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European Acedemy of Andrology (EAA) GUIDELINES ON KLINEFELTER SYNDROME

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Abstract

Background: Knowledge about Klinefelter Syndrome (KS) has increased substantially since its first description almost 80 years ago. A variety of treatment options concerning the spectrum of symptoms associated with KS exists, also regarding aspects beyond testicular dysfunction. Nevertheless, the diagnostic rate is still low in relation to prevalence and no international guidelines are available for KS.

Objective: To create the first European Academy of Andrology (EAA) guidelines on KS.

Methods: An expert group of academicians appointed by the EAA generated a consensus guideline according to the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system.

Results: Clinical features are highly variable among patients with KS, although common characteristics are severely attenuated spermatogenesis and Leydig cell impairment, resulting in azoospermia and hypergonadotropic hypogonadism. In addition, various manifestations of neurocognitive and psycho-social phenotypes have been described as well as an increased prevalence of adverse cardiovascular, metabolic and bone-related conditions which might explain the increased morbidity/mortality in KS. Moreover, compared to the general male population, a higher prevalence of dental, coagulation and autoimmune disorders is likely to exist in patients with KS. Both genetic and epigenetic effects due to the supernumerary X- chromosome as well as testosterone deficiency contribute to this pathological pattern. The majority of patients with KS is diagnosed during adulthood, but symptoms can already become obvious during infancy, childhood or adolescence. The paediatric and juvenile patients with KS require specific attention regarding their development and fertility.

Conclusion: These guidelines provide recommendations and suggestions to care for patients with KS in various developmental stages ranging from childhood and adolescence to adulthood. This advice is based on recent research data and respective evaluations as well as validations performed by a group of experts.

List of Recommendations and Suggestions

GENETIC ISSUES

1. We recommend that a prenatal diagnosis of KS is confirmed on a chromosome analysis on peripheral blood postnatally (1,⊕⊕⊕◯).
2. We recommend conventional karyotyping on peripheral blood cells for diagnosis of KS (1,⊕⊕⊕◯).
3. We recommend that patients with KS and/or their parents are offered genetic counselling; prenatal counselling should be non-directive (1,⊕⊕⊕◯).
4. We recommend karyotype analysis for detecting KS in men with non-obstructive azoospermia and (severe) oligozoospermia (total sperm count < 10 x 10^6/ejaculate or sperm concentration <5 x 10^6/ml (1,⊕⊕⊕◯).
5. We recommend karyotype analysis for detecting KS in men with primary hypogonadism (low serum levels of testosterone) and elevated serum levels of gonadotropins (LH and FSH) combined with small testicular volumes (<5 ml per testis) (1,⊕⊕⊕◯).

CHILDREN AND PRE-PUBERTAL BOYS WITH KS

6. We suggest karyotype analysis for detecting KS in boys born with cryptorchidism, especially the bilateral forms, who do not experience spontaneous descent of the testes at the first year (2,⊕⊕◯◯).
7. We recommend to treat cryptorchidism in children with KS according to the current treatment guidelines in children without KS (1,⊕⊕⊕◯).
8. We recommend general physical examinations in pre-pubertal children with KS including a testicular evaluation. These should be performed biennially or as deemed as appropriate. Suspected neurological or psychiatric deficits should be examined by respective specialists (1,⊕⊕⊕◯).

9. We suggest determination of LH and testosterone during the first 2-3 months after birth in children with prenatal diagnosis of KS when it might have a therapeutic consequence (i.e. diagnosis of micro-penis) (2,⊕○○○).
10. We recommend against testicular tissue cryopreservation or spermatogonial stem cell retrieval in pre-pubertal children with KS (1,⊕⊕⊕○).
11. We recommend against testosterone supplementation during early childhood in all patients with KS except in cases of micro-penis (1,⊕⊕⊕○).
12. We suggest measurement of height according to centiles or standard deviation scores as well as body proportions and determinations of bone age in pre-pubertal children with KS depending on individual growth patterns (2,⊕⊕○○).
13. We suggest determination of vitamin D blood levels and adequate vitamin D and calcium supplementation in pre-pubertal children with KS (2,⊕○○○).
14. We suggest assessment of bone mineral status during childhood in case of vitamin D deficiency biennially in patients with KS (DXA scan, size-corrected determinations using a three-step-method are required) (2,⊕○○○).
15. We recommend measurement of weight using centiles or standard deviation scores in pre-pubertal children with KS (1,⊕⊕⊕○).
16. We recommend against testosterone treatment in infants and pre-pubertal boys with KS (1,⊕⊕⊕○).
17. We recommend speech therapist control and therapy, monitoring learning disabilities, social training and psychological support, in pre-pubertal children with KS if needed (1,⊕⊕⊕○).

ADOLESCENTS WITH KS

18. We recommend that information on fertility issues is given to adolescent patients with KS and, if deemed adequate, his parents. There is no level of recommendation, as this can be considered good clinical practice.
19. We suggest testicular ultrasound during puberty of patients with KS and regularly at follow up visits (2,⊕○○○).

20. We suggest semen collection in adolescents with KS after careful information and assessment of the wish of the patient and cryopreservation if motile sperm are present (2,⊕⊕○○).
21. We suggest that adolescents with KS might undergo a testicular biopsy for testicular sperm extraction (TESE) either using multifocal (standard TESE) or microdissection-TESE (mTESE) and consequent sperm cryopreservation in selected cases requiring specific counselling, provided their physical and mental maturity is apt for this decision (2,⊕⊕○○).
22. We recommend assessment of Tanner stages, pubertal development, measurement of testosterone and gonadotropins, signs and symptoms of hypogonadism, height, weight, waist circumference and body proportions starting prior to the predicted start of puberty in patients with KS and at individually determined intervals thereafter (the time-window for the start of puberty does not differ from boys with a karyotype of 46,XY) (1,⊕⊕⊕○).
23. We recommend testosterone supplementation in case of delayed puberty and/or signs and symptoms of hypogonadism associated with low-normal testosterone and supra-normal LH serum concentrations (LH > 2 SD according to age-related references), after the fertility issues have been addressed (see above) (1,⊕⊕⊕○).
24. We suggest against testosterone therapy in adolescents with KS with compensated hypergonadotropic hypogonadism (2,⊕⊕○○).
25. We recommend speech therapist control, monitoring educational problems, social training and psychological support in adolescents with KS, if needed (1 ⊕⊕⊕○).

ADULTS WITH KS

26. We recommend initiation of testosterone substitution in patients with KS with hypogonadism as diagnosed according to established guidelines on hypogonadism, if possible once fertility issues have been addressed (1,⊕⊕⊕○).
27. We recommend that testosterone substitution in patients with KS should follow the established guidelines on hypogonadism using the usually suggested monitoring intervals for clinical assessment, safety parameters (hematocrit, PSA, other) and dose titration (1,⊕⊕⊕○).
28. We recommend endocrine evaluation every 12 months in adult patients with KS who are not on testosterone substitution (1,⊕⊕○○).

29. We recommend semen analysis and sperm cryopreservation in all adult patients with KS and a wish for paternity (1,⊕⊕⊕◯).
30. We recommend that all adult patients with KS and confirmed azoospermia and a current or putative future wish for paternity undergo a testicular biopsy for testicular sperm extraction (TESE) either using multifocal (standard TESE) or microdissection-TESE (mTESE) and consequent sperm cryopreservation (1,⊕⊕⊕◯).
31. We suggest against starting testosterone replacement therapy in patients with KS when a TESE is planned, due to the possible suppression of gonadotropins and further suppression of remnant spermatogenesis (2,⊕⊕◯◯)
32. We recommend education on lifestyle and yearly assessment of weight, waist circumference, blood pressure, fasting glucose, HbA1c and lipid profile and adequate treatment in all patients with KS (1,⊕⊕⊕◯).
33. We suggest thrombosis prophylaxis prior to long-term flights or exposure to other risks in patients with KS to attenuate the increased risk for deep vein thrombosis and/or pulmonary embolism (2,⊕⊕⊕◯).
34. We suggest assessment of 12-lead ECG QTc time at least once in patients with KS (2,⊕⊕⊕◯)
35. We recommend following the EAA clinical guidelines on management of bone health in the andrological outpatient clinic, bearing in mind that patients with KS are at risk of low bone mineral density (BMD) and fractures independently of their serum levels of testosterone (1,⊕⊕⊕◯).
36. We recommend DXA analysis at the lumbar and femoral levels and fracture risk assessment at the first visit of adult patients with KS and then on an individual basis (1,⊕⊕◯◯).
37. We suggest determination of vitamin D plasma levels in all adult patients with KS at the first visit and then on an individual basis, independently from their BMD, and proper vitamin D and calcium supplementation when needed (2,⊕⊕◯◯).
38. We recommend considering psychosexual and psychiatric issues in all adult patients with KS and to induce consultation by a specialist if required (1,⊕⊕◯◯).
39. We suggest attention to the putative existence of gender incongruence in patients with KS. The patient should then attend a respective specialist within a multidisciplinary setting (2,⊕◯◯◯) (2,⊕◯◯◯).

40. We recommend breast examination for patients with KS (including mammary gland ultrasonography if necessary) for detecting gynaecomastia at the first visit and then on an individual basis and eventual treatment as per guidelines (1,⊕⊕⊕◯).
41. We suggest clinical breast and axilla examinations every two years in adult patients with KS and eventual mammography and/or mammary gland ultrasonography especially in those patients with a family history of breast cancer or other reasons for suspicion thereof (1,⊕⊕◯◯).
42. We suggest ophthalmological assessments in patients with KS if the history points towards visual complaints (2,⊕◯◯◯).
43. We suggest examination of the dental status in patients with KS (2,⊕◯◯◯).
44. We suggest attention to possible autoimmune dysfunctions in patients with KS (2,⊕◯◯◯)

GENERAL DEMANDS

45. We recommend the setup of multidisciplinary centres or structures to care for patients with KS (1,⊕⊕⊕◯).
46. We recommend improving the transitional care for patients with KS from pediatric to adult endocrinologists/andrologists (1,⊕⊕⊕◯).
47. We recommend improving knowledge about KS among doctors and society, especially by structured graduate and postgraduate education (1,⊕⊕⊕◯).

1. INTRODUCTION

Klinefelter Syndrome (KS) is the most frequent chromosome disorder in men, exhibiting a karyotype of 47,XXY. Symptoms in KS are highly variable. Nevertheless, frequent characteristics are small testes, azoospermia and hypergonadotropic hypogonadism. Also neurocognitive and psycho-social manifestions can be seen as well as cardiovascular, metabolic and bone-related conditions of adverse nature. Generally, morbidity and mortality are increased in KS compared to men with a karyotype of 46,XY. Both hypogonadism and genetic effects seem to contribute to this clinical spectrum. Among physicians, knowledge about KS regarding diagnosis and treatment is distributed unevenly. KS is not fully known to many physicians and there is a marked need for a respective improvement of medical curricula.

Whereas KS is usually considered in all guidelines dealing with the management of hypogonadism, no specific guideline and recommendation has ever been published to care for patients with KS in various developmental stages. The aim of the present study is summarize available evidence on KS providing a list of suggestions and recommendations on behalf of European Academy of Andrology (EAA) and endorsed by the European Society of Endocrinology in order to correctly manage patients with KS from prenatal period to adulthood.

2. METHODOLOGY OF THE GUIDELINE COMPOSITION

2.1. Data identification

The EAA guidelines committee commissioned an expert task force of academicians with clinical expertise in the field to create new guidelines for the management of KS. All experts involved are andrologists with a various background (urology, genetics, paediatrics, endocrinology). PubMed was searched for articles in English with the search term “Klinefelter” (the search period involved all literature regarding KS starting from 1942 to June 2020). The text was drafted in a group effort following scrutiny and discussion of the best evidence from published literature. Thus, a series of consensus recommendations and suggestions according to the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system was generated (1) .

2.2. Levels of evidence and grades of recommendation

The GRADE system is a method of developing evidence-based guidelines, involving key recommendations and the use of a consistent language and graphical descriptions for standardizing the grading of both the strength of recommendation and the quality of the evidence (1), widely used from scientific societies including the EAA. According to GRADE, the task force used the following internationally shared coding system: [1] "we recommend" indicates a strong recommendation; [2] "we suggest" denotes a weak recommendation. As far as the evidence grading is concerned: ⊕◯◯◯ denotes "very low-quality evidence"; ⊕⊕◯◯ "low quality", ⊕⊕⊕◯ "moderate quality" and ⊕⊕⊕⊕ "high quality".

Largely, a "⊕⊕⊕⊕" score is awarded to evidence that is based on randomized controlled trials (RCTs) and meta-analyses, and a "⊕⊕◯◯" score to evidence that is based on observational studies. Specific methodological characteristics (quality, consistency, directness, effect size) increase or decrease this score.

Statements on AETIOLOGY AND PREVALENCE

I. **Klinefelter Syndrome (KS) is the most frequent sex chromosomal anomaly in males, described by a karyotype with one or more extra X Chromosome (most frequently 47,XXY) in men.**

II. **KS is associated with testicular malfunction resulting very often in hypogonadism and/or infertility**

III. **The classical description of the adult male with KS includes primary testicular failure with small testes (1-5 mL), hypergonadotropic hypogonadism, gynaecomastia, infertility (mostly azoospermia), sparse body hair, extended body length in relation to parentally derived target height, an increased risk for osteoporosis, metabolic syndrome and psychosocial disturbances**

IV. **Except for small testes, no consistent clinical features or specific abnormalities irrespective of age have been identified**

V. **The phenotypic spectrum is extremely wide, and the main clinical features are not present in infancy and childhood and may not become evident until after the onset of puberty**

VI. **The cohort of men with KS exhibits an increased morbidity and mortality compared to the general male population**

2.3. Aetiology

The supernumerary X of KS originates in 50% from paternal nondisjunction of sex chromosomes during meiosis I. The remaining 50% originate from maternal nondisjunction during meiosis I or II, or during early post-zygotic mitotic divisions (2,3). An association of the frequency of KS with increasing maternal age can be attributed to maternal meiosis I errors. An association of paternally derived 47,XXY with the father's age has been described, as well (4).

Gene-dosage effects, of which SHOX related to the tall stature in KS is a leading example (5), in combination with escapee genes from X-chromosomal inactivation, are most likely co- factors constituting the phenotype (4,6,7).

In addition, the androgen receptor (*AR*) gene, located in Xq11.2–q12, is of interest concerning genotype/phenotype correlations. The *AR* gene contains a highly polymorphic trinucleotide repeat

(CAG)n in exon 1, which is correlated with physiological androgen effects and might be associated with androgen-dependent features of KS (6,8,9). FSH polymorphisms might affect serum FSH in men with KS (10), as well as other genetic features of the X chromosome might contribute to phenotype variations (11).

2.4. Prevalence

The prevalence of KS is 1–2/1000 according to studies involving systematic screening of male newborns in the 1960-1970s (12-14).

Subclasses of KS are the classic 47,XXY form, which represent approximately 80–90% of the cases, higher-grade aneuploidies (48,XXXY, 49,XXXXY or 48,XXYY), mosaicisms (mainly 47,XXY/46,XY), and structurally abnormal X chromosomes (e.g. 47,iXq,Y) (15,16) that overall account for the remaining 10-20% of cases.

However, the prevalence of a 47,XXY karyotype among male blastocysts examined during pre-implantation genetic testing (PGT) has been reported as 0.9% (17/1794), with a significant correlation with maternal age. This discrepancy might suggest that 47,XXY blastocysts result in a lower implantation rate and/or higher early miscarriage rate than euploid embryos or that infertile patients of advanced maternal age and referred to IVF/PGT produce a higher rate of 47,XXY blastocysts (17).

2.5. Low diagnostic rate

The typical clinical phenotype of men with KS, as described in the earlier literature, is present in a minority of patients (Figure 1). Phenotypic variability, and especially a presentation with mild clinical features, often leads to diagnostic delay or non-diagnosis (8,15,18). It has been estimated that 50-75% of males with KS never obtain a diagnosis (19,20). To increase the diagnosis rate, screening all male newborns has been suggested (21). This, for example, can be facilitated by the detection of an increased gene copy number in DNA from dried blood spot samples (22). In general, efforts that improve our ability to reach an early diagnosis should be encouraged. Moreover, since the great majority of men with KS are diagnosed during infertility evaluation and pre- pubertal clinical signs are not specific for KS, diagnosis during childhood and adolescence is quite rare, representing about 10% of all the diagnoses

(19). Indeed, the advantages of a correct diagnosis and early diagnosis are evident for better management and prevention of diseases related to KS (15,21).

3. CLINICAL PICTURES, DIAGNOSTIC STEPS AND THERAPY

3.1. Genetics

Recommendations

1. We recommend that a prenatal diagnosis of KS is confirmed on a chromosome analysis on peripheral blood postnatally (1,⊕⊕⊕◯).
2. We recommend conventional karyotyping on peripheral blood cells for diagnosis of KS (1,⊕⊕⊕◯).
3. We recommend that patients with KS and/or their parents are offered genetic counselling; prenatal counselling should be non-directive (1,⊕⊕⊕◯).
4. We recommend karyotype analysis for detecting KS in men with non-obstructive azoospermia or (severe) oligozoospermia (total sperm count < 10 x 10^6/ejaculate or sperm concentration <5 x 10^6/ml (1,⊕⊕⊕◯).
5. We recommend karyotype analysis for detecting KS in men with primary hypogonadism (low serum levels of testosterone) and elevated serum levels of gonadotropins (LH and FSH) combined with small testicular volumes (<5 ml per testis) (1,⊕⊕⊕◯).

Evidence

Diagnosis of KS depends on determination of the karyotype (2,3,8). The prevalence of KS rises up to 3-4% among infertile males, to 6% among men with a total sperm count <10 million/ejaculate and to 10-15% in non-obstructive azoospermic subjects (23,24). The majority of men with KS are azoospermic and only 10% approximately present with severe oligozoospermia or cryptozoospermia, whereas the prevalence in men with normozoospermia is virtually zero (2,3,8). Low testosterone levels are described in about 50% of adults with KS (2,18), but nearly all patients have elevated LH levels which reflects the primary testicular (Leydig cell) damage in these patients. Therefore, overt or subclinical hypogonadism is almost a hallmark of KS (25) and clinical signs of hypogonadism are present in most

men. However, the real prevalence of KS among men with primary or subclinical hypogonadism is not known.

Values and Remarks

Determination of karyotype in patients with suspicion of KS or the exclusion thereof determines the pathway of counselling, further diagnostics and treatment. We place a high value on the recommendation on conventional karyotyping as the choice for confirming a clinical suspicion of KS.

Karyotyping should be considered in cases of low testicular volume (<5ml) which may also be present without overt primary hypogonadism, apart from non-obstructive azoospermia or severe oligozoospermia (which must be confirmed in more than one semen analysis). Other possible causes of non-obstructive azoospermia or severe oligospermia must be considered. Especially cases with central hypogonadism might present with small testes, non-obstructive azoospermia or severe oligozoospermia and not all of them are candidates for karyotype analysis.

KS may be diagnosed prenatally by cytogenetic evaluation [conventional karyotyping or array-CGH (array-comparative genomic hybridization)] of chorion villus tissue or amniotic fluid. Massively parallel sequencing of circulating cell-free fetal DNA in maternal plasma has allowed for the development of non-invasive prenatal testing (NIPT). Currently, NIPT is widely used for the screening for autosomal trisomies, such as trisomy 13, 18 and 21. However, data on sensitivity and specificity of the detection of sex chromosome aneuploidies (SCA) is sparse and there is a tendency towards increased false positive results. Importantly, NIPT is not a diagnostic test and requires cytogenetic confirmation (26).

In cases of pre-natal diagnosis of KS, genetic counselling is strongly advised and in some countries even mandatory according to law. Such counselling is non-directive and will inform the parents about the putative risks for patients with KS, up-to-date treatment options and experiences of clinicians treating children, adolescents and adults with KS. Contact to KS patient and parent groups may be provided. Any decision of the parents regarding the child must be absolutely individual

Additional screening for mosaicism on higher number of metaphases, potentially using fibroblasts or array-CGH, is suggested if the clinical suspicion is strong and the result of the chromosome analysis is normal. However, the more expensive analyses such as array-CGH are not recommended for routine use.

Determination of the length of the CAG repeat polymorphism of the *AR* gene (a parameter inversely related to androgen sensitivity) might be useful in decision-making regarding androgen replacement in cases of patients exhibiting features of hypogonadism and low-normal serum testosterone concentrations (6,9). Diagnostic procedures related to hypermethylation and differential gene expression remain experimental and do not have direct clinical consequences at this time.

3.2. Developmental issues in infants and pre-pubertal children

3.2.1. Testicular development and function of the hypothalamic-pituitary-testicular axis

Recommendations

6. We suggest karyotype analysis for detecting KS in boys born with cryptorchidism, especially the bilateral forms, who do not experience spontaneous descent of the testes at the first year (2,⊕⊕◯◯).
7. We recommend treating cryptorchidism in children with KS according to the current treatment guidelines in children without KS (1,⊕⊕⊕◯).
8. We recommend general physical examinations in pre-pubertal children with KS including a testicular evaluation. These should be performed biennially or as deemed as appropriate. Suspected neurological or psychiatric deficits should be examined by respective specialists (1,⊕⊕⊕◯).
9. We suggest determination of LH and testosterone during the first 2-3 months after birth in children with prenatal diagnosis of KS when it might have a therapeutic consequence (i.e. diagnosis of micro-penis) (2,⊕◯◯◯).
10. We recommend against cryopreservation of testicular tissue or spermatogonial stem cells retrieved from pre-pubertal children with KS (1,⊕⊕⊕◯).
11. We recommend against testosterone supplementation during early childhood in all patients with KS except in cases of micro-penis (1,⊕⊕⊕◯).

Evidence

In KS, testicular degeneration and abnormal testicular function may start during the fetal period of life and worsen with age, but no reliable data exist on ultrasound imaging of the testes in children with KS (27,28-31). Some boys with KS show clinical signs of intrauterine hypogonadism such as cryptorchidism, reduced penile length and testicular volume (32-35). Boys with KS rarely fulfil the criteria for micro-penis (<-2.5 SD), although, one study found a prevalence of 17% (35). In otherwise healthy patients with testicular failure and micro-penis, testosterone therapy in infancy is widely accepted (36-38).

Patients with cryptorchidism may have a prevalence of 3 - 4% of chromosomal anomalies, especially frequent in the bilateral form of cryptorchidism (39). Among chromosomal anomalies detected in cryptorchid boys, KS represents the most frequent alteration. A study on 600 newborns with isolated cryptorchidism found a prevalence of KS of 1.3% that increased to 2.6% when considering only boys who were still cryptorchid at 12-24 months (39). The prevalence of KS was even higher in persistent bilateral forms of cryptorchidism (4.2%) than in unilateral forms (1.6%) (39). However, the exact prevalence of cryptorchidism at birth in patients with KS is unclear, and few studies examined this aspect. The largest studies reported that 27-37% of patients with KS had a history of undescended testes (2,40,41). Orchidopexy before the age of twelve months is recommended as for non-KS cryptorchid boys (36,42). Treated cryptorchidism does not reduce the chances for positive sperm retrieval in patients with KS (43).

Conflicting data regarding the surge in testosterone during the first 3 months after birth (mini puberty) have been published. Some studies demonstrated low testicular testosterone levels in infants with KS (44,45). Another study reported testosterone concentrations within the normal range but significantly lower than controls (46). A recent investigation showed that during mini-puberty of infants with KS, serum levels of FSH and LH were significantly higher than in controls ($p < 0.05$), as were Inhibin-B and T (respectively $p < 0.0001$ and $p < 0.005$). No significant differences were found in height, weight, testicular volume, and penile length (47).

General pre-pubertal concentrations of testosterone, estradiol, FSH and LH are normal (48-57). However, some studies have shown that boys with KS may already be androgen deficient before puberty (34,35,58).

Values and Remarks

Preservation of testicular functions, especially sperm production, is closely linked to descended testes. As these testicular functions are impaired in KS anyway, these patients require high attention to management of undescended testes as early as possible (Table 1).

Testicular ultrasound may help to assess testicular development, but testicular palpation may also yield the required information. We recommend against testicular tissue cryopreservation and against spermatogonial stem cell retrieval in children with KS. So far, these procedures are not justified as there is no method of *in vitro* spermatogenesis, but this may change in future (59).

We place a moderate value on the recommendation regarding determination of LH and FSH during the first three months after birth because treatment of hypogonadism in this period of life is currently not recommended. We place a high value to the recommendation against testosterone supplementation during early childhood in all patients with KS, as it has currently no justification. However in cases of micro-penis, serum hormone assessment could provide a justification for short term low-dosed systemic testosterone treatment or local application of dihydrotestosterone (38; Table 1).

The postnatal surge of sex hormones during the so-called mini-puberty represents a diagnostic window for evaluating the hypothalamic-pituitary-testicular axis in infancy and recognize poor testicular function and hypogonadism (60). The result of poor testicular hormonal function may be cryptorchidism (61). Endocrine evaluation of the testicular function before puberty is challenged by very low concentrations of testosterone and low sensitivity of the generally available hormone-assays. Therefore, only very limited conflicting data exist (62) (also see 4.2.4).

3.2.2. Growth

Recommendation

12. We suggest measurement of height according to centiles or standard deviation scores (SDS) as well as body proportions and determinations of bone age in pre-pubertal children with KS depending on individual growth patterns (2,⊕⊕◯◯).

Evidence

During early infancy growth of the KS boys is usually within the normal range. By the age of 5-6 years growth velocity might be accelerated resulting in significantly taller stature than expected on the basis of parental target height (5,32, 63-67). In addition, significantly increased leg length already before puberty has been reported by several authors (63-65,67-69).

Values and Remarks

Growth should be monitored assessing height and body proportions in boys with Klinefelter syndrome, whenever possible according to centiles or SDS for the reference ethnic group of the patient and bone age should be determined in selected cases. However, the only parameters which would justify an intervention by testosterone supplementation are excessive growth compared to the estimated parental-derived target height or abnormal body proportions (5,64,65). In these cases determination of bone age is important (Table 1). The side effects on physical and psychological parameters can be rather significant and this form of therapy is rarely used nowadays. Since high dose T treatment is required to stop over-tall growth, and effects on testicular functions are unknown, such a procedure is generally seen with reluctance.

3.2.3. Bone mineralization

Recommendations

13. We suggest determination of vitamin D blood levels and adequate vitamin D and calcium supplementation in pre-pubertal children with KS (2,⊕○○○).
14. We suggest assessment of bone mineral status during childhood in case of vitamin D deficiency biennially in patients with KS (DXA scan, size-corrected determinations using a three-step-method are required) (2,⊕○○○).

Evidence

Normal lumbar bone mineral density (BMD), and whole body bone mineral content (BMC) as evaluated by whole body DXA scan in KS boys and adolescents (4.3 - 18.6 years of age) has been reported, indicating that the risk of osteopenia/osteoporosis may not be present until after puberty (70). It was found in a group of 7/40 KS boys (11.96 ± 3.97 years of age) that z-scores ≤−2.0 as evaluated by phalangeal amplitude-dependent speed of sound were present as well as higher parathyroid hormone levels and a significant reduction of 25-hydroxyvitamin D concentrations. This study is rather small and needs confirmation (71).

Values and Remarks

Studies evaluating the bone status and metabolism of children and adolescents with KS are very rare and not uniform. We place a moderate value on the recommendation to check vitamin D levels and, if needed, supplement with vitamin D preparations and calcium to maintain healthy bones. We put a moderate value on the recommendation to assessment of bone mineral status because it is important to diagnose and treat osteopenia/osteoporosis as soon as possible to allow achievement of peak bone mass, but probably only less than 20% of children with KS will suffer from osteopenia/osteoporosis (Table 1).

3.2.4. Body composition

Recommendations

15. We recommend measurement of weight using centiles or standard deviation scores in pre-pubertal children with KS (1,⊕⊕⊕◯).
16. We recommend against testosterone treatment in infants and pre-pubertal boys with KS (1,⊕⊕⊕◯).

Evidence

Findings of an unfavourable body composition already present in childhood and early adolescence with elevated body fat mass despite normal body mass index and normal lean body mass for age as determined by DXA (70) or triceps/subscapular skinfolds (32,40,66,72) point out causes other than low testosterone. Genetic causes (overexpression of X-linked genes, skewed X-chromosome inactivation or a longer-than-normal CAG-repeat polymorphism in the *AR* gene) and psychosocial aetiologies are both plausible: studies addressing these possible relations have to be further validated (4,6,73-76).

Testosterone supplementation in early childhood was proposed, particularly in the USA (77-79). An improved neurodevelopmental outcome in boys with KS treated with testosterone during infancy compared to non-treated boys with KS was claimed, but the treatment was not randomized or blinded (77,78). Two randomized trials have been conducted in children with KS. The effect on body composition in 20 infants aged 6 to 15 weeks who were randomized to testosterone injections or no treatment was also evaluated. A normal fat mass in treated boys compared to untreated boys was seen, the latter presenting with a significant accumulation of fat tissue as compared to 46,XY-controls (79). In addition, a double-blind, randomized, placebo-controlled study evaluated treatment with low-dose synthetic oral androgen (oxandrolone) in prepubertal boys aged 4 to 12 years (80-82). The authors reported improvements in areas of visual- motor performance, anxiety/depression, and social functioning, but no effect on cognition or attention (82). Furthermore, a significant reduction in body fat, triglycerides and high-density lipoprotein was found (80). Importantly, the oxandrolone treated boys had an increased risk of early gonadarche and pubarche, as well as advanced bone age, although no significant differences in T and gonadotropin concentrations between treated and untreated boys were found (81).

Values and Remarks

We place a high value on the recommendation to assess weight and possibly fat/lean proportions as well as keeping them within normal range as they are important for subsequent metabolic equilibrium (Table 1). To this end, also waist circumference and, where available, triceps/subscapular skinfolds can be assessed in pre-pubertal children with KS on an individual basis.

Further randomized controlled trials are required to confirm possible positive short- and long-term effects of testosterone treatment in infants and pre-pubertal boys with KS (Mason et al. 2020). Current knowledge does not support such a treatment.

3.2.5. Psychological aspects

Recommendations

17. We recommend speech therapist control and therapy, monitoring learning disabilities, social training and psychological support, in pre-pubertal children with KS if needed (1,⊕⊕⊕◯).

Evidence

Boys with KS are known to have an increased risk for psychosocial and educational problems (66,83). General intelligence in KS is preserved variably and verbal abilities might be decreased. KS can, albeit not mandatorily, present with lower general cognitive abilities, reduced knowledge achievements as well as some attenuated aspects of attention and executive functions (83-92). In patients with KS, the cognitive phenotype is often described by impaired performance on measures of language development, visuospatial and academic abilities. In younger boys with KS, a delay in speech development may be observed, whereas significant deficits in higher aspects of expressive language are more common in adolescents. In addition, it has been shown that KS is associated with difficulties in identifying and verbalizing emotions. Furthermore, patients with KS may be more easily emotionally aroused (93-98). There are also reports about an increased distractibility and an increased risk for hyperactivity and attention problems in patients with KS (90,99-101).

Values and Remarks

We place a high value to the recommendations to monitor speech development, learning abilities as well as psychosocial problems and give support, if necessary (Table 1). There are suggestions that early testosterone treatment may improve psychological development in patients with KS (66,77,78,102). However, data are lacking quality to recommend this procedure to all children with KS (103). So far, scientific evidence is not sufficient to support this procedure.

4.4. Developmental issues in puberty and adolescence

4.4.1. Spermatogenesis

Recommendations

18. We recommend that information on fertility issues is given to adolescent patients with KS and, if deemed adequate, his parents (Good Clinical Practice statement).
19. We suggest testicular ultrasound evaluation during puberty of patients with KS and regularly at follow-up visits (2,⊕○○○).
20. We suggest semen collection in adolescents with KS after careful information and assessment of the wish of the patient and cryopreservation if motile sperm are present (2,⊕⊕○○).
21. We suggest that adolescents with KS might undergo a testicular biopsy for testicular sperm extraction (TESE) either using multifocal (standard TESE) or microdissection-TESE (mTESE) and consequent sperm cryopreservation in selected cases requiring specific counselling, provided their physical and mental maturity is apt for this decision (2,⊕⊕○○).

Evidence

At the beginning of puberty, which in general occurs at a normal age, testes grow to a minor extent and subsequently shrink. In parallel with this, gonadotropin levels rise to the greatly elevated serum concentrations as seen in adults with KS. The degeneration of germ and Sertoli cells accelerates during puberty, and moreover, extensive fibrosis and hyalinization of the seminiferous tubules occur, as well as hyperplasia of Leydig cells and fibrosis of the interstitium (27-30). Spermatogenesis cannot be observed in the majority of seminiferous tubules. However, a few tubules with ongoing spermatogenesis and few spermatozoa can be found. In adolescents aged 13-14 years, spermatozoa were collected in only 10% of the TESE attempts, while in adolescents of 15–19 years, spermatozoa were found in 45% (104). Sperm retrieval rates by TESE in adolescents with KS aged 15-19 years are comparable with those reported in young adults who are 20-30 years old (105-107). It was also found

that adolescents with LH ≤17.5 IU/l, along with testosterone levels ≥7.5 nmol/l had the best success rate (54%), which fell to 44% with higher serum LH levels, whereas those with low serum testosterone concentrations (<7.5 nmol/l), had no sperm retrieval, irrespective of LH (106). See Figure 2a-c for an example of testicular tissue in KS.

Values and Remarks

We place a high value to the recommendation to provide the information on fertility status and treatment possibilities to the patients with KS and their parents. It is important that further medical treatment is undertaken within a setting of empowerment of the patient and his family. We place a lower value to the suggestion regarding semen collection and TESE in adolescents, because not all pubertal patients are psychologically ready to focus on fertility and because success rates are similar if these procedures are performed later. Adolescents might undergo a surgical procedure to attempt a TESE, provided they exhibit physical and mental maturity and the decision to preserve their fertility is clearly not made by the parents but the patient himself. We put also a moderate value to the recommendation to perform testicular ultrasound every year, because it may help to assess testicular development. Not all pubertal patients are mature enough to talk about their fertility. However, all patients with KS should be fully informed on their fertility status and possibilities of fertility preservation (108). Information should be provided at a level appropriate to the patient's age and cognitive level (table 1).

4.3.2. Function of the hypothalamic-pituitary-testicular axis

Recommendations

22. We recommend assessment of Tanner stages, pubertal development, measurement of testosterone and gonadotropins, signs and symptoms of hypogonadism, height, weight, waist circumference and body proportions starting prior to the predicted start of puberty in patients with KS and at individually determined intervals thereafter (the time-window for the start of puberty does not differ from boys with a karyotype of 46,XY) (1,⊕⊕⊕◯).

23. We recommend testosterone supplementation in case of delayed puberty and/or symptoms of hypogonadism associated with low-normal testosterone and supra-normal LH serum concentrations (LH > 2 SD according to age-related references), after the fertility issues have been addressed (see above) (1,⊕⊕⊕◯).
24. We suggest against testosterone therapy in adolescents with KS and with compensated hypergonadotropic hypogonadism (2,⊕⊕◯◯).

Evidence

From around mid-puberty testosterone and INSL3 concentrations stop to increase and remain in the low normal range in most patients with KS (48,49,51,109,110). Concomitantly, FSH and LH concentrations increase to hypergonadotropic levels (48,49,51,109,111). Inhibin B concentrations increase, but not as much as expected during puberty, and decline markedly within a year of pubertal onset and remain undetectable from the end of puberty onwards in the vast majority of patients with KS (28,50,111). The physiological pubertal decline in serum AMH occurs later in boys with KS than observed in healthy boys (53,55,112). Estradiol levels have been reported to be normal (113) or high (114).

A central issue is the normative age-related secretion of LH. Data are scarce and might be specific to local laboratories. There are some publications reporting such age-related values (56,115).

A hypergonadotropic state with compensated hypogonadism is found in over 60% of KS cases allowing for spontaneous accomplishment of pubertal development (104). However, about 25% of patients with KS have testosterone below normal levels and in some cases the diagnosis is made because of prolonged progression of pubertal development, which should not be confused with a delayed onset of puberty (111).

Values

We place a high value on the recommendation for peripubertal examinations. These should be based on an individual prediction of pubertal onset, which is derived from the parents' pubertal history. Assessment of the fertility status should ideally be performed before the start of testosterone substitution. We place a high value on the recommendation to treat patients with KS and low testosterone levels as well as accompanying symptoms of androgen deficiency with an adequately dosed testosterone replacement (Table 1).

Remarks

There are only a small number of studies on testosterone supplementation in patients with KS during puberty, and no controlled trials. However, such a regimen may ensure, as in other adolescents with hypogonadism, normal completion of puberty as well as improved physical and psychological development, educational achievements and social integration (116-122).

Randomized controlled trials are needed to elucidate the influence of testosterone replacement therapy on pubertal development and spermatogenesis in patients with KS. There are also no randomized controlled trials evaluating the possible deleterious impact of testosterone treatment on successful sperm retrieval or its possible effects on reproductive outcomes in men with KS. Testosterone preparations may theoretically suppress any remaining spermatogenesis if the endogenous LH drive is reduced. Therefore, it is better to assess the fertility status before this treatment. However, individual decisions should be made. The use of hCG or antiestrogens (aromatase inhibitors or selective estrogen receptor modulators) on compassionate care grounds may have a positive effect on gonadotropin secretion even in the hypergonadotropic state as seen in KS. Theoretically, this may result in higher levels of intra-testicular as well as serum testosterone and maintained (or even augmented) spermatogenesis. However, no controlled trials are available and evidence is poor. Hence, such therapies cannot be recommended in general.

No data have been published on the possible positive or negative effects of testosterone treatment in boys with KS and compensated hypergonadotropic hypogonadism. It is suggested that in the absence of clinical signs and symptoms of hypogonadism these boys should not be treated with testosterone.

4.3.3. Cognitive and psychological aspects

Recommendations

25. We recommend speech therapist control, monitoring educational problems, social training and psychological support in adolescents with KS, if needed (1,⊕⊕⊕◯).

Evidence

As younger children with KS, pubertal boys might have educational and psychosocial problems. Boys with KS might show deficits in verbal memory, verbal fluency, word retrieval, expressive/receptive vocabulary difficulties, as well as planning, organization and decision-making problems (90,91,123-126). Although all of these have been related to lower school/academic performance, patients with KS are not generally learning disabled. Limitations in communication might affect social adaptation and behavioural aspects, as well as the development of personality, which may result in social and professional problems (6,126,127).

Values and Remarks

We place a high value to the recommendation to monitor speech development, educational, social and psychosocial problems and give support, if necessary (Table 1). Notwithstanding, further studies are needed to evaluate the impact of educational and psychosocial problems of patients with KS on their final education, socio-economic status, psychological well-being and general quality of life in adulthood. Also see recommendation #16 against testosterone treatment during childhood in KS.

3.3. Pathophysiology and clinics in adults

3.4. 4.4.1. Hypogonadism

Recommendations

26. We recommend initiation of testosterone substitution in patients with KS with hypogonadism as diagnosed according to established guidelines on hypogonadism, if possible once fertility issues have been addressed (1,⊕⊕⊕◯).
27. We recommend that testosterone substitution in patients with KS should follow the established guidelines on hypogonadism using the usually suggested monitoring intervals for clinical assessment, safety parameters (haematocrit, PSA) and dose titration (1,⊕⊕⊕◯).
28. We recommend endocrine evaluation every 12 months in adult patients with KS who are not on testosterone substitution (1,⊕⊕◯◯).

Evidence

Most adults with KS have serum testosterone levels within the low-normal or sub-normal range, but some may have very low amounts of this steroid and others may even have normal levels. In adult patients with KS, not only levels of testosterone, but also insulin-like factor 3 (INSL3), and inhibin are decreased, whereas FSH and LH are highly elevated, while estradiol and SHBG are comparable to controls. Testosterone replacement therapy in adult men with KS should follow the established guidelines on diagnosis and treatment of hypogonadism (128).

Values and Remarks

Assessment of serum concentrations of sex steroids and gonadotropins is pivotal to understand the status of the patients with KS in terms of possible hypogonadism and fertility issues (table 2). It is paramount to monitor these values and safety parameters to tailor the hormone replacement therapy to establish the known benefits and avoid side-effects (2,8). Treatment of hypogonadism in KS should be started as soon as diagnosis is made. The effects of testosterone replacement might be modulated by the CAG-repeat polymorphism within the *AR* gene (6).

A recent meta-analysis including 21 observational and 22 interventional studies showed that non-treated patients with KS have worse metabolic profiles (fasting glycemia, HOMA index, HDL, LDL) and body composition (BMI, waist circumference) and reduced BMD with respect to controls. On the other hand, testosterone treatment in hypogonadal men with KS improved body composition and BMD at the spinal level. The effects on lipid and glucose profile were less significant evident (129). Larger and longer placebo-controlled RCT are necessary however to fully understand the benefits of testosterone treatment in KS.

4.4.2. Infertility

Recommendations

29. We recommend semen analysis and sperm cryopreservation in all adult patients with KS and a wish for paternity (1,⊕⊕⊕◯).

30. We recommend that all patients with KS and confirmed azoospermia as well as a current or putative future wish for paternity undergo a testicular biopsy for testicular sperm extraction (TESE) either using multifocal (standard TESE) or microdissection-TESE (mTESE) and consequent sperm cryopreservation (1,⊕⊕⊕◯).
31. We suggest against starting testosterone replacement therapy in patients with KS when a TESE is planned, due to the possible suppression of gonadotropins and further suppression of remnant spermatogenesis (2,⊕⊕◯◯)

Evidence

Motile sperm that can be cryopreserved from semen are found in approximately 10% of men with KS. In azoospermic men focal spermatogenesis may be discovered with the possibility of surgical extraction of viable sperm. See Figure 2a-c for an example of testicular tissue in KS. No differences in terms of sperm retrieval have been found between classic-TESE and micro-TESE (130). Sperm can be found in about 30-60% of patients with KS and the reported live birth rate by assisted reproduction is about 16%. Although no direct comparison is available, the success rate of ICSI seems to be reduced in KS when compared to the general population (131).

Values

We place high value on these recommendations because semen sampling is a non-invasive procedure and semen sampling should therefore be offered after puberty as soon as the patient can provide a sample (Table 2). Conventional TESE or micro-assisted TESE (mTESE) in azoospermic patients with KS is the next step to obtain sperm. These sperm can be used later for methods of assisted reproduction (ICSI).

Remarks

As offspring seems not to be affected by the genetic condition of the father, it is unclear whether offering PGT or prenatal genetic analyses is required. Genetic counselling however is mandatory. In fact it is the question whether 47,XXY spermatogonia are able to complete meiosis or, in contrast, some

spermatogonia lose the supernumerary X chromosome, hence becoming normal 46,XY cells and then proceed through meiosis.

It is still debated whether the chances to find foci within the testes with remnant intact spermatogenesis decrease with advancing age in KS (132-134). Fertility in KS may also be linked to their Leydig cell functionality (106). Nevertheless, in difference to adolescents and younger men with KS (see above), no clinical, biochemical or hormonal parameter has been clearly associated with higher or lower chances of finding sperm in adult men with KS. Similarly, it is unclear whether previous testosterone treatment decreases the success rate of TESE. Therefore, we cannot recommend for or against stopping testosterone therapy prior to TESE, but suggest against starting testosterone treatment when a TESE is planned. Hypogonadal men not receiving testosterone therapy might be treated on compassionate care grounds with hCG or antiestrogens (aromatase inhibitors or selective estrogen receptor modulators, see 4.3.2.). However, no controlled trials are available and evidence is poor. Hence, such approaches cannot be recommended in general.

4.4.3. Metabolic disorders, body composition, cardiovascular risk and thrombosis

Recommendations

32. We recommend education on lifestyle and yearly assessment of weight, waist circumference, blood pressure, fasting glucose, HbA1c and lipid profile and adequate treatment in all patients with KS(1,⊕⊕⊕◯).
33. We suggest thrombosis prophylaxis prior to long-term flights or exposure to other risks in patients with KS to attenuate the increased risk for deep vein thrombosis and/or pulmonary embolism (2,⊕⊕⊕◯).
34. We suggest assessment of 12-lead ECG QTc time at least once in patients with KS (2,⊕◯◯◯).

Evidence

Men with KS suffer from higher rates in various diseases and a higher mortality, which results in a shortened lifespan (about 2-3 years) (8,41,135-140).

KS is associated with increased fat mass and reduced lean mass, promoted by the intrinsic genetic background as well as hypogonadism. The resulting insulin resistance predisposes men with KS to a higher risk of developing type 2 diabetes mellitus (4,8,41,76,135,141). The conditions named above can explain, at least partially, the increased cardiovascular mortality and morbidity observed in patients with KS (8,41,135-140). These comorbidities are strongly related to hypogonadism (Corona et al. 2018). However, the latter cannot entirely explain the increased CV risk and other factors (such as epigenetic mechanisms and *AR* functionality) are most likely involved (4,6,9,142). Accordingly, testosterone replacement therapy does not completely attenuate the increased cardiometabolic risk (129).

There is some evidence that the cardiorrhythmic integrity of patients with KS is impaired, resulting in a reduced QTc time, which may eventually lead to sudden cardiac arrest. This impairment is most likely caused by gene over-dosage effects related to the additional X-chromosome. As this pathology may result in a life-threatening condition, we make suggestion No 33, although data are still based on smaller numbers of patients with KS (4, 143).

A higher risk of thrombosis has been described in men with KS. The risk of deep vein thrombosis and pulmonary embolism is elevated in KS, with an overall three- to six-fold increased risk as compared to the general population (8,41,135-140,144). The risk may be attributed to higher levels of the procoagulatory substance PAI-1, which is associated with the genetic setting in KS as well as with lower testosterone levels (4). Testosterone treatment may reduce this risk, but evidence is still low (145,146).

Value and Remarks

We place a high value to the recommendations to give the patients correct information on lifestyle intervention (physical activity, diet) to minimize cardiovascular risk factors, to monitor regularly cardiovascular risk factors, and treat obesity, diabetes and dyslipidemia (Table 2). Maintaining eugonadal testosterone levels is most likely important to attenuate the chances of promoting a metabolic syndrome with advancing age in patients with KS, but evidence is still poor. Randomized controlled trials are needed to elucidate the role of testosterone substitution in patients with KS in the prevention of an unfavourable body composition, metabolic syndrome and type 2 diabetes mellitus. A recent meta-analysis demonstrates that testosterone substitution ameliorates body composition, but not other cardiovascular risk factor (129). However, this conclusion is drawn mainly from observational studies (also see 4.4.1).

4.4.4. Bone disorders

Recommendations

35. We recommend following the EAA clinical guidelines on management of bone health in the andrological outpatient clinic, bearing in mind that patients with KS are at risk of low bone mineral density (BMD) and fractures independently of their serum levels of testosterone (1,⊕⊕⊕◯).
36. We recommend DXA analysis at the lumbar and femoral levels and fracture risk assessment at the first visit of adult patients with KS and then on an individual basis (1,⊕⊕◯◯).
37. We suggest determination of vitamin D plasma levels in all adult patients with KS at the first visit and then on an individual basis, independently from their BMD, and proper vitamin D and calcium supplementation when needed (2,⊕⊕◯◯).

Evidence

Bone density is likely to be reduced in hypogonadism in general, as outlined in the respective EAA guideline (147) and low bone mass and osteopenia/osteoporosis with a subsequent higher risk of fractures is present in up to 40% of patients with KS (122,148-150). However, no clear relation between serum levels of testosterone and BMD has been found in patients with KS and osteopenia/osteoporosis might also be present when testosterone concentrations are within the normal range (75). The decrease in bone mass is due to both reduced bone formation and higher bone resorption (148), and might be linked to a lack of peak bone mass at the end of puberty.

The higher fracture risk is linked also to other factors than hypogonadism and low bone mineral density (BMD) (hypovitaminosis D, unfavourable ratio of fat mass/lean mass, associated comorbidities, low INSL3, X inactivation, *AR* sensitivity) (6,148,149,151), as evidenced also by studies analyzing bone formation and bone resorption serum markers such as osteocalcin (152) and sclerostin (153). Patients with KS are likely to improve bone density upon testosterone replacement therapy (151), similar to observations in other patients with primary hypogonadism (129).

Correction of vitamin D deficiency is also fundamental to maintain BMD (150). Calcitriol is the double-hydroxylated-form of Vitamin D3 (1,25-dihydroxy-cholecalciferol) that does not require hydroxylation

at the hepatic and renal level. The direct substitution may be beneficial in cases of some chronic diseases, but requires close monitoring of calcium levels in the blood while during substitution with cholecalciferol, the synthesis of the required amount is regulated by the system itself. Nevertheless, also substitution with cholecalciferol requires control of serum levels of Vitamin D (154,155).

Values and Remarks

Young KS subjects may have normal BMD in childhood and at the beginning of pubertal development, while the risk of lower bone mass starts at mid puberty when testicular function progressively declines, therefore mitigating the achievement of optimal peak bone mass. Lifestyle interventions (physical activity, smoking, diet, sun exposure), vitamin D and calcium supplementation and specific anti-osteoporotic drugs are required based on individual assessments of both BMD and fracture risk (Table 2). Aside from BMD, other microarchitecture features of bone and bone strength have been described in KS using high-resolution peripheral quantitated computed tomography (pQCT) (156,157) that allows better definition of bone structure and strength than DXA (which permits determination of bone density only). No trial has been conducted in patients with KS on testosterone treatment using fractures as primary endpoint. In addition, the effects of anti-osteoporotic drugs on BMD and fracture risk in KS have not been studied.

4.4.5. Psychological and psychiatric conditions/ Gender incongruence

Recommendations

38. We recommend considering psychosexual and psychiatric issues in all adult patients with KS and to induce consultation by a specialist if required (1,⊕⊕◯◯).
39. We suggest attention to the putative existence of gender incongruence in patients with KS. The patient should then attend a respective specialist within a multidisciplinary setting (2,⊕◯◯◯).

Evidence

The intellectual ability of KS might be mitigated in some cases, but not generally (125). Characteristic impairments of language skills, such as in verbal processing speed, expressive grammar as well as word

retrieval have been reported (127). Patients with KS might have also exhibit impairments in executive functions related to attention, flexibility and planning as well as response inhibition (158). The risks of developing psychiatric conditions, such as schizophrenia, bipolar disorders, depression, anxiety, autism and ADHD are increased in patients with KS (159-163).

Psychosexual development is often delayed and sexual problems might be more frequent in KS (164). Most patients identify as male, but gender incongruence in KS has been reported in some studies (72,165-173).

Values

Attention to eventual psychological, sexual, psychiatric as well as gender incongruence aspects is suggested (Table 2). In patients with KS, a personality profile including lower levels of extraversion, insufficient emotional arousal, less openness and higher levels of neuroticism than in general is likely. Intelligence quotient scores are reported to be lower than average (6,159) leading to poor socioeconomic status for many.

Remarks

The neurocognitive phenotype of patients with KS is highly variable. Many studies suffer from selection bias and the current knowledge of these aspects in KS is mainly based upon diagnosed cases. The non-diagnosed cases may present a more neutral phenotype and may not express problems to the same extent as diagnosed patient populations.

The presence of various degrees of gender incongruence will have an important impact when testosterone substitution therapy is considered, as in such cases patients with KS may not wish for increased masculinisation (20,165-173) and aim for feminisation. Interdisciplinary work-up with mental health professionals will be mandatory (165-173). Feminising hormone treatment might augment the risk for thrombosis, which is already increased in KS (see above).

4.4.6. Risk of neoplasia

Recommendations

40. We recommend breast examination for patients with KS (including mammary gland ultrasonography if necessary) for detecting gynaecomastia at the first visit and then on an individual basis and eventual treatment as per guidelines (1,⊕⊕⊕◯).

Evidence

Patients with KS seem to have an increased mortality ratio for all cancers, especially regarding breast and lung cancer as well as non-Hodgkin lymphoma (137,138). The latter pathology exhibited an increased standardized incidence ratio (SIR) of 3.02 in a large cohort of patients with KS (174). Also various forms of leukaemia were found with an SIR 3.62 in this cohort (Ji et al. 2016). Nevertheless, a lower mortality from prostate cancer has been reported (137,138). Other studies demonstrated that patients with KS have an increased risk of breast cancer with a4-30-fold increased incidence compared with that in men with a karyotype of 46,XY (41,135-138,175-177). The mean age of breast cancer in patients with KS is 58 years, which is earlier compared to men with a 46,XY karyotype (67 years) (178). However, given the rarity of male breast cancer, the absolute risk remains low for patients with KS. The calculated cumulative risk of occurrence by the age 75 years is 1% (136-138).

Moreover, an increased incidence of extragonadal germ cell neoplasia in patients with KS, most commonly between an age of 15-30 years, has been reported. These tumours are located mainly within the mediastinum and are usually non-seminomas (mature teratomas or mixed tumours) (179). Clinically, younger boys may present precocious puberty due to hCG production, while older patients present with thorax symptoms (180).

A relationship between KS and testicular germ cell tumours is not documented (41,135-138,181,182). No cases of testicular germ cell neoplasia in situ (GCNIS) have been shown. The incidence of benign Leydig cell tumours in patients with KS has been reported higher than in the general population, although this may simply reflect a higher prevalence of Leydig cell hyperplasia rather than tumours. In a recent series, the frequency of Leydig cell tumours in KS does not seem higher than that found in infertile men (183,184).

Values and Remarks

Patients with KS have an increased risk of some forms of neoplasia, especially breast cancer and mediastinal germ cell tumours (table 2). Physicians should be aware of the neoplasia risk in patients with KS so as not to lose the opportunity for early detection and treatment. The current epidemiological studies should be interpreted with caution, because of the generally low diagnostic rate of KS and small number of cohort longitudinal studies.

4.4.7. Other disorders

Recommendations

41. We suggest clinical breast and axilla examinations every two years in adult patients with KS and eventual mammography and/or mammary gland ultrasonography especially in those patients with a family history of breast cancer or other reasons for suspicion thereof (1,⊕⊕○○).
42. We suggest ophthalmological assessments in patients with KS if the history points towards visual complaints (2,⊕○○○).
43. We suggest examination of the dental status in patients with KS (2,⊕○○○).
44. We suggest attention to possible autoimmune dysfunctions in patients with KS (2,⊕○○○)

Evidence

Gynaecomastia has been recognized as part of the cardinal stigmata of KS since its first description (185, 186). More recent studies have found gynaecomastia to be less common in KS, being present in about a third of adults, although prominent or persisting pubertal gynaecomastia remains an important sign of KS (122). Permanent gynaecomastia before and during pubertal transition is a clinical sign that should lead to suspicion of KS and eventual treatment, which should be surgical if required. The pathophysiology of gynaecomastia may be due to unbalanced serum levels of testosterone and

estradiol (15), but the length of the CAG repeat polymorphism of the *AR* gene and X-chromosome inactivation status have been also suggested as factors of influence (6,9,142). The exact prevalence of KS among boys and adults with gynaecomastia is unknown, also because gynaecomastia itself is frequently undetected and clinicians often do not suspect KS when addressing gynaecomastia as this is a frequent complaint in adolescents in general. The EAA guideline on gynaecomastia should be consulted (187).

There are reports on retinal dysfunction and impaired day vision / night vision in KS (188,189). Development of teeth may be affected, imposing as taurodontism (190) and an increased risk of caries has been reported (191-193). Also autoimmune disorders might be more frequent in KS (194-196), while thyroid functions do not seem to be affected in men with KS (197)

Values and Remarks

Adequate diagnosis and, if required, treatment of gynaecomastia is paramount in KS in order to maintain self-esteem and a positive body image which are essential for patients' mental health. The other conditions mentioned above should be diagnosed and treated, if possible (Table 2).

The reports on ophthalmologic, dental and autoimmune diseases are based on small numbers of patients with KS and need validation.

5. GENERAL DEMANDS

Recommendations

45. We recommend the setup of multidisciplinary centres or structures to care for patients with KS (1,⊕⊕⊕◯).
46. We recommend improving the transitional care for patients with KS from pediatric to adult endocrinologists/andrologists (1,⊕⊕⊕◯).
47. We recommend improving knowledge about KS among doctors and society, especially by structured graduate and postgraduate education (1,⊕⊕⊕◯).

Evidence

Actual data indicate that 21% of patients with KS are diagnosed prenatally, 10-12% during childhood, 16% at puberty and 51% during adulthood (19,172,198-200). Infants with KS usually present with a normal male phenotype, however KS may be suspected in infants with bilateral cryptorchidism and/or micro-penis (32-34,40,182,201-208).

During childhood, speech and behavioural disturbances, excessive growth as compared with parental predicted target height and abnormal body proportions may lead to a suspicion of KS (19). Delayed puberty, poor testicular development, gynaecomastia, excessive height, learning disabilities and psychosocial problems are symptoms that should cause suspicion of KS (20,65,172,204,205,209-212).

Nevertheless, early diagnosis is quite rare but the increasing use of prenatal tests will increase the number of patients with KS being diagnosed. This should be an advantage for the patient and a great opportunity to prevent possible associated comorbidities later in life (213-221). However, the empirical evidence that early diagnosis with subsequent intervention improves long-term adult outcomes is currently lacking (222-238). Physicians, especially paediatricians, should be aware of the relatively frequent incidence of KS and also the behavioural issues that might come with it (16,19,91,237). The information on KS and psychological support to the patient and his parents are of great importance for their confidence (222).

Values and Remarks

We put a high value on the recommendations to improve medical knowledge about KS among physicians and society in general (181,201). It is also paramount to provide patients with KS and their parents with specific information and support them psychologically as needed. We also put a high value on the recommendation to the set-up of multidisciplinary centres for the care of patients with KS. These should include all professionals involved (geneticists, paediatricians/paediatric endocrinologists, psychologists, speech therapists, adult endocrinologists/andrologists, urologists, reproductive gynaecologists, sexologists, psychiatrists). EAA centers and other specialized centers within Europe and

worldwide are recommended to serve as multidisciplinary units to be approached by KS patients and their physicians.

6. CONCLUSIONS AND FUTURE DIRECTIONS

KS is the most common sex chromosomal disorder in men. It affects patients with both hypogonadism and infertility. In addition, men with KS are afflicted with a higher risk of having cardiovascular, metabolic, psychiatric and other comorbidities. Providing the patient with KS and, if deemed adequate, his parents with suitable and balanced information as well as assistance for various aspects of his life after receiving the diagnosis is suggested. Prevention and treatment of the medical complications and comorbidities associated with KS should be standardized as far as possible. Also minimizing neurodevelopmental dysfunction, i.e. verbal deficits, learning difficulties, behavioural problems should be aimed at. These measures are likely to promote the patients' self-esteem, assure quality of life and improve his social adaption. Finally, preservation of the fertility potential, i.e. cryopreservation of spermatozoa from ejaculate or testicular tissue is an option now widely available.

The pathological conditions in patients with KS are most likely of a combined endocrine, genetic and epigenetic origin. Further research in a coordinated fashion is needed. KS is vastly underdiagnosed and an increment of general knowledge as well as establishment of standard care in multidisciplinary networks is mandatory. These are the first guidelines to take a step towards this goal.

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Legend to Figure 1

Signs and symptoms of Klinefelter Syndrome (KS) at various stages in life. It is indicated that these symptoms may be seen in some or many patients with KS, depending on their age. Most of these symptoms are not inherent to KS and are, therefore, not specific. Small firm testes can, however, be considered quite specific. Also the combination of symptoms in adults can be considered specific and promote the diagnosis of KS.

Legend to Figure 2a-c

Figure 2a

Testicular histology in an adult patient with Klinefelter Syndrome. The biopsy is dominated by Sertoli-cell-only tubules, Leydig cell hyperplasia with one large Leydig cell nodule (*) and completely hyalinized "ghost" tubules (white dotted circles). Note two types of Sertoli cell-only seminiferous tubules; type A which contain differentiated Sertoli cells (A), and type B which contain incompletely differentiated Sertoli cells (B).

Periodic acid-Schiff staining. The bar denotes 100 µ.
Courtesy of Lise Aksglaede

Figure 2b+c

Testicular histology (right testis) of a young adult/adolescent patient (age 18 years) with non-mosaic 47,XXY Klinefelter Syndrome.

b: section with various types of tubules, containing elongated spermatids but also a „ghost" tubule c: section with almost intact tubules, containing more elongated spermatids.
PAS-staining. The bar denotes 100µ

Further patient data: testis volume right: 2.5 ml. LH 6.3 IU/L, FSH 29 IU/L, total testosterone 7.5 nmol/L,

Inh B 15.4 pg/mL (normal value: >100 pg/mL), as the lower limit of detection is 10 pg/mL, this finding might indicate remnant processes of spermatogenesis.

AMH 2.6 ng/mL (normal range for age 18 y: 1.3-4.8 ng/mL).

Courtesy of Sabine Kliesch. Joachim Wistuba and Heidi Kerseboom helped with the histological work up

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Parameter to be tested and/or treated	**Infancy and childhood**	**Puberty and adolescence**
Cryptorchidism	Treated according to the current guidelines	-
Physical examination including testis and mammary gland evaluation	Biennially or as deemed as appropriate *Testicular ultrasound if required*	Annually or as deemed as appropriate *Testicular ultrasound in all patients*
Cognitive evaluation and or specialist consultation	Monitor speech development, learning abilities psychosocial problems. *Support, if necessary*	Monitor educational problems, social training and psychological problems *Support, if necessary*
Growth, body proportion and weight	Evaluated according to SD or centiles: pay attention to body proportions and overweigh or obesity	Evaluated according to SD or centiles: pay attention to body proportions and overweigh or obesity
Fertility issues		
Information	Inform parents	Inform patients and their parents if deemed adequate
Semen collection and cryopreservation	-	Selected cases after careful information
Testicular biopsy and cryopreservation		Selected cases in azoospermia

Assessment of vitamin D and calcium	In all patients *Support, if necessary* *Bone mineral status in case of vitamin D deficiency*	In all patients *Support, if necessary* *Bone mineral status in case of vitamin D deficiency*
Assessment of LH and testosterone	Only in the presence of micro-penis	In all patients
Testosterone therapy	Selected cases with micropenis	In the presence of hypogonadism

Table 1. Summary of the most important parameters to be tested and/or related to therapeutical approaches from childhood to adolescence in patients with KS

Table 2. Summary of the most important parameters to be tested and/or related therapeutical approach in adult with KS

Parameter to be tested	**Frequency**
Physical examination including testes and mammary gland evaluation	At least annually *Mammary gland ultrasound if required* *Testis ultrasound on individual basis* *Follow guidelines for gynecomastia*
Cognitive evaluation and or specialist consultation	Selective cases *Support if necessary*
Metabolic profile	At least annually *Adequate treatment if necessary*
Hormonal profile: testosterone and LH	At least annually *Testosterone therapy only in the presence of hypogonadism (follow guidelines for hypogonadism)*
Vitamin D and calcium determination	At the first visit and then on an individual basis *Support if required*
Fertility issues *Information*	Inform if not already notified if deemed adequate

Semen collection and cryopreservation *Testicular biopsy and cryopreservation*	In all patients desiring fertility If fertility desidered in azoospermia
Thrombosis prophylaxis	Prior to long-term flights or exposure to other risks
12-lead ECG QTc time	At the first visit and then on individual basis
DXA analysis at the lumbar and femoral levels and fracture risk assessment	At the first visit and then on individual basis *Treat if required (follow guidelines for osteoporosis)*

Figure 1

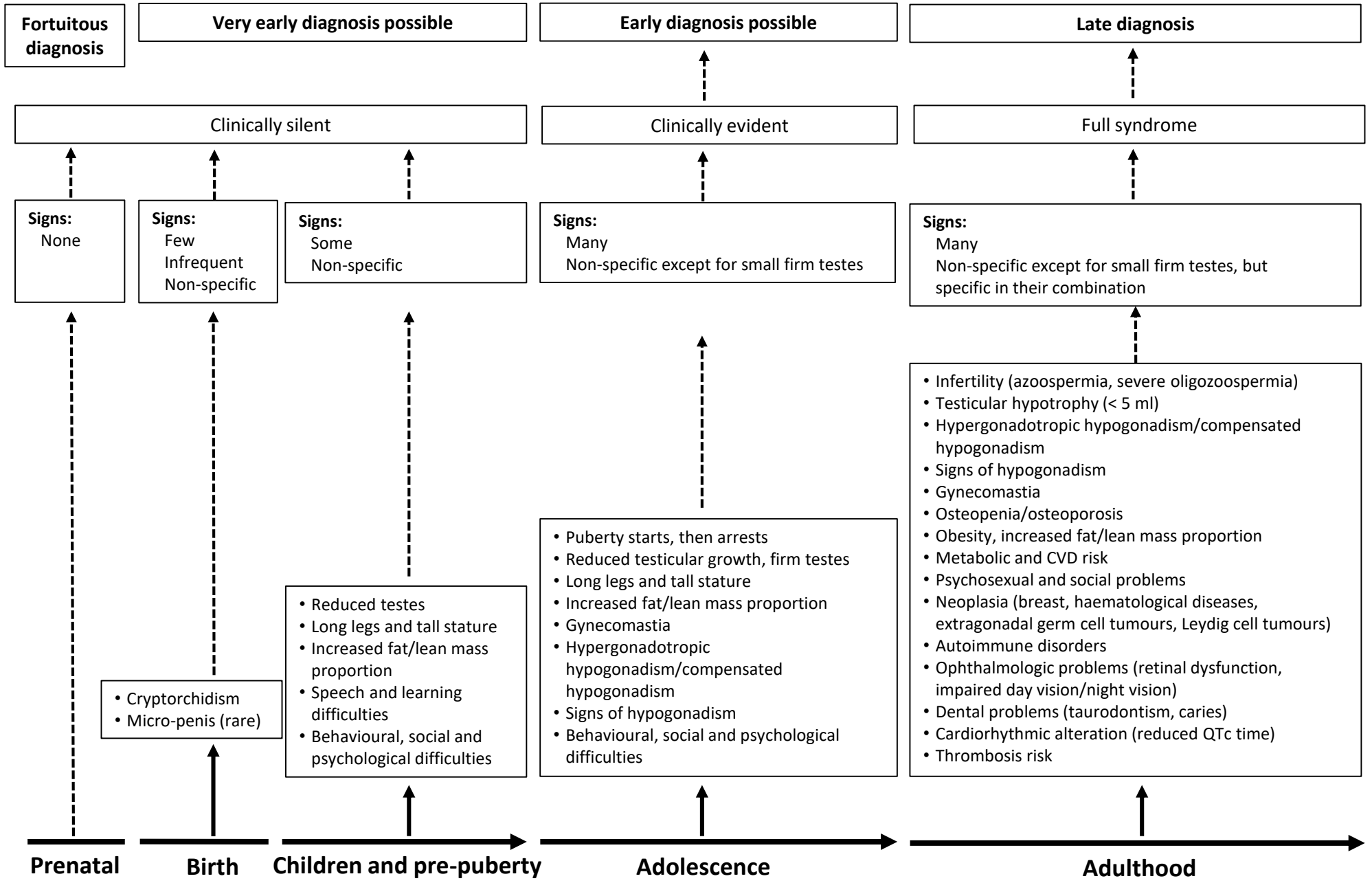

Fortuitous diagnosis
Very early diagnosis possible
Early diagnosis possible
Late diagnosis
Clinically silent
Clinically evident
Full syndrome
Signs: None
Signs: Few Infrequent Non-specific
Signs: Some Non-specific
Signs: Many Non-specific except for small firm testes
Signs: Many Non-specific except for small firm testes, but specific in their combination
• Cryptorchidism
• Micro-penis (rare)
• Reduced testes
• Long legs and tall stature
• Increased fat/lean mass proportion
• Speech and learning difficulties
• Behavioural, social and psychological difficulties
• Puberty starts, then arrests
• Reduced testicular growth, firm testes
• Long legs and tall stature
• Increased fat/lean mass proportion
• Gynecomastia
• Hypergonadotropic hypogonadism/compensated hypogonadism
• Signs of hypogonadism
• Behavioural, social and psychological difficulties
• Infertility (azoospermia, severe oligozoospermia)
• Testicular hypotrophy (< 5 ml)
• Hypergonadotropic hypogonadism/compensated hypogonadism
• Signs of hypogonadism
• Gynecomastia
• Osteopenia/osteoporosis
• Obesity, increased fat/lean mass proportion
• Metabolic and CVD risk
• Psychosexual and social problems
• Neoplasia (breast, haematological diseases, extragonadal germ cell tumours, Leydig cell tumours)
• Autoimmune disorders
• Ophthalmologic problems (retinal dysfunction, impaired day vision/night vision)
• Dental problems (taurodontism, caries)
• Cardiorhythmic alteration (reduced QTc time)
• Thrombosis risk
Prenatal
Birth
Children and pre-puberty
Adolescence
Adulthood

Fig 2a

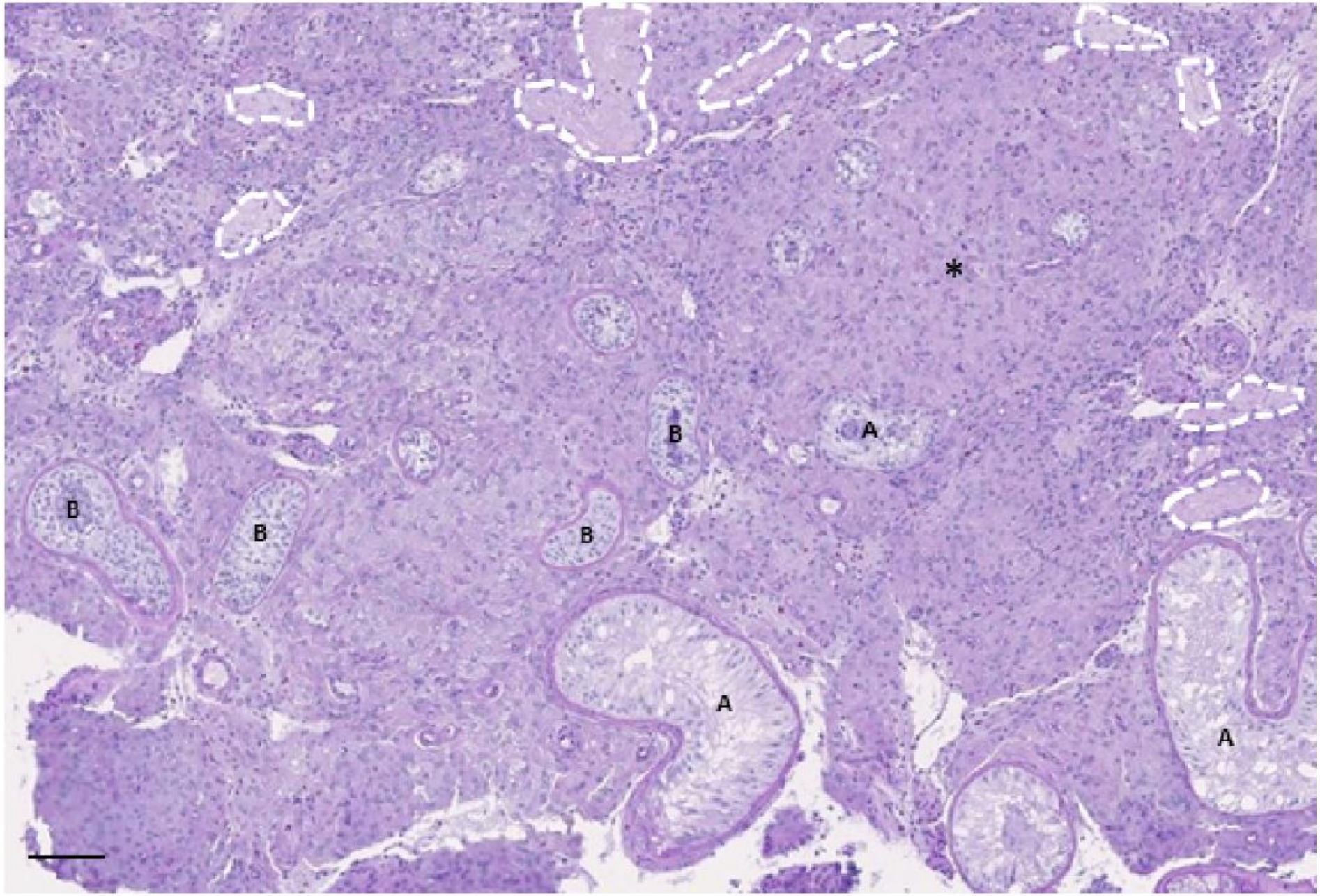
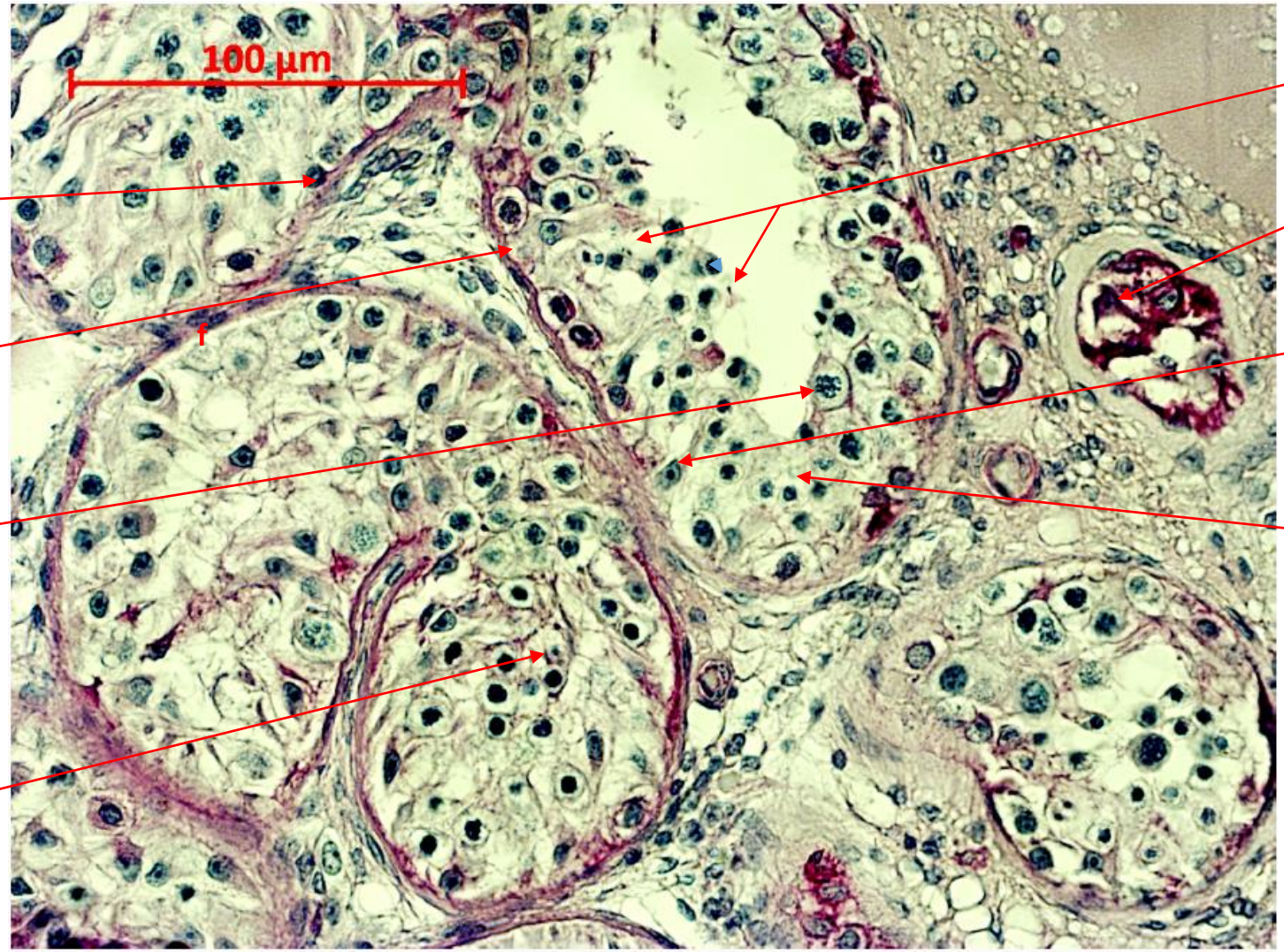


Fig 2b



a-dark spermatogonium

a-pale spermatogonium

pachytene spermatocyte

Pre-meiotic spermatocyte

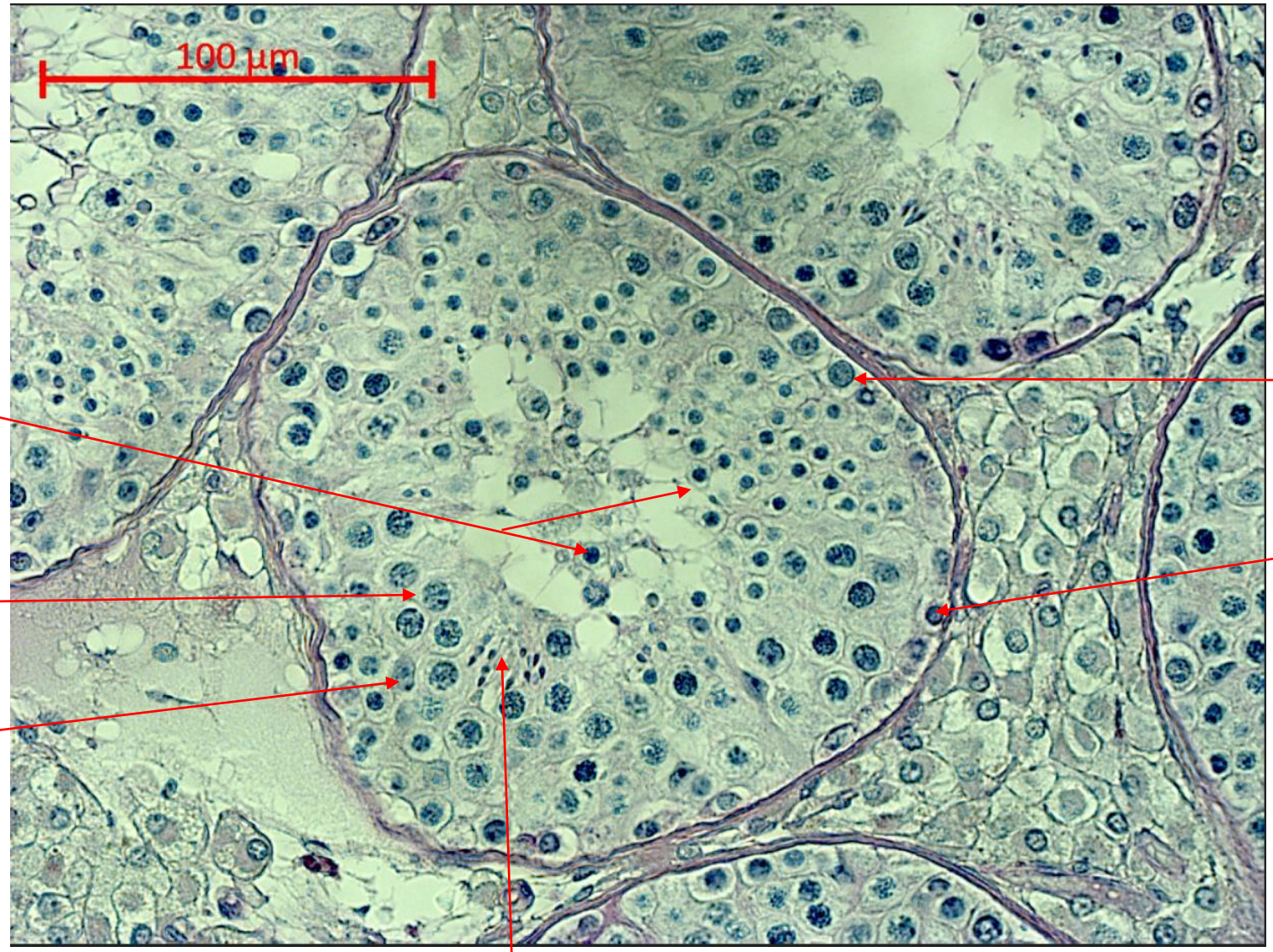
elongated spermatids

„ghost“

Sertoli cell

round spermatids

Fig 2c



round spermatids

pachytene spermatocytes

Sertoli cell

elongated spermatids

a-pale spermatogonium

a-dark spermatogonium