



Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Klinefelter syndrome: more than hypogonadism

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ARTICLE INFO

Article history:

Received 3 June 2017

Received in revised form 24 September 2017

Accepted 26 September 2017

Available online xxxx

Keywords:

Klinefelter syndrome

Hypogonadism

Azoospermia

ABSTRACT

Klinefelter syndrome (KS) is the most frequent chromosome disorder in males (1:650 newborn males), defined by 47,XXY karyotype. The classical phenotype is that of a tall male with relatively long legs, small, firm testes and gynecomastia. Azoospermia and infertility are almost inevitably present, but may be overcome by TESE and ICSI. Nevertheless, a broad spectrum of phenotypes has been described and more than 70% of the actually existing KS men may remain undiagnosed throughout their lifespan. Accordingly, hypogonadism is usually not evident until early adulthood and progresses with ageing. KS patients present a series of comorbidities that increase morbidity and mortality by 40%. Such disturbances are the impaired metabolic profile (obesity, dyslipidemia, insulin resistance) and a tendency to thrombosis, which all favor cardiovascular disease. They also present susceptibility for specific neoplasias (breast cancer, extragonadal germ cell tumors), autoimmune diseases as well as osteoporosis and bone fractures. Moreover, KS has been associated with verbal processing and attention deficits as well as social skill impairments, leading KS individuals to academic and professional achievements inferior to those of their peers of comparable socio-economic status. Nevertheless, the majority fall within the average range regarding their intellectual abilities and adaptive functioning. Testosterone replacement therapy (TRT) is the mainstay of treatment in hypogonadal KS patients; however, randomized trials are needed to determine optimal therapeutic regimens and follow-up schedules.

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Contents

1.	Introduction	0
2.	Insights into the Pathogenesis of KS	0
3.	Disease Pattern	0
3.1.	General Features	0
3.2.	Disruption of Spermatogenesis	0
3.3.	Endocrine Dysfunction	0
3.4.	The Spectrum of Neuropsychological Phenotypes	0
3.4.1.	The Cognitive Phenotype	0
3.4.2.	Executive Function.	0
3.4.3.	Behavioral Features and Social Responsiveness	0
4.	Comorbidities and Increased Mortality	0
4.1.	Metabolic Abnormalities	0
4.2.	Cardiovascular Morbidity	0
4.3.	Thrombosis	0
4.4.	KS and Cancer	0
4.4.1.	Breast Cancer Risk among Patients with KS.	0
4.4.2.	Extragenital Germ Cell Tumors	0
4.4.3.	Prostate Cancer	0
4.5.	Bone Density and Risk of Osteoporosis in KS	0
4.6.	Autoimmunity in KS	0

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5. Presence of Supernumerary X vs. Hypogonadism	0
6. Considerations for Management	0
Funding	0
Conflicts of Interest.	0
References.	0

1. Introduction

Klinefelter syndrome (KS) is the most frequent sex chromosome disorder of the male population, accounting for almost 1 in every 650 newborn males and the most frequent form of male hypogonadism. Although KS was first described seventy-five years ago [1], many issues still remain to be elucidated regarding the phenotypic variability observed. The initial report included 9 male patients with gynecomastia, sparse facial and body hair, small testes and azoospermia, whereas it was not before 1959 that the presence of a supernumerary X chromosome was described in the karyotype of KS patients [2]. This breakthrough has allowed the detection and subsequent follow-up of KS patients even since intrauterine life, revealing a broad spectrum of corresponding phenotypes.

In 80–90% of the cases the defining karyotype (47,XXY) is universally observed among the patient's cells, whereas various grades of mosaicism (47,XXY/46,XY) or a structurally abnormal X chromosome (e.g. X isochromosome) may be detected in the remaining cases. Higher-grade X chromosome aneuploidies (e.g. 48,XXX or 48,XXYY polysomies) are often referred to as KS variants; however, their occurrence is far more rare (1:18,000–1:100,000 male births) and they are associated with more severe abnormalities, particularly regarding cognitive and behavioral impairments [3].

According to studies carried out in the USA and later in Denmark, the estimated prevalence of KS among newborns ranges from 153 to 173 per 100,000 males (1 in every 650 newborn males) [4,5]. Currently, 10% of KS cases are detected prenatally, 3% are identified before the age of 20 due to developmental delays or behavioral problems, whereas only 2% are diagnosed due to delayed puberty or gynecomastia [5]. The corresponding proportion of cases diagnosed in adulthood due to hypogonadism or infertility is 17%. These data imply that with the current diagnostic strategies more than two thirds of the actually existing KS men may remain undiagnosed throughout their lifespan [6]. Indeed, comparison of the epidemiological data retrieved from neonatal screening with those obtained from men who were diagnosed at later stages indicates that there is a broad phenotypic spectrum with indolent characteristics that might escape the sensitivity of established diagnostic screening [4]. However, the clinical characteristics stressed in textbooks often lack the required specificity, as was evidenced in a specialized center where the clinical suspicion of KS was confirmed by a corresponding karyotype only in 25% of cases [7].

Infertility is a condition almost inevitably present among KS men, frequently leading to its diagnosis. Consequently, the prevalence of KS rises up to 3–4% among infertile males and surpasses 10% in azoospermic patients [8]. Nevertheless, one should not overlook the fact that, apart from causing hypogonadism, this aneuploidy is also responsible for a multifactorial disease, related to metabolic disorders, with a propensity for particular malignancies and psychosocial problems [9].

2. Insights into the Pathogenesis of KS

The aneuploidy in KS is the result of non-disjunction (e.g. the failure of homologous X chromosomes to separate at anaphase) that can take place both in meiosis I and meiosis II of maternal oogenesis or during meiosis I of paternal spermatogenesis (non-disjunction of paternal

meiosis II results in either 47,XXX or 47,XYY karyotypes) (Fig. 1). The possibility that the supernumerary X chromosome in KS is either of paternal or maternal origin seems to be almost equal. Less frequently (~3%), non-disjunction occurs during mitosis of the early post-zygotic divisions [10].

Advanced maternal age is the only evidence-based risk factor for KS. In particular, maternal age > 40 was associated with a 4-fold increase in the risk of conceiving a Klinefelter fetus as compared to maternal age < 24 [4,11]. The evidence relating advanced paternal age to sex chromosome aneuploidies is less robust [12].

Several theories try to explain the impact of a supernumerary X chromosome on the phenotypic features of KS. In normal females, the transcription of one of the two X chromosomes is randomly inactivated in order to compensate for the minimal gene content of the Y chromosome of the male cells. This inactivated X chromosome can be microscopically visualized in female cells as the Barr body (sex chromatin) [13] and has been shown to be regulated by X inactive specific transcript (XIST) gene. XIST lies on the X chromosome and expresses a long non-coding RNA that literally coats the X chromosome selected for inactivation [14]. In a similar way, in the somatic cells of KS subjects the supernumerary X chromosome is inactivated and, until recently the detection of a Barr body has been used for the diagnosis of KS [7,15]. This inactivation of redundant genetic material is probably the reason that KS is not characterized by severe phenotypic abnormalities compared to other aneuploidies. Nevertheless, it was later demonstrated that actually only approximately 65% of genes on the X chromosome of normal females are inactivated, whereas the remaining 35% may escape this transcription inactivation either globally (20%) or randomly in a cell-type specific manner (15%) [16]. Ultimately, the tissues of females are mosaics of cells with a different X inactivation pattern. Such a mosaicism does not exist in XY subjects, whereas it is present in KS. Existence of X inactivation has been recently supported in KS patients in a population-wide study from Denmark, which related the most frequent comorbidities of KS with corresponding gene deregulations and found that the most abundant differentially expressed and up-regulated gene in KS was XIST [17]. The increased prevalence in KS of several conditions which are usually more common in women than in men (gynoid proportions, breast cancer, and autoimmune diseases) may be explained by such genetic peculiarities [18].

The androgen receptor (AR) gene is among these genes that undergo inactivation, and is implicated in the variability of the phenotype observed among KS patients. Of particular importance is the length of a stretch containing polyglutamine coding (CAG)_n repeats. The shorter this stretch is, the higher is the activity of the receptor. Selective inactivation of the shorter of the two alleles would be associated with a more severe phenotype and diminished response to TRT [18,19]. In a recent study of anthropometric characteristics in KS, the length of the (CAG)_n region correlated positively to arm length, arm span and leg length [20]. Moreover, there are terminal regions on the X and Y chromosomes that exhibit identical haplotypes, which recombine during meiosis and therefore are inherited in an autosomal way. For this reason they are referred to as pseudoautosomal regions (PAR1 and PAR2) [21]. Most of the genes on PAR escape X inactivation, resulting in two active copies in normal males, but in three active copies in KS. Such a gene is the Short-stature Homeobox on chromosome X (SHOX), which is located on PAR1 and, via an increased gene-dose effect, is probably responsible for the tall stature and long legs observed in KS [22]. Nevertheless, it has

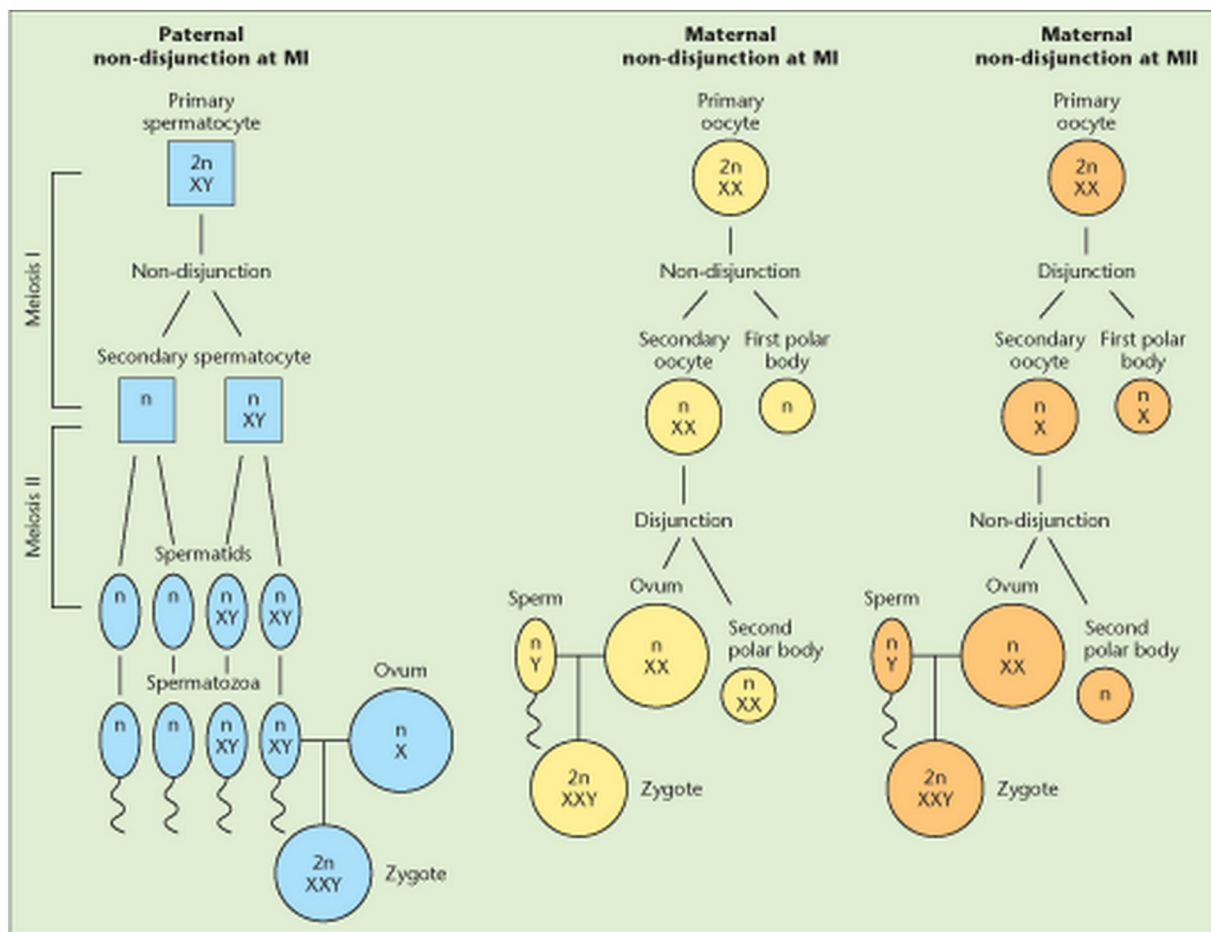


Fig. 1. Pathogenesis of Klinefelter syndrome. Comparisons of events leading to non-disjunction of homologous X chromosomes during paternal or maternal gametogenesis (adapted from ref. [9]).

to be stressed that the majority of genes that have been demonstrated to be deregulated in KS lie outside the X chromosome. This fact implies that the presence of a supernumerary chromosome may affect the expression of several genes throughout the genome [17]. Accordingly, differential methylation of multiple loci relative to both male and female controls has been shown in KS. The majority of these genes are related to energy balance and regulation of immunity [23,24].

3. Disease Pattern

3.1. General Features

The classical clinical description of KS patients is that of a subject of male appearance with very small, firm testes, gynecomastia and tall stature with long legs and a relatively short trunk. The mean height and leg length difference compared with controls has been estimated to be 5.1 and 5.7 cm respectively [20]. Of note, in KS the arm span does not exceed height, in contrast to the eunuchoid proportions observed in other forms of hypogonadism. These features are usually accompanied by signs of hypogonadism, including infertility as diagnosed by azoospermia in more than 90% of cases and severe oligozoospermia in the rest [9,25]. In addition, disorders of testicular descent are observed more frequently than in the general population, ranging from 6 to 25% of cases [26,27]. Nevertheless, it has to be stressed that this classical description of KS may suffer from ascertainment bias, since it is mostly based on patients seeking medical attention and who tend to present the most severe phenotype. Longitudinal studies performed on cohorts diagnosed prenatally have demonstrated that there is a broad spectrum of phenotypes with subjects presenting less severe

signs and symptoms and with others who present such a mild form of the syndrome that they can escape diagnosis and live an apparently normal life [4,28]. Individuals with mosaic KS in particular tend to present milder forms of the syndrome, both with respect to testosterone deficiency and sperm production [29].

3.2. Disruption of Spermatogenesis

Testicular atrophy and dysfunction is a constant feature of KS patients affecting primarily the tubular compartment and spermatogenesis. Markers that reflect the integrity of the blastic epithelium such as serum inhibin B, and anti-müllerian hormone start to decrease already from puberty and are undetectable until adulthood [30,31], whereas follicular stimulating hormone (FSH) tends to increase [32]. Concerning testicular volume, an initial increase during pubertal onset has been reported, which, however soon ceases, followed by rapid shrinkage and establishment of the hallmark of small and firm testes [33]. Moreover, the degeneration of the seminiferous epithelium has been associated with a reduction in testicular vascular density and flow [34]. Nevertheless, isolated foci with intact spermatogenesis can be present in a selected number of KS patients. These tubuli may be obtained by biopsy and testicular sperm extraction (TESE) may result in viable sperm for intracytoplasmic sperm injection (ICSI) and pregnancies [6].

3.3. Endocrine Dysfunction

Premature failure of Leydig cell function is observed in KS subjects, however, at later stages. Thus, despite detecting a compensational elevation of luteinizing hormone (LH) [32] and low INSL3 levels already

from early puberty [35], hypogonadism is usually not evident before early adulthood [36]. In line with this, congenital anomalies of the genital organs driven by hypogonadism such as micropenis, bifid scrotum or hypospadias, although more frequent in KS than in the general population, have an overall low prevalence [26]. Accordingly, the temporary surge in gonadotropins observed in early infancy, also known as “mini-puberty”, is usually present with FSH levels peaking at 2–3 months of age, followed by a subsequent rapid decline [37]. This pattern agrees with that observed in 46,XY males and contrasts with the prolonged elevation observed in females [38].

There is a paucity of data regarding sex steroid secretion in KS patients during childhood [36], however, one should anticipate similarities to normal boys as this is in general a period of minimal Leydig cell function. On the other hand, puberty in KS subjects seem to occur in a timely fashion and follows a more or less normal course, with testosterone levels sufficient for satisfactory development of the secondary sexual characteristics and able to trigger a growth spurt [8,39]. Nevertheless, T levels are usually suboptimal to promote epiphyseal closure as in eugonadal boys, a fact that may contribute to the preponderance of tall stature among KS subjects and exacerbate the ratio between the trunk and the disproportionately long lower extremities [20].

As KS patients progress to adulthood, hypogonadism tends to become more prevalent. Serum T levels fall into the low normal range beginning in early adulthood and eventually 65–85% of KS patients present overt hypogonadism after the age of 25 [36]. Moreover, the compensational elevation of LH activates aromatase, causing a relative increase in estrogen levels, which may contribute to the development of gynecomastia [40]. The great variability that characterizes the phenotype of KS is also evident regarding the virilization of adult patients, e.g. penile size, sexual hair density and fat distribution. This variability may be attributed to the magnitude of androgen deficiency or inherent differences in the sensitivity of AR and, particularly, the length of the CAG repeats polymorphism [18]. Recent studies have suggested that the observed low testosterone levels of KS are not the result of diminished production, but of defective release into the testicular bloodstream due to aberrations of the testicular vasculature [41].

3.4. The Spectrum of Neuropsychological Phenotypes

KS has been traditionally associated with a particular neuropsychological phenotype, including deficits in specific domains of cognition, mainly regarding verbal processing abilities and adaptation problems as well as attention deficits and social skill impairments [42,43]. As a consequence, individuals with KS are prone to suffering from learning difficulties and their achievements regarding academic performance and professional status are reported to be inferior to those of their peers of comparable socio-economic status [44]. However, it should be underscored that much of the related literature has been based on earlier studies, when only KS patients presenting more severe phenotypes could be identified [45]. In order to avoid such bias, subsequent studies were carried out on cohorts of newborns screened for 46,XXY and followed up prospectively until young adulthood [46,47]. These and subsequent studies included a broader spectrum of KS cases and demonstrated that the majority of KS individuals fall within the average range concerning their intellectual abilities, behavior, attention, social skills and adaptive functioning [18,48]. Nevertheless, even these studies have methodological flaws as boys identified in this way usually have undergone pro-active tutoring intervention that has probably modified their final outcome.

Neuroimaging studies have shown characteristic structural and functioning differences between KS individuals and age/educational level matched controls. These differences concern mainly the gray matter of the frontal lobe, which is crucially involved in executive functioning as well as the amygdala and the hippocampus, regions associated with social responsiveness [49]. A more recent voxel-based morphometric study demonstrated a decrease of the total brain volume in KS,

which concerned both the gray and white matter, however correlation with data from neuropsychological test scores did not reveal significant associations between these differences and cognitive impairment [50].

3.4.1. The Cognitive Phenotype

The overall cognitive ability of patients with KS falls in the average to low average range [43,46,48]. In particular, the distribution of the estimated Full-Scale IQ (FSIQ) shows a significant overlap with controls, but with a small leftward shift of the curve with a mean FSIQ around 90. A mild impairment of cognitive development is found in 4.2% of cases and it is mainly driven by deficits in the verbal conceptual domain (Verbal IQ, VIQ), rather than the nonverbal or spatial domain (Performance IQ, PIQ) [42]. The VIQ deficit consists of delayed expressive as well as receptive language skills, starting at an early developmental stage and may result in academic difficulties which surpass the area of literacy and may expand in math calculation and problem solving [51,52]. Academic difficulties may also persist in adulthood, however, over time, an increase is observed in VIQ relative to PIQ [53].

3.4.2. Executive Function

Despite their average cognitive functioning, it has been demonstrated that occupational achievements of KS individuals still fall behind compared to their peers, suggesting that normal cognition is not adequate to compete in a complex social and working environment [18,54]. The term “executive function” (EF) has been introduced to describe the ability to coordinate a variety of higher order processes in order to achieve goal-directed problem-solving [55]. Successful EF often requires the control of behavioral and emotional responses as well as the ability to hold attention while completing a specific task. Attention deficits have been demonstrated in KS and increased rates of Attention-Deficit/Hyperactivity Disorder (ADHD) have been reported in cohorts of KS children [48,56]. In a recent study of 57 KS children and adolescents, 36% met the criteria for a diagnosis of ADHD based on parent report, with the vast majority presenting predominantly inattentive symptoms without significant hyperactivity or impulsivity, suggesting that attention deficits may probably emerge as a consequence of verbal processing difficulties [56]. Moreover, such problems are usually detected in childhood and abate as KS individuals progress to adulthood with subsequent improvement of VIQ [52].

3.4.3. Behavioral Features and Social Responsiveness

KS has been steadily associated with socio-emotional impairment as 32% of patients have been reported to present anxiety and 24% depressive disorders [57]. Nevertheless, the behavioral and emotional functioning of KS individuals generally falls in the average range regarding both internalizing and externalizing behaviors [42]. Particularly regarding internalizing behaviors such as anxiety, depression and withdrawal, a recent study of KS children reports that at least 25% of individuals obtain scores in the at-risk or clinically significant ranges for each domain, with this figure surpassing 50% regarding the withdrawal domain [56]. Concerning externalizing behaviors, over 25% of KS children obtained scores in the mild-to-moderate or severe range in most domains of social responsiveness. These scores indicate that KS individuals may present an increased risk for internalizing distress and for social difficulties and raise the question if a significant proportion of KS patients meet the criteria for an autism spectrum disorder (ASD). According to recent studies, the prevalence of ASD in KS ranges from 5 to 48% depending on the population studied and the tools used for assessment [49,56,58] and KS individuals show difficulties in cognitive flexibility, reduced empathic understanding and decreased visual fixation to the eye region [58,59].

4. Comorbidities and Increased Mortality

Apart from the apparent phenotypical aberrations, KS is accompanied by a series of comorbidities leading to more frequent hospitalization and

Table 1

Comorbidities in KS and related mortality, followed by evidence for the possible etiopathogenesis (attributed to supernumerary X chromosome or hypogonadism or both) and the effect of testosterone replacement therapy (TRT) on each specific manifestation/comorbidity (modified from ref. [6]).

	Incidence (%)	Mortality (SMR)	Etiopathogenesis	Effects of TRT
Verbal disorders	70–80	–	Extra X chromosome associated with:	<ul style="list-style-type: none"> Improvement of developmental and behavioral issues Partial amelioration [130,132]
Mental retardation	4.2	–		
Metabolic syndrome	44	–	<ul style="list-style-type: none"> Abnormal structure of brain areas [53] Executive and social dysfunction [117] Prevalence of T2 diabetes and MetS is higher in KS than in other forms of hypogonadism [69] Obesity precedes puberty [25] No significant relationship has been demonstrated between testosterone levels and bone mass [106] 	<ul style="list-style-type: none"> Improvement of body composition and measures of cardiometabolic risk [131]
Type 2 diabetes	10	6		
Osteoporosis	10	–		
Osteopenia	40	–		
Hip fracture	?	39		
Gynecomastia	38	–	<ul style="list-style-type: none"> Not present before puberty – associated with hypogonadism 	<ul style="list-style-type: none"> Does not seem to normalize BMD in KS adults Prevents bone loss in patients <20 years [107]
Maldescended testes	27	–		
Thrombosis	4.7	8	<ul style="list-style-type: none"> Increased 2D:4D suggests a relative androgen deficiency in the intrauterine milieu [114] 	<ul style="list-style-type: none"> Improved if gynecomastia is of recent onset
Pulmonary embolism	2.3	6		
			<ul style="list-style-type: none"> Combination of imbalance between thrombosis and hemostasis with increased platelet aggregation rate [85,86] Gynecomastia does not present a risk factor [94] 	<ul style="list-style-type: none"> No effect Not assessed in KS
Mediastinal tumors	0.4	–		
Breast cancer	0.3	29		

contributing to a significant increase in mortality risk by almost 50% (Hazard ratio (HR) for all-cause mortality = 1.40–1.70). The lifespan of KS patients appears to be truncated by approximately 2.1 years [60]. In a recent study that scrutinized hospital records of KS patients, a total of 78 significant comorbidities were identified [17]. The main disturbances associated with increased morbidity and elevated mortality risk are the impaired metabolic risk profiles characterized by an increased prevalence of type 2 diabetes and a propensity to thrombosis/embolism, both of which are risk factors for cardiovascular (CV) disease [61]. Other conditions increasing the morbidity of KS patients are osteoporosis and bone fractures, as well as the higher prevalence of specific types of cancer, autoimmune diseases, infectious diseases and epilepsy [62](Table 1). Last but not least, the increased mortality of KS individuals seems to be affected by their lower socio-economic achievements [63].

4.1. Metabolic Abnormalities

Epidemiological studies have demonstrated a 5-fold higher prevalence of metabolic syndrome (MetS) among KS males compared to age-matched controls [64]. This percentage is higher even when compared to patients presenting hypogonadism of other etiology (46% vs. 27%) [65]. The role of abdominal obesity is central in the definition of MetS and it has been consistently shown to be more prevalent in adults with KS [20]. The excess of truncal fat causes insulin resistance, which in turn impairs carbohydrate metabolism and is probably responsible for the increased incidence of diabetes mellitus (DM) found in KS. Thus, a KS patient may present an increased relative risk of between 1.64 and 7.07 to develop DM during his lifetime [62,66], which is also higher when compared to hypogonadal patients of other etiology [67]. Similarly, an increased prevalence of dyslipidemia exists, which is mostly of the mixed type; e.g. consisting of high levels of total and low-density lipoprotein (LDL) cholesterol as well as triglycerides [68,69].

4.2. Cardiovascular Morbidity

The role of CV morbidity is pivotal for the increased overall morbidity and mortality observed in KS [61]. Interestingly, data on the prevalence of coronary artery disease are conflicting and definitely not as frequent as might have been expected in relation to the reported metabolic derangement [62]. Moreover, alterations in biomarkers of CV dysfunction such as increased CRP levels [64] have not always been confirmed by other parameters such as carotid intima media thickness or flow-mediated dilation of the brachial artery [34,70].

Consideration of these findings should be cautious as the same study by Foresta et al. has shown that the main arteries of KS subjects are smaller in diameter compared to controls independently of hormonal status, metabolic parameters, anthropometric measures and the length of CAG(n) repeats. Moreover, of note KS is not associated with arterial hypertension, a finding that has been attributed to the protective role of normal levels of adiponectin that these patients have despite the presence of MetS [66].

In search of factors that may contribute to increased CV morbidity one may note the impaired cardiopulmonary performance occasionally observed in KS. This may be a result of subclinical systolic and diastolic dysfunction, especially when associated with chronotropic incompetence i.e. the inability of the heart to increase its rate as a response to increased activity or demand [70,71]. This anomaly is associated with exercise intolerance as evident by a reduced peak oxygen uptake (VO₂ max) in KS patients and is an independent predictor of major adverse cardiovascular events and overall mortality in an asymptomatic population [70,72]. Moreover, resting ECG evaluation may reveal a QTc interval shorter than in euploid men and women, which may be associated with fatal arrhythmias [73,74]. This phenomenon is more pronounced in KS men with paternal origin of the excessive X-chromosome and is related to the presence of metabolic syndrome. KS patients also appear to be at a higher risk of congenital heart diseases (HR 4.71) [60,75], which may lead to higher mortality (SRM 7.3%) [62]. Several abnormalities have been reported to co-exist with KS, including a higher prevalence of mitral valve prolapse, atrial and ventricular septal defects with patent ductus arteriosus, bilateral hypoplasia of the internal carotid arteries with dilatation of the vertebral arteries and anomalous pulmonary venous connection [74,76].

4.3. Thrombosis

KS patients exhibit an increased risk of venous thromboembolism, consisting of both minor phenotypic features such as recurrent venous ulcers (7–13%) and venous insufficiency (20%) [77], as well as major events like deep venous thrombosis (DVT) and pulmonary embolism (PE), with a HR of 6.63 for DVT and 3.60 for PE respectively [60,78]. Regarding the risk of arterial thromboembolism it has also been shown to be increased in KS, though the data are robust solely regarding cerebrovascular disease (SMR 2.2) in comparison to CAD (SMR 0.7–2.12) [62]. This proneness to thrombosis is probably the result of a combination of increased plasminogen activator inhibitor-1 (PAI-1) activity with deficits in C and S proteins, antithrombin III as well as factor V Leiden

alterations, which account for an imbalance between thrombosis and hemostasis. These aberrations may be aggravated by an increased platelet aggregation rate [79,80]. Possible confounders in the assessment of coagulation/fibrinolysis function in KS are the relatively high levels of circulating estrogens and the effects of TRT per se [81].

4.4. KS and Cancer

There is no significant difference between the overall cancer risk in KS and that of the general male population; however, there is a propensity for specific types of malignancies such as germ cell tumors and breast cancer [82].

4.4.1. Breast Cancer Risk among Patients with KS

The incidence of breast cancer, an otherwise rare type of cancer among males (1:100,000) is increased up to 30-fold in KS [83]. A recent review of the literature including case series and epidemiologic studies that have evaluated breast cancer risk among KS patients has estimated this risk to be 19-fold [84]. This figure is lower than previously believed and still 30% inferior to the corresponding risk among females, in contrast to older studies suggesting a propensity to breast cancer equal to that of females [61]. This figure might be even lower if KS cases identified after a diagnosis of male breast cancer were excluded [85]. Nevertheless, KS remains the strongest independent risk factor for male breast cancer and this risk is particularly elevated among patients with mosaic karyotypes [86]. Breast cancer is a late event in the evolution of the KS phenotype as it is usually diagnosed in the 7th decade of life (range 58–82) [87] and is associated with increased mortality compared to normal men [84]. Relatively elevated levels of estrogens have been implicated in the pathogenesis of breast cancer; however gynecomastia does not present a risk factor despite being observed in almost 40% of KS patients [88]. On the other hand, long-term TRT has also been associated with an increased risk for male breast cancer [89], whereas genetic reasons attributed to the supernumerary X chromosome cannot be excluded.

4.4.2. Extragonadal Germ Cell Tumors

A higher prevalence (30–40 fold) of extragonadal germ cell tumors, primarily of the non-seminomatous subtype, is observed in KS and they tend to present at a younger age (15–30 years) than in normal males [90]. The most frequent localization is the mediastinum, and the most frequent type of tumor is teratoma associated with yolk sac tumor, followed by choriocarcinoma [91]. Several theories have been suggested for the origin of these tumors: abnormal migration of germ cells due to abnormalities of the embryonic hormonal milieu has been considered, whereas other investigators suggest a neoplastic disorder of primordial thymic cells [92].

4.4.3. Prostate Cancer

In contrast to the aforementioned malignancies, the prevalence of prostate cancer (SIR 0.24) as well as associated mortality have been reported to be lower in KS compared to 46,XY [85,93]. This may be attributed to the presence of hypogonadism, although recent studies cast doubt on the correlation between androgen levels and prostatic malignancies [94]. Moreover, in KS the levels of prostate specific antigen and prostate volume fall into the lower normal range regardless of the presence of TRT [95].

Nonetheless, an increased risk for hematological malignancies has been reported in KS [82]. Recent data retrieved from the Swedish Cancer Register confirm this high propensity for hematological malignancies (SIR 2.72) in KS, especially leukemia and Non-Hodgkin lymphoma (SIR 3.62 and 3.02 respectively), while the risk for solid tumors appears to be decreased (SIR 0.66) [85]. This peculiarity is proposed to be associated with the higher rate of gene fusions and chromosome translocations observed in the dividing cells of patients with excessive

chromosomes, and which are common in hematological malignancies, whereas they are relatively rare in solid tumors [96].

4.5. Bone Density and Risk of Osteoporosis in KS

KS patients present an increased prevalence of metabolic bone disorders, particularly reduced bone mineral density (BMD). A recent study found the combined proportion of KS patients with osteopenia and/or osteoporosis to be 42.5%, a figure which is 8 times more frequent than in 46,XY age-matched males [97]. Furthermore, two studies have assessed volumetric BMD, microarchitecture and estimated bone strength of the radius and the tibia, using quantitative computed tomography (pQCT). Their findings regarding volumetric BMD are divergent, however both demonstrated reduced tibial cortical area and reduced trabecular density that in combination compromise the estimated bone strength [98,99]. Reduced BMD is attributed to increased bone turnover and is accompanied by increased risk of bone fractures, especially regarding the femoral area [100]. Moreover, hip fractures in KS are associated with an elevated mortality rate [60,101]. Although hypogonadism is a condition strongly related to BMD reduction, this may not be the case in KS as no significant relationship has been demonstrated between testosterone levels and bone mass [97]. In addition, TRT does not seem to normalize BMD in KS adults, in contrast to patients younger than 20 years old who seem to gain significant benefits from such treatment [100,102]. These findings imply that variable degrees of hypogonadism occurring during the critical pubertal stages of bone development, might account for an equally variable accrual of peak bone mass that cannot be modified later in adulthood. Finally, KS patients seem to be particularly prone to 25OH-vitamin D fluctuations, as they present insufficient levels more frequently than controls, whereas vitamin D repletion has been demonstrated to be superior to TRT in improving BMD [103].

4.6. Autoimmunity in KS

As early as the 1960s and 1970s, several reports described the concurrence of KS with autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), juvenile idiopathic arthritis, psoriatic arthritis, polymyositis/dermatomyositis, systemic sclerosis and mixed connective tissue disease [104,105], however, there is no substantial systematic evidence. A recent retrospective study from England has demonstrated in KS a significant increased risk over controls regarding Addison's disease (RR 11.7), diabetes mellitus type 1 (RR 6.1), multiple sclerosis (RR 4.3), acquired hypothyroidism (RR 2.7), RA (RR 3.3), Sjogren's syndrome (RR 19.3) and SLE (18.1) [106]. Interestingly, the majority of the above conditions are more frequent among females, whereas no increased risk was found regarding autoimmune diseases that predominantly occur in men, such as ankylosing spondylitis and Goodpasture's syndrome. Regarding hypothyroidism, it has to be noted that an increased presence of thyroid antibodies is debated in KS, whereas there are studies suggesting a central component of hypothyroidism [107].

5. Presence of Supernumerary X vs. Hypogonadism

As evident from the preceding description, phenotypic heterogeneity is a significant feature of KS, while its manifestations can be attributed either to the aneuploidy and the impact of increased gene dosage by the supernumerary X or the presence of hypogonadism per se [108]. Distinguishing the relative impact of each component on the phenotypic expression of KS is a difficult task that is complicated by the fact that ageing modifies many aspects of the syndrome, as symptoms and comorbidities accumulate.

Since in KS, hypogonadism usually emerges in early adulthood, it is justified to consider signs and symptoms that are already present before puberty and prior to the onset of the hormonal derangement, as sequels

of aneuploidy rather than hypogonadism per se. Accordingly, the particular habitus with disproportionately long legs accompanied by abdominal obesity is observable since infancy, suggesting a genetic base [20]. In contrast, there are studies that have demonstrated an increased second to fourth finger ratio in KS patients, similar to that observed in females, suggesting a relative androgen deficiency in the intrauterine milieu that may have impact on KS already from fetal life [109]. The same conclusion may be drawn by the higher prevalence of anomalies of the development of the genital organs such as cryptorchidism and hypospadias [27]. Indeed there are studies demonstrating evidence of androgen deficiency already from early infancy in KS [110].

The fact that verbal and attention deficits become evident in early childhood also suggests a genetic origin. Moreover, most of the cognitive traits observed in KS are also seen in 47,XXX trisomy which is not typically associated with hypogonadism, as well as in high-grade X chromosome aneuploidies, with a severity that depends on the number of supernumerary X chromosomes present in the karyotype [42,111,112]. This concept is supported by evidence from neuroanatomical studies revealing differences in temporal and frontal lobe volume between KS prepubertal children and controls [113]. Comparisons between KS, Turner syndrome and controls suggest that this effect depends on the presence of an additional sex chromosome in a linear dose-dependent fashion [114]. Such differences have also been identified in genomic level, by comparing microarray expression profiles that show deregulation of X-linked genes correlated to verbal cognition [115]. Additional evidence from mouse models of KS that show behavioral traits similar to human KS demonstrate that differences from wild type mice persist after castration and do not improve with TRT [116]. Nonetheless, cognitive profiles of mosaic KS do not differ significantly from patients with classical KS, while androgen levels at early developmental stages have been shown to play a role in neurodevelopment and brain lateralization, making it difficult to distinguish hormonal from genomic contribution in the phenotype [46].

Concerning metabolic abnormalities in KS, the role of hypogonadism is central as it may perpetuate a vicious circle of insulin resistance, dyslipidemia and obesity, which in turn is responsible for a progression of hypogonadism itself [65]. Nevertheless, abdominal obesity usually precedes puberty and in KS subjects is only partially ameliorated by TRT [70,99], suggesting that a genetic component is also to be considered. There is a body of evidence that this relative resistance to TRT may be attributed to a high prevalence of aberrations of the CAG polymorphism of the AR gene reported in KS [18].

6. Considerations for Management

The challenges that a physician has to face in the management of KS may even emerge during intrauterine life, as the diagnosis is often established by prenatal screening, a situation requiring thorough genetic counseling of the parents. The recent introduction of non-invasive prenatal screening is anticipated to increase the number of couples seeking counseling and it is largely admitted that their decision whether to abort or not relies heavily on the information received during counseling [117–119]. The termination rate in KS pregnancies varies from 11.6% to 87.5% in different countries. In certain societies, the way research has changed our understanding of the natural history and prognosis of this condition has led to a significant decrease in the rate of termination [119,120]; whereas in other societies it still remains high, depending on local traditions, religious beliefs and legislation [121].

Subsequently, the management of KS children warrants the collaboration of a multidisciplinary team consisting of pediatric endocrinologists as well as developmental and behavioral specialists, in order to ameliorate the developmental defects of early life [56]. In addition, an initial echocardiographic study should be offered in order to reveal congenital cardiovascular abnormalities [68]. As the boy transits to adulthood, follow-up should be conveyed to a specialized team consisting

of adult endocrinologists/andrologists, cardiologists and occasionally psychologists to aid in facing the challenges of adult life.

In the presence of hypogonadism, TRT remains the medical treatment of choice until robust evidence and feasible therapies are found to compensate for the genetic defects of KS. Although there is evidence that TRT in KS is not as effective as in 46,XY hypogonadal males, at least regarding body proportions and BMD [70,102], the association of hypogonadism with both increased morbidity and mortality is well established and should be addressed accordingly [122,123]. Besides, many of the anticipated benefits of TRT such as improvement in energy level/stamina, attention span, mood, measures of cardiometabolic health and general well-being are also observed in young KS patients [124,125]. Moreover, it has been demonstrated that early TRT may have a beneficial effect on KS boys' developmental and behavioral issues without serious adverse effects [126,127]; however it should be stressed that TRT is not a "cure" for the neurodevelopmental deficits of KS that may exist due to the possible effects of the extra X chromosome on brain development [117]. In contrast, there is concern about the negative impact TRT might have on the risk of comorbidities prevalent among patients with KS, such as venous thromboembolism and obstructive sleep apnea [128,129]. Since specific data regarding therapeutic targets are not available for KS patients, the general guidelines on TRT should be employed [130]; however, randomized controlled trials are needed to evaluate the efficacy of TRT on different aspects of the syndrome, to determine optimal dose regimens and to assess the suitability of the available testosterone formulations for different subgroups of KS patients [117].

As TRT would suppress any spermatogenesis, the option and chances of TESE should be discussed with the patient before initiation of TRT. If paternity is desired by a patient who is already on TRT this therapy has to be suspended for at least 6 months to allow spermatogenesis to recover [131].

There are no universally accepted guidelines on the follow-up of KS patients. Apart from TRT that should be monitored according to current guidelines for hypogonadism, including T, PSA and hematocrit evaluation [132], patients should be followed up for their related comorbidities. Regarding CV morbidity, the initial evaluation has been proposed to include risk assessment for MetS and thromboembolic disease as well as echocardiography focused on systolic and diastolic dysfunction. The subsequent follow-up should be based on the relevant findings [68]. Concerning screening for related malignancies, the level of absolute risk of breast cancer does not justify regular examination with mammography, but warrants monthly breast self-examination and periodic physical examination by a specialized physician [84], while a recent Italian consensus [133] suggests bi-annual chest X-ray to address the risk for extragonadal germ cell tumors. The same consensus proposes bi-annual assessment of BMD and 25OH-vitamin D status to address the risk of osteoporosis.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

None.

Acknowledgements

We express our acknowledgements to Susan Nieschlag for her language editing of the manuscript.

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