Endocrinología, Diabetes y Nutrición 69 (2023) 629–645

Endocrinología, Diabetes y Nutrición

www.elsevier.es/endo

CONSENSUS DOCUMENT

Consensus guide on prophylactic gonadectomy in different sex development

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KEYWORDS
Differences/Disorders of sex development;
Intersex;
Gonadectomy;
Germ cell tumor;
Gonadoblastoma;
Germ cell neoplasia in situ

Abstract The risk of suffering from gonadal germ cell tumors (GCT) is increased in some patients with different sexual development (DSD), mainly in those with Y chromosome material. This risk, however, varies considerably depending on a multitude of factors that make the decision for prophylactic gonadectomy extremely difficult. In order to make informed recommendations on the convenience of this procedure in cases where there is potential for malignancy, this consensus guide evaluates the latest clinical evidence, which is generally low, and updates the existing knowledge in this field.

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PALABRAS CLAVE
Desarrollo sexual diferente;
Anomalías de la diferenciación sexual;
Intersexo;
Gonadectomía;
Tumor de células germinales;
Gonadoblastoma;
Neoplasia in situ de células germinales

Guía de consenso sobre la gonadectomía profiláctica en el desarrollo sexual diferente

Resumen El riesgo de padecer tumores gonadales de células germinales (TCG) se encuentra incrementado en algunos pacientes con desarrollo sexual diferente (DSD), fundamentalmente en aquellos que presentan material de cromosoma Y. Dicho riesgo, sin embargo, varía considerablemente en función de multitud de factores que dificultan enormemente la decisión de una gonadectomía profiláctica. Con el fin de hacer recomendaciones fundamentadas sobre la conveniencia de este procedimiento en los casos donde existe potencial de malignización, esta guía de consenso evalúa la última evidencia clínica existente, en general escasa, y actualiza los conocimientos en este terreno.

Introduction

A 2006 consensus statement established the term ¨*disorders of sex development*¨(or, more commonly used now, ¨*differences of sex development*¨[DSDs]), and reorganised diagnoses into three broad groups based primarily on sex chromosomes and secondarily on hormonal aetiology and genetics.[1]

Conditions considered DSDs have wide-ranging aetiologies and phenotypic expressions; however, just some of them in particular, especially those with Y chromosome material, feature a predisposition towards developing neoplasms. These are, essentially, gonadal germ cell tumours (GGCTs) (≃14.9% of all DSDs; 0.8%–40% depending on age and diagnosis) and, with a lower prevalence (0.9%) and only in some DSD subtypes, gonadal stromal tumours and smooth-muscle leiomyomas/hamartomas.[2,3]

With advances in molecular diagnostics technology, numbers of identified DSD-causing genes have expanded considerably, allowing for a more accurate taxonomy. Nevertheless, determining the personalised risk of gonadal neoplasia (essentially a GGCT) in a person with DSD remains a daunting task, considering that said risk varies considerably depending on multiple other non-genetic factors.

In order to make substantiated recommendations on the advisability of prophylactic gonadectomy in cases in which the potential for malignant transformation is non-negligible, this article offers an update on knowledge in this regard and evaluates the latest existing clinical evidence, which is generally limited, on this potential in each group or condition included among DSDs.

Everything set out below will exclusively refer to the risk of GGCTs, since these represent the most common strains of gonadal malignancies in DSD patients, and since histopathological tests can predict the risk of suffering from them.

Germ cell ontogeny

Germ cell development is a very complex process strictly organised in time and space on a genetic basis that is not entirely well understood. It starts in week two with somatic cells that, through a process of specification driven by *BLIMP1* and *SOX17*, transform into *primordial germ cells* (PGCs). Guided by *KIT*/*KITLG* signalling, they migrate in groups along the midline from the proximal epiblast towards the gonadal ridge; this process occurs between weeks five and six, and with it, they become gonocytes (testicles) or oogonia (ovaries) and remain morphologically indistinguishable from PGCs. During this period, epigenetic reprogramming takes place which grants them the ability to transfer the characteristic of pluripotentiality to the next generation. This phenomenon is due to factors including genes of parental origin subjected to genomic imprinting involving expression of placental alkaline phosphatase (PLAP), c-KIT, octamer-binding transcription factor 3/4 (OCT3/4) and other markers.[4–6]

Once the undifferentiated gonad (gonadal ridge and germ cells) is formed, initiation of a signalling route around week seven will determine the differentiation thereof as a testicle or an ovary. In particular, the *SRY* and *SOX9* genes will be the main drivers of testicular differentiation, working through multiple transcription factors such as NR5A1 and ZFPM2 to form Sertoli cells, while the development of the ovary will require the absence of *SRY* expression at the same time as initiation of *WNT4*/β-catenin, *FOXL2* and *RSPO1*, leading to differentiation of stromal cells in granulosa cells. In the testicle, due to interaction with pre-Sertoli cells, gonocytes will differentiate into pre-spermatogonia which, as part of their maturation process, will be displaced from the center to the basal lamina of the seminifeous tubule, losing expression of embryonic markers OCT3/4 and c-KIT to then mature into spermatogonia and acquire expression of *DDX4* and *TSPY*. With all this, testicular differentiation will end in week nine, when the Leydig cells are capable of producing testosterone (T) and insulin-like factor 3 (INSL3) and the Sertoli cells are capable of producing anti-Müllerian hormone (AMH). Full ovarian differentiation, for its part, will end around week 11 with theca and granulosa cells.

Ultimately, the presence of testicular hormones (T and AMH) will determine male (internal and external) genital differentiation, and the absence thereof will determine female (internal and external) genital differentiation.[5,7]

Pathogenesis of precursor lesions to germ cell tumours in differences of sex development

By definition, primordial germ cells are the most pluripotent cells in the body following embryogenesis, in which hypomethylation and gene expression are similar to those of embryonic stem cells. In most patients with DSDs, the germ cells disappear as the body grows by means of a process of apoptosis. Some may persist in a state of immaturity; these are generally believed to be the cells that are vulnerable to malignant transformation, becoming a pre-invasive lesion or a precursor to an invasive GGCT.

DSD patients at risk of GGCTs are those who, in the presence of germ cells, have Y chromosome material in their gonadal karyotype; this occurs regardless of whether or not any degree of testicular differentiation is present. This Y chromosome material includes certain genes involved in male gonadal differentiation found in the region around the centromere of the Y chromosome (GBY region): *TSPY* (a gene that encodes testis-specific protein on Y chromosome, located in Yp11.2) and other candidate genes including *SRY* and *DYZ3*.[8]

Starting from these premises, the problem seems to lie in the fact that, in the absence of duly developed Sertoli cells, the primordial germ cells that migrate to the gonad in development retain their foetal phenotype. Under these conditions of biological immaturity, germ cells can undergo malignant transformation by the action of the above-mentioned genes of the GBY region, heightened expression of which promotes cell proliferation, as well as prolonged expression of OCT3/4.[2,4,6–12] Another essential requirement is for any abnormalities that may occur over the course of gonadal development to have a sufficient impact on the location or environment of these germ cells, but not ultimately affect their survival; otherwise, the risk of GGCTs will be considered null if said environment is hostile enough to induce their death.[4,12]

It can be concluded, then, that the oncogenic risk of these germ cells in the presence of gonadal Y chromosome material depends more on supporting cells (Sertoli cells) and on the microenvironment in which germ cells themselves are found — or, amounting to the same, that said risk is linked to degree of gonadal differentiation, such that the greater the gonadal immaturity, the greater the oncogenesis risk.

Considering all this, it has been estimated that the risk of such abnormalities being maintained after birth and resulting in a precursor lesion to infiltrating GGCT (whether or not it ultimately progresses to an infiltrating GGCT) is 14.9% on average in DSD patients with Y chromosome material. This essentially occurs in patients in whom the underlying genetic defect leads to early blockage of gonadal development (e.g. mutation or deletions of *SRY* or *WT1*). This risk is also present, though somewhat lower, in 45X0/46XY mosaicism (gonadal karyotype), while it is significantly lower in DSDs that do not interfere with normal gonadal development but do affect germ cell maturation (e.g. abnormalities in androgen action).[2,13]

To these intrinsic risk factors should be added others such as the possible role of gonadotropins (especially follicle-stimulating hormone [FSH]), oestrogens and, above all, androgens, which account for the higher risk of oncogenic development at two specific points in life: minipuberty and, in particular, puberty/youth (reinitiation of previously arrested mitosis). Patient age (older age being associated with higher risk) and gonadal location (intra-abdominal gonads carrying a higher risk) are also risk factors.[2,7,14]

Histopathological diagnosis of precursor lesions in patients with differences of sex development and course of the risk of germ cell tumour based on these

Histological study of the gonads in DSD patients enables classification of the degree of development and maturity thereof, grouping them as follows: (1) classic streak and hypoplastic ovary in gonads with ovarian differentiation; (2) streak with epithelial cords, dysgenetic testicle and structurally normal testicle in gonads with progressive differentiation towards a testicle; and, finally, (3) streak testicle and ovotestis in gonads with bipotential differentiation (Fig. 1).[15] This degree of development plus some of the above-mentioned circumstances represent the factors that can contribute in certain DSD patients to their gonads developing precursor lesions (less common in fully developed gonads such as the testicle, ovary or ovotestis) and that, ultimately, can progress late or early to infiltrating GGCT. These precursor lesions are usually generically called *germ cell neoplasia* in situ/*gonadoblastoma* (GCNIS/GB). Their diagnosis is based on certain combined histopathological findings (morphology), for cases of DSD featuring testicular tissue and expression of certain immunohistochemistry markers of pluripotentiality.

Markers of pluripotentiality in the diagnosis of precursor lesion of testicular origin: delayed maturation versus precursor lesion to a germ cell tumour

As mentioned, germ cells in initial stages of ontogenesis express certain immunohistochemistry markers such as OCT3/4 encoded by *POU5F1*; stem cell factor (SCF), also known as c-KIT ligand (encoded by *KITLG*); and PLAP. After birth, cells with sustained ''delayed maturation,'' essentially those of testicular origin, tend to retain positivity for these markers of pluripotentiality until they enter apoptosis. This explains why it is advisable to not rely on them exclusively in histological diagnosis of a precursor lesion to a GGCT of testicular origin, at least in the first 6–12 months of life, and thus prevent overdiagnosis during the postnatal period. To this end, it is crucial to take into account other differentiating histological criteria particular to such lesions such as the distribution of OCT3/4-positive cells in the gonad, the location of these germ cells in the seminiferous tubule, cellular atypia and expression of *KITLG*. In particular, germ cells with delayed maturation do not express the latter marker (c-KITLG or SCF), tend to be located in the central and suprabasal segment of the seminiferous tubules and are diffusely distributed throughout the gonad, whereas the cells of precursor lesions to GGCTs (GCNIS) express such a factor, will have characteristic atypia, will be in a basal

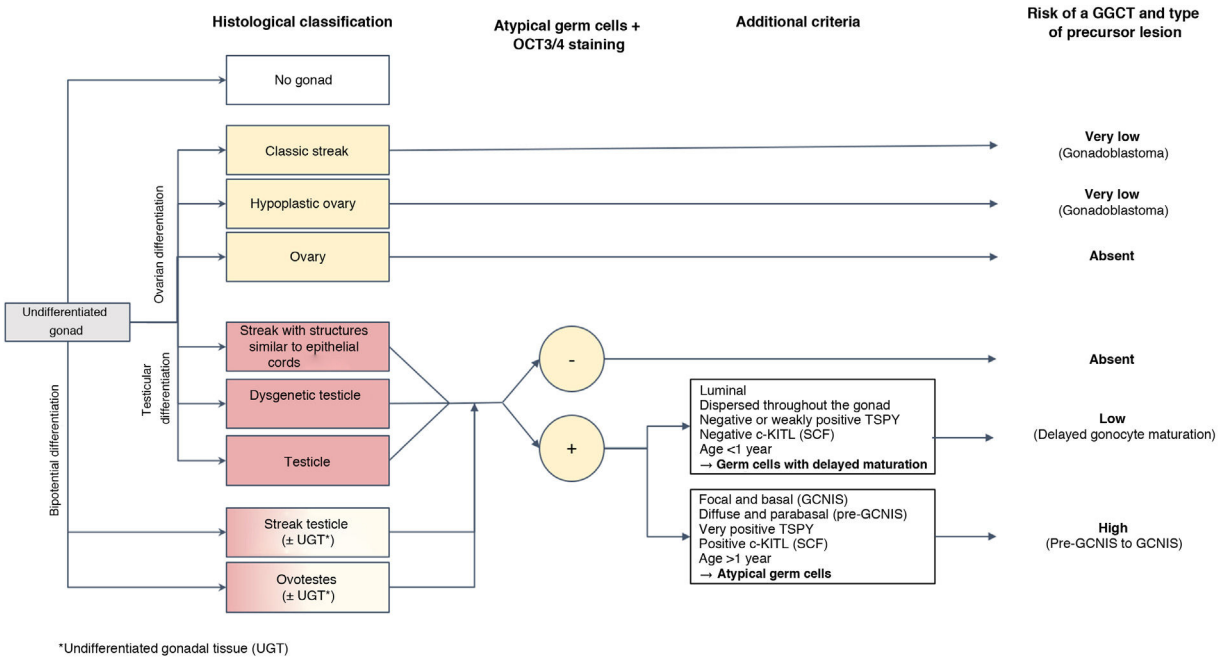

Figure 1 Histological classification of the gonad and risk of an associated GGCT. Adapted from two sources: Pleskacova et al., 2010,[21] and Nistal et al., 2017.[22].
GCNIS: *germ cell neoplasia* in situ; GGCTs: gonadal germ cell tumours; UGT: undifferentiated gonadal tissue.

location within the seminiferous tubules in the so-called spermatogonial niche and will have a patchy distribution.[5,13]

To be precise, dysgenetic gonads that include testicular parenchyma (dysgenetic testicles, streak testicles, streaks with epithelial cords and ovotestes) have a higher incidence of a delayed maturation of germ cells, rarely persisting beyond the first year of life.[5,13,16] Therefore, this distinction not exclusively based on the OCT3/4 marker is of paramount importance to prevent erroneous identification as precursor lesions to immature cells in dysgenetic gonads which in the past accounted for incorrect rates of risk of malignant transformation and, therefore, progression to GGCTs.[6,7,10,13,17]

In the case of dysgenetic gonads featuring Y chromosome material but not testicular tissue (classic streak or streak with epithelial cords), morphology is usually sufficient for diagnosis of a precursor lesion, and the use of immunohistochemistry techniques is not necessary; furthermore, the above-mentioned problem of delayed maturation would not be raised.

Histological classification of precursor lesions to germ cell neoplasia in situ/gonadoblastoma (GCNIS/GB) and risk of progression to a germ cell tumour

Based on histopathological study of the gonadal biopsy specimen and, if applicable, the immunohistochemistry markers mentioned, a diagnosis of a precursor lesion to a GGCT can be made (Fig. 1). These lesions, generically called GCNIS/GB, are subdivided into two types depending on their supporting cells (Sertoli cells or granulosa cells)[4,5,7,9–11,13,18–22]:

Gonadoblastoma (GB): this usually occurs in DSD patients with streak gonads or hypoplastic ovary; rarely, it occurs in testicular tissue. These patients have an XY karyotype (mosaic or not) but do not express the *SRY* gene, which would explain why, in many cases, they are phenotypically women. Its development is specifically linked to the GBY, region, which includes the above-mentioned *TSPY* gene. Thus, 45X0/46XY patients and women with Turner syndrome in the presence of Y chromosome material are at higher risk of GB. Its supporting cells are considered granulosa cells (positive *FOXL2*) and it is classified in the gropus of non-invasive germ cell neoplasias. Its diagnosis is primarily morphological, and immunohistochemistry is usually not required for diagnosis. It consists of circumscribed nests comprising a mixture of germ cells in different degrees of maturation from very similar to GCNIS (positive for OCT3/4) to similar to spermatogonia and sex cord cells, sometimes surrounded by hyaline deposits of basal membrane material composed of laminin.

Progression from a GB to an infiltrating GGCT is impossible to predict. The actual incidence of this course is unknown given the trend in past decades towards performing prophylactic gonadectomy in these patients, although it has been reported that it may be present at birth or develop later on in life. Some series have found that just 50% become an infiltrating malignant tumour, primarily dysgerminoma, over the course of a patient's life.[9,23]

GCNIS, as it is called in the latest (2016) WHO classification[24]; in prior medical literature, other terms were used such as *carcinoma* in situ (CIS) (which is incorrect as they are not epithelial cells) and intratubular germ cell neoplasia, unclassified type (IGCNU), (which is erroneous in relation to the meaning of unclassifiable). This lesion is considered a precursor lesion to infiltrating GGCT of testic-

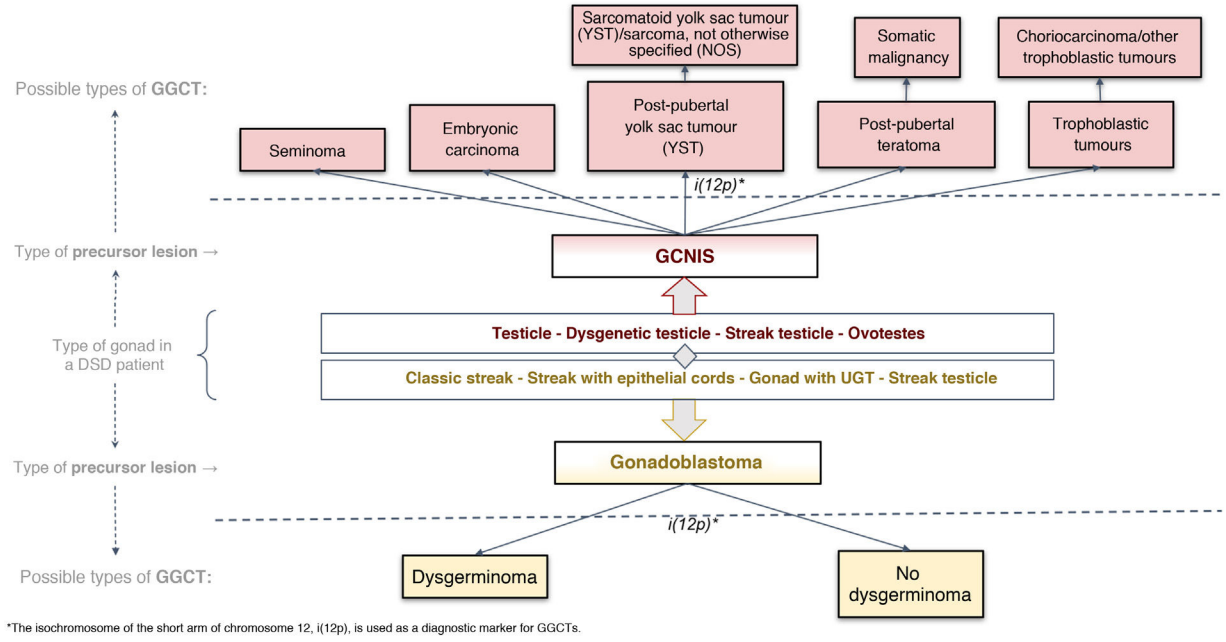

Figure 2 Histological classification of GGCTs according to the 2016 WHO classification[24] applied to dysgenetic gonads. DSDs: *differences of sex development*; GCNIS: *germ cell neoplasia* in situ; GGCTs: gonadal germ cell tumours; UGT: undifferentiated gonadal tissue.

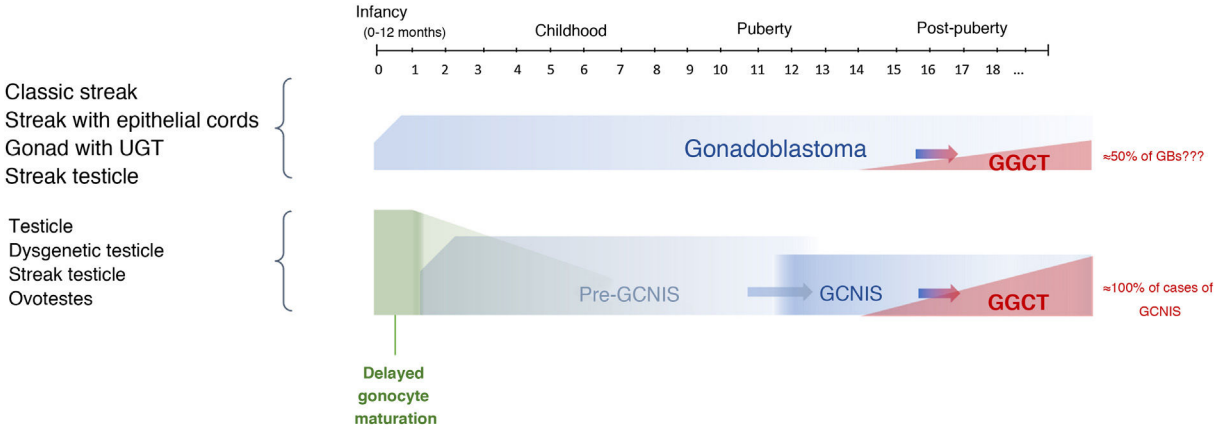

Figure 3 Course of preneoplastic risks (GCNIS/GB) and neoplastic risks (GGCTs) in DSD gonads with Y chromosome material. GCNIS: *germ cell neoplasia* in situ; GGCTs: gonadal germ cell tumours; UGT: undifferentiated gonadal tissue.

ular origin which is located in the seminiferous tubules and which, therefore, takes place in more or less virilised XY patients, due to the presence of the *SRY* gene. The supporting cells are Sertoli cells (positive for *SOX9*). GCNIS cells are large and atypical, morphologically similar to a gonocyte/primordial germ cell and found in a basal location in the seminiferous tubule, with extensive clear cytoplasm and a hyperchromatic angulated nucleus with a prominent nucleolus. Its diagnosis may require, in addition to this morphology, immunohistochemistry markers (OCT3/4, c-KIT and PLAP).

The potential for developing GCNIS is already present at birth as this is a foetal defect in gonocyte maturation, and nearly 100% of cases are predestined (some authors have placed this figure at 70%) to develop as a malignant tumour (GGCT) with a mean age of onset of 14–44 years of age (its onset being most common between age 20 and age 35).[9,10,13,23]

The term *pre-GCNIS* or *childhood GCNIS* was recently proposed to describe an intermediate lesion between delayed maturation and GCNIS that, though suggested as a precursor to the latter, does not always progress to it.[25]

When one of these precursor lesions (GCNIS or GB) progresses to an invasive tumour, it progresses only to a type 2 GGCT pathways (Fig. 2). According to the latest (2016) WHO classification,[23] two pathways include, in the testicle with GCNIS, seminoma and non-seminomatous tumours (embryonic carcinoma; yolk sac tumor, post-pubertal type; teratoma, post-pubertal type; and trophoblastic tumours, the most common of which is choriocarcinoma); and, in

Table 1 Indications for gonadal biopsy in DSD patients.[a]

- In *pre-puberty*, as of one year of age (in the absence of a clear recommendations, between one year and two years of age is proposed), for diagnosis and classification of the gonad ± search for a precursor lesion (risk of GB in very dysgenetic gonads):
  - o 46XY DSD patients with clinical/hormonal suspicion of gonadal dysgenesis
  - o 45X0/46XY DSD patients
  - o 46XX DSD patients with hypervirilisation of probable gonadal origin, i.e., having ruled out congenital adrenal hyperplasia (CAH) and a virilising tumour
- In *puberty* or *post-puberty* for diagnosis of a GCNIS/GB or GGCTs precursor lesion in the following situations:
  - o In the above-mentioned DSD patients, a second biopsy in puberty or after the end of puberty for immunohistochemistry testing will be considered
  - o In 46XX/46XY DSD patients, in puberty at the same time as removal of the undesired part of the gonadal tissue, or in post-puberty for immunohistochemistry testing
  - o To be considered in post-puberty if there is no desire for gonadectomy in 46XY DSD patients due to androgen receptor abnormalities, for immunohistochemistry testing

DSDs: differences of sex development; GCNIS/GB: germ cell neoplasia in situ/gonadoblastoma; GGCTs: gonadal germ cell tumours.
[a] The goal of biopsy is histopathological study and classification of the gonad and, in the case of testicular tissue, depending on morphology, consideration of immunohistochemistry testing that detects a delayed gonocyte maturation (0–12 months of age) versus pre-GCNIS (as of one year of age and before puberty) or GCNIS (usually as of puberty).

streak gonads or ovaries with GB, dysgerminoma (equivalent to testicular seminoma).[9,13,23]

The timeline of this course from initial gonadal tissue to precursor lesions to GCNIS/GB and ultimately to a GGCT is shown in Figure 3.

The risk/benefit balance of prophylactic gonadectomy and decision-making in patients with differences of sex development at risk of germ cell tumours

Prophylactic gonadectomy can only be proposed in DSD patients at risk of GGCTs. The risk of suffering from other neoplastic lineage such as gonadal stromal tumours (referring to Sertoli–Leydig hamartomas, Sertoli cell adenomas and smooth-muscle leiomyomas/hamartomas, characteristic of partial androgen sensitivity syndrome), does not in itself justify prophylactic gonadectomy given that they are uncommon and nearly always benign.

Classically, in decision-making in relation to performing gonadectomy in patients at true risk of GGCTs, three factors have to be taken into account: underlying diagnosis, sex and age at presentation, and molecular histopathological and immunohistochemistry findings (the latter having been recently added).[3,8,19] Despite the latest advances in these fields, the above-mentioned risks of malignant transformation (essentially in GBs) amount to mere speculation, as their actual natural history is unknown, in terms of both rates of malignant transformation and times to develop them.[4,5,7,17] These limitations mandate consideration for the fact that gonadectomy means infertility, a need for hormone supplementation and a subjective experience reported by some patients of poorer acquisition of secondary sex characteristics, as well as a worsening in quality of life despite suitable replacement therapy. On the other hand, if it is not pursued, despite the fact that the survival rate of a malignant GGCT is high (95% at five years), chemotherapy for these entails lifelong side effects such as a risk of metabolic syndrome and cardiovascular risk.[5]

That said, today, in DSD patients with gonadal activity (usually testicles), there is a growing trend towards postponing gonadectomy or even avoiding it altogether, carefully balancing the risk of developing a GGCT on the one hand and maintenance of endocrine function and fertility potential on the other hand. In this endeavour, gonadal biopsy remains the gold standard for evaluating this risk in certain DSDs (Table 1), and as a general action plan, it is recommended that the biopsy sample be 3 mm × 3 mm × 2 mm in order to have enough tissue for pathology examination; at the same time, pathologists with experience in this area are needed to ensure suitable interpretation of the lesions. It cannot be overlooked, however, that biopsy does not necessarily represent the entire gonad and that diagnosis of a precursor lesion can be missed; therefore, in gonads with a heterogeneous macroscopic appearance, such as ovotestes, samples should be taken from different areas. In addition, some authors have recommended that biopsy be combined with other procedures such as orchidopexy or sperm extraction in the case of a testicular DSD.[7,8,10]

Similarly, with the exception of 45X0/46XX or 45X0 Turner syndrome with no Y chromosome material (demonstrated by means of a conventional karyotype, fluorescence in situ hybridisation [FISH] and/or other molecular techniques), if the gonads are suspected to be streaks when they are rudimentary, they should always undergo histological examination to rule out the presence of undifferentiated gonadal tissue (UGT) (a precursor lesion to GB), which has a high likelihood of developing a GGCT over time. For this reason, since from a functional point of view these tissues do not have any sort of hormonal activity, they should always then be removed.[10]

Depending on the results of the histological examination, before a decision is made as to whether to perform gonadectomy (Table 2), the patient's risks of developing a GGCT should be stratified. In addition, other factors, some of which have already been mentioned, should be considered; these include the patient's chromosome set, underlying diagnosis, age (the older the age, the higher the risk), race (higher risk in Caucasians), anatomical location of the gonad (higher risk in an intra-abdominal location), genital

Table 2 Criteria rendering gonadectomy advisable in DSD patients.

- Histopathology diagnosis of GCNIS[a]/GB (required in GGCTs):
  - o In cases of GCNIS, the parents should be informed that the presence of this lesion in biopsy predicts progression to an infiltrating GGCT in virtually 100% of cases (according to some authors, this figure is lower) with a probable time of onset of 5–10 years later
  - o In the case of GB, although there is a risk of GGCTs, this is considered minor (the exact figure is unknown)
- Histopathology diagnosis of UGT. Considered a precursor to GB by some authors
- Blood detection of Y chromosome material through karyotype/FISH or molecular testing for genes of the GBY region (TSPY, SRY and DYZ3, among others) in patients with Turner syndrome. Although it is not routinely done, FISH could be proposed in search of Y chromosome material in the gonad
- To be considered when felt gender differs from gonadal sex or the gonad is not functioning

DSDs: differences of sex development; GCNIS/GB: germ cell neoplasia in situ/gonadoblastoma.
[a] GCNIS is usually diagnosed in puberty. Pre-GCNIS is usually a finding reported before puberty (repeat biopsy in puberty to confirm progression to GCNIS and, if confirmed, perform gonadectomy).

Table 3 Proposed follow-up in DSD patients at risk of GGCTs[a] from the start of puberty to gonadectomy (or indefinitely, if there is a desire to keep the gonads).

- Lifelong gonadal self-examination, and regular palpation by a paediatrician/general practitioner
- Yearly gonadal ultrasound as of puberty (pre-puberty if at high risk) in those in an inguinal or scrotal location. Inguinal or scrotal orchidopexy is advised in those in an intra-abdominal location
- Yearly detection of alpha-fetoprotein, beta-hCG and LDH as of puberty (in pre-puberty if at high risk) as markers of some GGCTs.[b] Alternative that still lacks clinical validity: detection of micro-RNA in plasma

beta-hCG: chorionic gonadotropin; DSDs: *differences of sex development*; GGCTs: gonadal germ cell tumours; LDH: lactate dehydrogenase.
[a] This group can include patients in whom biopsy showed no histopathological risk of GGCTs (GCNIS/GB) but was concluded to be potentially non-representative and in whom, furthermore, there are any risk factors such as the presence of testicular tissue, Y chromosome material or intra-abdominal gonad location.
[b] Very significant increases in beta-hCG (by several thousand units) are associated with the presence of choriocarcinoma, while mild increases (by hundreds to one or two thousand units) are associated with the presence of syncytiotrophoblast cells. High levels of alpha-fetoprotein, for their part, are characteristic of yolk sac tumour. There is no increase in such markers in seminoma (or dysgerminoma in the ovary), embryonic carcinoma or teratoma, post-pubertal type.

phenotype, potential fertility, quality of gonadal endocrine activity, potential for performing self-examination, risks inherent to surgery and possible side effects of hormone replacement therapy.[3,8,10,14] To these factors must be added other, no less important factors of a psychological nature, such as the patient's degree of understanding of risk and cooperation, as well as congruence with the patient's gender identity. All these things must considered together, on the fundamental basis of an evaluation of the degree of development of this identity, when establishing strategies for action and monitoring. Therefore, from the start of the medical approach to a DSD, and essentially during adolescence, the gender identity of these patients should be examined and worked on so that all surgical decisions may be made with the greatest assurances of success.[26] Bear in mind, however, that recent studies have shown that most adult individuals with DSDs identify with the gender assigned at birth, at rates close to those of the general population, although when such congruence is not present, rates and degrees of gender dysphoria have been reported as very high.[27]

When a patient with a DSD, or the patient's parents, ultimately wish to keep the gonads despite the genotype and/or histopathological study and immunohistochemistry testing showing a non-negligible potential for malignant transformation, they can be offered a strategy based on early, proactive detection of localised lesions suggestive of GGCTs through inguinal or scrotal ultrasound imaging and/or through self-examination of the gonads if they are easily palpable (in a scrotal location). If, by contrast, the gonads have an intra-abdominal location, given that imaging has proven insufficient here, alternatives have been proposed such as relocation thereof to the inguinal canal for better visualisation. Whether the reasons for keeping the gonads are these or others, follow-up thereof is advised as of the start of puberty as set out in Table 3.[5,8]

Finally, whatever the decision made with respect to gonadectomy, the patient and the patient's parents should be informed as to the patient's prospects for fertility. First of all, it is necessary to assess the risk of transmitting the genetically determined DSD condition to one's descendants. It is also necessary to look for germ cells in a gonadal tissue

Table 4 Proposed consensus for performing gonadectomy by type of DSD.

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
DSD with abnormalities in the karyotype	**45X0/46XY** (mixed gonadal dysgenesis)	**Intermediate to high** for GGCTs (15%–35%)	**Gonadectomy is recommended**, although postponing it can be considered depending on degree of virilisation, gender identity and reproductive wishes (if potential for fertility is confirmed):[2,8,30,31] • ***Mild hypovirilisation***: - **Bilateral pre-pubertal biopsy** is required to classify the gonad. Orchidopexy in cases of non-palpable (intra-abdominal) gonads between six months and one year: ■ In cases of streak gonads or hypoplastic ovaries, the presence of GB should be ruled out to decide upon **early bilateral gonadectomy** ■ In cases of testicular tissue, assess the presence and maturity of germ cells (delayed maturation versus pre-GCNIS) and propose follow-up as of puberty: • Self-examination every three months and yearly ultrasound as of the start of puberty • Consider annual or biannual laboratory follow-up of markers of GGCTs (beta-hCG, LDH and alpha-fetoprotein) during puberty (see Table 3) • Repeat **biopsy** after puberty (see below) - Repeat biopsy after puberty (17—25 years of age) in patients not having undergone early gonadectomy. At this time it is possible to evaluate the risk of GGCTs with specific immunohistochemistry → In cases of **GCNIS** perform **gonadectomy** (as an alternative, gonadal radiation therapy can be proposed to preserve hormone function in Leydig cells, which are more resistant than germ cells) • ***Limited virilisation (ambiguous genitals):*** - Functional testing of the gonad (baseline laboratory testing during minipuberty or beta-hCG testing subsequently) followed by... - Bilateral pre-pubertal biopsy to classify the gonad ± orchidopexy if applicable (see previous regimen): consider **bilateral gonadectomy before or during puberty** in cases of (1) insufficient hormone production requiring hormone replacement therapy, (2) impossibility of bringing the gonad into a stable scrotal position, (3) suspicion of malignant transformation on physical examination or ultrasound, or (4) morphological or immunohistochemistry abnormalities related to GCNIS • ***Feminine phenotype***: consider **bilateral gonadectomy performed early**, i.e. before puberty (in cases of signs of virilisation it must be performed at the time of diagnosis). If the patient or the patient's parents are reluctant to pursue gonadectomy, consider leaving the gonads in place and weighing the possible effects of hormone production during puberty

Table 4 (*Continued*)

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
	46XY/46XX (chimerism: streak testicle or ovotestes)	**Low or very low** for GGCTs (2.6%–3%)	**Gonadectomy** will be indicated in puberty, **only on the gonad** (testis or ovary) inconsistent with assigned gender[5,8,32–35]: 1. If the tissues are separated, this surgical procedure will be feasible 2. In the ovotestes (both tissues in a single gonad) the separating surgical procedure would be possible only if there are two differentiated areas (bilobed shape) of ovary and testicle. Some have advocated for the use of intraoperative gonadal ultrasound during laparoscopic externalisation thereof to reliably identify ovarian and testicular tissue[32,35]: - Gonadectomy of the ovary will be done in patients assigned as male to avoid gynaecomastia and cyst formation due to FSH stimulation during puberty; Müllerian structures will also be removed. Temporary alternative at the start of puberty: GnRH analogues, which would slow down both the testicular and the ovarian component - Gonadectomy of the testicle will be done in patients assigned as female to prevent virilisation during puberty. Subsequently functional testing (baseline hormones and β-hCG testing) can be performed to assess the efficacy of the procedure. Temporary alternative at the start of puberty: GnRH analogues, which would slow down both the testicular and the ovarian component If the ovotestis is macroscopically indistinguishable mixed tissue, the use of GnRH analogues at the start of puberty will be required and initiation of hormone replacement therapy must be considered. Performing **bilateral gonadectomy after puberty** can be considered
	45X0/46XX (Turner syndrome)	**Nearly null or intermediate** for GGCTs (GB) depending on whether or not Y chromosome material is detected (0%–1% and 12%–40%, respectively)	All types of Turner syndrome, essentially non-mosaic 45X0 Turner syndrome, require **detection of Y chromosome material** in the blood (karyotype/FISH followed by molecular testing of genes of the GBY region such as *TSPY, SRY* and *DYZ3*)[36–41]: - Neither gonadectomy nor follow-up will be required in the absence of Y chromosome material. Bear in mind, however, that if only blood testing was done for karyotype, the results thereof do not rule out the possibility of the presence of Y chromosome material in the gonads; this situation arises in 6%–11% of cases and carries a risk of GB - **Should said material be detected**, **bilateral gonadectomy at the start of puberty** would be advisable, although some would propose biopsy with histopathological study and immunohistochemistry testing to make this decision

Table 4 (*Continued*)

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
	47XXY (Klinefelter syndrome)	**Null** for GGCTs	**Gonadectomy is not indicated**, and neither biopsy nor specific follow-up in relation to the gonads is required. The risk of GGCTs is reported exclusively extragonadally. There have been rare reports of Leydig cell tumours[2]
	47XYY	**Null** for GGCTs	**Gonadectomy is not indicated**, and neither biopsy nor specific follow-up in relation to the gonads is required
XX DSD with hyperviril-isation	Hypervirilisation of **NON**-gonadal origin (CAH, virilising tumours, etc.)	**Null** or **nearly null** for GGCTs	In general terms, **gonadectomy is not indicated**, and neither biopsy nor specific follow-up in relation to the gonads is required: - In the specific case of CAH due to mutations with loss of *CYP21A2* function, there is no increased risk of GGCTs (GB). Gonadectomy would be performed rarely if the male gender were assigned in 46XX CAH with extreme virilisation - There have been isolated published cases of GGCTs in other rarer forms of CAH due to mutations in *CYP17A1* and *HSD17B3*[42,43] - In cases of nodules of tumours of adrenogenital syndrome when they become autonomous testicular biopsy of the nodules would be indicated for differential diagnosis with Leydig cell tumours
	Hypervirilisation of **gonadal** origin (46XX ovotesticular DSD and 46XX testicular DSD)	In the absence of *TSPY* (± *SRY*), possibly **low** or **very low** risk (2.6%–3%) of GGCTs	Gonadal biopsy is usually necessary for a definitive diagnosis in which, furthermore, a karyotype is suggested to rule out gonadal mosaicism, as well as molecular testing of Y chromosome material to determine risk of GGCTs (at least *TSPY* and *SRY*) This 46XX gonadal dysgenesis group usually has a genetic cause such as translocation of the *SRY* gene to the X chromosome or to an autosome (10%–15% of cases of ovotesticular DSD, or 80% of cases of testicular DSD), or, more occasionally, duplication of *SOX9*, mutations in *RSPO1*, *SOX10*, *NR5A1*, *NR2F2*, *WNT4* or *WT1*. Few cases feature underlying 46XX/46XY gonadal mosaicism in which the risk of GGCTs seems to be equally low (see 46XY/46XX entity)[5,8,32–35]
		In the presence of *TSPY* possibly intermediate risk of testicular tissue for GGCTs	**Gonadectomy** will be indicated in puberty, only on the gonad (testis or ovary) inconsistent with assigned gender. See prior 46XY/46XX section: - In cases in which there is no underlying genetic cause (negative *SRY)*, the steps outlined in the above-mentioned section are to be followed - In cases in which there is an underlying genetic cause (*SRY* ± *TSPY*), we propose follow-up with markers ± gonadal ultrasound during puberty until a decision is made with respect to bilateral gonadectomy. Meanwhile, puberty can be blocked with GnRH analogues and hormone replacement therapy can be started

Table 4 (*Continued*)

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
XY DSD	**Complete testicular dysgenesis and partial testicular dysgenesis** (complete gonadal dysgenesis [CGD] and partial gonadal dysgenesis [PGD])	**Intermediate to high** for GGCTs (12%–60%) depending on molecular abnormalities	Most cases require **gonadal biopsy** for a definitive diagnosis. **Decision-making concerning gonadectomy** in this broad subgroup **is complicated** since the risk of GGCTs varies widely depending on various factors: (1) the presence of the *TSPY* gene (Yp.11.2) [detection of *SRY* solely indicates that there is Y chromosome material; it is not useful as a marker of tumour predisposition] and of the causative gene (when found, even though the risks are not usually well established), as well as (2) the characteristics of the gonad: anatomical position (scrotal, inguinal or abdominal) and stage of maturation (undifferentiated or UGT, ovary, testicle or ovotestes): the more mature the gonad and the lower its location, the lower the tumour risk.[5,8,12,13,17,44] For more details, the following is proposed: 1. If the ***female gender*** is assigned or the ***phenotype is feminine***, **bilateral gonadectomy performed early** or **around puberty** is advised depending on gonad type and considerations in the previous paragraph, because: (a) precursor lesions and invasive tumours can develop even in pre-puberty, and (b) given that the gonad is completely dysgenetic (CGD), androgens and oestrogens are not going to be synthesised. In cases of UGT, gonadectomy should be performed at the time of diagnosis 2. When there is ***genital virilisation*** and the ***assigned gender*** is ***male*** or ***there are reasonable doubts about gender***, the option of postponing gonadectomy to after puberty can be proposed since the testes, though dysgenetic, can produce testosterone and enable a certain degree of virilisation in puberty in 46XY males with PGD. Notwithstanding everything, in these patients, the risk of developing an invasive tumour should be weighed against the benefits of possible hormone production. Therefore, the **decision to perform gonadectomy should be based on** (1) *the results of a pre-pubertal biopsy* (to rule out GCNIS/GB) and one or more post-pubertal biopsies, both with detailed immunohistochemistry testing, and (2) *the results of molecular testing*, where available, as set out in detail below:
	Ovotesticular XY DSD	**Low for** GGCTs (2.6%–3%)	
	Ovarian XY DSD	Not reported. Possibly **low**	

Table 4 (*Continued*)

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
			a. In ***WT1*** gene mutations (Frasier syndrome and Denys–Drash syndrome), the risk of developing GCNIS/GB ranges from 40% to 60%. **Early bilateral gonadectomy** is recommended[5] b. In mutations of the ***SRY*** gene with CGD or PGD, development of gonadal tumours has ranged in different series from 20% to 52.5% of patients.[5] In such cases, **early bilateral gonadectomy** is recommended, although some authors have proposed performing gonadal biopsy in cases of limited virilisation with immunohistochemistry testing in pre-puberty to rule out the presence of GCNIS/GB* c. In mutations that predominantly affect the ***MAP3K1*** gene, which were recently reported but have a relatively high prevalence among single-gene causes of XY gonadal dysgenesis, the risk of GGCTs could be elevated.[5] In such cases **early bilateral gonadectomy** could be recommended, although performing gonadal biopsy with immunohistochemistry testing in pre-puberty to rule out the presence of GCNIS/GB would seem to be a suitable alternative* d. Two 46XY sisters with CGD and a homozygotic mutation in the ***DHH*** gene were reported to have a seminoma at age 30 and a seminoma and GB at age 17.[45] Decision-making concerning gonadectomy could be based on morphological study and immunohistochemistry testing of a pre-pubertal biopsy* e. GB has been reported in patients with 9p deletion affecting the ***DMRT1*** gene.[46] Decision-making concerning gonadectomy could be based on morphological study and immunohistochemistry testing of a pre-pubertal biopsy to rule out GCNIS/GB* f. All the patients of the same pedigree with a mutation in the ***FTHL17*** gene (Xp21.2) presented CGD and a gonadal tumour (GB or dysgerminoma at age 15).[47] Decision-making concerning gonadectomy could be based on morphological study and immunohistochemistry testing of a pre-pubertal biopsy*xxxxx Dominant mutations in ***SOX9*** can develop GB and dysgerminoma.[48] Decision-making concerning gonadectomy could be based on morphological study and immunohistochemistry testing of a pre-pubertal biopsy* g. A mutation with a dominant effect on ***WWOX*** and PGD showed the presence of GB precursor germ cells at two years of age.[49] Decision-making concerning gonadectomy could be based on morphological study and immunohistochemistry testing of a pre-pubertal biopsy* although it is not possible to make an evidence-based recommendation and therefore early gonadectomy could be considered h. For mutations in the ***NR5A1*** gene (SF1), pre-puberty and post-puberty abnormalities in the interstitium and tubule have been reported in various patients, as has the presence of a GCNIS lesion in a 13-year-old pubertal patient.[50] Therefore, decision-making concerning gonadectomy could be based on morphological study and immunohistochemistry testing of a pre-pubertal biopsy to rule out GCNIS/GB* i. For all other genes not mentioned, or if the cause is unknown, morphological study or immunohistochemistry testing of a pre-pubertal biopsy is proposed*

Table 4 (*Continued*)

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
			**Ruling out GCNIS/GB before puberty makes it possible to postpone gonadectomy or wait for a second biopsy in puberty/post-puberty. As an alternative to gonadectomy some authors have proposed gonadal radiotherapy*
	Abnormalities in androgen synthesis	Not well known (varies by condition)	Although the risk seems generally low or very low, certain conditions seem to carry a risk of GGCTs. Decision-making concerning gonadectomy will depend, therefore, on molecular diagnosis as well as assigned gender[5,8,51,52]: 1. If the ***female gender*** is assigned there may be virilisation in puberty; therefore, **bilateral gonadectomy** is recommended 2. In those assigned ***male gender***, tumour risk is unknown but probably low because testicular differentiation is normal. Some factors such as cryptorchidism, variable degrees of delayed maturation of germ cells and other unknown factors contribute to a higher risk (cases of invasive GGCTs have been reported in this population): a. In 17-ketoreductase (*HSD17B3*) **deficiency**, the risk appears to be intermediate (17%–28%); hence, **post-pubertal bilateral gonadectomy** is recommended. If the testes are kept, they should be brought into a scrotal position, biopsy should be performed and their course should be rigorously monitored (Table 3) b. In **5-α-reductase** deficiency (*5⟨R2*) and in **Leydig cell aplasia/hypoplasia**, the risk is unknown, though not null (possibly low). In 5-α-reductase deficiency, the frequent acquisition of a male gender identity, the strong possibility of spontaneous masculinisation during puberty and even the possibility of fertility render keeping the gonads advisable, although follow-up is needed (Table 3), as there have been rare reports of GGCTs (seminoma in a young adult)

Table 4 (*Continued*)

Group	Condition	Risk	Recommendation for gonadectomy and follow-up
	Androgen insensitivity syndrome	*Complete forms (CAIS) with no residual AR receptor activity:* **Low risk of GGCTs**[#] (variable risk across studies: 1%–3% with a cumulative risk of 3.6% at 25 years of age and 33% at 50 years of age,[48] other authors have estimated a risk of 15% after puberty[49])	As the risk of malignant transformation in adulthood is largely unknown, decision-making with respect to gonadectomy in CAIS remains enmeshed in a great deal of debate. In any case, at present, the most commonly accepted recommendation is **gonadectomy in late puberty** since: (1) GGCTs (seminoma) present after puberty and (2) if the gonads are kept until post-puberty then spontaneous pubertal feminisation will occur (due to androgen aromatisation) with no need for exogenous oestrogens, thus achieving suitable optimisation of both breast development and bone mineralisation. In addition, (3) postponing gonadectomy enables the patient to take part in decision-making after being duly informed[8,53–56]: • *If a decision is made to perform* ***gonadectomy after the end of puberty*** (18—20 years of age), the need for replacement hormone therapy should be discussed with the patient and the patient's family. In general, the oestrogen doses that are required to maintain bone mass and prevent symptoms of oestrogen deficiency are higher than those used in menopause, and should be adapted to each patient • By contrast, *if a decision is made to postpone or not perform gonadectomy*, one option for follow-up is gonadal biopsy with immunohistochemistry staining. Often, laparoscopic orchidopexy is needed for the gonad to be accessible. Tumour markers such as β-hCG and LDH, which are high in patients with seminomas (β-hCG is elevated in 15%–20% of seminomas with syncytiotrophoblst cells, and LDH is elevated in 40%–60% of seminomas). In follow-up using imaging techniques, magnetic resonance imaging (MRI) does not appear to be useful for detecting pre-malignant microscopic lesions[57]; ultrasound and testicular computed tomography (CT) have been used to detect GGCTs in males with maldescended testicles with abdominal or inguinal masses,[58] although this type of follow-up has not been performed in women with CAIS, and some authors have deemed follow-up by means of ultrasound every six months of the gonads in CAIS following orchidopexy to be suitable.[59] One variant on this proposal is that of Patel, who recommended performing an initial MRI to locate the gonads[55]: - If the testes are visualised, follow-up should be performed with tumour markers yearly and testicular ultrasound every six months - If the testes are not seen on MRI, then laparoscopic orchidopexy and gonadal biopsy with assessment of OCT3/4 at the end of puberty (18—20 years of age) are recommended [#] *In CAIS, in addition to the (low) risk of GGCTs, the risk of gonadal stromal tumours and, more commonly, smooth-muscle leiomyomas/hamartomas should not be overlooked*
		Partial forms (PAIS) and CAIS with residual AR receptor activity: **Intermediate to high risk** of GGCTs (15%–20%) Minor, if a scrotal location; recent studies have substantially reduced this risk	No studies have performed follow-up in patients with PAIS in whom gonadectomy was postponed; therefore, the predominant approach is **early bilateral gonadectomy**[56] 1. In *women*, **pre-pubertal gonadectomy** is generally recommended without too many reservations to prevent virilisation and the risk of malignant transformation 2. In ***males***, although the recommendation is the same, should there be a desire to postpone gonadectomy, **orchidopexy** should then be performed to facilitate follow-up. In such cases, the strategy may be similar to that recommended in CAIS (see above)
	Patent Müllerian duct syndrome	**Low or very low** for GGCTs	**Gonadectomy is not indicated**, nor is biopsy required, although rare cases of GGCTs reported could render follow-up as of puberty or later advisable (see Table 3)[3]

beta-hCG: chorionic gonadotropin; DSDs: *differences of sex development*; FSH: follicle-stimulating hormone; GBY: region around the centromere of the Y chromosome; GCNIS/GB: *germ cell neoplasia* in situ/*gonadoblastoma*; GGCTs: gonadal germ cell tumours; GnRH: gonadotropin-releasing hormone; LDH: lactate dehydrogenase.

biopsy. Numbers thereof decrease considerably with age, such that they are absent or very scarce after puberty in most of these patients. This cellularity, however, is highly dependent on the patient's condition, such that it may be absent or limited as of birth in a large proportion of cases of gonadal dysgenesis, or it may be normal or almost normal in the beginning, but gradually decrease and exhibit a predominance of immature forms, in patients with complete androgen insensitivity and in patients with mild defects of androgen synthesis such as 5-alpha reductase deficiency. In any case, although the techniques for preserving germ tissue applicable today are feasible (cryopreservation of precursor tissue in early stages of life or, rarely, of sperm or oocytes in some mild cases of DSD with spontaneous pubertal onset), very strict research protocols are required for techniques of induction of future fertility of immature germ cells (maturation in vitro), which have already been achieved for ovarian germ tissue, but are not currently available for those of testicular origin except experimentally.[28,29]

Proposed indication for prophylactic gonadectomy in differences of sex development

In summary, the current recommendations on prophylactic gonadectomy, listed in Table 4, are based on type of DSD. Gonadal radiation has been proposed as an alternative, but experience is limited.[8]

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