life Aksglaede et al. (22) found normal-high T and elevated levels of FSH and LH in comparison with healthy controls.

Childhood and adolescence

Patients with KS have an increased incidence of cryptorchidism over the normal population (23). The frequency of genetic alterations in boys with persistent cryptorchidism in the general population is 5.3%, and KS is the most common genetic finding (24); for this reason, careful evaluation of the presence of bilateral cryptorchidism is recommended for the correct diagnosis and management of KS (Table 1).
It is estimated that only 10% of adolescents with KS are diagnosed before puberty due to presentation of specific features such as severe dyslexia, speech problems, and scholastic difficulties (25). A smaller penile length, lower testicular volume, taller stature, and reduced upper to lower segment ratio might also be observed during childhood. The main studies in pre-pubertal KS boys found normal T, FSH, LH, and Inhibin B (InhB) levels (26-30), with a normal androgenic response to hCG stimulation (27).
Testicular biopsies of pre-pubertal KS boys have shown preservation of seminiferous tubules with reduced numbers of germ cells, but Sertoli and Leydig cells appeared normal (31).
Boys with KS usually start pubertal development at the same age as normal population (32, 33). Low testicular volume and a firm consistency are often specific signs of KS. Testicular size can be assessed by palpation and comparison with testis-shaped models of defined sizes (Prader orchidometer) or, more precisely, by ultrasonography (34).
Another classic feature of KS is gynecomastia. Although this is a common problem in puberty, occurring in up to 65% of adolescent boys (35), its prevalence in KS boys is higher, with reported incidences from 50 to 88% (25, 36, 37). Gynecomastia is thought to be a sex steroid imbalance, with a reduced testosterone-to-estradiol ratio (T/E2) that might directly stimulate breast growth. It is normally considered a benign condition which resolves spontaneously in about 6 months. Sympathetic reassurance and watchful waiting are thus the mainstays of treatment when the T level is in the normal range. Diet modification, weight loss, and increased physical activity are indicated when weight gain or obesity is associated with gynecomastia. If the problem persists, systematic treatment with anti-estrogens and local treatment with a dihydrotestosterone (DHT) gel could be useful.
During puberty, after an initial physiological rise T concentrations tend to fall to the mid-low range and stay there throughout the maturation process (26-30, 36, 37). The hormone pattern is clearly confirmed by clinical evidence: in contrast with other forms of hypogonadism, in most KS patients androgen concentrations seem to be sufficient to allow the regular onset and progression of puberty until completion of epiphyseal closure, with satisfactory, though variable, development of secondary sexual characteristics, such as penile size, scrotum morphology, and pubic hair distribution, even though the testes fail to grow (25-27, 29, 38, 39) (Table 1).
Dramatic changes take place in the tubular compartment of the testis during mid-puberty (G3). These include stem cell apoptosis and fibro-hyalinosis of the tubular wall, frequently leading to infertility and becoming clinically evident with gonadal hypotrophy (normal subjects show a mean testicular volume in G3 of approximately 8-10 ml). In the euploid male, InhB levels are correlated with gonadal tubular compartment maturation and function. In late pre-puberty and early puberty (G2) there is a rise in InhB and FSH. From the intermediate stage on (G3/G4), InhB produces a negative feedback on FSH synthesis and

secretion, causing an inverse relationship between these two hormones and a direct correlation between InhB and increasing testicular volume (30, 40). Longitudinal studies of InhB and FSH levels in KS boys revealed that during childhood and pre-puberty, gonadal hormone concentration was comparable to the control group, suggesting regular Sertoli cell activity. Later, 6-12 months after the onset of puberty, InhB begins to decrease and there is a parallel rise in FSH, with striking differences in comparison with the control group (19, 28). During G2/G3 InhB falls rapidly, reflecting the progressive damage to the gonadal tubular compartment, consisting in disruption of germ cells and of the majority of Sertoli cells (41). In these patients, E2 levels are generally high from the very beginning of puberty, irrespective of the presence or absence of gynecomastia (27, 29). Throughout the pubertal phase, the E2/T ratio tends to remain high, notwithstanding the physiological decrease in SHBG levels (29).
From mid-puberty onwards, at about 13 yr of age, KS patients show a progressive rise in both FSH and LH (27, 29, 42), which is usually more pronounced for FSH. The hormonal panel is also characterised by a normal decrease in AMH values, low or very low T concentration and low or absent insulin-like factor 3 (INSL3) (30). INSL3 is produced by the Leydig cells during fetal and adult life and is considered a sensitive marker of interstitial cell function and differentiation (43, 44). Like T, INSL3 increases during puberty in normal subjects (45).
A large number of germ cells are lost during puberty in KS. Semen analysis should thus be performed at this stage, and any sperm present should be cryopreserved. Moreover, the dramatic acceleration of germ cell degeneration at the onset of puberty makes retrieval of germ cells at an early age for banking and future use [intracytoplasmic sperm injection (ICSI)] an attractive option (46, 47).

Adulthood

Most patients who are diagnosed with KS in adulthood show infertility and/or hypogonadism. Signs of hormonal testicular failure, such as sexual dysfunction, and the presence of co-morbidities such as diabetes, metabolic syndrome (MetS), osteoporosis, and cardiovascular diseases may also lead to diagnosis (48).
The hypergonadotropic hypogonadism phenotype in KS adults is often characterised by tall stature, narrow shoulders, broad hips, sparse body hair, gynecomastia, and small, firm testes. However, its high variability and the fact that symptoms rarely present simultaneously can make diagnosis more difficult. Many cases remain undiagnosed and only 26% of the estimated number of KS adults are detected late in adult life, leading to severe complications and a more difficult clinical management (48).
According to the scientific literature, between 65% and 85% of adults with KS showed lower than normal serum T concentrations, although they can sometimes be in the normal range (14). However, gonadotropin values are always high (36, 49). In 2004, Nieschlag's group in Germany presented the results of the largest study (in terms of sample size) carried out to date: 63% of patients (118 of the 186 analysed) showed hypogonadism, with T<12 nmol/l.
E2 and SHBG concentrations are usually higher than normal (14) and serum InhB is virtually absent, demonstrating tubular damage (28, 50, 51). More recent studies have provided evidence of lower INSL3 levels in comparison with normal subjects (43, 44).
Controlled studies showing a different age-related hypogonadism in patients with KS are currently lacking, so the use of inter-society guideline criteria for late-onset hypogonadism seems appropriate (52). In any case, on the basis of the latest testicular physiopathology data, our own clinical experience and recent findings from molecular biology studies (especially on AR polymorphism) for subjects with normal T levels we recommend the monitoring of both clinical signs and symptoms and serum T levels, including calculated free T and SHBG levels. Additional instrumental and laboratory markers [such as bone densitometry, insulin-resistance (IR) index, and potential ejaculate volume reduction] could also be considered for a proper assessment of androgenisation.

METABOLIC SYNDROME

Epidemiological studies on both morbidity and mortality in patients with KS have reported a significant increased risk of diabetes and cardiovascular diseases (7, 48). KS is frequently associated with IR and MetS, which are themselves linked to abdominal adiposity (48). The prevalence of MetS in subjects with KS is 33-46% (48, 53) compared to about 20% in the general population (54). Patients with KS have increased amounts of body fat and especially of truncal fat, with a reduction in lean mass and increased levels of fasting glucose, insulin, total cholesterol, LDL cholesterol, and triglycerides, and reduced levels of HDL cholesterol and insulin sensitivity (48). The pathogenesis of this association is not completely clear although there is increasing evidence that low T affects body composition, MetS and IR (55). In fact, low T induces a reduction in muscle mass and an increase in visceral fat (56, 57), causing an increase in free fatty acid production – the central pathogenetic mechanism for IR and thus MetS (58). In addition, the adipose tissue produces adipokines that are implicated in the pathogenesis of dyslipidemia, IR, and MetS (58).
Data on the prevalence of MetS in subjects with KS usually refer to young adults (mean age 35 yr) who are diagnosed after consultation for infertility or sexual dysfunction associated with hypogonadism. In these subjects the onset of MetS, with the vicious circle of central obesity-hypogonadism, seems to be more frequent and more precocious than in the general population. Even for a similar body mass index (BMI), infants and adolescents (4-18 yr) with KS have a higher level of body fat, and especially of truncal fat, than the general population (59). These early body modifications are associated with metabolic alterations, as demonstrated in a study of 13 Klinefelter subjects (mean age 22 yr) who underwent euglycemic hyperinsulinemic clamp, showing higher levels of fasting insulinemia and lower peripheral glucose uptake than age- and BMI-matched non-KS subjects (60). These data suggest that reduced insulin sensitivity, already present in pre-puberty, could be an initial sign of subsequent MetS as a consequence of the altered muscle/fat ratio.

Table 1 - *Management and follow-up of Klinefelter syndrome at different ages.*

Infancy and childhood	Physical examination, including assessment of height, weight, and testes Language and scholastic difficulties: educational support, logopedic assessments and therapeutic support Provision of information to the family
Adolescence and adulthood	Physical examination, including assessment of blood pressure, height, weight, waist size, testes size, gynecomastia and varicose veins Measurement of sex hormone levels: testosterone, estradiol, SHBG, FSH, LH Measurement of fasting glucose and lipids Thyroid function, CBC, and serum PSA Measurement of serum βhCG Chest X-ray Semen analysis Bone densitometry (dual-energy X-ray absorbtiometry), vitamin D status, and bone metabolism parameters Testes and breast ultrasonography Echocardiography Psychoneurological evaluation Provision of information to the patient (and family) Initiation of testosterone replacement therapy (injected, transdermal or oral administration) Answering patient's questions about wellbeing, physical activity, energy, sexual activity, libido Diet and lifestyle modification, if necessary Osteoporosis treatment, if necessary
Follow-up assessment (initially every 3 months, then annually)	Physical examination, including assessment of blood pressure, height, weight, waist size, testes size, gynecomastia and varicose veins Measurement of sex hormone levels: testosterone, oestrogen, SHBG, FSH, LH Measurement of fasting glucose and lipids Hemoglobin, hematocrit, and serum PSA Answe ring patient's questions about wellbeing, physical activity, energy, sexual activity, libido
Every 2 years	Bone densitometry (dual-energy X-ray absorbtiometry), vitamin D status and serum calcium phosphate levels Chest X-ray Mammary gland ultrasonography

CBC: complete blood count; PSA: prostate specific antigen.

According to the NCEP-APTII criteria for MetS, Klinefelter subjects have a high prevalence of central obesity (waist ≥102 cm) and alterations in glucose and lipid metabolism, but blood pressure is rarely affected (48, 61). MetS is usually associated with increased levels of leptin (62) and C-reactive protein (CRP) (63) and reduced levels of adiponectin (64); Klinefelter subjects with MetS have increased levels of leptin and CRP, but normal levels of adiponectin (48), probably because the low T level results in its increased production regardless of the BMI (14). In KS patients, adiponectin should thus counteract the negative effects of the metabolic and glycolipid changes seen in ischemic heart diseases.

Although it is well known that T replacement therapy (TRT) in hypogonadal men improves insulin sensitivity and lipid profile and reduces fat mass (65-67), little data are available on the long-term effect of T replacement on the MetS of Klinefelter subjects (48). This study showed that TRT reduces adiponectin and LDL levels as well as total fat, truncal fat and glucose, cholesterol, leptin, and CRP levels.

OSTEOPOROSIS

Osteoporosis is a systemic disorder characterised by reduced bone mass and micro-architectural deterioration of bone tissue, leading to bone fragility and increased susceptibility to fractures of the hip, spine, and wrist. Until a few years ago it was commonly considered a female disorder. According to the World Health Organization (WHO), osteoporosis is 4 times more common in women than men (68), for various physiological and pathophysiological reasons: peak bone mass tends to be higher in men (8-10%) than in women (69); T helps to limit osteoclast activity and to promote bone growth, by increasing periosteal bone apposition (70); reduced bone formation is the predominant mechanism of age-related bone loss in men.

It was recently demonstrated that male osteoporosis is responsible for a significant rise in morbidity, mortality, and health and social service expenditure (66, 71).

The International Society for Clinical Densitometry recommends the evaluation of absolute bone mineral density (BMD) and defines osteoporosis as the condition characterised by a T-score of –2.5 SD or lower, using the male reference database. As with women, the fracture risk also increases with age in men, but the incidence peaks about 10 yr later. Excessive alcohol consumption, glucocorticoid excess, and hypogonadism are the most commonly identified causes of osteoporosis (68). Since the first articles published on KS, several studies have demonstrated that hypogonadism is frequently associated with accelerated bone loss and osteoporosis (73, 74). It is now known that while young KS subjects have normal bone density until pubertal development (59), BMD is reduced in 25-48% of adult patients (75) and 6-15% suffer from osteoporosis (76). Reduced T production during puberty is the most important risk factor for bone loss (77).

There is a direct correlation between BMD and T in patients with non-mosaic KS, suggesting that low T levels cause inadequate bone development and low BMD in these patients. However, reduced bone mass does not seem to be necessarily related to low T blood levels, as it can also be found in KS patients with normal T levels, and androgen replacement therapy does not always restore bone density in KS patients with low T.
Published data suggest that patients who do not achieve normal bone density during puberty are not able to achieve it later through TRT. T replacement should therefore be started as early as possible in order to prevent bone mineral deficiency and future risk of fractures in KS men with a low serum T level (78).
The demonstrated correlation between INSL3 and bone mass, confirmed by the fact that human osteoblasts express the INSL3 receptor and young adults with *RXFP2* gene mutations frequently show low bone mass or osteoporosis, even when T levels are normal (25), could partly explain why adult KS patients with very low INSL3 concentrations suffer osteoporosis (43).
A relationship between T and the vitamin D pathway has also been demonstrated, as T seems to play a significant role in the renal synthesis of 1,25-hydroxy vitamin D. It has been observed that in KS subjects 25-hydroxy and 1,25-hydroxy vitamin D are in the low-normal range and there is evidence that the rate of bone mass gain after treatment is inversely correlated with blood vitamin D concentration (79).
In children and adolescents with KS the evaluation of body composition and bone mineral content (BMC) showed an increase in body fat mass and body fat percentage despite normal lumbar BMD, suggesting an unfavorable muscle/fat ratio. Lumbar BMD and whole body BMC were normal. These findings suggest that the unfavorable metabolic profile seen in adult KS may already be present in childhood, as evidenced by the increased fat mass, whereas the reported low BMD seems to develop after puberty (59).
Even though low T concentration seems to be the most important determinant leading to a pathological decrease in BMD, other factors such as non-random X chromosome inactivation, CAG length polymorphism of the AR gene and low INSL3 levels may also have an effect on bone structure.
Apart from androgen replacement therapy, anti-resorption drugs such as amino-bisphosphonate with proven efficacy in increasing BMD in men with osteoporosis may be necessary (76, 79-83). Selected cases of severe bisphosphonate-resistant osteoporosis may be treated with teriparatide (1-34 recombinant human PTH), the first anabolic treatment registered by the US Food and Drug Administration for use in osteoporosis (84).

COGNITIVE PROFILE

The cognitive profile of classic KS is characterised by some specific features common to most subjects that have been described over the past 20 years from a developmental perspective (85). The typical difficulties of the KS cognitive profile are increasingly serious and striking in clinical and cytogenetic variations of the syndrome, the most common of which are 48,XXXY, 48,XXYY, and 49,XXXXY.
Although KS subjects have a general cognitive level around the normal range, it is about 10 points lower than their siblings or peers (86). The typical cognitive profile is characterised mainly by a reduced score in speech-related tests in comparison with performance tasks.
The literature is unanimous in describing language difficulties as one of the more distinctive traits in the cognitive function of people with KS. It has also been suggested that these communication limitations strongly affect social adaptation and behavioural aspects, as well as the development of personality (87). Language difficulties include a delay in production of the first words and in the acquisition of the main stages of language development, but also problems in some more specific aspects. Individuals with KS require more time to process information and have a limited ability to memorise auditory verbal material, due to their difficulties in decoding words.
Some authors have documented an executive impairment in inhibitory functions in subjects affected by KS, although other executive functions addressing planning, concept formation, problem solving, task switching and speeded responding are intact (even though in speeded responding, the pattern of responses also indicates inhibitory difficulties) (88). The specificity of this impairment has been questioned more recently and the issue remains unresolved (89).
Few studies have been conducted to date on motor skills in KS, but the little literature data available have revealed their generally low level.
The developmental characteristics of the KS child strongly resemble the profile of older children with developmental dyspraxia (90). The Klinefelter phenotype is thought to be due to X chromosomal genes that escape inactivation and thus are expressed in excess (91).
The X chromosome has accumulated a disproportionate number of genes linked to mental functions and is thought to play a crucial general role in intelligence. X-linked genes are supposedly involved in social-cognition and emotional regulation. Most X-linked genes are inactivated on one X chromosome in the presence of an additional X chromosome. Cognitive deficits have been confirmed in male mice carrying a supernumerary X-chromosome (92, 93).
Difficulties in learning language also appear to be related to changes in manual dominance and functional lateralisation, making KS subjects a suitable model for studying genetic lateralisation abnormalities. A psychopathological risk is associated with this abnormality.
Epidemiological studies have reported a higher incidence of psychiatric disorders (anxiety, depression, behavioral disorders, and schizophrenia) in people with 47,XXY karyotype compared with the general population (94, 95), although studies have mainly been conducted on limited samples (91).
Behavioral problems such as closure and anxiety arise in childhood. School-age children and adolescents with XXY often show low self-esteem, anxiety and mood disorders, and socialisation problems (94). The risk of hospitalisation for psychosis in adults with KS is significantly greater than in control subjects (3, 95).

An early diagnosis is useful in order to plan different types of rehabilitation, where the need is documented. KS subjects diagnosed prenatally develop a lower proportion of learning and language disabilities than patients diagnosed by chance (23). The potential benefits of T therapy on cognition should be evaluated prospectively.

ONCOLOGICAL ASPECTS

There is little information about the long-term risk of cancer in KS men (96-98), reflecting the lack of studies in large cohorts. In the only very large cohort study, of 4806 cases of KS followed up for up to 40 yr, a significantly higher mortality rate was found for several causes, including cancer (6).

Breast cancer

The evidence that KS patients have an elevated risk of breast cancer comes largely from studies that karyotyped men with breast cancer (99-101). Male breast cancer is rare (0.6% of all breast carcinomas and <1% of all malignancies in men). A meta-analysis of published case-control studies reported that the prevalence of breast cancer in KS was about 3% (102), placing KS subjects at a 50-times higher risk than the general male population (6, 103). A possible explanation for this could be an E2-to-T ratio several times higher than that of karyotypically normal men (104). Another possibility is that the presence of 2 X chromosomes *per se* might increase the genetic risk of breast cancer (103, 105). Thus, evaluation of patients' reported symptoms and clinical breast and axilla examinations are recommended to detect any suspicious breast (painless subareolar lump, nipple retraction, and bleeding from the nipple) or lymph node abnormalities. In the presence of any suspicious clinical signs, a mammography and/or breast/axilla ultrasound examination is recommended. In addition, any family history of breast cancer or personal history of alcohol dependence, liver disease, obesity, and exposure to chest wall radiation should be carefully evaluated.

Germ cell tumors

Recent data reported that young KS men have a 65-times higher risk of developing extragonadal germ cell tumors (GCT) (106, 107). KS-associated GTC are mainly in the mediastinum (108, 109) but have also been reported in the retroperitonenum, abdominal cavity, and pineal gland (110, 111). Hence, the presence of mediastinal GCT (M-GCT) in young men should raise the suspicion of KS. The majority (75%) are mature teratomas (109). A small subgroup of KS children could present with precocious puberty due to hCG-producing M-GCT. These might originate from misplaced primordial germ cells, becoming malignant due to still-unknown genetic factors on the X chromosome. Interestingly, cohort studies have failed to demonstrate any association between KS and the occurrence of testicular cancer (100, 112). Similarly, analysis of testicular biopsy samples from KS found no cases of in situ testicular carcinoma (113).

Other malignancies

A recent large cohort study reported a statistically significant increased incidence of and mortality from lung cancer and a higher mortality from non-Hodgkin lymphoma in KS men when compared with the general population. A statistically significant reduced incidence of prostate cancer was also observed (103). However, in light of the general under-diagnosis of KS, the current epidemiological studies should be interpreted with caution.

KS has been associated with a statistically significant increased incidence of and mortality from certain malignancies. Patients affected by KS should be carefully evaluated and informed about these risks, which however remain limited on the whole.

FERTILITY

KS, usually viewed as the prototypic form of primary male hypogonadism, frequently causes azoospermia (about 90% of cases) and patients' seminiferous tubules appear fibrotic and hyalinised (1, 114, 115) as a direct consequence of the extra X chromosome. However, there might be sperm in the ejaculate, albeit at low concentrations (severe oligozoospermia) (14, 116-118), and rare cases of spontaneous fertility have even been reported (116, 119). Azoospermic patients might also have residual foci of spermatogenesis in the testes (118, 120). On the basis of these data several studies have reported successful pregnancies in the partners of patients with no sperm in the ejaculate, using testicular sperm extraction (TESE) and intracytoplasmic sperm injection techniques. TESE success has been reported in 16% to 69% of cases (44, 121-127), but there are no clinical or hormonal parameters clearly predictive of successful sperm retrieval (128). The severity of spermatogenic impairment is related to the number of supernumerary X chromosomes, and mosaic forms are less frequently azoospermic. Testicular involution in KS subjects starts in mid- or post-puberty and is progressive. Younger men are therefore more likely to have sperm in the ejaculate than older men (120). These data highlight the importance of early diagnosis and the cryopreservation of ejaculated or intra-testicular sperm before KS patients become azoospermic or TRT is begun.

Full spermatogenesis in KS might be explained by two mechanisms: testicular mosaicism in which 46,XY spermatogonia are able to differentiate normally into mature sperm, and spermatogenesis arising from 47,XXY spermatogonia. It has previously been demonstrated that 47,XXY spermatogonia and spermatocytes are able to complete the spermatogenic process, leading to the formation of mature spermatozoa (115). Consistent with the hypothesis that 47,XXY germ cells may undergo and complete meiosis, the aneuploidy rate for XX- and XY-disomies in ejaculated sperm is also higher in KS subjects than in controls, whereas the percentage of YY-disomies is normal (120).

Although the great majority of children born by intracytoplasmic sperm injection from KS subjects are chromosomally normal, the risk of producing offspring with chromosome aneuploidies and the higher risk of spontaneous abortion should be discussed with the couple during ge-

netic counselling. Although few studies have examined pre-implantation embryo diagnosis, they support the hypothesis that embryos generated with assisted reproduction techniques are frequently aneuploid (40-50% of aneuploidy) (129-131).

SEXUAL FUNCTION

It has been recently reported that in adulthood, most KS subjects seek medical attention not only for infertility but also due to sexual dysfunction (132). However, very few studies have specifically investigated this issue (133-135). Yoshida and colleagues (133) compared the sexual features of 40 KS patients with 40 non-azoospermic infertile 47,XY patients, using a non-validated questionnaire on sexual function. Among their KS patients the only sexual disorders reported were slightly reduced sexual desire (10%), erectile dysfunction (ED) (normal erection with shrinkage after entering the vagina) (2.5%), and a lower orgasm frequency (19%). However, there was no statistically significant increase in the incidences when compared with the control group. The only significant difference was the mean frequency of intercourse, which was significantly higher in KS patients. In contrast, another study did not find any significant difference in coital activity between married KS men and men with a varicocele investigated for infertility (135). One of the main limitations of these studies is that both were performed in a population sample of subjects whose chief complaint was infertility and not sexual dysfunction.

In a large cohort of 1386 male patients attending an Outpatient Clinic for sexual problems, Corona et al. analysed the prevalence and specific sexual correlates of KS. A relatively higher prevalence (1.7%) of KS than that reported in general population (14) was observed (134). This suggests that sexual dysfunction is a common feature of KS and that clinical settings for the treatment of sexual problems are convenient sites for its diagnosis. When sexual parameters were evaluated, 22.7% of KS patients reported severe ED (an erection not sufficient for penetration in >75% of cases), 60.9% hypoactive sexual desire (HSD), 9.5% premature ejaculation, and 9.5% delayed ejaculation. In a multiple logistic regression model only a higher risk for HSD and overt primary hypogonadism retained statistical significance (134). Interestingly, KS was also associated with higher risk of hypertriglyceridemia, the most common factor associated with MetS and hypogonadism in subjects with sexual dysfunction. When KS patients were compared to T-matched control subjects, all the above sexual and non-sexual problems were no longer associated with the syndrome (134). This case-control study demonstrated that any HSD in KS subjects was related to the T deficiency. Similarly, a small placebo-controlled study found an increase in sexual desire in KS subjects during TRT (136).

In conclusion, although limited, the literature data indicates that the sexual features present in KS are not specifically associated with the syndrome but are more likely to be related to underlying hypogonadism. Finally, it can be speculated that TRT could revert hypogonadism-related signs and symptoms (including sexual dysfunction) and prevent the unwanted consequences of androgen deficiency (including MetS) in KS patients.

EARLY DIAGNOSIS: BENEFITS AND DRAWBACKS

Given the lack of pathognomonic phenotypic evidence, KS is often not diagnosed until adulthood. From the methodological point of view, diagnosis is quite simple: the characteristic chromosomal pattern 47XXY, or one of its varieties, can be demonstrated through the standard analysis of blood lymphocytes, amniocytes or foetal chorial villi karyotype. The main difficulty is in defining the target group of subjects who should undergo genetic screening. If we consider the high percentage of individuals whose KS is unknown or only diagnosed in adulthood, early identification of the condition is clearly necessary to enable appropriate treatment and prevent possible complications.

Advantages

The main advantage of early diagnosis is the prevention of both complications and associated conditions. Regular monitoring of patients from the very beginning allows prompt recognition of any variation in their clinical picture and thus directs the appropriate intervention. Furthermore, the earlier the relationship between patients and doctors begins, the better the quality of communication will be, leading KS individuals to easily voice their worries and thoughts.

When early diagnosis is possible, KS boys can receive prompt educational support, to prevent any difficulties that might compromise their scholastic performance (137). Several studies have demonstrated that patients with KS have a mild cognitive impairment and that fewer learning difficulties are seen in patients diagnosed prenatally than in subjects diagnosed following the onset of symptoms (23).

Early detection is also important in order to start TRT as soon as patients need it. T should be given when its lack is clinically evident, continued lifelong and constantly adapted to the patients' symptoms and wishes.

Although KS men have long been considered sterile, the introduction of new fertilisation techniques, such as TESE and ICSI, offers the opportunity of reproduction even when no spermatozoa are found in the ejaculate. Recent findings show the presence of rare seminiferous tubules with ongoing spermatogenesis that seems to stop when germinal apoptosis occurs, probably at puberty (138). Semen cryopreservation should therefore be performed as soon as possible, even before T therapy begins. Cryopreservation of testicular tissue could also be performed, again before puberty, as this seems to be the age when testicular damage starts (139, 140).

Appropriate genetic counselling is always recommended in order to evaluate the risk of chromosomal abnormalities in children fathered by KS subjects. As KS patients have an increased risk of metabolic diseases, such as obesity, glucose intolerance and diabetes (48), early diagnosis would also enable subsequent nutritional counselling, to treat the tendency to gain fat mass and help prevent any cardiovascular consequences.

Drawbacks

A couple whose son has been prenatally diagnosed with KS may develop anxiety about his physical appearance, sexual development and fertility (141). When the syndrome is diagnosed during childhood or adolescence it is harder for the family to accept it. As the family always influences children's psychophysical wellbeing, it is important for parents to be accurately and thoroughly informed about the syndrome (142). It is also essential that they are given appropriate support to prevent excessive worrying or overprotective behaviors (143).

With early diagnosis, both the parents' anxiety and medical attention to the follow-up may lead to an exaggerated medicalisation, possibly increasing the young patient's concerns about his condition. Furthermore, early TRT may compromise fertility, as spermatogenesis is influenced by T treatment.

It is also important to establish the best timing for the parents to tell their child of the diagnosis. This is not easy, and appropriate cooperation between parents and doctors is always recommended. In any case, the consequences of this information should be considered. As adolescence is a crucial period in which boys are growing up and establishing their social and sexual role, the patient's discovery of his condition may cause an unhealthy approach to his sexual and social relationships. The young KS boy's anxiety about his condition should not be ignored, but treated through the cooperative intervention of psychologists, endocrinologists and sexologists.

The main benefits and drawbacks of early KS diagnosis are summarised in Table 2.

TESTOSTERONE REPLACEMENT THERAPY

During pubertal development, it is considered rational to start TRT when a pathological increase in gonadotropin levels is found, in order to allow the regular development of secondary sexual characteristics and muscle mass and achieve a normal peak bone mass. Literature data show that androgen therapy during puberty enhances muscle strength, improves mood and concentration and is useful in developing relational skills (144), while psychosocial problems may arise in the absence of treatment (145).

TRT should be considered lifelong, in order to prevent hypogonadism complications such as osteoporosis, obesity, diabetes and MetS and obtain probable cardiovascular benefits. In treated young hypogonadal patients with KS, positive effects such as decreased fat mass and increased lean mass, improved muscle strength, intensified sexual activity and improved mood are evident (146). Experience in older KS subjects also points to positive cognitive results (147).

Many KS patients show a characteristic hormonal profile: high FSH and LH levels and low to normal concentrations of T. TRT might be useful when signs or symptoms of hypogonadism (asthenia, low sexual desire, increased abdominal adiposity) become evident: the negative feedback suggests that receptor sensitivity is probably reduced, representing sub-clinical hypogonadism. However, to date there are no conclusive evidence-based studies supporting this hypothesis.

From current pharmacogenetic knowledge of T and T receptor sensitivity (148), we suggest that restoration of normal blood T and LH concentrations should be the aim of replacement therapy. However, not everybody agrees on this point. According to some authors, an alteration of the LH-T axis could be present in KS patients, with LH-producing cells having reduced sensitivity to T negative feedback. Some gonadotropic cell adenoma or hyperplasia has been reported in adult KS subjects, especially those aged over 50 (149); it could be hypothesised that this condition boosts excessive LH synthesis and secretion and thus a rise in blood levels. Reduced feedback would therefore be more frequent in older patients.

As in other hypogonadisms, we should focus on the laboratory and clinical signs that act as markers of response to the replacement therapy (52). Follow-up examinations should be effected 3 and 6-12 months after the start of treatment, if clinical response is adequate and no significant side effects have arisen. Transrectal prostate examination (and possibly prostate ultrasonography) matched with prostate specific antigen levels should be carried out both before starting treatment and after 3 months; annual checks, depending on the patient's age, are recommended thereafter. In osteoporotic hypogonadal patients, lumbar and femoral dual-emission X-ray absorptiometry follow-up is suggested 1 or 2 years after the start of treatment; a total body composition scan could also be useful to assess any modifications, and especially any increase in lean mass. Hematocrit should be kept in the normal range: this, together with erythrocytes and hemoglobin, is another important parameter to be monitored.

Table 2 - *Benefits and drawbacks of early diagnosis in Klinefelter syndrome.*

Benefits	Drawbacks
Early follow-up	Parental anxiety and possible negative effects in pregnancy (pre-natal diagnosis)
Fast identification of clinical changes (e.g. onset of hypogonadism)	Parental anxiety and possible effects on child's psychosocial development
Patient's better consciousness and awareness of his condition	Risk of excessive "medicalisation"
Early start and/or adaptation of therapy	Patient's anxiety about his own health
Prevention and medical care of co-morbidities	Ethical aspects concerning sex and fertility (when patient is a minor)
Prevention, treatment and proper approach to and support of neurolinguistic disabilities	
Early sperm retrieval and cryopreservation	
Better scientific awareness of condition and its natural history	

CONCLUSION

In this consensus we have highlighted the features that may prompt doctors to look for both KS and related diseases. We have provided evidence of possible benefits in establishing a diagnosis and starting proper follow-up and treatment at various phases of life.

As there is a high prevalence of undiagnosed patients, it is also important to improve our ability to promptly reach a diagnosis. This should be achieved through raising medical awareness of the condition and its clinical signs: not only in pediatricians, gynecologists and fertility specialists, but also in general practitioners. For this reason a strategy to improve diagnosis would be to provide GPs with information on the variety of pictures that KS may present at all stages in life, through a continuous exchange of information through conferences and training courses. It is in fact necessary to improve medical students' knowledge of rare conditions, and KS in particular. In our situation, we have in fact produced significant benefits by cooperating with family doctors and patients associations.

Many of the benefits of an early diagnosis strategy relate to the improved care of patients and their quality of life. Increased awareness among physicians and better patient education could lead to improved care.

Finally, more research is needed to establish the best interventional approaches to prevent complications and correlated diseases and improve knowledge of the underlying genetic mechanisms. Another open issue remains the early use of T therapy during puberty and the preservation of testicular sperm through retrieval for reproductive purposes later in life.

For all these reasons, the authors have planned more meetings on KS under the auspices of SIAMS with the participation of patients associations and general practitioners, with the aim of spreading clinical guidelines for KS management. Another consensus conference has been planned to take place in about two years, in order to evaluate the latest results.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Emanuela De Marco and Dr. Simona Granato for technical revision of the text and references and Marie-Hélène Hayles for language revision.

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REFERENCES

1. Klinefelter HF, Reifenstein EC, Albright F. Syndrome characterized by gynecomastia, aspermatogenesis without a leydigism and increased excretion of follicle-stimulating hormone. J Clin Endocrinol Metab 1942, 2: 615-27.
2. Jacobs PA, Strong JA. A case of Human intersexuality having a possible XXY sex-determining mechanism. Nature 1959, 183: 302-3.
3. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab 2003, 88: 622-6.
4. Abramsky L, Chapple J. 47, XXY (Klinefelter syndrome) and 47,XYY: estimated rates of and indication for postnatal diagnosis with implications for prenatal counseling. Prenat Diagn 1997, 17: 363-68.
5. Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Birth Defects Orig Artic Ser 1990, 26: 209-23.
6. Swerdlow AJ, Higgins CD, Schoemaker MJ, et al. Mortality in patients with Klinefelter syndrome in Britain: a cohort study. J Clin Endocrinol Metab 2005, 90: 6516-22.
7. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. Reprod Biomed Online 2007, 14: 734-45.
8. Stemkens D, Broekmans FJ, Kastrop PM, Hochstenbach R, Smith BG, Giltay JC. Variant Klinefelter Syndrome 47,X,i(X)(q10),Y and Normal 47, XY Karyotype in Monozygotic Adult Twins. Am J Med Genet 2007; Part A 143A:1906–1911
9. Linden MG, Bender BG, Robinson A. Sex chromosome tetrasomy and pentasomy. Pediatrics 1995, 96: 672-82.
10. Hassold TJ, Sherman SL, Pettay D, Page DC, Jacobs PA. XY chromosome nondisjunction in man is associated with diminished recombination in the pseudoautosomal region. Am J Hum Genet 1991, 49: 253-60.
11. MacDonald M, Hassold T, Harvey J, Wang LH, Morton NE, Jacobs P. The origin of 47, XXY and 47,XXX aneuploidy: heterogeneous mechanisms and role of aberrant recombination. Hum Mol Genet 1994, 3: 1365-71.
12. Iitsuka Y, Bock A, Nguyen DD, Samango-Sprouse CA, Simpson JL, Bischoff FZ. Evidence of skewed X-chromosome inactivation in 47, XXY and 48,XXYY Klinefelter patients. Am J Med Genet 2001, 98: 25-31.
13. Bojesen A, Gravholt CH. Klinefelter syndrome in clinical practice. Nat Clin Pract Urol 2007, 4: 192-204.
14. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. Lancet 2004, 364: 273-83.
15. Simpson JL, de la Cruz F, Swerdloff RS, et al. Klinefelter syndrome: expanding the phenotype and identifying new research directions. Genet Med 2003, 5: 460-8.
16. Ratcliffe SG. The sexual development of boys with the chromosome constitution 47, XXY (Klinefelter's syndrome). Clin Endocrinol Metab 1982, 11: 703-16.
17. Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47, XXY Klinefelter syndrome. Horm Res 2005, 64: 39-45.
18. Linden. MG, Bender BG. Fifty-one prenatally diagnosed children and adolescents with sex chromosome abnormalities. Am J Med Genet 2002, 110: 11-8.
19. Lee YS, Cheng AW, Ahmed SF, Shaw NJ, Hughes IA. Genital anomalies in Klinefelter's syndrome. Horm Res 2007, 68: 150-5.
20. Zeger MP, Zinn AR, Lahlou N, et al. Effect of ascertainment and genetic features on the phenotype of Klinefelter syndrome. J Pediatr 2008, 152: 716-22.
21. Lahlou N, Roger M. Inhibin B in pubertal development and pubertal disorders. Semin Reprod Med 2004, 22: 165-75.
22. Aksglaede L, Petersen JH, Main KM, Skakkebæk NE, Juul A. High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. Eur J Endocrinol 2007, 157: 345-50.
23. Girardin CM, Lemyre E, Alos N, Deal C, Huot C, Van Vliet G. Comparison of Adolescents with Klinefelter Syndrome according to the circumstances of diagnosis: amniocentesis versus clinical signs. Horm Res 2009, 72: 98-105.
24. Ferlin A, Zuccarello D, Zuccarello B, Chirico MR, Zanon GF, Foresta C. Genetic alterations associated with cryptorchidism. JAMA 2008, 300: 2271-6.
25. Ratcliffe S. Long-term outcome in children of sex chromosome abnormalities. Arch Dis Child 1999; 80: 192-5.
26. Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z, Laron Z. Puberty in 24 patients with Klinefelter syndrome. Eur J Pediatr 1982, 139: 8-12.
27. Salbenblatt JA, Bender BG, Puck MH, Robinson A, Faiman C, Winter JS. Pituitary-gonadal function in Klinefelter syndrome before and during puberty. Pediatr Res 1985, 19: 82-6.

28. Christiansen P, Andersson AM, Skakkebaek NE. Longitudinal studies of inhibin B levels in boys and young adults with Klinefelter syndrome. J Clin Endocrinol Metab 2003, 88: 888-91.
29. Wikström AM, Dunkel L, Wickman S, Norjavaara E, Ankarberg-Lindgren C, Raivio T. Are adolescent boys with Klinefelter syndrome androgen deficient? A longitudinal study of Finnish 47, XXY boys. Pediatric Research 2006, 59: 854-9.
30. Wikström AM, Bay K, Hero M, et al. Serum insulin-like factor 3 levels during puberty in healthy boys and boys with Klinefelter syndrome. J Clin Endocrinol Metab 2006, 91: 4705-8.
31. Aksglaede L, Wikström AM, Rajpert-De Meyts E, Dunkel L, Skakkebaek NE, Juul A. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. Hum Reprod Update 2006, 12: 39-48.
32. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child 1970, 45: 13-23.
33. Castellino N, Bellone S, Rapa A, et al. Puberty onset in Northern Italy: a random sample of 3597 Italian children. J Endocrinol Invest 2005, 28: 589-94.
34. Behre HM, Yeung CH, Holstein AF, Weinbauer GF, Gassner P, Nieschlag E. Diagnosis of male infertility and hypogonadism. In: Nieschlag E, Behre HM, Nieschlag S, eds. Andrology: male reproductive health and dysfunction, 2nd ed. Berlin, Heidelberg, New York: Springer 2000, 90-124.
35. Ma NS, Geffner ME. Gynecomastia in prepubertal and pubertal men. Curr Opin Pediatr 2008, 20: 465-70.
36. Smyth CM, Bremner WJ. Klinefelter syndrome. Arch Intern Med 1998, 158: 1309-14.
37. Visootsak J, Aylstock M, Graham JM Jr. Kilnefelter syndrome and its variants: an update and review for the primary pediatrician clin pediatr (Phila) 2001, 40: 639-43.
38. Winter JS Androgen therapy in Klinefelter syndrome during adolescence. Birth Defects Orig Artic Ser 1990, 26: 235-45.
39. Aksglaede L, Skakkebaek NE, Juul A. Abnormal sex chromosome constitution and longitudinal growth: serum levels of insulin-like growth factor (IGF)-I, IGF binding protein-3, luteinizing hormone, and testosterone in 109 males with 47, XXY, 47,XYY, or sex-determining region of the Y chromosome (SRY)-positive 46,XX karyotypes. J Clin Endocrinol Metab 2008, 93: 169-76.
40. Radicioni AF, Anzuini A, De Marco E, Nofroni I, Castracane VD, Lenzi A. Changes in serum inhibin B during normal male puberty. Eur J Endocrinol 2005, 152: 403-9.
41. Wikström AM, Raivio T, Hadziselimovic F, Wikström S, Tuuri T, Dunkel L. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. J Clin Endocrinol Metab 2004, 89: 2263-70.
42. Ratcliffe SG, Murray L, Teague P. Edinburgh study of growth and development of children with sex chromosome abnormalities. III. Birth Defects Orig Artic Ser 1986, 22: 73-118.
43. Foresta C, Bettella A, Vinanzi C, et al. A novel circulating hormone of testis origin in humans. J Clin Endocrinol Metab 2004, 89: 5952-8.
44. Bay K, Hartung S, Ivell R, et al. Insulin-like factor 3 serum levels in 135 normal men and 85 men with testicular disorders: relationship to the luteinizing hormone-testosterone axis. J Clin Endocrinol Metab 2005, 90: 3410-8.
45. Ferlin A, Garolla A, Rigon F, Rasi Caldogno L, Lenzi A, Foresta C. Changes in serum insulin-like factor 3 during normal male puberty. J Clin Endocrinol Metab 2006, 91: 3426-31.
46. Selice R, Di Mambro A, Garolla A, Ficarra V, Iafrate M, Ferlin A, Foresta C. Spermatogenesis in Klinefelter syndrome. J Endocrinol Invest 2010, Mar 22 [Epub ahead of print].
47. Fullerton G, Hamilton M, Maheshwari A. Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009? Hum Reprod 2010, 25: 588-97.
48. Bojesen A, Kristensen K, Birkebaek NH, et al. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. Diabetes Care 2006, 29: 1591-8.
49. Kamischke A, Baumgardt A, Horst J, Nieschlag E. Clinical and diagnostic features of patients with suspected Klinefelter syndrome. J Androl 2003, 24: 41-8.
50. Anawalt BD, Bebb RA, Matsumoto AM, et al. Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab 1996, 81: 3341-5.
51. Klingmüller D, Haidl G. Inhibin B in men with normal and disturbed spermatogenesis. Hum Reprod 1997, 12: 2376-8.
52. Wang C, Nieschlag E, Swerdloff R, et al. Investigation, treatment and monitoring of late-onset hypogonadism in males. Int J Androl 2009, 32: 1-10.
53. Ishikawa T, Yamaguchi K, Kondo Y, Takenaka A, Fujisawa M. Metabolic syndrome in men with Klinefelter's syndrome. Urology 2008, 71: 1109-13.
54. Ravaglia G, Forti P, Maioli F, et al. Metabolic Syndrome: prevalence and prediction of mortality in elderly individuals. Diabetes Care 2006, 29: 2471-6.
55. Corona G, Mannucci E, Forti G, Maggi M. Hypogonadism, ED, metabolic syndrome and obesity: a patological link supporting cardiovascular diseases. Int J Androl 2009, 32: 587-98.
56. Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY. Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. Int J Obes Relat Metab Disord 2000, 24: 485-91.
57. Mårin P, Holmäng S, Jönsson L, et al. The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. Int J Obes Relat Metab Disord 1992, 16: 991-7.
58. Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature 2006, 444: 881-7.
59. Aksglaede L, Molgaard C, Skakkebaek NE, Juul A. Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome. Arch Dis Child 2008, 93: 30-34.
60. Yesilova Z, Oktenli C, Sanisoglu SY, et al. Evaluation of insulin sensitivity in patients with Klinefelter's syndrome: a hyperinsulinemic euglycemic clamp study. Endocr 2005, 27: 11-5.
61. Andersen NH, Bojesen A, Kristensen K, et al. Left ventricular dysfunction in Klinefelter syndrome is associated to insulin resistance, abdominal adiposity and hypogonadism. Clin Endocrinol (Oxf) 2008, 69: 785-91.
62. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998, 395: 763-70.
63. Laaksonen DE, Niskanen L, Punnonen K, et al. Sex hormones, inflammation and the metabolic syndrome: a population-based study. Eur J Endocrinol 2003, 149: 601-8.
64. Díez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. Eur J Endocrinol 2003, 148: 293-300.
65. Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. Eur J Endocrinol 2006, 154: 899-906.
66. Saad F, Gooren LJ, Haider A, Yassin A. A dose-response study of testosterone on sexual dysfunction and features of the metabolic syndrome using testosterone gel and parenteral testosterone undecanoate. J Androl 2008, 29: 102-5.
67. Corona G, Monami M, Rastrelli G, et al. Testosterone and metabolic syndrome: a meta-analysis study. J Sex Med 2010, in press.
68. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. Osteoporosis prevention, diagnosis and therapy. JAMA 2001, 285: 785-95.
69. Lombardi A, Ross PD. The assessment of bone mass in men. Calcif Tissue Int 2001, 69: 222-4.
70. Jackson JA, Kleerekoper M. Osteoporosis in men: diagnosis, pathophysiology, and prevention. Medicine (Baltimore) 1990, 69: 137-52.
71. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994, 9: 1137-41.
72. Kamel HK. Update on osteoporosis management in long-term care: focus on bisphosphonates. J Am Med Dir Assoc 2007, 8: 434-40.
73. Horowitz M, Mordin BEC, Aaron JE (eds). Klinefelter's Syndrome. Berlin: Springer-Verlag. 1984, 51-61.
74. Stepán JJ, Lachman M, Zv rina J, Pacovský V, Baylink DJ. Castrated men exhibit bone loss: effect of calcitonin treatment on biochemical indices of bone remodeling. J Clin Endocrinol Metab 1989, 69: 523-7.
75. Breuil V, Euller-Ziegler L. Gonadal dysgenesis and bone metabolism. Joint, bone, spine: revue du rhumatisme 2001, 68: 26-33.

76. van den Bergh JP, Hermus AR, Spruyt AI, Sweep CG, Corstens FH, Smals AG. Bone mineral density and quantitative ultrasound parameters in patients with Klinefelter's syndrome after long-term testosterone substitution. Osteoporos Int 2001, 12: 55-62.
77. Fintini D, Grossi A, Brufani C, et al. Bone mineral density and body composition inmale children with hypogonadism. J Endocrinol Invest 2009, 32: 585-9.
78. Seo JT, Lee JS, Oh TH, Joo KJ. The clinical significance of bone mineral density and testosterone levels in Korean men with nonmosaic Klinefelter's syndrome. BJU Int 2007, 99: 141-6.
79. Ferlin A, Schipilliti M, Di Mambro A, Vinanzi C, Foresta C. Osteoporosis in Klinefelter's syndrome. Mol Hum Reprod 2010, 16: 402-10.
80. Ringe JD, Faber H, Dorst A. Alendronate treatment of established primary osteoporosis in men: results of a 2-year prospective study. J Clin Endocrinol Metab 2001, 86: 5252-5.
81. Shimon I, Eshed V, Doolman R, Sela BA, Karasik A, Vered I. Alendronate for osteoporosis in men with androgen-repleted hypogonadism. Osteoporos Int 2005, 16: 1591-6.
82. Ringe JD, Faber H, Farahmand P, Dorst A. Efficacy of risedronate in men with primary and secondary osteoporosis: results of a 1-year study. Rheumatol Int 2006, 26: 427-31.
83. Lyles KW, Colón-Emeric CS, Magaziner JS, et al; HORIZON Recurrent Fracture Trial. Zoledronic acid and clinical fracture and mortality after hip fracture. N Engl J Med 2007, 357: 1799-808.
84. Orwoll ES, Scheele WH, Paul S, et al. The effect of teriparatide [human parathyroid hormone (1-34)] therapy on bone density in men with osteoporosis. J Bone Miner Res 2003, 18: 9-17.
85. Geschwind DH, Boone KB, Miller BL, Swerdloff RS. Neurobehavioural phenotype of Klinefelter syndrome. Ment Retard Dev Disabil Res Rev 2000, 6: 107-16.
86. Ratcliffe SG, Jenkins J, Teague P. Cognitive and behavioural development of the 47,XYY child. In: Berch DB, Bender BG, editors. Sex chromosome abnormalities and behavior: psychological studies. Boulder, CO, USA: Westview Press; 1990. pp. 161–84.
87. Bancroft J, Axworthy D, Ratcliffe S. The personality and psychosexual development of boys with 47, XXY chromosome constitution. J Child Psychol Psychiatry 1982, 23: 169-80.
88. Temple CM, Sanfilippo PM. Executive skills in Klinefelter's syndrome. Neuropsychologia 2003, 41: 1547-59.
89. Liss M, Fein D, Allen D, et al. Executive functioning in high-functioning children with autism. J Child Psychol Psychiatry 2001, 42: 261-70.
90. Samango-Sprouse CA, Rogol A. XXY: the hidden disability and a prototype for an infantile presentation of developmental dyspraxia (IDD). Infants Young Child 2002, 15: 11-18.
91. DeLisi LE, Maurizio AM, Svetina C, et al. Klinefelter's syndrome (XXY) as a genetic model for psychotic disorders. Am J Med Genet B Neuropsychiatr Genet 2005, 135B: 15-23.
92. Lue Y, Jentsch JD, Wang C, et al. XXY mice exhibit gonadal and behavioral phenotypes similar to Klinefelter syndrome. Endocrinology 2005, 146: 4148-54.
93. Lewejohann L, Damm OS, Luetjens CM, et al. Impaired recognition memory in male mice with a supernumerary X chromosome. Physiol Behav 2009, 96: 23-9.
94. Bender BG, Harmon RJ, Linden MG, Robinson A. Psychosocial adaptation of 39 adolescents with sex chromosome abnormalities. Pediatrics 1995, 96 (2 Pt 1): 302-308.
95. DeLisi LE, Friedrich U, Wahlstrom J, et al. Schizophrenia and sex chromosome anomalies. Schizophr Bull 1994, 20: 495-505.
96. Price WH, Clayton JF, Wilson J, Collyer S, De Mey R. Causes of death in X chromatin positive males (Klinefelter's syndrome). J Epidemiol Community Health 1985, 39: 330-6.
97. Swerdlow AJ, Hermon C, Jacobs PA, et al. Mortality and cancer incidence in persons with numerical sex chromosome abnormalities: a cohort study. Ann Hum Genet 2001, 65: 177-88.
98. Bojesen A, Juul S, Birkebaek N, Gravholt CH. Increased mortality in Klinefelter syndrome. J Clin Endocrinol Metab 2004, 89: 3830-4.
99. Harnden DG, Maclean N, Langlands AO. Carcinoma of the breast and Klinefelter's syndrome. J Med Genet 1971, 8: 460-1.
100. Scheike O, Visfeldt J, Petersen B. Male breast cancer. 3. Breast carcinoma in association with the Klinefelter syndrome. Acta Pathol Microbiol Scand A 1973, 81: 352-8.
101. Hultborn R, Hanson C, Köpf I, Verbiené I, Warnhammar E, Weimarck A. Prevalence of Klinefelter's syndrome in male breast cancer patients. Anticancer Res 1997, 17: 4293-7.
102. Sasco AJ, Lowenfels AB, Pasker-de Jong P. Review articles: epidemiology of male breast cancer. A mate-analysis of published case-control studies and discussion of selected aetiological factors. Int J Cancer 1993, 53: 538-49.
103. Swerdlow AJ, Schoemaker MJ, Higgins CD, Wright AF, Jacobs PA; UK Clinical Cytogenetics Group. Cancer incidence and mortality in men with Klinefelter syndrome: a cohort study. J Natl Cancer Inst 2005, 97: 1204-10.
104. Wang C, Baker HW, Burger HG, De Kretser DM, Hudson B. Hormonal studies in Klinefelter's syndrome. Clin Endocrinol 1975, 4: 399-411.
105. Lynch HT, Kaplan AR, Lynch JF. Klinefelter syndrome and cancer: a family study. JAMA 1974, 229: 809-11.
106. Schneider DT, Schuster AE, Fritsch MK, et al. Genetic analysis of mediastinal nonseminomatous germ cell tumors in children and adolescents. Genes Primary mediastinal germ cell tumors in children and adolescents: results of the German cooperative protocols MAKEI 83/86, 89, and 96. J Clin Oncol 2000, 18: 832-9.
108. Hasle H, Jacobsen BB, Asschenfeldt P, Andersen K. Mediastinal germ cell tumour associated with Klinefelter syndrome. A report of case and review of the literature. Eur J Pediatr 1992, 151: 735-9.
109. Nichols CR, Heerema NA, Palmer C, Loehrer PJ Sr, Williams SD, Einhorn LH. Klinefelter's syndrome associated with mediastinal germ cell neoplasms. J Clin Oncol 1987, 5: 1290-4.
110. Hachimi-Idrissi S, Desmyttere S, Goossens A, Desprechins B, Otten J. Retroperitoneal teratoma as first sign of Klinefelter's syndrome. Arch Dis Child 1995, 72: 163-4.
111. Sato K, Takeuchi H, Kubota T. Pathology of intracranial germ cell tumors. Prog Neurol Surg 2009, 23: 59-75.
112. Hasle H, Mellemgaard A, Nielsen J, Hansen J. Cancer incidence in men with Klinefelter syndrome. Br J Cancer 1995, 71: 416-20.
113. Müller J, Skakkebaek NE. Gonadal malignancy in individuals with sex chromosome anomalies. In: Evans JA, Hamerton JL, Robinson A (eds). Children and young adults with sex chromosome aneuploidy: follow-up, clinical, and molecular Studies. Proceedings of the 5th International Workshop on Sex Chromosome anomalies. Minaki, Ontario, Canada, June 7-10, 1989.
114. Gordon DL, Krmpotic E, Thomas W, Gandy HM, Paulsen CA. Pathologic testicular findings in Klinefelter's syndrome. 47, XXY vs 47, XY-47, XXY. Arch Intern Med 1972, 130: 726-9.
115. Skakkebaek NE. Two types of tubules containing only Sertoli cells in adults with Klinefelter's syndrome. Nature 1969, 223: 643-5.
116. Laron Z, Dickerman Z, Zamir R, Galatzer A. Paternity in Klinefelter's syndrome – a case report. Arch Androl 1982, 8: 149-51.
117. Lin YM, Huang WJ, Lin JS, Kuo PL. Progressive depletion of germ cells in a man with nonmosaic Klinefelter's syndrome: optimal time for sperm recovery. Urology 2004, 63: 380-1.
118. Foresta C, Galeazzi C, Bettella A, Stella M, Scandellari C. High incidence of sperm sex chromosomes aneuploidies in two patients with Klinefelter's syndrome. J Clin Endocrinol Metab 1998, 83: 203-5.
119. Terzoli G, Lalatta F, Lobbiani A, Simoni G, Colucci G. Fertility in a 47, XXY patient: assessment of biological paternity by deoxyribonucleic acid fingerprinting. Fertil Steril 1992, 58: 821-2.
120. Foresta C, Galeazzi C, Bettella A, et al. Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. J Clin Endocrinol Metab 1999, 84: 3807-10.
121. Tournaye H, Staessen C, Liebaers I, Van Assche E, Devroey P, Bonduelle M, Van Steirteghem A. Testicular sperm recovery in nine 47, XXY Klinefelter patients. Hum Reprod 1996, 11: 1644-9.
122. Friedler S, Raziel A, Strassburger D, Schachter M, Bern O, Ron-El R. Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's syndrome. Hum Reprod 2001, 16: 2616-20.
123. Ulug U, Bener F, Akman MA, Bahceci M. Partners of men with Klinefelter syndrome can benefit from assisted reproductive technologies. Fertil Steril 2003, 80: 903-6.

124. Seo JT, Park YS, Lee JS. Successful testicular sperm extraction in Korean Klinefelter syndrome. Urology 2004, 64: 1208-11.
125. Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab 2005, 90: 6263-7.
126. Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47, XXY Klinefelter's syndrome patients? Hum Reprod 2004, 19: 1135-9.
127. Okada H, Goda K, Yamamoto Y, et al. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. Fertil Steril 2005, 84: 1662-4.
128. Selice R, Di Mambro A, Garolla A, et al. Spermatogenesis in Klinefelter syndrome. J Endocrinol Invest 2010 [Epub ahead of print].
129. Yarali H, Polat M, Bozdag G, et al. TESE-ICSI in patients with non-mosaic Klinefelter syndrome: a comparative study. Reprod Biomed Online 2009, 18: 756-60.
130. Bielanska M, Tan SL, Ao A. Fluorescence in-situ hybridization of sex chromosomes in spermatozoa and spare preimplantation embryos of a Klinefelter 47, XY/47, XXY male. Hum Reprod 2000, 15: 440-4.
131. Staessen C, Tournaye H, Van Assche E, et al. PGD in 47, XXY Klinefelter's syndrome patients. Hum Reprod Update 2003, 9: 319-30.
132. Paduch DA, Fine RG, Bolyakov A, Kiper J. New concepts in Klinefelter syndrome. Curr Opin Urol 2008, 18: 621-7.
133. Yoshida A, Miura K, Nagao K, Hara H, Ishii N, Shirai M. Sexual function and clinical features of patients with Klinefelter's syndrome with the chief complaint of male infertility. Int J Androl 1997, 20: 80-5.
134. Corona G, Petrone L, Paggi F, et al. Sexual dysfunction in subjects with Klinefelter's syndrome. Int J Androl 2009 Sep 25 (epub ahead of print).
135. Raboch J, Pietrucha S, Raboch J. Serum testosterone levels and coital activity in men with somatosexual disorders. Neuro Endocrinol Lett 2003, 24: 321-4.
136. Wu FC, Bancroft J, Davidson DW, Nicol K. The behavioural effects of testosterone undecanoate in adult men with Klinefelter's syndrome: a controlled study. Clin Endocrinol (Oxf) 1982, 16: 489-97.
137. Manning MA, Hoyme HE. Diagnosis and management of the adolescent boy with Klinefelter syndrome. Adolesc Med 2002, 13: 367-74.
138. Wikström AM, Dunkel L. Testicular function in Klinefelter syndrome. Horm Res 2008, 69: 317-26.
139. Kamischke A, Jürgens H, Hertle L, Berdel WE, Nieschlag E. Cryopreservation of sperm from adolescents and adults with malignancies. J Androl 2004, 25: 586-92.
140. Damani MN, Mittal R, Oates RD. Testicular tissue extraction in a young male with 47,XXY Klinefelter's syndrome: potential strategy for preservation of fertility Fertil Steril 2001, 76: 1054-6.
141. Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, Zeyneloglu HB. Chromosome heteromorphisms: an impact on infertility. J Assist Reprod Genet 2008, 25: 191-5.
142. Schor EL; American Academy of Pediatrics Task Force on the Family. Family pediatrics: report of the Task Force on the Family. Pediatrics 2003, 111: 1541-71.
143. Sørensen K. Physical and mental development of adolescent males with Klinefelter syndrome. Hormone Research 1992, 37 (Suppl 3): 55-61.
144. Nielsen J, Pelsen B, Sørensen K. Follow-up of 30 Klinefelter males treated with testosterone. Clin Genet 1988, 33: 262-9.
145. Simm PJ, Zacharin MR. The psychosocial impact of Klinefelter syndrome-a 10 year review. J Pediatr Endocrinol Metab 2006, 19: 499-505.
146. Wang C, Swerdloff RS, Iranmanesh A, et al. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. J Clin Endocrinol Metab 2000, 85: 2839-53.
147. Cherrier MM, Asthana S, Plymate S, et al. Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology 2001, 57: 80-8.
148. Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E. X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab 2004, 89: 6208-17.
149. Scheithauer BW, Moschopulos M, Kovacs K, Jhaveri BS, Percek T, Lloyd RV. The pituitary in Klinefelter syndrome. Endocr Pathol 2005, 16: 133-8.