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NEW RESEARCH HORIZON *Review*

Strategies and advantages of early diagnosis in Klinefelter's syndrome

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ABSTRACT: Nearly 70 years after its description, Klinefelter's syndrome (KS) remains a largely undiagnosed condition. In addition to its typical characteristics of increased follicle-stimulating hormone secretion and small and firm testes, the syndrome presents an extremely wide spectrum of phenotypes. This could be explained by the possible presence of chromosomal mosaicism, androgen receptor polymorphisms and related heterogeneous endocrine abnormalities. The varied but relatively mild physical abnormalities also explain why many patients do not receive clinical attention until adulthood, when they seek medical advice on small testes or infertility. Diagnosis is also hindered by the low awareness of the disease among health professionals. This paper aims to review the possible signs of KS at different stages of life that could help achieve an early (or at least earlier) diagnosis. It has been demonstrated that the early diagnosis of KS improves patients' quality of life and enables better medical treatment. To achieve this, it is crucial to increase both medical and general awareness of the disease, including through use of the media and patients' associations.

Key words: Klinefelter / hypogonadism / aneuploidy / testosterone / diagnosis

Introduction

Klinefelter's syndrome (KS) was first described by Harry Klinefelter in 1942 (Klinefelter *et al.*, 1942) as a condition characterized by gynaeco-mastia, small, firm testes, hypogonadism and a high level of FSH. It is the most common chromosomal disorder, affecting 1 in 500 men (Lanfranco *et al.*, 2004), and is a frequent cause of hypogonadism and infertility. It is caused by the presence of extra X chromosomes, usually acquired through non-disjunction during maternal or paternal gametogenesis. The most common karyotype is 47,XXY. Recent find-ings suggest that the prevalence of diagnosed KS, but not of XXX or XYY aneuploidies, has increased in recent decades, probably due to both the increased prevalence of paternal meiotic alterations and a greater number of routinely performed prenatal investigations (Morris *et al.*, 2008). Nevertheless, the true prevalence of this aneu-ploidy is still considered to be underestimated (Abramsky and Chapple, 1997).

As its clinical presentation may be subtle, many of those affected may be unaware or diagnosed only during evaluation for hypogonad-ism and/or infertility. It has been demonstrated that KS is one of the most frequent causes of infertility, affecting ~11% of azoospermic and ~3.1% of all infertile men (Lanfranco *et al.*, 2004).

KS is often not diagnosed until adulthood, due to the extreme varia-bility of its clinical presentation and because the milder forms are very common but lack obvious signs, with patients reporting few subjective symptoms. Furthermore, awareness of KS among general practitioners is probably insufficient to enable its diagnosis (Visootsak *et al.*, 2001; Lanfranco *et al.*, 2004).

Abramsky and Chapple (1997) showed that up to 64% of KS patients are never diagnosed, whereas 10% of those affected are diag-nosed prenatally and only 26% are diagnosed in prepuberty or adult-hood. A very large study of the Danish national registry recently confirmed these data and showed that KS is often undiagnosed and seldom predicted before puberty (Bojesen *et al.*, 2003).

The aim of this paper is to review the possible signs of KS in the different phases of life, to help achieve an early diagnosis.

Prenatal diagnosis

Only a marginal percentage of males with KS are diagnosed prenatally, when amniocentesis is performed due to advanced maternal age. Since the maternal age effect is weaker in KS than in other chromo-some disorders, such as Down syndrome (Hook, 1981; Visootsak *et al.*, 2001), prenatal KS is mainly a chance finding. So far, no specific ultrasound (US) features or serum screening markers suggesting the presence of KS have been found, meaning that there is no effective way to screen the population prenatally, apart from karyotype analysis (Abramsky and Chapple, 1997). Moreover, it has been observed that

chromosome analyses are usually performed due to suspicions other than KS and their frequency increases with maternal age.

The techniques that can be used for prenatal diagnosis—chorionic villus sampling, amniocentesis and cordocentesis—are all invasive procedures and carry some risks. They all need to be accompanied by suitable genetic counselling and clinical support.

The benefit of prenatal identification of KS mainly lies in the opportunity to prepare the parents and provide a dedicated clinical path for the affected infant. The aim is to make the parents aware of their son's disorder, reduce their anxiety and provide appropriate social and educational support. Early diagnosis thus allows physicians and parents to treat the affected boys in time and prevent the majority of complications associated with late intervention.

Parents will also be able to:

(i) have their KS son start any necessary speech therapy as soon as possible, aiming for early and complete rehabilitation, and plan an educational strategy, given that the percentage of patients with learning or language difficulties tends to be lower when patients are diagnosed by amniocentesis (Girardin et al., 2009);

(ii) promote physical activity in order to contain the effects of any motor dyspraxia;

(iii) monitor pubertal development with the help of an endocrinologist;

(iv) plan, with the help of a psychologist, the best time to tell their son about his condition.

However, some aspects of prenatal diagnosis are the object of discussion, such as the resulting increase in terminations, estimated in some reports as up to 70% of prenatally diagnosed KS (Bojesen et al., 2003). The decision to terminate a pregnancy due to sexual abnormalities and aneuploidies with a low risk of abnormal phenotype seems to be related to the parents' social and cultural background, local laws and the genetic counselling strategy (Table I). The way that information about the condition is conveyed by medical staff also plays a critical role in this decision (Quadrelli et al., 2007). This is why parents should be given adequate time to reflect and all decisions should be made only after they have been given thorough information and any prejudices and mistaken affirmations have been addressed.

If the diagnosis is not made prenatally, it is unlikely to be made during the first 10 years of life and ∼75% of the estimated cases are never diagnosed at all (Abramsky and Chapple, 1997). The rare

Table I Percentage of abortion after prenatal diagnosis of KS in the various countries.

Country	% of abortions	Reference
Canada	87.5	Christian et al. (2000)
France	41.7	Perrotin et al. (2000)
Israel	85.0	Sagi et al. (2001)
Denmark	70.0	Bojesen et al. (2003)
Switzerland	73.9	Hamamy and Dahoun (2004)
Hawaii	39.0	Forrester and Merz (2003)
USA	70.0	Shaffer et al. (2006)
Uruguay	23.0	Quadrelli et al. (2007)

early diagnosis of KS highlights the need to identify the early features of typical and atypical post-natal presentation, with the aim of appropriate treatment and prevention of possible complications.

Diagnosis at birth

At birth, affected males rarely present clinical signs and the diagnosis of KS at this stage is uncommon. Nevertheless, some congenital malformations have occasionally been reported as more frequent in KS than in healthy boys. The most common are: genital abnormalities, such as cryptochidism, small penis, scrotum bifidus and hypospadias, or other types of malformation such as: fifth finger clinodactyly, cleft palate and inguinal hernia (Lee et al., 2007). A few patients show a variable degree of hypotonia: muscle tone is reduced in a high percentage of assessed patients (Zeger et al., 2008). Detection of these features should motivate physicians to request karyotype analysis.

Diagnosis in infancy

KS infants have variable phenotypic features and do not have obvious facial dysmorphology; thus they are usually indistinguishable from other boys with normal karyotype.

Infants with 47,XXY aged 1–23 months may exhibit smaller testicular and penile size. As growth of the penis in-uterus and during childhood is a good bioassay for endogenous testosterone as well as androgen receptor responsiveness, reduced penile growth in early childhood may indicate androgen deficiency (Ross et al., 2005).

Even though the prevalence of cryptorchidism is still debated, given that few studies have examined this feature, it appeared more frequent among KS boys than healthy controls in the series of patients from the Danish registry (6.25%; 95% CI, 3.97–9.84%; Bojesen et al., 2006a). Ferlin et al. (2008) found that KS was the most frequent genetic abnormality detected in a population with isolated cryptorchidism, with this association being stronger for the persistent forms (1.6% in the unilateral forms and 4.2% in the bilateral forms).

It has been assumed that genital development is due to the activation of the hypothalamus–pituitary–gonad (HPG) axis during the first months of life. In the healthy male infant, gonadotrophins, testosterone (T) and inhibin B increase to pubertal or even adult levels at about 3 months of age (minipuberty); serum levels of FSH, LH and T then decrease in the following 3–6 months until the HPG axis reactivates in puberty. Although serum inhibin B levels decrease from 3 (or 6) months of age, they remain elevated even after levels of the other three hormones decrease to within the range observed later in childhood (Andersson et al., 1998a, b). The described post-natal increase in sex hormones represents a window where it is possible to study the function of the axis (Main et al., 2002).

A very early reduction in Leydig cell activity has been postulated in KS, based on initial findings of low T serum levels in umbilical cord (Sørensen et al., 1981). However, subsequent studies showed that the differences in umbilical cord T levels between healthy and affected boys were not statistically significant (Ratcliffe, 1982).

Conflicting data have been reported on hormone levels in KS infants. Aksglæde et al. (2007) found high-normal serum levels of total and free-T and elevated levels of gonadotrophins in 3-month-old KS infants when compared with healthy controls. However, Lahlou et al. (2004) observed that the T surge in the first 3 months of life

was somewhat lower than in normal infants; in contrast, LH levels were considered similar to those found in normal infants, suggesting that KS causes the Leydig cells to develop some degree of LH resistance. As with the controls, inhibin B levels increased significantly from birth to 4 months and then decreased progressively. At the same time, anti-Müllerian hormone (AMH) levels increased progressively from birth to 8 months and then remained stable for the next 2–3 years. The normal level of gonadotrophins, inhibin B and AMH suggests that the Sertoli cells are qualitatively and quantitatively normal in KS children during infancy (Lahlou et al., 2004).

In summary, as hormonal variations are similar to those of healthy infants, at this stage KS is unlikely to be suspected, unless on the basis of clinical signs.

Diagnosis in childhood

The school-aged child with KS presents learning disabilities, verbal cognitive deficits, speech difficulties and trouble in spelling, reading and mathematics. A variable degree of difficulty in expressing feelings and socializing is often observed. In this respect, KS males are at higher risk of behavioural disorders and psychiatric problems than the general population (Visootsak et al., 2001). Early detection of the syndrome is important because it allows prompt identification of speech problems and scholastic difficulties that require speech therapy and educational support.

Genital abnormalities associated with KS have been reported sporadically, but they are not mentioned as features of the condition in the reference textbooks. However, the older child or adolescent should be evaluated for signs of delayed or incomplete pubertal development. Physical abnormalities such as a slightly smaller penile length, a lower than normal testicular volume, a taller stature and a reduced upper to lower segment ratio could be the basis for a vague suspicion.

Diagnosing the condition in childhood offers important advantages:

(i) it enables early initiation of language therapy; this is particularly important in helping the child develop more advanced language comprehension and expression skills, in order to prevent social isolation (Visootsak et al., 2001; Girardin et al., 2009);

(ii) it enables the child to be monitored and supported during the peripubertal period;

(iii) it enables better management of these patients during puberty, the preservation of fertility and the initiation of androgen replacement therapy before signs and symptoms of hypogonadism develop (Ferlin et al., 2008).

Diagnosis in puberty

The onset of puberty in KS boys is usually spontaneous and occurs at the expected age. However, from adolescence onwards, the testes become increasingly firm and never reach the size observed in normal adults.

It should be clarified that according to Tanner's definition of puberty in normal subjects, various elements need to be taken into account: pubic hair distribution (P) and morphology and size of external genitals (G). The beginning of puberty is conventionally defined as the attainment of a testicular volume of 4 ml (Marshall and Tanner, 1970). In KS subjects testicular volume generally stops at about 4–5 ml (Wikström

et al., 2004; Aksglæde et al., 2008a, b). However, T production is sufficient for the development of normal primary and secondary sexual characteristics, attainment of puberty stage P5 and completion of epiphyseal closure, even if this tends to take place 3–4 years later than in 46,XY males (Hall et al., 2001). At some stage during pubertal maturation, these compensatory efforts may fail to maintain sufficient circulating T levels and a normal estrogen (E2)/T ratio. This can cause features of eunuchoidism and gynaecomastia to develop. This situation results in sparse facial, body and genital hair. The described incidence of gynaecomastia in KS varies between 50 and 88%, depending on how it is evaluated (Smyth and Bremner, 1998; Ratcliffe, 1999; Visootsak et al., 2001); the differences between the caseloads could be due to the difficulty in distinguishing between gynaecomastia and pseudo-gynaecomastia, unilateral and bilateral, transient and stable and painful and non-painful forms. Gynaecomastia may also be caused by an imbalance between estrogen receptors and androgen receptors, leading to excessive estrogen action, deficient androgen action or a combination of these effects. However, the development of unilateral or bilateral gynaecomastia could be one of the most common signs leading boys and their parents to consult a doctor (Visootsak and Graham, 2006).

During puberty it is easier to come to a correct diagnosis. Certain tubular changes, such as germ cell apoptosis and sclerosis and hyalinization of the seminiferous tubules, take place at an intermediate stage of puberty (Wikström et al., 2004), with a small, firm testis being observed. Physicians who examine patients in the G3 stage could easily suspect this syndrome, as the testicular volume is lower than 8 ml, the lower limit of normal at this stage (Radicioni et al., 2005).

It has recently been demonstrated that gonadal transformation can be followed by US. Several observations demonstrate that prepubertal testes appear identical in affected and unaffected subjects. However, with the onset of puberty, the increase in gonadotrophins alters the echotexture of the testicles, which become inhomogeneous with scattered hypoechoic areas. In this respect, US may suggest KS as early as puberty stage G2, before any signs are seen on clinical examination (Isidori and Lenzi, 2008).

There is a critical time during pubertal development when a sufficient androgen level is necessary for normal bone mineralization. This may explain the gravity of impaired androgen secretion at the critical stage of bone maturation, which leads to such a deficiency of mineralization that subsequent androgen replacement in adults may not be sufficient to restore normal bone mass (Seo et al., 2007). This highlights the importance of a preventive study of body composition and bone mineral content in adolescents with KS before and around the time of puberty.

The hormonal pattern of adolescent KS boys is quite distinctive, showing a progressively increasing difference from euploid boys. Mid-puberty is characterized by a gradual increase in blood LH, which reflects the compensatory feedback mechanism in maintaining sufficient Leydig cell function, and a surge in FSH, which indicates progressive tubular damage, resulting in a hypergonadotropic hormonal pattern by late puberty. Abnormally high concentrations of serum FSH and LH should without doubt prompt the general practitioner to request supplementary investigations, ultimately leading to diagnosis.

At puberty, after activation of the HPG axis the serum T concentration rises and reaches normal or near-normal levels, but as T levels increase, inhibin B levels drop significantly and soon become

undetectable (Christiansen et al., 2003). During this phase, AMH also decreases and a strong inverse nonlinear relationship between T and both AMH and inhibin B has been described (Wikström et al., 2004). During prepuberty and early puberty, this hormonal pattern shows the functional integrity of the Sertoli cells, with normal down-regulation of AMH and inhibin βB-subunit expression that becomes germ cell dependent (Andersson et al., 1998a, b). The dramatic drop in inhibin B demonstrates germ cell apoptosis.

In conclusion, quantification of AMH (Lee et al., 1996; Wikström and Dunkel, 2008) and inhibin B (Aksglæde et al., 2008a, b) could identify the best time for cryopreservation of testicular tissue.

Diagnosis in puberty has the following important advantages:

(i) prevention of the long-term consequences of gonadal insufficiency;
(ii) attainment of the correct peak bone density (Seo et al., 2007);
(iii) development of psychological and sexual identity;
(iv) prevention of infertility, if possible, through cryopreservation of testicular tissue at early puberty and of semen samples containing very low numbers of spermatozoa as soon as possible (Lanfranco et al., 2004; Aksglæde et al., 2008a, b);
(v) prevention of metabolic syndrome signs and symptoms (Ishikawa et al., 2008).

Diagnosis in adulthood

In early adulthood the most frequent problems leading to diagnosis may be infertility and/or azoospermia (Lanfranco et al., 2004). Later in life signs of hormonal testicular failure, such as sexual dysfunction, and co-morbidities such as endocrine, metabolic and cardiovascular diseases, osteoporosis and autoimmune diseases may also lead to diagnosis (Bojesen et al., 2006a).

The adult KS phenotype is typically characterized by tall stature, narrow shoulders, broad hips, sparse body hair, gynaecomastia, small and 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 by late adulthood, leading to severe complications and a more difficult clinical management (Bojesen et al., 2006b). The hormonal patterns and the timing and severity of the androgen deficiency could in part explain the high variability of the clinical picture, with putative alterations in secretory activity, or receptor responsiveness at multiple endocrine sites. In adult patients, physical examination and serum hormone quantification definitely help confirm the diagnosis of hypergonadotropic hypogonadism.

As the testicles are invariably small and firm, US can be used to confirm any substantial changes in their echotexture. They are generally hypoechoic and inhomogeneous, with multiple scattered hypoechoic foci. These changes are often proportionate to the length of exposure to elevated gonadotrophin levels and are the result of the changes beginning during pubertal development (Isidori and Lenzi, 2008; Fig. 1).

In 65–85% of adult KS patients, serum T concentrations progressively fall below the normal reference range, although some cases may retain normal values. In contrast, serum concentrations of E2 and SHBG are generally higher than normal, causing a relative reduction in unbound T (Lanfranco et al., 2004). Serum inhibin B in most adult KS subjects is undetectable (Christiansen et al., 2003).

Investigation of the hormonal pattern and a chromosome analysis is recommended for all patients with small testes attending an infertility clinic (Lanfranco et al., 2004).

With regard to fertility, although a complete absence of spermatozoa is usually observed in the ejaculate, focal spermatogenesis has occasionally been reported. Testicular sperm extraction associated with intra-cytoplasmic sperm injection offers KS patients the possibility of fatherhood (Ramasamy et al., 2009) as reported by Palermo et al. (1998), when the first pregnancy was described. Unsuccessful sperm recovery has an important emotional impact on patients with KS, so predictive factors for sperm recovery need to be established. Clinical and biological parameters such as testicular volume, patient age, serum FSH, LH, T and inhibin B have been studied as predictive factors, but the results of various series were contradictory. Patient age is one of the few parameters that has a significant correlation with successful sperm retrieval (Ferhi et al., 2009; Emre et al., 2006; Ramasamy et al., 2009). KS patients may benefit from early diagnosis, giving them the chance to bank their sperm cells before the onset of azoospermia. It was recently debated whether early treatment of hypogonadism in KS patients affects sperm cell retrieval. Incomplete data from mixed populations (including non-KS subjects) seem to suggest that testosterone treatment may have a negative effect on fertility; however, this is unlikely to apply to KS subjects and further studies are warranted (Ramasamy et al., 2009).

Knowledge of co-morbidity related to KS could help diagnosis. Several cross-sectional studies found a strong correlation between circulating T and cardiovascular risk factors due to the effects of androgens on adipose tissue, insulin sensitivity, endothelial function, vascular tone, atherosclerosis and left ventricular dysfunction. Hypogonadism in KS may lead to an elevated incidence of metabolic syndrome, diabetes mellitus and cardiovascular diseases (Swerdlow et al., 2005; Bojesen et al., 2006a,b; Ishikawa et al., 2008).

Moreover, sex hormones play an important role in the development of muscle, bone and joint mass; KS patients have increased body fat mass and decreased muscle mass (Bojesen et al., 2006b), as well as an increased risk of osteoporosis and a precocious onset of bone diseases: up to 40% of patients may have osteopenia (Breuil and Euller-Ziegler, 2001). A significantly lower BMD has been found in patients who had never received T treatment or when therapy was commenced late in life, suggesting that bone loss is related to androgen deficiency rather than the chromosome abnormality (Seo et al., 2007). For this reason the finding of altered bone density and body composition should prompt further evaluations to investigate KS.

Finally, hypoandrogenism developing later in life in undiagnosed KS could precipitate sexual dysfunction and mood disorders and deeply affect quality of life (QoL). Even though few studies have examined the relationship between KS and QoL, the negative impact of long-term hypogonadism on physical, social, emotional, cognitive and sexual function has been amply described (Maggi et al., 2007).

The advantage of early diagnosis and careful follow-up in KS patients is that it enables earlier detection of any co-morbidities and interventional strategies to be established to counteract late-onset complications. Early lifelong replacement therapy is thus indicated to prevent related symptoms and improve QoL of patients with androgen deficiency. Therapy has a positive effect on mood and
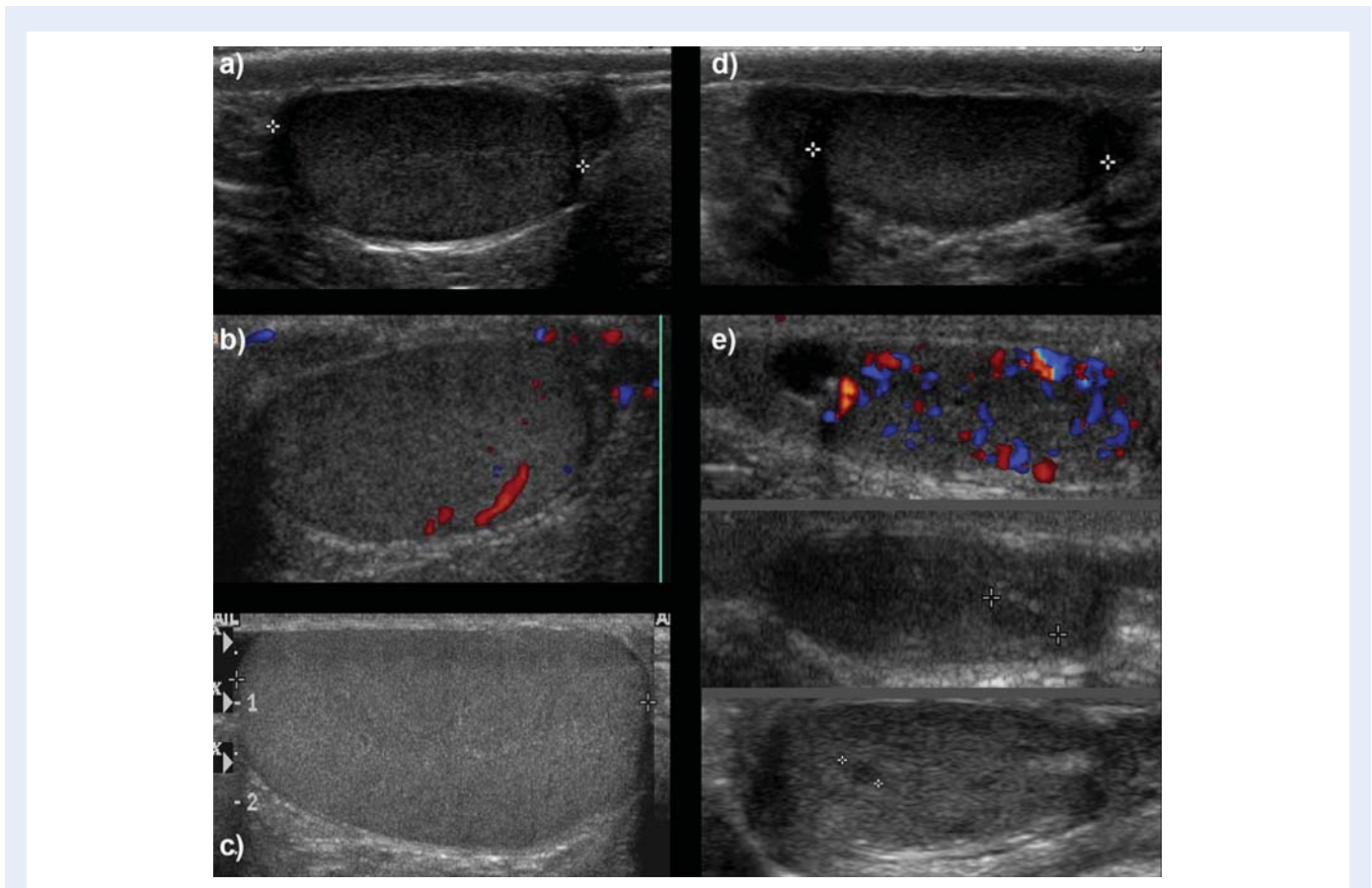
Figure 1 US of the testis during pubertal development. On the left side the normal pattern is represented (**a**, **b** and **c**); on the right side the pattern commonly seen in KS patients is showed (**d** and **e**). Prepubertal testes in KS patients (d) are undistinguishable from normal prepubertal (a). In normal puberty (b), vascular activation occurs, testicular volumes grow and brightness of the echotexture increases progressively to reach the typical aspect of the adult testes (c). In KS, increased gonadotrophin levels and testicular growth failure are responsible for the increased vascularization density (e, upper) and acquisition of a non-homogeneous echotexture. KS adults' testes often develop multiple scattered hypoechoic foci (e, lower).

behaviour, improves goal-directed thinking and self-esteem and reduces fatigue and irritability (Nielsen et al., 1988).

Even though no specific studies have evaluated the economic impact of increased morbidity and mortality in KS, early diagnosis and treatment of androgen deficiency may have a positive effect on the healthcare system (Maggi et al., 2007).

Diagnosis in early adulthood could offer the following advantages:

  (i) adequate prematrimonial family planning;
 (ii) prevention of co-morbidities and late complications;
(iii) improvement of QoL.

Conclusions

This review highlighted the features that may prompt physicians to suspect KS at various ages of presentation (Table II). It has been demonstrated that there are benefits in diagnosis and proper follow-up and treatment at all stages and that 'it is never too late'. Clearly, the earlier the diagnosis is made, the greater the benefits.

In this respect, the most productive strategies are to increase awareness: not only in paediatricians, gynaecologists and fertility doctors, but also in general practitioners.

One of the most common erroneous beliefs is that KS is diagnosed only at puberty. This review shows that various hormonal and clinical features do exist that can lead to diagnosis of a 47,XXY male earlier in life.

It has been shown that KS males may come to medical attention through bone fractures or sexual dysfunctions. For this reason diagnosis could be improved by informing general practitioners of the different pictures that it can present, at all stages in life. Our experiences highlight the importance of strong cooperation between affected males and both family doctors and patient associations.

Many of the advantages of strategies to boost early diagnosis strategies lie in the opportunity to boost the medical care of patients with KS and their QoL.

Finally, more research is needed on the best interventional approaches to prevent complications. A trial addressing the differential contribution of behavioural approaches versus androgen therapy in the prevention of cardio-metabolic complications is currently in progress.

The early use of androgen treatment is still an open issue, as it is strongly related to the preservation of testicular sperm retrieval later in life. Although this aspect will be clarified through future controlled randomized trials, very early diagnosis still seems the most appropriate strategy at the current time, and can be achieved

Table II KS: most prominent signs according to period of life.

AGE	SIGNS
Prenatal age	–
Birth	Cryptorchidism Small penis Scrotum bifidus Hypospadia Fifth finger clinodactily Cleft palate Inguinal hernia Hypotonia
Infancy	Small testes Small penis size Speech and language deficits Long legs and harms
Childhood	Speech and language deficits Learning disabilities Psychological difficulties Behavioral issues
Puberty	Long legs and harms Gynaecomastia Sparse facial, body and pubic hair Gradual increase of FSH and LH
Adulthood	Small and firm testes Gynaecomastia Sparse facial, body and pubic hair Infertility Sexual dysfunction Metabolic syndrome

through increasing awareness of the condition among specialists and general practitioners.

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