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Sexual Developmental Disorders in Pediatrics

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Abstract

Disorders of sex development (DSD) are a heterogeneous group of pathologies that result in an alteration in sex determination or differentiation. DSD are estimated to affect 1: 4,500 newborns and according to the 2006 Chicago Consensus classification, DSD can be divided into three categories: those with a 46 XX karyotype, those with a 46 XY karyotype and those relating to sex chromosomes. It is crucial to correctly identify the pathology already in the first days of life to direct the patient and his family to the best path of care. For this reason, the role of the pediatrician is fundamental in the correct identification of the clinical picture and in supporting the family during the long process that involves the management of these patients. To make a diagnosis, it is necessary to follow a path led by a multidisciplinary team that includes several steps such as the execution of the genetic analysis, the evaluation with diagnostic imaging methods and laboratory evaluations. The therapeutic management, on the other hand, is still very complex even if in recent years we have moved from an attitude of early gender reassignment to an approach of watchful waiting to let the patient choose when she/he is mature enough to do so, which gender she/he feels to belong. It should not be forgotten that throughout this process the pediatrician must be both supportive and clinically active in the management of the child and his family. ***Clin Ter 2022; 173 (5):475-488 doi: 10.7417/CT.2022.2466***

Key words: *Ambiguous genitalia, disorders of sex development, newborn, genetic, pediatrician, hormonal therapy, surgery, gender assignment*

Introduction

Disorders of sex development (DSD) are a heterogeneous group of pathologies that leads to an alteration in the determination of sex and its respective differentiation with atypical development of the internal and external genitalia (1). This clinical condition may be due to genetic and/or hormonal alterations that lead to anomalies during the sex determination process (2). However, it is necessary to differentiate sexual development disorders from ambiguous genitalia which instead represent a phenotypic alteration of the urogenital system (3). In numerical terms, it is estimated that the incidence of DSD is about 1 per 4500 live births (4). The main aim of this report is to propose the pediatrician as a key figure in the diagnostic and therapeutic path of patients with DSD. For this reason, it is essential that the pediatrician is fully aware of the latest scientific evidence in the DSD field disorders in order to propose the most appropriate path for the individual patient (Fig. 1).

Embryology

Before talking about the embryological processes that define sexual development, it is necessary to explain ***the genetic or chromosomal sex*** that develops at the moment of fertilization, determining the growth of a XX or XY gamete (1,5). Subsequently, the genetic sex defined by **sexual differentiation** will determine the development of the gonad, between the 7th and 13th week of fetal life, defining ***the gonadal sex*** (6).

The gonadal sex corresponds to the primary sexual characteristics that will determine, both during the prenatal and postnatal life, the development of the secondary sexual characteristics that usually occur during puberty. The set of anatomical, functional and behavioral aspects, determined by the primary and secondary sexual characteristics, define ***the phenotypic sex*** (7). The formation of the gonads begins from the 5th week of gestational life. Indeed, the gonadal crests originate from the lateral mesoderm also called splanchnopleure which represents the sketches of the gonads (8). Until the beginning of the 6th week of development, the testicles and ovaries are morphologically indistinguishable, but starting from the 7th week the differentiation of the testicles begins (9).

Around this week of gestation, the seminiferous cords are formed which will host the gonocytes or prospermatogonia, that will be surrounded by the Sertoli cells (10). The seminiferous tubules will branch outreaching the hilum of the testis and giving rise to the rete testis. At the same

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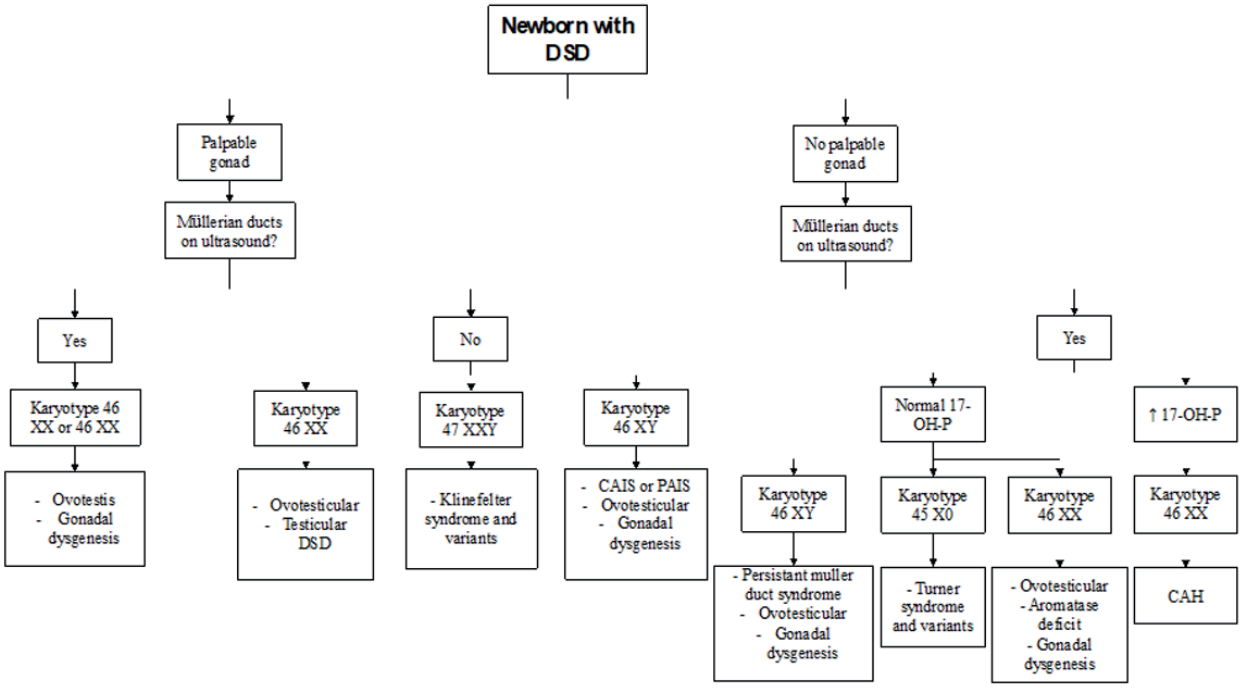

Fig. 1. The figure shows the diagnostic procedure to follow for the newborn with sexual developmental disorders (DSD). CAH (congenital adrenal hyperplasia): PAIS (partial androgen insensitivity syndrome): CAIS (partial androgen insensitivity syndrome).

time, the interstitial cells of Leydig are arranged between the seminiferous tubules (11). Around the 20th week of pregnancy, the testes migrate from their intra-abdominal position to the scrotum passing through the inguinal canals (12). The ovaries, on the other hand, are not identifiable until the 10th week of gestation, the germ cells differentiate into oogonia within the secondary sexual cords. Oogonia proliferate intensely around the 5th month of pregnancy with a production of about 5-7 million gametes. Once the proliferative phase is over, the ovogonium degenerates or undertakes the meiotic division (13). The cells that started the first meiotic division are called primary oocytes, when the oocytes reach the diplotene stage they are surrounded by the follicular cells. In this way, between 5 and 9 months of embryonic development, the primordial follicles develop with about 4-5 follicular cells that enclose a primary oocyte in diplotene. Usually, before birth, the ovaries descend from the abdominal wall to the pelvis. At birth there are about 1 million primordial follicles, considering both two ovaries, each containing a primary oocyte still at the diplotene stage (14).

During the 4th week of gestation the mesonephric or Wolff duct develops, at the 6th week the paramesonephric or Müller duct develops outside the Wolff duct, both ducts open into the cloaca (15). Starting from week 8th, Leydig cells produce testosterone and dihydrotestosterone (DHT) which will determine the differentiation of Wolff's duct into the epididymis, seminal vesicles and vas deferens; the Sertoli cells instead produce the anti-Müllerian hormone or AMH which inhibits the development of Müller's ducts (16,17). It should be remembered that the testicle begins to produce testosterone from the 8th week of gestation with a progressive increase up to about the 14th week of gestation, the testosterone is then partially converted into DHT by the 5-alpha-reductase enzyme (18). The DHT is required for the fusion of the urethral and labioscrotal folds, elongation of the genital tubercle, and regression of the urogenital sinus (19,20). In females, the fusion of the two Müller's ducts will give life to the fallopian tubes, uterus and the upper portion of the vagina (15).

At the end of the 5th week, the genital tubercle is formed which, thanks to hormonal production, will form the penis in males. In females, the genital tubercle increases, but in a limited way compared to the male, turning into the clitoris (21,22). Similarly, the genital swellings formed at the end of the 5th week will give rise to the scrotum in the male and the labia majora in the females (19).

Genetics

The formation and growth of the undifferentiated gonad depend on the expression of a group of genes such as the steroidogenic factor 1 (Sf1), GATA binding protein 4 (GATA4), Wilms tumor 1 (Wt1), chromobox homolog2 (CBX2), LIM homeobox factor 9 (LHX9), sine oculis-related homeobox 1/4 (SIX 1/4) and empty spiracles homeobox2 (EMX2) (5,23–29). These genes favor the expression of certain proteins necessary for the development of the gonad. The

differentiation of the gonad in the male sense occurs thanks to the presence of the Y chromosome which encodes the SRY gene (30). The latter in association with SF1 and Sry-box transcription factor 9 (SOX9) stabilizes the environment allowing the development of Leydig cells and male germ cells (30). The fibroblastic growth factor (FGF-9) determines the production of SOX9 which antagonizes the production of Wnt family member 4 (WNT4) (31,32).

As for the female gonad, the growth factor determining its development is WNT4, this promotes the production of follistatin which inhibits activin B and therefore the correct formation of the vascular network of the testicle (33,34). Another factor that appears to be involved is Forkhead box ligand 2 (FOXL2) which is fundamental in the differentiation and maintenance of ovarian differentiation. Furthermore, WNT4 inhibits SOX9 production in pre-granulosa cells (35). In conclusion, it would seem that the two factors determining the transformation of the undifferentiated gonad into testis or ovary are FGF-9 and WNT4 as one antagonizes the function of the other (32,36).

Classification of disorders of sexual development

According to the Chicago Consensus of 2006, the term DSD refers to all those congenital conditions in which the chromosomal, gonadal, or anatomical sex is not in conformity with the usual processes of embryonic development of the gonads and/or genitals (37). This classification allows to divide the DSD into three categories, as shown in table 1:

– DSD with 46, XX karyotype
– DSD with 46, XY karyotype
– DSD related to sex chromosomes

DSD with 46, XX karyotype. The 46,XX DSD cause virilization of the female fetus. The final common pathway of all 46,XX DSD is an excess of dihydrotestosterone (DHT) in the genital tissue during the critical period of sexual differentiation (38). The most common form of DSD belonging to this group is congenital adrenal hyperplasia (CAH) (39). This pathology occurs due to the deficiency of 21-hydroxylase, related to the deficit of the CYP21A2 gene, which is the most common form of congenital adrenal hyperplasia (CAH) (40). Two subtypes are recognized: the simple virilizing form or the form with salt loss; it has a prevalence of about 1 case in 14:000 and it clinically manifests already at birth (40–43).

Females have ambiguous genitalia characterized by: clitoromegaly, labia majora partially fused with wrinkles, and common urogenital sinus (22,44). The degree of virilization can range from an almost masculine appearance to minimal clitoromegaly, the uterus is normal and the developmental abnormalities of the vagina are variable (3,40).

The salt-losing forms of CAH involve dehydration and hypotension in the first two to three weeks of life due to aldosterone deficiency (43,45). The patients may develop growth retardation, hyponatremia, hyperkalemia, acidosis and hypoglycemia (40). Hyperandrogenism can manifest itself in pediatric age with accelerated growth and skeletal maturation, advanced bone age, and premature puberty. In adulthood, hyperandrogenism can manifest with acne, hirsutism, menstrual problems, infertility, metabolic syndrome and obesity (39,43).

Non-classical 21-hydroxylase deficiency is more common than classical 21-hydroxylase deficiency. The incidence varies from 1/1000 to 1/2000 live births. Non-classical 21-hydroxylase deficiency causes a less severe form of the disease as there is residual activity of the enzyme from 20% to 50% of normal. In the classic form of the deficit, however, the residual activity fluctuates between 0 and 5% of the total (46,47). The loss of salts is absent because the levels of aldosterone and cortisol are normal; however, adrenal androgen levels are elevated, resulting in slight excess of androgens in childhood or adulthood (39).

Table 1. The table shows the classification of sexual developmental disorders (DSD) according to the Chicago Consensus of 2006.

DSD with 46 XY karyotype [16,37,38,46,52]				
Disorders of gonadal development	Disorders in androgen synthesis	Disorders in androgen action	Disorders of AMH or AMH receptor	Unclassified disorders
Complete gonadal dysgenesis (Swyer syndrome) Partial gonadal dysgenesis Ovotesticular Testicular regression	Androgen biosynthesis defect	Androgen insensibility (CAIS)	Persistent Mullerian duct syndrome (PMDS)	Severe hypospadias Epispadias Cloacal extrophy
DSD with 46 XX karyotype [35,37,39,46,48,93,132]				
Disorders of gonadal development		Disorders of androgen excess		Unclassified disorders
Gonadal dysgenesis Ovotesticular Primary ovarian insufficiency		Congenital adrenal hyperplasia (CAH) Aromatase deficiency		MRKH I and II syndrome Cloacal extrophy Vaginal atresia
DSD related to sex chromosomes [37,38,68,74,75,132,160]				
45, X		47, XXY		45 X/ 46 XY and 46 XX/ 46 XY Chimerism
Turner syndrome and variants		Klinefelter syndrome and variants		Mixed gonadal dysgenesis

Other forms of DSD belonging to this group include 11-beta hydroxylase deficiency, 3-beta-hydroxysteroid dehydrogenase deficiency, P450 oxidoreductase deficiency, and aromatase deficiency due to mutations in CYP11B1, HSD3B2, POR, and CYP19A1 (1). All these pathologies involve an increase in androgens with more or less severe forms of virilization. Another clinical condition belonging to this family is ovotesticular karyotype 46,XX characterized, from the histological point of view, by the presence of both testicular and ovarian tissue; the estimated prevalence is approximately 1:20.000 births (48,49).

The diagnosis is often made in the neonatal period due to the presence of anomalies of the genitals, but some patients have anomalies of pubertal development. Clinical symptoms include abdominal pain, gynecomastia, inguinal hernias, presence of an inguinoscrotal mass, cryptorchidism, or periodic amenorrhea and hematuria, depending on the sex assigned (28). Most patients have female internal genitalia such as the uterus, hemi-uterus, or rudimentary uterus. The development of the external genitalia varies from apparently female genitalia to male genitalia with curved penis and hypospadias (50). Rarely, patients have a normal or near-normal penis and ovotestis, gonads containing ovarian and testicular elements, bilaterally descended (49). The appearance of the gonads is variable. Infertility is common in males, while females are of potential fertility (35).

DSD with 46,XY karyotype. This group of DSD includes various groups of pathologies such as alterations in gonadal development, alterations in androgen synthesis, alterations in androgen functionality and the persistence of the Müller ducts syndrome (4). One of the most common pathologies belonging to this group is complete Androgen Insensitivity Syndrome (CAIS) or Morris Syndrome (51). This is a form of androgen insensitivity syndrome in individuals who have normal, but undescended testes, and androgen levels that do not match normal for age (52). The incidence is estimated between 1/20,000 and 1/99,000 live birth males (53). At birth, CAIS can be characterized by an inguinal hernia or the presence of labial protuberances, which contain the testicles (54). A typical sign is primary amenorrhea during adolescence at the same time breast development is normal, even if axillary and pubic hair is absent or sparse (55). The external female genitalia is often normal, while the internal ones are absent (56). The disease is due to mutations in the androgen receptor (AR), which is located on the short arm of the X chromosome (Xq11-12) (35). The CAIS variant is associated with a mutation in the AR gene that completely blocks its function; target cells do not respond to testosterone or dihydrotestosterone (DHT) (57).

Another pathology belonging to this group is the persistence of the Müllerian ducts syndrome (PMDS). This is a rare DSD, characterized by the persistence of Müllerian derivatives and by the presence of the uterus and/or fallopian tubes in male subjects who, for the rest, appear normally virilized (14,35). The exact prevalence in the general population is unknown; the patients are considered to be genotypically and phenotypically male and usually the affected individuals have cryptorchidism or inguinal hernia (58). The testes are normally differentiated and, in the absence of persistent cryptorchidism, usually contain germ cells; however, affected males can be sterile, as the testes are often not properly connected to the excretory ducts due to aplasia of the epididymis and the upper part of the vas deferens (59). Testosterone levels are usually normal unless testicular degeneration is present. PMDS is transmitted in an autosomal recessive manner and is due to the mutation of the anti-Müllerian hormone gene (AMH; 19p13.3) (16,60).

DSD of sex chromosomes. This group of DSD determines anomalies in the sex chromosomes and includes the Turner syndrome, Klinefelter syndrome and mixed gonadal dysgenesis (61). Turner syndrome is due to the partial or total monosomy of one of the two X chromosomes. It has a prevalence of 1:2,000-1:2,500 female infants (62). This syndrome is characterized by stature growth deficit, with a short final stature on average of 145-150 cm, and primary amenorrhea as a result of the involution of the ovaries (63). Renal anomalies such as horseshoe kidney, and total or partial duplication of the collecting system may also be present in the syndrome (64). Cardiac anomalies such as the bicuspid aortic valve, coarctation of the aorta, stenosis of the aortic valve and prolapse of the mitral valve, aortic dissection may occur in 15% -50% of cases (65–67). Affected patients have an increased risk of developing: hearing loss, thyroid disease, type 1 diabetes mellitus and celiac disease (68). In adulthood, however, they are at risk of developing obesity and type 2 diabetes mellitus (69). The syndrome is not normally associated with mental retardation, but learning difficulties have been reported (70).

Another DSD belonging to this group is Klinefelter syndrome (KS), which is a chromosomal disorder due to the presence of one or more Xs in a male's karyotype (71). In the neonatal period, there is a small penis with the possibility of hypospadias and/or cryptorchidism (72). Subsequently, language delay may occur around 3-4 years of age, which if not treated can be associated with reading defects, poor academic performance and a lack of self-confidence, which in adolescence can promote psycho-social discomfort (70,73). From an intellectual point of view, they are perfectly normal. Mental retardation in KS has the same incidence as the general population, about 3% (74,75).

At puberty, there may be a delay in sexual development with a definitive testicular volume that hardly exceeds 10ml, while the penis has a normal development (76). If the condition is not identified early, some degree of overweight can occur, with female-type fat deposition and, at puberty, mammary gland development or gynecomastia (77). These children are more prone to autoimmune diseases, metabolic syndrome and in 92% of cases, they have azoospermia with consequent infertility (75,78).

Physical examination

Physical anomalies may be arguably framed by a careful physical examination of the newborn (38). Such evaluation should be carried out by a multi-disciplinary team that includes a pediatrician, endocrinologist, urologist or gynecologist, surgeon, geneticist and psychologist or psychiatrist if it is considered appropriate (79,80). Social workers and experts in medical ethics could be of great help, if their presence is

allowed by the local resources, depending on the location and the social development. The most recent guidelines advise the specialists to chart a clinical pathway taking into consideration the possible diagnosis and gender assignment treatment before making any recommendations to the family. Thirdly we must stress the importance of possessing appropriate and effective communication skills, especially when discussing with the family which most commonly lacks any proper tool to process the delicate situation their newborn is going through (37,81).

The dysmorphisms included in the wide group of the DSD are often caused by a genetic pathology, this being the case for 7% of children with hypospadias, 3% of those affected by cryptorchidism and 13% of patients presenting both conditions (82). The pediatrician to perform a physical examination should start by taking an accurate family history, taking into account several conditions such as the presence of eventual parental consanguinity, multiple abortions, cases of infertility, Sudden Infant Death syndrome or a previous genital anomaly (83). The clinician has to ask the mother questions about her health, both in general and during the pregnancy to expose signs of virilization such as hirsutism which could be related to congenital adrenal hyperplasia (39). Furthermore, the ingestion of drugs and the exposure to specific environmental factors should be carefully taken into account since those elements could have led to the inhibition of virilization of the fetus during the pregnancy (84). An adequate history should also include the results of any prenatal tests performed and concentrate on what are the current concerns of the parents (85). It is pretty common among them to refuse to acknowledge the presence of atypical genitals usually for a social and cultural disadvantage, indeed, they show express confidence about the actual sex of the baby probably as a defense mechanism of self-persuasion (86).

Most parents, being a genuine reflection of contemporary society, are usually unaware of the existence of DSD and correlated medical conditions before the diagnosis of their child (87). An ongoing conflict that has been traced by the experts is the one between the desire to preserve the privacy of the affected child to avoid stigmatization and the sharing of the condition to social support access (88). The clinical evaluation must start with inspection and palpation to define the degree of genital anomaly, quantified by using the External Genitalia Score (EGS), a useful tool to identify the patients who could benefit from further medical investigations (89). This score is a modified more recent gender-neutral version of EMS, the External Masculinisation Score whose effectiveness has been proven repeatedly since it is objective, standardized and widely used, but that undoubtedly lacks a validated purpose when it comes to assigned females affected by a DSD (90). The non-binary EGS can instead be applied in both typical male and female babies and those affected by an alteration in their genital characteristics, making it a reliable and easy-to-use tool for a detailed description of external genitalia in neonates and infants (90).

While the EMS goes from 0 to 12, this being the score corresponding to the absence of any DSD in a typical boy, the EGS uses a gradual scale whose range of 0-12 goes from female to male (81,89).

The five characteristics of the external genitalia whose phenotype leads to the allocation of points are the ones listed in Table 2 (90). For legal medical reasons and for an accurate medical history, which would be available to the patient and the future health care professional dealing with the case, the detailed physical examination must be documented thoroughly (70). In the individuals presenting normal female external genitalia, the palpation allows the research of the gonads, the localization of the urethral meatus must be noted, together with the presence of an additional orifice that would correspond to the vaginal one (3). After defining the presence of eventual hypospadias or the much rarer, ones according to the localization respectively in the ventral or dorsal surface of the genital tubercle, the pediatrician may proceed to the careful measurement (91). It is important to describe the degree of labioscrotal fusion, whose range goes from the complete absence to a posterior fusion of labia majora to a complete fused scrotum (79,81).

Table 2. The table shows the corresponding score for each conformation of the analyzed anatomical landmarks (GT = Genital Tubercle).

	3	2.5	2	1.5	1	0.5	0
Labioscrotal Fusion	Fused			Posterior Fusion			Unfused
Genital Tubercle Length (mm)	>31	26-30		21-25	10-20		<10
Urethral Meatus	Top of the GT	Coronal Glandular	Along with the GT	At the GT base	Labioscrotal		Perineal
Right Gonad				Labioscrotal		Inguinal	Impalpable
Left Gonad							

The children whose physical examination can lead to a suspicion of DSD and who should be investigated by a specialist are the following:

– those with a combination of genital anomalies and a resulting EMS lower than 11, since typical boys in several studies almost entirely received the highest score of 12;
– those with isolated perineal hypospadias, micropenis and/or clitoromegaly;
– those affected by any form of familial hypospadias.

It is worth stating that this will avoid further medical evaluations for boys with isolated glandular hypospadias and boys with unilateral inguinal testis, which only result in a loss of 1 point for each mentioned alteration when it comes to define the EMS (92).

The diagnostic process

The correct diagnostic process includes:

1. **Karyotype**, whose results are usually available within 24/48 hours from the sampling and represent a mandatory step in the management of DSD since it defines one of the three major diagnostic subclasses (93). The initial management could benefit from the fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) techniques which involve the use of X- and Y-specific probes, the latter having largely replaced the first one (94). It has to be reminded that the mosaicisms could be tissue-dependent and that the test should be repeated in cases of prenatal karyotype mismatch (95,96).
3. **The ultrasound examination** is the most widely used imaging technique for internal sex organs, that unfortunately doesn't lack common misleading results, since it is operator dependent and requires a full bladder (85). Valid alternatives include a magnetic resonance imaging (MRI), likewise aimed to evaluate and demonstrate internal gonads and genitalia, and a genogram, used to assess the urethra, vagina, and any fistulas or complex tracts. Further investigation would include the laparoscopy which undoubtedly allows the straightforward identification of gonads with an appropriate grade of invasivity and it also makes possible intervention in the same procedure (97,98).
3. **Laboratory investigations**, including 17OH-progesterone, serum testosterone, Anti-Müllerian hormone (AMH), cortisol, androstenedione, gonadotrophins and uranalysis, are usually available within one week (81). Serial measurements have been advised strongly since the value of steroid hormones and gonadotrophins fluctuate over the first few weeks of life and abnormalities of electrolytes in patients with salt-losing conditions are not to be found until the second to the third week of life (39). Even if the physiological role of the testosterone is indisputable as a marker of well-functioning testes, the available laboratory technique usually lacks an acceptable specificity grade with many cases of cross-reaction with other conjugated steroids (97). For this reason, the definition of the serum AMH level is a more reliable tool when it comes to defining the functionality of testes (60). Moreover, AMH can be of great help in order to assess cases of anorchia, 46 XY complete gonadal dysgenesis and cases of persistent Mullerian duct syndrome (PMDS) with a defect of the AMH gene. It has been proven that the AMH concentration is completely different in boys and girls, especially in early childhood, with 200 ng/ml being the most widely accepted cut-off to define normality (99). Neuroinflammatory markers are also affected in DSD because immunocompetent cells in the brain are responsive to steroid hormones (100,101). Interleukin (IL)-1β, IL-6, IL-10 and tumor necrosis factor-alpha (TNF-α) and other neuropeptides regulate our immune system (102–106). Altered levels in neuroinflammation are indeed linked to the history of DSD (100,101). However, the neurobiological causes behind neuroinflammation and DSC are not yet fully interpreted.

Even if the first-line investigations are usually enough to determine the sex of rearing, the multidisciplinary team taking care of the child could consider it appropriate to run second-line tests as a gonadal biopsy (107). In the end, the experts agree on the importance of the human chorionic gonadotropin (HCG) stimulation test as a reliable diagnostic instrument used to evaluate testicular function during childhood (108). For instance, if there are viable testicular Leydig cells, a single injection of HCG at an effective dosage of 100 iu/kg can lightly increase the testosterone level, but noticeably for 72 to 120 hours (108).

Life-threatening conditions

One of the main objectives of the pediatrician in the management of a patient with DSD is to exclude life-threatening conditions such as adrenal and/or renal failure or tumors (109). Those conditions could be caused by the DSD itself or be the result of a linked non-genital malformation that makes the management of the patient more complex (97,110).

The congenital disorders associated with adrenal dysfunctions can be found in individuals with both typical karyotypes, i.e. 46,XX and 46,XY, but in the first case the genital anomalies are caused directly by the adrenal steroidogenic disorder, while the latter is due to the correlated testicular dysfunction (36). In the case of primary adrenal insufficiency, the reduction in cortisol secretion and the associated overproduction of ACTH cause the trophic impact on the genitals, while the eventual aldosterone deficiency may lead to salt-wasting crises, which have to be carefully considered for their deadly potential (111).

Renal failure can be found in the Denys–Drash syndrome, due to the progressive glomerulosclerosis, in the strictly associated Frasier syndrome and, with a lower prevalence, in the Wilms Tumor, whose incidence among the patients affected by the Denys-Drash almost reaches the 75% of the total and often requires urological surgery such as nephrectomy and renal transplantation (112,113). That is why, as we mentioned above, a pediatrician should investigate the proper functioning of the renal structure in all patients affected by DSD (97,112,114,115).

The tumor risk for specific types of cancers is significantly increased, resulting clinically relevant. The most

frequent forms of malignancies are the seminomatous and non-seminomatous types of tumors, which are classified as type-II Germ Cell Tumor (25,28). The proposed underlying reason for the increased risk of cancer is the testicular dysgenesis syndrome, which also links infertility and cryptorchidism (116). For the development of type-II Germ Cell Tumor in the patients affected by DSD a crucial element is the presence of the gonadoblastoma locus of the Y chromosome, shortened GBY, whose likely candidate gene could be the testis-specific protein on the Y chromosome, shortened to TSPY (117–119).

Since the GBY is a prerogative for malignant transformation, its presence in the karyotype of a patient with DSD is the discriminating factor for the risk classification at different levels: high, intermediate and low as shown in Table 3. It should be noted that some entities are quite rare and, as a consequence, the collected data are insufficient, which would otherwise allow the specific definition of the risk: for this reason, there must be added the unknown category, which includes 5 alpha-reductase deficiency and Leydig-cell hypoplasia (109,120). The recommended action for each group may differ, ranging from the gonadectomy in the high-risk one to the strict monitoring or the execution of a biopsy (120).

Gender assignment

The pediatrician is involved in the medical care of infants, children and adolescents, and one of the main objectives as a public health provider is to hand over to the community well-adjusted, functional members of society (70,121). Since sexual activity, gender identity and reproductive health are three fundamental aspects of the well-being of an individual, what has been said about pediatrics is valid in the management of patients with DSD (81). The DSD definition has replaced the terms "hermaphrodite", which results imprecise and mythological, and "intersex", which for some carried a stigma: patients with DSD aren't in between sexes, but just show variations in some parts of the body scientifically used to assign one's gender (122). The latest proposed term is "variations in sex characteristics", which seem to empower the patients with DSD. Nevertheless, in some cases the obsoletes terms have been used by the affected individuals or their advocates, it being the case of the Intersex Society of North America which, during the nineties, used the motto "Hermaphrodites with Attitude" (123). Anyway, health care providers must choose carefully the terms taking into consideration the preference of the individuals affected (123).

In the light of the above, one must consider how in the latest period the strictly binary structure of society which has been the rule throughout the history of Western culture is rapidly blurring. Unlike Europe and America, Southern Indian countries such as Pakistan have long been tolerant towards the third gender, there called Khawaja Sira, which forms part of the country's established culture (124). Even if Pakistani citizens forming part of this third gender aren't entitled to the same civil rights people have in the European liberal society, the example may be of help to understand how gender non-conforming attitudes have always formed part of the human behavior and must be accepted and respected (37,125). In the past decade, an increase in the societal acceptance of non-stereotypic gender presentation can be clearly perceived (126). A growing number of countries now recognize non-binary or third gender classifications, either as voluntary opt-in, as is the case for Australia, Canada and Denmark, or for intersex people only, as it happens in Germany and Austria. Furthermore, many national jurisdictions have been providing explicit protection against unnecessary and harmful modifications to the sex characteristics of children, the first being Malta in 2015 which prohibited forced surgical intervention on intersex minors through a law that recognized the right to bodily integrity and physical autonomy (37,127–129).

It is important to assess if the damage caused by irreversible surgical treatment is wider than the one caused by a delayed gender assignment (129). One of the bioethicist principles considers "ethical" essential for medical practice the respect for the autonomy of the patient, which is legally reified by informed consent (130). Pediatric health care implies a more complex situation where perhaps the best approach is the parental permission combined with childhood assent, for this reason, many pediatricians and mental health

Table 3. The table shows the different types of interventions depending on the risk of tumor of sexual developmental disorders (DSD).

RISK GROUP	DISORDER	INTERVENTION
HIGH	Gonadal Dysgenesis (+y) intrabdominal	Gonadectomy
	Partial Androgen Insensitivity Non-Scrotal	
	Frasier; Denys-Drash	
INTERMEDIATE	Y+ (GBY+) Turner syndrome	
	Gonadal Dysgenesis (+y)	Biopsy and Irradiation
	Partial Androgen Insensitivity Non-Scrotal	
	17b-hydroxysteroid dehydrogenase deficiency	Monitor
LOW	Complete Androgen Insensitivity	Biopsy
	Ovotestis DSD	Testis removal
	Y- Turner	None

providers, experienced in DSD treatment, advise deferring any irreversible sex assignment treatment until the person being treated can decide autonomously, focusing instead on the protection of the child's physical, psychological and social integrity (127,130–132). Likewise, multiple studies have estimated that the prevalence of gender dysphoria in patients with DSD ranges between 8.5% and 20%, showing a rejection of the sex which has been assigned at birth with major consequences for the patient's psychological integrity (133). Nonetheless, gender uncertainty can undoubtedly be a source of stress for the family of the affected child, the uncomfortable initial situation must be assessed through psychological support which should be offered during the finalization of the diagnosis and families are processing the diagnosis as well for further decision-making (134).

It becomes clear how many factors can influence the eventual gender assignment which follows the diagnosis, those are the disorder itself, since a similar gender role behavior can be outlined in each group of patients, the genital appearance and their surgical management, the hormone replacement therapy which shall be necessary and for how long, the potential for a satisfying sexual activity and the preservation of fertility (89). One should rely partly upon the familial views and cultural circumstances or biases, too (81,135). Historically the fertility potential was the strongest reason for any decision regarding the sex assignment in patients with DSD, since it relates to the instinct to procreate for species preservation (136). To fulfill one's desire to have a family, other ways can be equally rewarding such as adoption, which doesn't carry any risk of germ cell cancer or unwanted hormone production (132,136).

What has been mentioned above can be applied specifically to any DSD whereby the following example may be illustrating the topic. Children with 46,XY affected by 5α-reductase-type 2 deficiency or 17β-hydroxysteroid dehydrogenase-type 3 deficiency, which both have a non-functioning androgen biosynthesis, are usually raised as girls since they possess female-appearing genitalia (3,43). When they hit puberty, because of the virilization due to the increase in testosterone level, approximately 60% of the patients choose to live as males. Nowadays an increased rate of XY patients with a normal level of testosterone at birth, regardless of the genital malformation, consisting for example of a micropenis which, due to the absence of a labioscrotal fusion, is often misperceived as a hypertrophic clitoris, is assigned to the male sex, for the majoritarian male gender identity combined with the fertility preservation (137,138).

In the case of Congenital Adrenal Hyperplasia (CAH), nine out of ten patients that have been assigned female at birth identify as female in adulthood, anyway gender dysphoria's prevalence is calculated as being approximately five percent, much higher than the general population. Girls with CAH undoubtedly show a behavioral pathway typical of boys, due to androgen exposure (139–141). Contradicting pieces of evidence regarding markedly virilized 46,XX affected by CAH advise either to assign them as females, already a long-established guideline, or as males, despite the loss of fertility and the necessity of life-long replacement therapy. This avoids the risk of a feminizing genital surgery and those reared male seem to have a satisfactory social and sexual life as male adults (142–144) .

Indeed, the sex of rearing is the best predictor of long-term gender development in patients with 46,XX affected by congenital adrenal hyperplasia (129,139). Practically all patients with complete androgen insensitivity syndrome are born with female-appearing external genitalia, are reared female and express a female-typical behavior in long-term follow-up virtually without any case of gender dysphoria, regardless of the karyotype being XY (128). Such reinsuring results are not the case for partial androgen insensitivity syndrome, since a dissatisfaction of roughly 25% has been proved when the patient is assigned male as an infant and 12% when they are raised as females (145).

The role of surgery and hormonal replacement therapy

In the management of a patient with DSD, hormone replacement therapy plays a key role in helping boost the growth and pubertal development and for maintaining the secondary sex characteristics as well as for preserving psychosocial and sexual health (146). Clearly, it is important to monitor the clinical response to the therapy and the level of the hormone (110). Raised male individuals with 46, XY have an undebatable need for testosterone replacement therapy in order to induct puberty, preserve a physiological sexual drive and stimulate the development of secondary sexual traits (147,148). To optimize virilization, a valid alternative for patients with 5α reductase-type 2 at higher doses can be topical dihydrotestosterone which helps promote the virilization, e.g. increasing the penile length, without undesired side effects such as gynecomastia (84,149,150). In girls with 46,XY estrogen replacement is needed around 9 to 11 years of age to promote breast increase and general feminization, taking into account the risk for excessive bone maturation (110,151). Testosterone can improve the libido of patients with complete androgen insensitivity (152). Progesterone replacement is recommended to induce endometrial maturation and cycling in patients with the uterus (109,110). In conclusion, glucocorticoids are necessary in the case of 46,XY patients with classical congenital adrenal hyperplasia to avoid adrenal insufficiency and hypertensive crisis and mineralocorticoids for those who are salt-losing (110,153).

Since the discordance between the sex of rearing and the atypical genital appearance can cause distress to the family as well as the patient during adolescence, surgery was considered in the past the most determining factor for the outcome in patients with DSD (87). This has changed due to the increasing surgical reintervention rate, the possible infectious and non-infectious complications and the patients being dissatisfied with function or appearance ranging respectively from 20% to 22% (124,132,154). The surgeon, outlining the surgical sequence and the timing of the interventions, aims to allow future sexual activity and optimize fertility potential, in order to preserve the quality of life of the individual (81,110,155).

Masculinizing surgical procedures are to be performed in 46,XY patients who have been reared as males. Those may include:

- *Correction of hypospadias*, through ortophalloplasty and urethroplasty, with a prior administration of testosterone

which may increase the penile length;
- *Scrotoplasty*, whose simultaneous performance with the urethroplasty has been disadvised because of vascular concerns, consisting either of the relocation of the testes or orchidopexy when a conservative approach is not possible and orchiectomy is necessary in order to prevent malignancies;
- *Resection of Müllerian remnants*, usually through laparoscopy, especially when the patient is affected by urinary tract infection or stones.(98,110,156,157).

Feminization can be much more complex and imaging techniques are usually necessary to fully understand the actual patient's anatomy. Feminizing genitoplasty includes:
- *Clitoriplasty*, with reduction of phallic size, whose innervation has to be kept intact to preserve orgasmic function and erectile sensation;
- *Vaginoplasty*, with vaginal dilatation usually performed when the patient hits puberty, which often implies the separation of the urethra from the vaginal introitus since a urogenital sinus is present.
- *Constructing labioscrotal folds*, which are usually fused at a variated grade (81,110,158,159).

Conclusions

Considering what emerges from the literature, the pediatrician should identify the pathologies that are part of the disorders of sexual development. He will care the patient at birth or in the first days of life and guide the clinical and diagnostic paths of these patients. The most important thing is certainly being able to communicate the diagnosis to the family trying to support it over time by sustaining its fears and doubts. It will also be crucial that the patient suffering from DSD is framed as early as possible to avoid suffering marginalization and the social stigma deriving from his condition. Fortunately, compared to the past, these patients can lead a life comparable to that of their peers and, unlike what happened in past decades, they try to have an attitude of watchful waiting. What emerges from clinical experience is that in cases where it is necessary to perform a surgical reassignment of sex this is not done in early childhood, but subsequently to make sure that the patient can choose based on the feeling of her/his gender. This attitude of expectation and support of the affected patients once again highlights the importance of the role of the pediatrician that should follow the patients for several years, supporting their choices and favoring the transition from pediatric to adulthood in the best possible way.

Although a meticulous review of the currently existing literature has been performed, there are no up-to-date and unambiguous guidelines to follow in the management of children with DSD. Furthermore, further studies are needed to draw up the correct therapeutic diagnostic process to be carried out with these patients.

Abbreviations' List:
AMH: anti-Müllerian hormone
AR: androgen receptor
CAH: congenital adrenal hyperplasia
CAIS: Androgen Insensitivity Syndrome
CBX2: chromobox homolog2
DSD: disorders of sex development
DHT: dihydrotestosterone
EGS: External Genitalia Score
EMX2: empty spiracles homeobox2
EMS: External Masculinisation Score
FGF-9: fibroblastic growth factor
FISH: fluorescence in situ hybridization
GATA4: GATA binding protein 4
GBY: gonadoblastoma locus on the Y chromosome
HCG: human chorionic gonadotropin
IL: Interleukin
KS: Klinefelter syndrome
LHX9: LIM homeobox factor 9
MRI: magnetic resonance imaging
PCR: polymerase chain reaction
PMDS: persistence of the Müllerian ducts syndrome
SF1: steroidogenic factor 1
SIX 1/4: sine oculis-related homeobox 1/4
SOX9: Sry-box transcription factor 9
TNF-α: tumor necrosis factor-alpha
TSPY: testis-specific protein on the Y chromosome
WNT4: Wnt family member 4
Wt1: Wilms tumor 1

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