TESTICULAR CANCER: PATHOGENESIS, DIAGNOSIS AND MANAGEMENT WITH FOCUS ON ENDOCRINE ASPECTS

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ABSTRACT

Testicular cancer comprises different neoplasms, depending on the cell of origin and the typical age at presentation, but germ cell-derived tumors constitute the vast majority of cases. Testicular germ cell tumors (TGCT) can be diagnosed in every age group, but more than 90% of cases occur in young men. These tumors, comprising seminoma and nonseminoma, are derived from germ cell neoplasia in situ (GCNIS). Pathogenesis of TGCT associated with GCNIS partly overlaps with that of other developmental disorders of the male reproductive system within the testicular dysgenesis syndrome (TDS). Testicular somatic cell neoplasms, known as sex cord-stromal tumors, are relatively rare, but can have endocrine manifestations, such as precocious puberty or gynecomastia. In addition to its malignant features, cancer of the testis represents a developmental, endocrine, and reproductive problem. These issues are the focus of this chapter, and emphasis is given to aspects that are of interest to endocrinologists, including pediatric endocrinologists and andrologists. Management of invasive testicular tumors is largely handled by urologists and oncologists, thus only general information on surgical treatment, radiotherapy, and chemotherapy is presented. Impact of cancer treatment on the endocrine system, co-morbidities, fertility issues, and quality of life issues are also briefly reviewed.

INTRODUCTION

Testicular cancer comprises a number of different neoplasms, depending on the cell of origin and the typical age at presentation (1, 2). Although several cell types in the testis can undergo neoplastic transformation, germ cell-derived tumors constitute the vast majority of cases of testicular neoplasms. The relative distribution depends on age: in young adult men nearly all tumors are germ cell tumors, whereas in patients aged 70 years or older, a large proportion of lymphomas and secondary carcinomas can be expected (2). In other words, as explained in

detail further in the text, germ cell cancer can be diagnosed in every age group, but more than 90% of cases occur in young men, and this subgroup is the main focus of this chapter. Pathogenesis of testicular germ cell tumors (TGCT) of young adults partly overlaps with that of other developmental disorders of the male reproductive system, within the testicular dysgenesis syndrome (TDS) (3, 4, 5). Somatic cell tumors in the testis, known as sex cord-stromal neoplasms are relatively rare, but are also discussed in this chapter. These neoplasms can have endocrine manifestations due to their origin from endocrine cells.

In addition to its malignant features, cancer of the testis represents a developmental, endocrine, and reproductive problem. These issues are the focus of this chapter, and emphasis is given to aspects that are of interest to endocrinologists, including pediatric endocrinologists and andrologists. The contribution of andrologists and endocrinologists to the overall management of patients with testicular cancer is very important, especially concerning early diagnosis, fertility issues, testosterone deficiency, and the impact of treatment on the quality of life of the patients, the majority of whom are young adults. We have to emphasize that only very general information on surgical and oncological treatment options (e.g., chemotherapy) is presented here. However, urologists and oncologists, who are responsible for the clinical management of testicular tumors, would also benefit from learning about the pathogenesis and andrological aspects of testicular cancer summarized in this chapter.

GERM CELL TUMORS (GCT)

Germ cell tumors (GCT) are characterized by extreme phenotypic heterogeneity (2). They display features of pluripotency and about half of them (nonseminomas) can differentiate into virtually any somatic tissue type within the so-called teratomas that recapitulate early embryonic differentiation (6, 7, 8). Although in comparison to other solid tissue cancers, GCTs are relatively rare, they constitute the most common form of solid tissue cancer of young age. The typical localization of GCT is the testis in males and ovary in females, but GCTs may also be found outside the gonads, thus these tumors are named extragonadal GCTs (8, 9). Extragonadal GCTs occur most often in children of both genders, preferentially along the body midline (intracranial, pineal, mediastinal) but can occur also in adults (8, 9). An increased frequency of extragonadal GCTs, especially in mediastinum, has been associated with Klinefelter syndrome (10). It is important to remember that a large proportion of cases of extragonadal GCT in retroperitoneal locations are associated with pre-malignant changes in testicles, and can be assumed early metastases of testicular neoplasms, although a multi-site development of GCT cannot be completely excluded (8, 9, 11, 12). This chapter focuses on the testicular GCT. For the extragonadal GCT in children and adults, the reader should consult specialized literature.

Classification and Histopathology of Testicular Germ Cell Tumors (TGCT)

Testicular germ cell tumors (TGCT) are by far the most frequent neoplasms of the testis and comprise approximately 90-95% of cases. They may affect infants (rarely), young men (commonly) and elderly men (rarely). The TGCTs of childhood are mentioned only briefly at the end of this section. The diagnosis and management aspects of TGCT in adult men is the focus of this chapter.

The most commonly accepted and currently used classification is the WHO classification of testicular tumors. The main changes occurred in 2016 when the WHO consensus panel proposed a thorough revision of the classification, which for the first time was based on biological evidence (2). The most radical change was the new division of TGCT into two major groups according to the origin from *germ cell neoplasia in situ* (GCNIS), and coining the term GCNIS, replacing several previously used and confusing names (2, 13). The latest 2022 WHO classification update proposed only minor

adjustments; the most important is recognition of gonadoblastoma as a precursor similar to GCNIS and proposing placing seminoma under the major category of germinomas (14).

For use by non-pathologists, a simplified division of TGCT shown in Table 1.

Table 1. Simplified Classification of Testicular Germ Cell Tumors (TGCT), Based on the WHO Classification.

- **GCT derived from germ cell neoplasia in situ (GCNIS)**
  - Non-invasive lesions: GCNIS (9064/2)* and gonadoblastoma (9073/1)
  - Germinomas:
    - Seminoma, pure (9061/3)
    - Seminoma with syncythiotrophoblastic cells
  - Nonseminomatous (non-germinomatous) GCT, pure
    - Embryonal carcinoma (9070/3)
    - Yolk sac tumor, postpubertal type (9071/3)
    - Trophoblastic tumors, incl. choriocarcinoma (9100/3)
    - Teratoma, postpubertal type (9080/3), incl. teratoma with somatic-type transformation (9084/3)
  - Nonseminomatous (non-germinomatous) mixed germ cell tumors (9085/3)
  - Regressed GCTs (9080/1)
- **GCT unrelated to GCNIS**
  - Spermatocytic tumor (9063/3)
  - Prepubertal (pediatric) tumors
    - Teratoma, prepubertal type (9084/0)
      - Dermoid cyst
      - Epidermoid cyst
    - Yolk sac tumor, prepubertal type (9071/3)
    - Prepubertal type testicular neuroendocrine tumor (8240/3)
    - Mixed prepubertal type tumors (9085/3)

*Footnote: The codes in parentheses are from the International Classification of Diseases for Oncology (ICD-O-3).

Precursor Lesions

The TGCT of young adults originate from a common precursor, *germ cell neoplasia in situ* (GCNIS), initially termed *carcinoma in situ* (CIS) testis (13, 15). ***GCNIS*** is considered to originate from developmentally arrested immature germ cells (gonocytes) that fail to differentiate to spermatogonia (4, 16, 17). Accordingly, morphology of GCNIS cells resembles closely that of fetal gonocytes, but with more irregular chromatin in the nuclei. GCNIS cells are located inside seminiferous tubules, most frequently in a single row along the basement membrane (Figure 1).

Gonadoblastoma is a preinvasive lesion which occurs almost exclusively in individuals with disorders of sexual development (DSD). This lesion is most often found in female patients with mixed gonadal dysgenesis (45,X/46,XY) or Turner Syndrome but can also occur in males (2, 18, 19). Gonadoblastoma cells and GCNIS cells have a very similar gonocyte/oogonium-like phenotype, but the surrounding somatic cells are different; GCNIS cells

are present inside seminiferous tubules, which are usually well developed but may be hypoplastic and contain immature Sertoli cells, while gonadoblastoma consists of groups of germ cells, which are nested in small stromal cells similar to granulosa cells (19, 20, 21, 22). GCNIS and gonadoblastoma can be present in the same gonad and there are also lesions with morphology in between the two entities (18, 19). The clinical course of pure gonadoblastoma may be benign, but it has a potential to transform into a malignant germ cell tumor, especially if accompanied by GCNIS and greater virilization of the patient (19, 22, 23, 24).

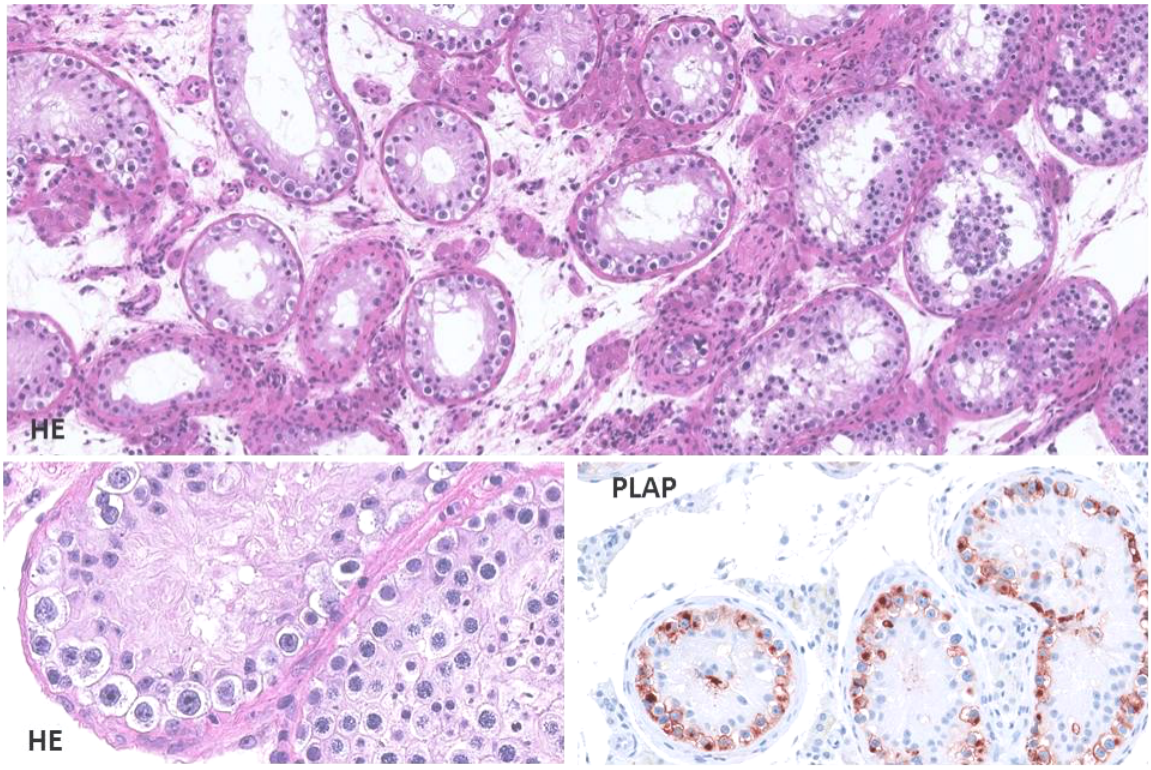

Figure 1. Histology of germ cell neoplasia in situ (GCNIS). The upper panel (hematoxyllin-eosin, HE staining shows a low magnification view of GCNIS in a typical pattern with only GCNIS cells and Sertoli cells present inside tubules. The tubules with neoplasia have a smaller diameter than normal seminiferous tubules. On the right side of this image a few tubules with decreased spermatogenesis are visible. The lower left image shows a fragment of a tubule with GCNIS side-by-side with a tubule with preserved spermatogenesis; note the large GCNIS nuclei. The lower right image displays GCNIS cells visualized by immunohistochemical staining for placental-like alkaline phosphatase (PLAP).

The immunohistochemical profiles of GCNIS and gonadoblastoma cells are virtually identical and resemble very closely those of primordial germ cells and fetal gonocytes (21, 25, 26). This was subsequently confirmed by comparative studies at the transcriptional level using microarrays (17, 27, 28). Among many genes highly expressed in GCNIS cells (as well as gonadoblastoma, normal fetal germ cells and several TGCTs) the following should be mentioned because of their interesting biological function (germ cell survival or maintenance of embryonic stem cell pluripotency) and usefulness in histopathological diagnosis (24): OCT4 (29), AP2-gamma/TFAP2C (27, 30), NANOG (27, 31, 32), KIT (25, 33), TP53 (34), and LIN28 (35, 36).

In addition to protein-coding genes, GCNIS cells display a specific profile of embryonic-type micro-RNAs (miRNA); miR-371-3 cluster, miR-302 and miR-367 (37, 38). This miRNA profile is also detectable in overt TGCT, except teratoma (38), see the description in the section on serum markers.

Testicular Germ Cell Tumors (TGCT) Derived From GCNIS

As mentioned in the introduction, TGCT of young adult men display very variable histology (some examples are shown in Figure 2) and are divided into seminomas (under the main GCT category of germinomas which occur in men and women) and nonseminomas (non-germinomas) (2, 14, 39).

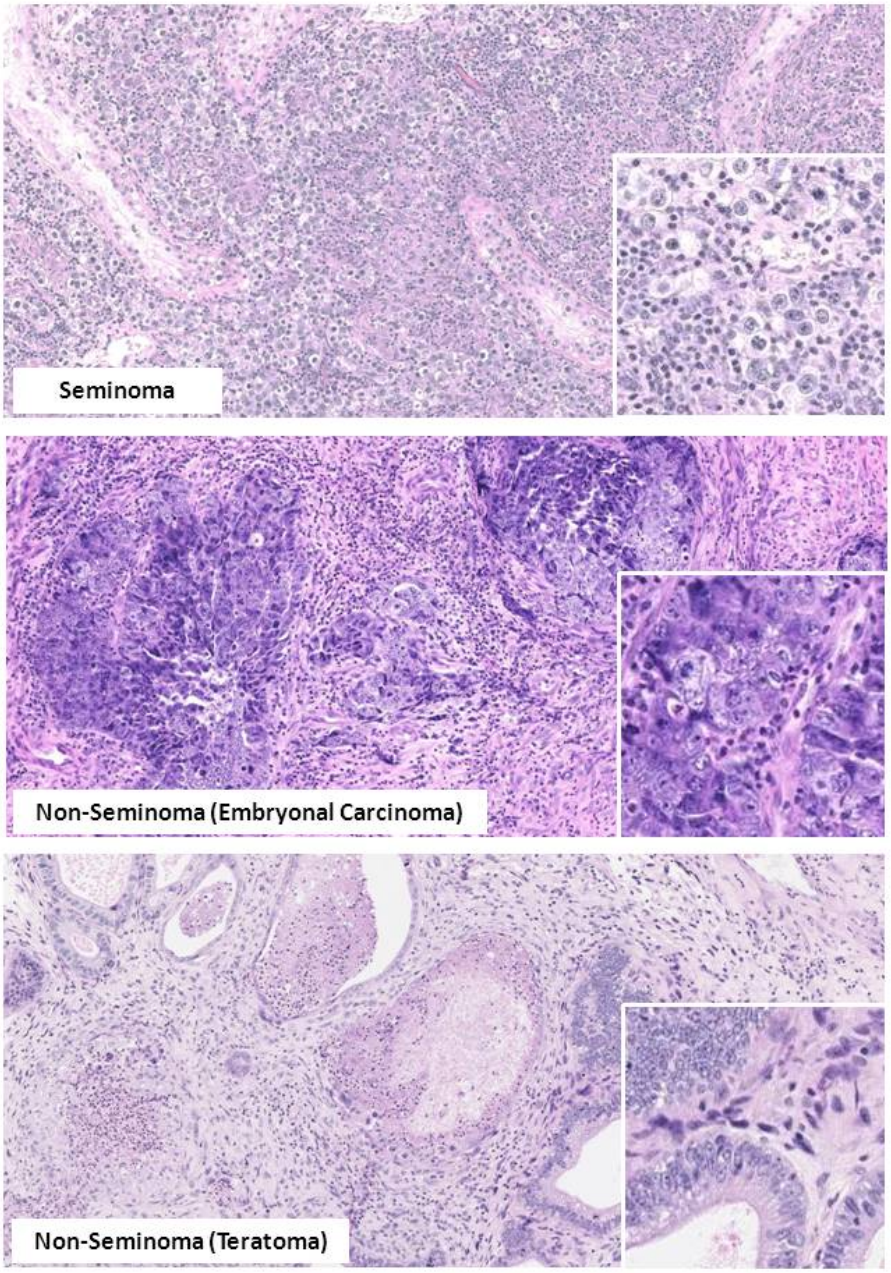

Figure 2. Histology of main types of testicular germ cell tumors. The large images show a general histology pattern of a seminoma (upper panel) and two most often seen types of nonseminoma: undifferentiated embryonal carcinoma (middle panel) and teratoma, a tumor displaying differentiation into various somatic tissues (bottom panel). Small square pictures on the right show cellular characteristics in a greater magnification. All sections are stained with hematoxylin-eosin (HE).

Seminomas are most often diagnosed in the 25- to 40-year-old age group, whereas nonseminomatous tumors occur in even younger men (adolescence to 30 years). Both types originate from GCNIS (2, 16). Seminoma resembles a mass of immature germ cells; the tumor cells are morphologically very close to GCNIS cells and proliferate as a homogeneous tumor, which retains features of germinal lineage. The gene expression profile of seminoma is similar to that of GCNIS and fetal gonocytes, and virtually identical to the female equivalent of germinoma, called dysgerminoma (26, 39).

Nonseminomatous tumors display a variety of histological forms and contain undifferentiated embryonal carcinoma and somatic components partly differentiated along embryonic lineage of any tissue type (1, 2, 8). Nonseminomas also contain extra-embryonic tissue components (yolk sac tumor and choriocarcinoma). The combined or mixed tumors contain elements of seminoma and nonseminoma but are classified and clinically treated as nonseminoma, which usually has more severe clinical course than seminoma (1, 2).

Testicular Germ Cell Tumors Not Associated with GCNIS

PREPUBERTAL (PEDIATRIC) TGCTs.

These tumors occur in early childhood (between birth and approximately 5 years of age) and comprise two main histological types: yolk sac tumor of the prepubertal type and mature teratoma (including dermoid cyst) (Table 1). Prepubertal teratomas can contain secondary neuroendocrine elements, which can overgrow the tumor, hence, the latest WHO classification added this entity as the third subtype (14). The histology of the prepubertal tumors does not differ from the adult equivalents, which are components of nonseminoma (2). Likewise, these tumors display similar characteristic transcriptome and micro-RNA profiles (40, 41). In contrast to the TGCT derived from GCNIS, the tumor genome does not display isochromosome 12p. The etiology and pathogenesis of infantile TGCT remain unknown. These tumors are assumed to originate from primordial germ cells (PGC) but there is no known precursor lesion of GCNIS/gonadoblastoma type, neither are there signs of testicular dysgenesis (2, 42, 43).

SPERMATOCYTIC TUMOR

This rare tumor occurs primarily in older men (median age at diagnosis around 50-55 years, range 25-94) but occasionally can be diagnosed in men in in the third decade of life (44). Spermatocytic tumor has no extragonadal or ovarian counterpart, and occurs exclusively in post-pubertal testis (1, 2, 8). This tumor is not derived from GCNIS (45) and has the expression profile similar to spermatogonia B or early primary spermatocytes (26, 46, 47, 48). Spermatocytic tumors appear to grow from clonally expanding germ cells, which are committed to but have not yet entered meiosis. The following *de novo* genetic aberrations causing increased spermatogonial proliferation have been identified; amplifications in chromosome 9p encompassing *DMRT1* locus (46), rare gain-of-function mutations in genes encoding *FGFR2, FGFR3, HRAS, NRAS, PTPN11,* and simultaneous whole chromosome gains of chromosome 9 and chromosome 20 combined with a loss of chromosome 7 (49, 50). Some of these mutations that occur spontaneously in germ cells of aging men have been depicted as “selfish mutations” because - if transmitted to next generation - can cause severe inborn skeletal

abnormalities in the offspring, such as achondroplasia, hypochondroplasia, thanatophoric dysplasia or Costello syndrome (51).

Etiology and Pathogenesis of Testicular Germ Cell Cancer of Young Adults (Derived From GCNIS)

INCIDENCE TRENDS AND RISK FACTORS

Epidemiology of testicular germ cell tumors (TGCT) has attracted the growing attention of researchers, because of the steadily rising incidence (51). The incidence is remarkably variable geographically and dependent on the ethnic background (Figure 3) (52). TGCT is currently the most common malignancy of young men of white European ancestry, while it is relatively rare among Asians and Africans (52, 53, 54). Interestingly, indigenous Maoris of New Zealand have a higher incidence of testicular cancer than the white population (55).

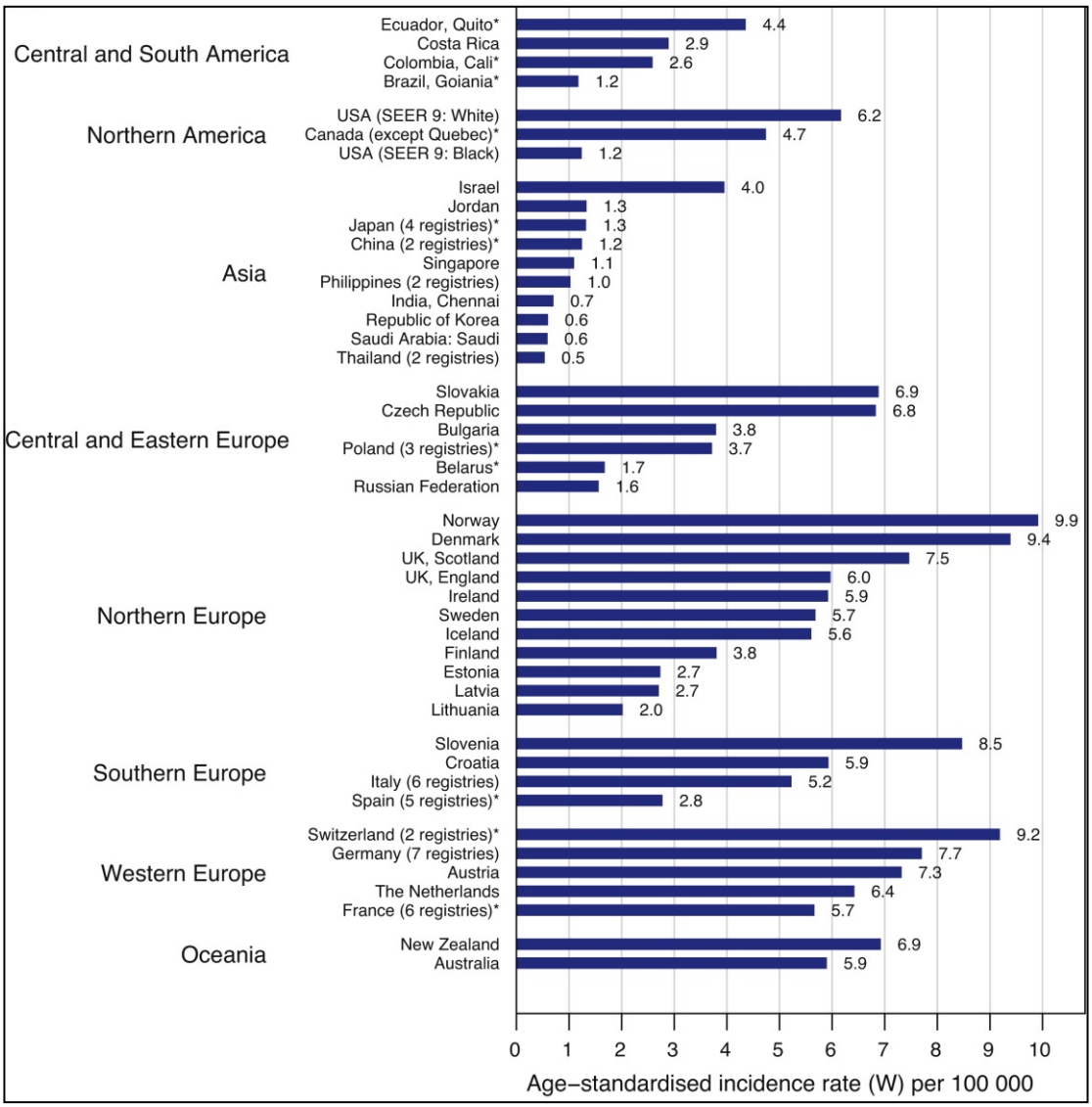

Figure 3. Global incidence rates of testicular cancer. Age-standardized incidence rates for testicular cancer for men of all ages in selected countries of all regions of the world, extracted from national registries. National reporting systems varied by country, and data quality may have fluctuated between regions. Reprinted from Znaor et al., Eur Urol 2014 (52).

However, the incidence rates are not stable and have been changing over time. Within populations rapid changes have been observed in recent decades. For example, the highest rates of testicular cancer have historically been observed in Norway and Denmark but much lower in the Eastern and Southern Europe. While the incidence in Denmark has been attenuating since the 1970s, an alarming rise has been noted in Finland and in Slavic Balkan countries, especially Slovenia and Croatia (52, 53, 54, 56). Similarly, a rapid increase has been noted among the Hispanic/Latin population in the United States (57). Another interesting feature of epidemiology of testicular cancer is the so-called birth cohort effect; the rise in the incidence correlated with the calendar year of birth rather than with the age at diagnosis (58). For unexplained reasons, cohorts born at wartime had lower incidence than men born just before or after the war (58).

As mentioned before, testicular cancer has an unusual age-specific incidence rate with a small peak in the postnatal period and a major peak in young adult age, starting at puberty. These periods coincide with an activation of gonadal hormones, indicating there may be a possible connection between the hormonal factors and invasive transformation of germ cells. In certain risk groups, the incidence of testicular cancer is much increased. Individuals with developmental abnormalities of the gonads and sex differentiation (DSD) are at high risk of developing germ cell neoplasia. Among these, individuals with the so-called mixed gonadal dysgenesis and the 45,X/46,XY karyotype are at particular risk of harboring GCNIS or gonadoblastoma and developing TGCT in adolescence (18, 19, 20, 23, 59, 60). An association with testicular cancer has been noted in a number of developmental abnormalities, such as cryptorchidism (61, 62), Down syndrome (63) but also low birth weight and unspecific perinatal factors, e.g., premature birth, birth order, high levels of maternal estrogens or bleeding during pregnancy, high maternal age and neonatal jaundice (62, 64). A late age at puberty and tall stature were also associated with lower and higher risk of testicular cancer, respectively (65, 66).

For endocrinologists and andrologists it is essential to know that male infertility (the type linked to TDS) is one of the commonest risk factors for testicular cancer (67). Men with TGCT have poorer semen quality and significantly fewer children than controls prior to development of their tumor (68, 69), see also the section on TDS below.

Patients with a unilateral TGCT are also at an increased risk to develop a new primary testicular tumor of the contralateral testis. Depending on the studied population and the interval from the primary diagnosis, approximately 2 - 5% patients will be diagnosed with a second TGCT during their lifetime (70, 71, 72, 73). The majority of bilateral TGCT occur metachronously (> 6 months after the primary

TGCT), some as late as 20-40 years after the primary TGCT (74). The histology of the primary tumor influences the risk only marginally, with the predominance of seminomas among the synchronous TGCTs but primary nonseminomas observed most often in younger patients with metachronous tumors developing after shorter intervals (70, 72). The presence of testicular atrophy increases the risk of bilateral neoplasia considerably (75, 76).

Testicular Dysgenesis Syndrome (TDS)

The association of testicular cancer with poor testicular function, cryptorchidism, hypospadias, and abnormal testicular development led to a hypothesis that poor gonadal development and testicular neoplasia are etiologically linked. A concept of TDS was proposed, in which testicular cancer is one of the symptoms, in addition to other phenotypes, including cryptorchidism, hypospadias, shortened anogenital distance (AGD), reduced Leydig cell function, and decreased spermatogenesis (3, 5). This hypothesis is supported by histological studies showing that dysgenetic features, such as undifferentiated tubules with visibly immature Sertoli cells, clusters of poorly differentiated tubules, or hyaline bodies, often seen in association with testicular cancer, are not uncommon among men referred to andrology clinics because of fertility problems (3, 5, 18, 71). It is important to underline that not all cases of genital malformations and infertility are a part of TDS. Milder phenotypes linked to impaired development of the testis in fetal life due to diverse environmental or lifestyle-related factors can be considered part of TDS (5, 77). Severe genital malformations caused by genetic disorders are clearly a part of DSD but there is a partial overlap between the two syndromes (18). However, TDS severity and prevalence are undoubtedly modulated by the genetic variability (polymorphisms) and epigenetics (5). A schematic representation of the pathogenesis and manifestations of TDS is depicted in Figure 4.

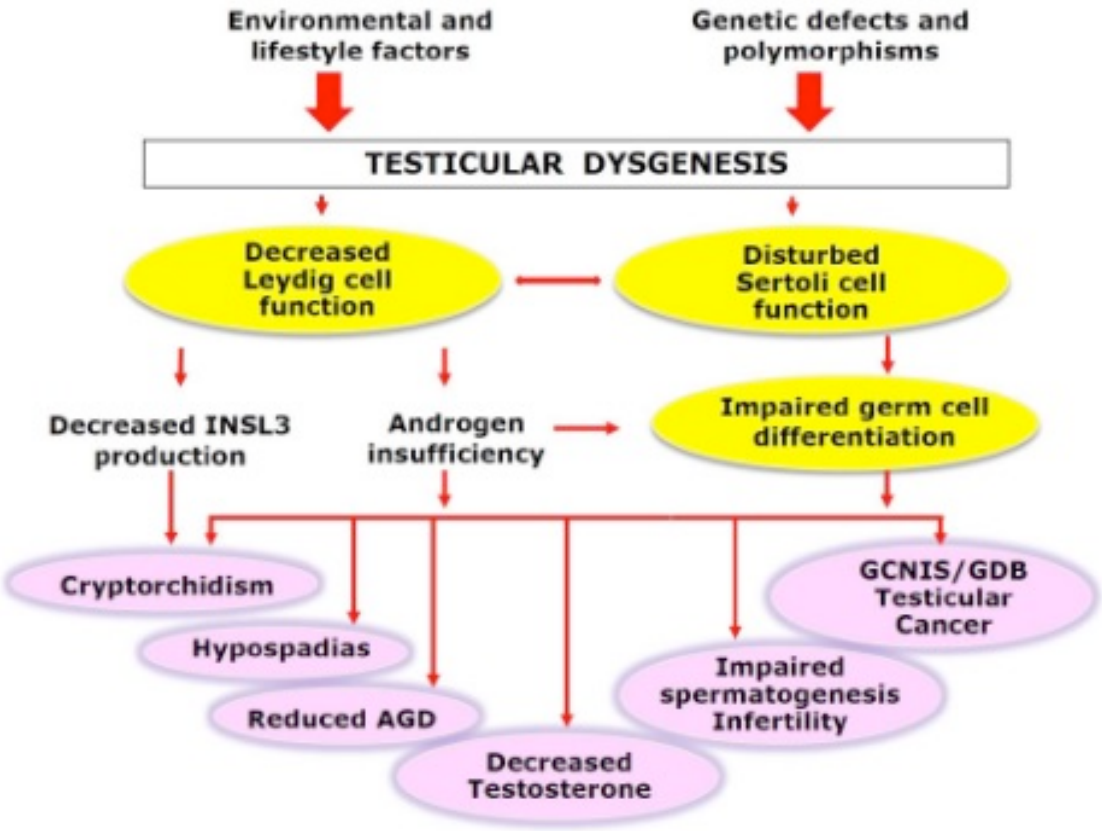

Figure 4. Schematic illustration of aetiology and pathogenesis of disorders grouped within Testicular Dysgenesis Syndrome (TDS). The TDS concept implicates disturbed function of testicular somatic cells (Leydig- and Sertoli cells) caused by inherited genetic variation (gene polymorphisms) in combination with environmental /lifestyle factors acting during early development. Dysfunction of the somatic cells results in disturbed hormonal homeostasis and causes impaired germ cell differentiation. Depending on the severity of the impairment, multiple outcomes or phenotypes may occur, ranging from reduced anogenital distance (AGD), genital malformations to testicular cancer. Note that the most severe forms of TDS (disorders of sex development with gonadoblastoma (GDB) or GCNIS are the least frequently seen, whereas the mildest forms, such as impaired spermatogenesis are quite common. Modified from Skakkebæk et al., Hum Reprod 2001 (3) and Physiol Rev 2016 (5).

Genomics of TGCT and Predisposing Polymorphisms

Tumors derived from germ cells via GCNIS stage are characterized by the presence of a nearly universal aneuploidy (polyploidization) and amplification of chromosome 12p, often in the form of an isochromosome (8, 78). Additional secondary genomic aberrations in invasive TGCT include rare recurring chromosomal gains or losses (in particular loss of chromosome Y), and fusion transcripts (79, 80). Strikingly few oncogenic mutations have been reported in TGCTs, and the short list of affected genes includes only *KIT* (predominantly in seminomas) and *KRAS (*mainly in nonseminomas) (79, 81, 83).

Although testicular cancer in most cases is a sporadic disease, the familial risk of testicular GCT is among the highest when compared to other cancers; brothers and sons of TGCT cases have an 8-10-fold and 4-6-fold increased risk, respectively (84). A twin study from Nordic countries estimated the heritability of testicular cancer as 37%, with 24% attributed to shared environment (85). Recent studies using next generation sequencing technology confirmed the absence of specific oncogenic driver mutations that would explain a high heritable risk of testicular cancer (86). The bulk of inherited risk is instead caused by a constellation of unfavorable gene variants, which jointly account for estimated 44% of testicular cancer heritability (87). Men with a polygenic risk score in the 95th percentile have a 6.8-fold increased risk of TGCT compared to men with median scores (87). This estimation is based on numerous genome-wide association studies (GWAS) performed in recent decades, which revealed a large number of significant gene variants (markers) associated with TGCT risk (87, 88, 89, 90, 91, 92, 93). The strongest association, which was identified in all GWAS, and most interesting from the biological point of view, is with a cluster of single nucleotide polymorphisms (SNPs) within or near KIT ligand gene (*KITLG)* and in genes downstream of the KIT/KITLG/MAPK signaling pathway, e.g., *SPRY4*. This pathway is essential for germ cell migration and survival, is highly active in GCNIS, with the frequent secondary gain-of-function mutations identified in seminomas (33, 81, 82, 83, 89, 94, 95). Among other significant SNPs associated with TGCT risk, the most interesting are *DMRT1*, a transcription factor involved in sex differentiation and regulation of meiosis (96, 97), *PRDM14* and *DAZL,* factors involved in primordial germ cell specification and differentiation, *AMHR2*, the receptor for anti-Mullerian hormone (AMH), *AR,* the androgen receptor, and several genes in the telomerase and DNA repair pathway (87, 90, 91, 92, 93). It is biologically important that several predisposing variants are potentially associated with germ cell differentiation and testis development, including hormone regulation, and there is an overlap with some pathways involved in TDS.

Current Views on the Environmental Etiology of TGCT

In spite of significant recent inroads into the understanding of the pathogenesis of TGCT, etiology

of these tumors, and especially the reason for the rise in incidence, remains obscure. The high incidence of testicular cancer in subjects with congenital errors of gonadal development strongly implicates the involvement of intrauterine factors and perinatal factors. We believe that the neoplastic transformation of male germ cells occurs during their pre-meiotic development, and this happens preferentially in individuals with genetic susceptibility. GCNIS cells and primordial germ cells share some distinct features, such as expression of embryonic pluripotency factors, low DNA methylation and constellation of miRNAs and histone modifications (4, 5, 17, 27, 28, 37, 39, 99, 100).

The mechanisms of neoplastic transformation of early germ cell are not known. There is a growing consensus that there may be multiple mechanisms and testicular cancer is a multifactorial and polygenic disorder. A disturbance in the fetal programming of gonadal development may be a result of an intrauterine hormonal imbalance, which in turn may be caused by a genetic disorder or by an impact of an exogenous factor targeting a key pathway, e.g., androgen signaling, KIT-KITLG signaling, DMRT1 signaling and regulation of meiosis, the TGF-beta superfamily regulation (including Nodal pathway) or the WNT pathway, leading to a delay in the testis development and maturation of fetal gonocytes (3, 4, 91, 98, 94, 101, 102, 103).

As mentioned above, the rising and quickly changing incidence of testicular cancer in well-developed countries suggests a possible adverse influence of environmental or lifestyle-related causative factors. In recent decades a great number of potent natural and synthetic hormones and hormone antagonists have been identified in environment. Observations in wildlife and experiments in laboratory animals exposed to synthetic hormones and a broad range of endocrine disrupters suggest that these substances can cause a disturbance of hormonal milieu of the developing gonad and disturb differentiation of early germ cells (3, 4, 5, 104, 105, 106). Whether or not the endocrine disrupters have contributed to the rise in testicular cancer remains to be demonstrated. Human exposure studies are difficult, mainly because of the long interval between the vulnerable period of early development and manifestation of cancer development in adulthood. There is also the problem of the plethora of confounding factors, including mixtures of possible contaminating factors present in food, packaging, cosmetics, and other products of daily life. Therefore, the evidence for the role of endocrine disrupters in the etiology of TGCT remains scarce. Among few existing data one can mention a higher serum level of some persistent organic pollutants found in mothers of men with testicular cancer (107), and a greater burden of p,p′-DDE (a DDT metabolite) in serum of testicular cancer patients (108). For more information on the existing evidence and possible mechanisms the reader is referred to recent review articles on the subject (109, 110). It is clear that further studies in large cohorts of patients and controls are needed to identify the causative factors behind the observed testicular cancer epidemics. Few data exist concerning postnatal exposures and risk of TGCT. A heavy use of marijuana (cannabis) has been consistently reported as a risk factor for nonseminoma but the mechanism remains to be elucidated (111, 112).

Diagnosis and Staging of Testicular Germ Cell Neoplasms

PREINVASIVE LESIONS AND TESTICULAR BIOPSY

All attending physicians should consider a possibility of incipient germ cell neoplasia in men with infertility, especially those with small testes, a history of cryptorchidism, or previous testicular tumors, or in individuals from the high-risk group of DSD (5, 68, 69). The preinvasive stage of GCNIS is asymptomatic, so diagnosis at this early stage is sporadic. Ultrasonographic examination can be helpful in some cases. GCNIS or incipient TGCT are frequently associated with an irregular echo pattern or microcalcifications /microlithiasis (113, 114, 115, 116). However, ultrasonic microlithiasis is very

common and can be present in normal men or patients with mild disorders, so it alone is not an indication for a testis biopsy. A biopsy is indicated if microlithiasis is accompanied by additional risk factors, e.g., history of cryptorchidism, a family history of testicular cancer, atrophic testis (<12 mL), or poor semen quality, especially azoospermia (117, 118). Surgical testicular biopsy is currently the only sure diagnostic procedure for GCNIS diagnosis (119). Experience in the use of needle biopsies for GCNIS diagnosis is limited and this method is not commonly used in the clinic. Regardless of the method of obtaining the testis tissue, examination for GCNIS by immunohistochemistry in a diagnostic biopsy or any leftover material after testicular sperm extraction in fertility clinics is now considered mandatory (119, 120, 121).

GCNIS is present in the testis contralateral to a testicular tumor in approximately 5% of cases, with a range of 4% to 8%, depending on the studied cohort (71, 75, 121, 122). Screening for the contralateral GCNIS to prevent the appearance of metachronous bilateral TGCT has been practiced in several countries, including nationwide in Denmark and in some centers in other European countries (Germany, Austria, the Netherlands). Most of other centers adopted a recommendation of the European Association of Urology (123) to take contralateral biopsies only in patients at risk for GCNIS presence, such as by a history of cryptorchidism, a small testis volume (<12 ml), or testicular microlithiasis (75, 76). In the USA and Canada, the contralateral biopsy is rarely practiced, and American guidelines recommend this procedure only if the contralateral testis is cryptorchid, shows marked atrophy, or suspicious ultrasonic changes, but not microlithiasis alone (124, 125). Our recommendation is to offer a contralateral biopsy to all patients at the time of surgery for the primary TGCT, perhaps with the exception of men older than 50, because of a minimal risk of GCNIS. Added benefits of a biopsy are a good assessment of the fertility potential of the other testicle, and the peace of mind for the patient concerning the possibility of bilateral cancer (126). Mild post-biopsy complications (3%) are rare and the procedure is well accepted by the patients (127). In young men with normal size testicles, two-site biopsies may be considered to decrease a chance of a false-negative biopsy (126). False-negative cases are relatively rare (128) but the procedure does not completely eliminate the risk of the second TGCT. In the Danish nationwide study, the cumulative incidence of the metachronous TGCT was 1.9% (after median 20-year follow-up) compared to 3.1% in a non-biopsied earlier cohort of patients (121). However, in a sub-cohort of Danish patients, in whom the contralateral biopsy was performed with a quality control and obligatory immunohistochemistry with GCNIS markers, the cumulative rate of the second cancer (after similar follow-up period rate) decreased to 0.95% (121).

Technical aspects of surgical biopsy are important. The tissue fragment has to be sufficiently large (approx. 3x3x3 mm) and care has to be taken to avoid damage to the specimen, which should be dropped directly to a container with a fixative solution. For morphology evaluation Bouin's or similar solutions are preferred because formalin causes shrinkage artefacts, while for immunohistochemistry buffered formalin is preferred. As for any diagnostic testis biopsy, the contralateral biopsy evaluation must include immunohistochemical staining for at least one (better two) of the known markers for GCNIS, e.g., placental-like alkaline phosphatase (PLAP), OCT-3/4 *(POU5F1)*, PDPN/M2A/D2-40, or AP2γ-*TFP2AC* (120, 122, 129). An example of immunohistochemical PLAP staining for GCNIS detection is shown in Figure 1.

SEMEN ANALYSIS

Poor spermatogenesis is a good indication that the patient may be at risk of harboring GCNIS (5, 68, 69, 75, 76, 130, 131). Semen analysis cannot as yet be used alone for detection of early stages of testicular cancer. However, it has been known for a long time that GCNIS cells may be occasionally found in semen (132). Over the years, progress has been

made towards establishing better quality immunocytochemical detection of GCNIS or early invasive intratubular tumor cells in semen, and an improved automated double-staining assay has been developed (133, 134, 135). Unfortunately, the sensitivity of the cytological method is not high enough for screening, but it can be used as an additional part of semen analysis in experienced andrological centers performing immunohistochemistry (135). It is also not possible to use a micro-RNA (miRNA)-based assay, especially miRNA-371a-3p, a very promising serum marker for invasive TGCT, for detection of GCNIS in seminal fluid, despite that this embryonic-type miRNA is secreted by GCNIS cells (37). Even though some patients with GCNIS can have measurable miR-371a-3p in serum (136), the presence of the same miRNAs in normal germ cells in control patients with non-malignant conditions precludes using this assay in seminal fluid (137, 138, 139).

SERUM TUMOR MARKERS, DIAGNOSIS AND MONITORING OF OVERT TGCT

In the vast majority of cases a scrotal mass is usually the first presentation of testicular cancer, with tenderness reported by very few patients. In a few percent of testicular cancer cases, the presenting symptoms are the result of metastatic disease. They are usually uncharacteristic and may include lumbar pain, palpable abdominal mass, supra-clavicular lymph node enlargement, and in rare cases pulmonary symptoms (1).

The majority of primary and metastatic germ cell tumors secrete proteins and other biochemical products that can be detected in circulating blood. These biochemical serum tumor markers are very helpful in diagnosis and monitoring of testicular cancer (38, 124, 140).

The most important serum markers established in clinical practice are human chorionic gonadotropin (HCG), alpha-fetoprotein (AFP), and lactate dehydrogenase (LDH), the first two mainly useful to detect nonseminomas (1, 140). Among nonseminomatous tumors, choriocarcinoma, which resembles a gestational trophoblast, produces large quantities of HCG, while yolk sac tumor, which is similar in morphology to the embryonic yolk sac, secretes AFP (38, 140, 141). In addition, LDH may be secreted by both seminomas and nonseminomas. LDH levels in serum tend to be higher in patients harboring tumors with an increased copy number of chromosome 12p, consistent with the genomic location of the *LDHB* gene (142). Increased concentrations of LDH in the absence of AFP and HCG suggests the presence of seminoma. It is important to keep in mind that TGCT rarely occur in pure histological forms, and a subtype of seminoma with syncytiotrophoblastic cells can secrete HCG, which can be measurable in patients' blood (14, 143). In preinvasive GCNIS and in most cases of pure seminoma, none of the above-mentioned markers are detectable in serum.

The most promising currently emerging serum markers are micro-RNAs (miRNA), among which miRNA 371a-3p is the best studied and most robust marker, and has already been proven valuable for detection of seminomas and nonseminomas, except for differentiated pure teratomas, in children and adults (38, 144, 145). Several large clinical studies clearly demonstrated that miRNA 371a-3p test has a much better specificity than the classical serum markers, and is especially useful for detection and monitoring of HCG- and AFP-negative seminomas (146, 147).

After diagnosis, careful clinical staging is necessary in each patient to decide for the most appropriate treatment strategy, and the measurements of circulating tumor markers are an important part of this process (1, 123, 124). There is a tendency for higher levels of tumor markers to be associated with a poorer prognosis. For example, the presence of syncytiotrophoblastic cells in the subtype of seminoma can lead to a mild increase of serum HCG, but the tumor responds well to treatment

regardless of the presence of these cells, so more aggressive management should be avoided.

In addition to serum markers, scrotal ultrasonography is the first line diagnostic procedure. In general, seminoma is a homogenous tumor, whereas nonseminomas usually display heterogeneous patterns, with frequent hyperechoic calcified areas (148, 149). Additional imaging procedures help to evaluate the spread of disease, including CT scan of the chest and abdomen, or MRI (148, 149, 150).

Histopathological evaluation of the orchiectomy specimen is an essential part of the clinical staging. The important risk factors are tumor size, vascular/lymphatic invasion or the presence of tumor cells in rete testis, and the presence of pure embryonal carcinoma (152, 153).

The most commonly used staging and prognostic classification system is the TNM (tumor, node, metastases) System of the International Germ Cell Cancer Collaborative Group (GCCCG) and the American Joint Committee on Cancer (AJCC) (154). The stage grouping updated by the WHO Consensus (2), is shown in Table 2.

Table 2. Stage Grouping According to TNM Classification
Stage 0 (pTis): Germ cell neoplasia in situ
Stage IA (pT1, N0, M0, S0): Tumor limited to testis and epididymis
Stage IB (pT2-4): as IA but with vascular/lymphatic, tunica or scrotal invasion
Stage II (any pT, N1-3, M0, S0-1): Metastasis in lymph nodes, serum markers normal or moderately increased
IIA (N1): lymph nodes <2 cm
IIB (N2, S1): lymph nodes ≥2 cm but <5 cm
IIC (N3, S1): lymph nodes ≥5 cm
Stage III (any pT, any N, M1, S0-3): Distant metastasis (spread beyond regional nodes)
IIIA (M1a, S0-1): Spread to non-regional nodes or lung
IIIB (M1a, S2): as IIIA but high serum markers
IIIC (M1a-b, S3): distant metastasis to sites other than IIIA, or very high serum markers

Footnote: pT=primary tumor, N=regional lymph nodes, M=distant metastasis, S=serum tumor markers (X=unknown, not available)

Management of GCNIS and Testicular Germ Cell Cancer

Early diagnosis of testicular neoplasia at the stage of GCNIS, followed by adequate treatment of GCNIS is capable of preventing progression to invasive tumors. Unfortunately, the vast majority of cases progress unnoticed to overt tumors. However, germ cell tumors are extremely radio- and chemo-sensitive and have apparently very high propensity to apoptosis, likely mediated by p53 (155, 156). Because of this sensitivity, and thanks to cisplatin-based chemotherapy regimens, TGCT is a highly curable malignancy, with more than 90% of patients reaching a sustained complete remission (157).

We give here only very general information concerning management of testicular cancer. The reader should consult specialized oncologic and urologic literature for management options pertinent to treatment-resistant tumors and metastatic disease.

TREATMENT OF GCNIS

Spontaneous regression of GCNIS has never been described and it is anticipated that all cases of GCNIS will eventually develop into TCGT. The following management options for GCNIS are available depending on a specific situation (158):

- Orchiectomy - is the curative treatment with the highest assured success rate. It should always be performed on a testis with GCNIS or localized tumor when the second testis is not affected by malignancy (including GCNIS).
- Radiotherapy - low dose radiotherapy is a good alternative to orchiectomy when GCNIS is present bilaterally or in the contralateral testis, so the patient can be spared a bilateral castration and lifelong androgen replacement therapy. The efficacy of radiotherapy with doses as low as 16 Gy was demonstrated in the early studies (159). Even though the lower dose better preserves the function of Leydig cells (160), more recent studies and current EAU guidelines recommend a dose of 18 Gy, given in fractions of 2Gy (123, 161). This dose of radiation will almost always destroy normal germ cells, so radiotherapy may be delayed in patients who wish to secure natural conception of a child.
- Chemotherapy - is not an option to treat GCNIS, because a persistence or relapse has been reported in a high proportion of patients (121, 161). Furthermore, in some cases of extragonadal germ cell tumors treated with chemotherapy, testicular GCNIS progressed to metachronous overt testicular tumors (162). However, in patients with disseminated disease receiving chemotherapy, the risk of metachronous bilateral TGCT is lower (73, 163, 164).
- Surveillance - is potentially hazardous since it increases the risk of metachronous bilateral TGCT (164) and GCNIS may progress to invasive cancer at any time. However, watchful surveillance may be an option after careful informed discussion of risks and monitoring with ultrasound examinations, especially if the patient wishes to defer treatment temporarily for the purpose of paternity.

In men who desire fatherhood, and in all very young men, cryopreservation of semen samples should be offered, if viable spermatozoa are detected.

TREATMENT OF OVERT SEMINOMAS AND NONSEMINOMAS

As far as the treatment of invasive germ cell tumors is concerned, the reader should consult the specialized urology and oncology guidelines.

Prior to surgery, all patients with TGCT should be offered full andrological evaluation and cryopreservation of semen. Endocrinologic evaluation of the patient should also preferably be done before surgery, with the reproductive hormone profile, including serum testosterone, gonadotropins, and inhibin B, if possible. This can be done together with the above-mentioned measurements of serum tumor markers. Pre-operative semen analysis and cryopreservation is especially important in patients with bilateral tumors or only one testicle. In the patients who have azoospermia or cryptozoospermia, and in whom it was impossible to retrieve sperm pre-operatively, testicular sperm extraction (TESE) at the time of orchiectomy (‘onco-TESE’) may be attempted in specialized centers (165). The best predicting factor of sperm retrieval is a small size of the tumor, because a chance of finding tubules with ongoing spermatogenesis increases with the distance from the tumor (166).

Radical orchiectomy remains the primary surgical treatment method of choice. Primary dissection of retroperitoneal lymph nodes (RPLND) was previously commonly practiced, especially in North America. The current consensus is to perform RPLND in experienced high-volume centers, and in selected cases, mainly in patients with nonseminoma and intermediate prognosis (1, 167, 168, 169). Nerve-sparing technique is preferred in order to avoid the loss of antegrade ejaculation, which is the commonest long-term complication of RPLND (169).

Methods of post-surgical management of overt TGCT are variable, depending upon the histological type of tumor (seminoma vs nonseminoma), levels of serum markers and stage of disease and the presence of residual retroperitoneal masses (1, 115, 151, 167). Adjuvant radiotherapy, e.g., of the retroperitoneal/para-aortic field, which was previously routinely used, is no longer recommended, because of the long-term risk of secondary malignancies (1, 167, 168).

Pure seminomas have a good prognosis and around 80% of patients with stage I seminoma (tumor confined to the testis) do not require any treatment after the surgery, thus most of the centers practice a surveillance strategy (151, 168, 170). Nonseminomas have a somewhat poorer prognosis (relapse rate is around 30%) and in some centers, patients with nonseminoma and high risk of relapse are treated with one course of adjuvant chemotherapy with cisplatin and etoposide (171, 172), but most centers currently recommend a surveillance strategy (1, 151, 152, 168, 172).

The most common post-surgical management of disseminated disease is systemic chemotherapy with a combination of cytotoxic drugs. The standard first line chemotherapy regimen is BEP (bleomycin, etoposide and cisplatin), administered in 3 or 4 cycles, depending on the patient's prognosis (173, 174). It is very important to make a dynamic assessment of the progress of treatment through the early stages of chemotherapy, thus monitoring of serum markers is obligatory. Post-chemotherapy retroperitoneal surgery should be performed in patients with a residual tumor after BEP (151). In patients with relapse after first line treatment, salvage regimens and complex surgery for residual tumors need to be performed. Overall, the management is difficult, thus it should be carried out in specialized tertiary centers (1, 167).

The majority of relapses occur within 2 years of the initial treatment but late relapses are observed in some cases, therefore individual management of each patient and lifetime follow-up is advocated in some patients (1, 151, 167). Andrological follow-up should be coordinated with the post-surgery oncological management and controls (see the section on 'Endocrine problems and late effects').

SEX CORD-STROMAL TUMORS OF THE TESTIS

In adults, sex cord-stromal tumors of the testis are found in less than 5% of all testicular tumors, whereas in children, these tumors are more common and account for approximately 25% of cases (123, 175, 176, 177, 178). Most of these tumors are benign, only around 5% have malignant characteristics (2). The classification of the tumors by WHO (2016) is shown in a simplified form in Table 3 (2).

Sex cord-stromal tumors are derived from testicular somatic cells; Leydig cells, and Sertoli/granulosa cells. Despite a different cell of origin, some stromal tumors are sometimes misinterpreted as seminoma. A number of features can be used to distinguish sex cord-stromal tumors from germ cell tumors; Inhibin A and B are the best serum markers for this purpose (179, 180, 181). Inhibin A appears to be a common immunohistochemical marker for sex cord-stromal tumors, including Leydig cell, Sertoli cell and juvenile granulosa cell tumors (179, 182). Sertoli cell tumors are positive for anti-Mullerian hormone (183) and GATA-4 (184), while Leydig cell tumors express steroidogenic enzymes and INSL3 (185). Other useful immunohistochemical markers, include SOX9, calretinin, CD99, SF1 (2).

Table 3. Sex Cord-Stromal Tumors of the Testis, Adapted from WHO Classification (ref. 2)

• Leydig cell tumor (8650/1) Malignant Leydig cell tumor (8650/3)
• Sertoli cell tumor (SCT) (8640/1) Malignant SCT (8640/3) Large cell calcifying SCT (8642/1) Intratubular large cell calcifying SCT (8643/1)
• Granulosa cell tumors Juvenile-type granulosa cell tumor (8622/1) Adult-type granulosa cell tumor (8620/1)
• The fibroma-thecoma tumors (8600/0)
• Mixed sex cord-stromal tumors (8592/1)
• Unclassified sex cord-stromal tumors (8591/1)

*Footnote to Table 2: The codes in parentheses are from the International Classification of Diseases for Oncology (ICD-O-3).

Leydig Cell Hyperplasia and Tumors

Leydig cells are located in the interstitial compartment of the testis and are involved in the development of secondary male characteristics and maintenance of spermatogenesis by secretion of testosterone. Although Leydig cells in adult men are considered to be a terminally differentiated and mitotically quiescent cell type, in various disorders of testicular function, focal or diffuse Leydig cell hyperplasia is very common. Micronodules of Leydig cells are frequently seen in certain conditions associated with severe decrease of spermatogenesis or germinal aplasia, such as the Sertoli-cell-only syndrome (Del Castillo syndrome), cryptorchidism, or Klinefelter syndrome, when the nodules can be particularly florid (186, 187). A term 'Leydig cell adenoma' is used when the size of a nodule exceeds several- fold the diameter of a seminiferous tubule. It is unknown whether Leydig cell adenomas can progress further to form overt Leydig cell tumors, but even if it were the case, it is exceedingly rare. Morphological heterogeneity of hyperplastic Leydig cells is noticeable in some cases, and it has been shown that the micronodules contain a large proportion of immature Leydig cells (186, 187).

The mechanism of Leydig cell hyperplasia and larger nodules in the human male is still poorly understood. Leydig cell nodules of variable-size are common in patients with TDS and infertility, and the functional insufficiency of Leydig cells is often reflected by decreased testosterone/LH ratio whereas patients with overt Leydig cell tumors usually have an increased testosterone/LH ratio and high estradiol (186, 188). The disruption of hypothalamo-pituitary-testicular axis leading to an excessive stimulation of Leydig cells by LH can play a central role (186). This in turn leads to an increased renewal of immature, adult-type Leydig cells from their precursors. The immature cells are characterized by low numbers of Reinke crystals, a relatively high expression of a mesenchymal factor DLK1 and low amounts of INSL3 (187, 189, 190). However, Leydig cell hyperplasia is distinct from tumors that are usually solitary. Leydig cell hyperplasia and adenomas can be easily induced in rodents by administration of estrogens, gonadotropins, and a wide range of chemical compounds. Whether or not humans would be similarly susceptible to environmental effects remains to be elucidated.

Leydig cell tumors account for up to 5% of testicular neoplasms and occur in all age groups (2, 178, 191). Approximately 20% are found in boys, most often between five and ten years of age. There is perception that the prevalence of benign Leydig cell tumors among the adults is greater than previously

thought, possibly thanks to improved detection of small nonpalpable nodules by modern imaging methods. Higher incidence numbers have also been reported in fertility centers that actively scan the testes of infertile patients (192).

In a subset of cases of Leydig cell tumors, activating mutations of the LH receptor (193, 194, 195) or G proteins (196, 197) can be detected. Constitutively activating mutations of LH receptor cause early Leydig cell hyperplasia and precocious puberty (193, 195, 197). Similarly, constitutively activating mutations of Gs-protein in Leydig cells or inactivation of PKAR1A (protein kinase cyclic adenosine monophosphate-dependent regulatory type 1 alpha) lead to hyperplasia and endocrine hyperactivity (178, 197). In adult Leydig cell tumors, germline fumarate hydratase mutations have been identified in a few cases which also had hereditary leiomyomatosis and renal cell cancer (199).

Precocious puberty (so-called testotoxicosis) is the presenting symptom in most of cases of Leydig cell adenomas or tumors in children, due to the excessive androgen production, mainly testosterone that causes growth of penis, pubic hair, accelerated skeletal and muscle growth, advancement of bone age, skin changes (acne, comedos, hair greasing), and adult-type odor of sweat. Androgen secretion in the pediatric cases is gonadotropin independent, and therefore LH and FSH remain low in spite of external signs of puberty (195). Approximately 10% of the boys also have gynecomastia that is caused by estrogens produced in excess due to aromatase activity in some of the tumors or peripheral aromatization of testosterone. It is important to keep in mind that transient gynecomastia or pseudo-gynecomastia can occur in newborns, in obese boys, and at puberty as a non-pathological condition (200). In adults, gynecomastia is a frequent condition with a reported prevalence of 32–65%, depending on the age and the criteria used for definition (summarized in 201). Gynecomastia is sometimes associated with loss of libido, impotence, and infertility (201). However, Leydig cell tumor is the cause in only 1-2% of cases, and the excessive androgen secretion by these tumors rarely causes notable effects in adults (202). Malignant tumors are hormonally active only in exceptional cases (2, 178).

In children, Leydig cell tumors are always benign and can be treated with surgical enucleation when the tumor is encapsulated (203). In adults malignant Leydig cell tumors have been found in 10-15 % of patients, and inguinal orchiectomy is often used (204), while testis-sparing enucleation remains an option, if there are no signs of malignancy in the frozen preparation (205). The presence of cytologic atypia, necrosis, angiolymphatic invasion, increased mitotic activity, atypical mitotic figures, infiltrative margins, extension beyond testicular parenchyma, and DNA aneuploidy are associated with metastatic behavior in Leydig cell tumors (178, 202, 206) (see Figure 5).

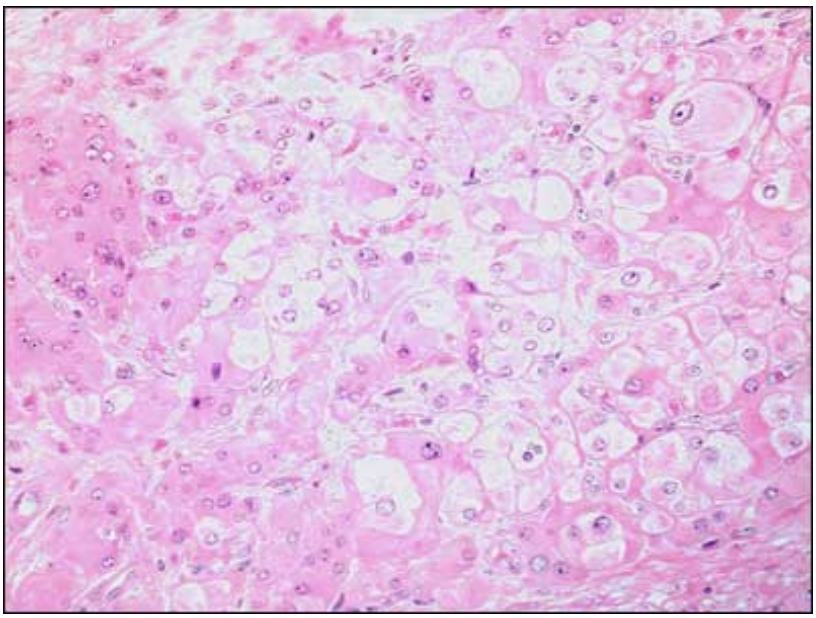

Figure 5. Histology of a Leydig cell tumor. The appearance of tumor cells resembles normal Leydig cells. A section stained with hematoxylin-eosin (HE).

Malignant Leydig cell tumors do not respond favorably to conventional chemotherapy and irradiation (204). Survival time has ranged from 2 months to 17 years (median, 2 years), and metastases have been detected as late as nine years after the diagnosis (178, 202). Metastatic Leydig cell tumors require salvage surgery, radiotherapy and intensive chemotherapy regimens but in most cases the outcome is poor (188, 207). Therefore, follow-up of patients with malignant Leydig cell tumors has to be life-long. The remaining testis may be irreversibly damaged by longstanding high estrogen levels, resulting in both permanent infertility and hypoandrogenism (202, 206).

Testicular Adrenal Rest Tumors (TART)

Excessive secretion of adrenocorticotropin (ACTH) in 21-hydroxylase deficiency (congenital adrenal hyperplasia, CAH) or Nelson syndrome (post adrenalectomy status) may lead to development of hyperplastic interstitial nodules called testicular adrenal rests tumors (TART) resembling Leydig cell tumor or hyperplasia (191, 208, 209). These cells are hormonally active in secreting androgens. It is important to remember that the adrenal rests are almost invariably bilateral, whereas the Leydig cell tumors are usually unilateral. Adrenal rests can be treated by appropriate glucocorticoid substitution of the patient, which leads to gradual regression of the 'tumor' in 75 percent of cases (208, 209). TART have to be distinguished from benign hyperplasia, adenomas and malignant Leydig cell tumors (185).

Histopathological and endocrine evaluation covering both testicular and adrenal steroids and pituitary gonadotropins and ACTH are important in the differential diagnosis (185, 208). It would be an error to orchidectomize the CAH patients with TART, since the tumors are always benign and only some of them continue to be active after appropriate glucocorticoid substitution. In the patients who do not respond well to glucocorticoid replacement, or develop fibrosis, testis sparing surgery with enucleation of the larger nodules can be considered, if the testicles have become so large that they cause discomfort for the patient (209).

Sertoli Cell Tumors

Sertoli cells are the somatic cells in the seminiferous epithelium giving structural, metabolic, and hormonal

support to spermatogenic cells. Sertoli cells cease their proliferation at puberty. In rare infantile cases, multiple foci of proliferating Sertoli cells have been described and proposed to be early intratubular forms of Sertoli cell tumors (210). The classification of these tumors according to the WHO (2, 178), is shown in Table 3.

Sertoli cell tumors often occur as a part of multiple neoplasia syndromes (see below). Rare Sertoli cell tumors which do not belong to a syndrome are called 'not otherwise specified' (NOS). About 5% of these tumors are malignant and can metastasize. The age of patients ranges from 18 to 80 years, but most of them are young adults (median age 30). Out of 60 patients, only four were younger than 20 years old in the series reviewed by Young et al. (211). The tumors typically are composed of sex cord cells with tubular differentiation, with a subset of tumors hyalinized, previously classified as sclerosing variant (2). Some tumors often contain lipid droplets but do not show any endocrine activity. The molecular origin is known only in a small proportion of tumors with sclerosing appearance, in which *CTNNB1* mutations causing nuclear accumulation of β-catenin were identified (212). The tumors occurred in descended testes and were always unilateral. An infiltrative margin was found in four cases, but most of the tumors were well demarcated. The tumors were hormonally inactive, and only two patients with alcoholic cirrhosis also had gynecomastia. Eighteen pediatric cases were reported from the Kiel Pediatric Tumor Registry (176), but perhaps the histopathologic pattern was somewhat different, because the age of the children was very young, ranging from 0 to 14 months (median 4 months). Juvenile Sertoli cell tumors often showed infiltrative growth into adjacent tissue, dense cellularity and considerable proliferative activity. However, after surgical excision no local recurrences and no metastases occurred. Thus, these patients have a good prognosis. These Sertoli cell tumors can be treated by orchiectomy, and retroperitoneal lymphadenectomy is indicated only when there is radiographically detected retroperitoneal involvement (213).

Calcifying Sertoli cell tumors, are frequently found in association with two distinct multiple neoplasm syndromes, Carney complex and Peutz-Jeghers syndrome, however in the latter syndrome, the tumors belong to a distinct 'intratubular' morphological group (2, 178, 214). Association of large-cell calcifying Sertoli cell tumors with other neoplasms, particularly heart myxomas in Carney complex and gastrointestinal tumors in Peutz-Jeghers syndrome, should be kept in mind to reach an early diagnosis of these potentially fatal diseases.

The Carney complex is characterized by skin myxomas, heart myxomas, skin pigmentations, adrenal and testicular tumors, but other tumors can also occur (215). The testicular tumors are *large-cell calcifying Sertoli cell tumors* that are multifocal and bilateral, and should be distinguished from teratomas (Figure 6) (178, 216, 217). The tumors appear usually during the second decade of life (218). Only one malignant case has been reported in association with Carney complex (in an adult patient), whereas seven malignant tumors were reported in other patients with large-cell calcifying Sertoli cell tumors (218). These patients were older than 25 years. The malignant cases were unilateral and solitary in contrast to bilateral and multifocal occurrence of testicular tumors in Carney complex. Large-cell calcifying Sertoli cell tumors are usually not hormonally active, although elevated levels of serum inhibin B or testosterone have been reported, but other tumors of Carney complex, including Leydig cell tumors, can cause endocrine manifestations (181, 213, 214).

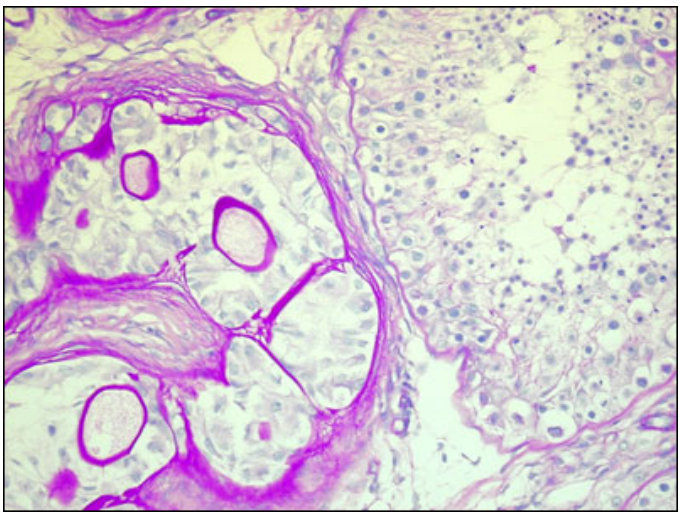

Figure 6. Large cell calcifying Sertoli cell tumor isolated from a 12-year-old boy. The neoplastic tubules contain only large pale Sertoli cells and visible calcifications in the lumen (stained with PAS). Adjacent normal tubules show advanced spermatogenesis.

Two genetic loci for Carney complex have been identified on chromosome 2p16 (196) and 17q23-24 (219, 220). Germline mutations identified in Carney complex most often occur in type I-alpha regulatory subunit of protein kinase A, *PRKAR1A* (221, 222). Inactivating mutations of phosphodiesterase 11A212 and phosphodiesterase 8B213 are associated with bilateral adrenocortical hyperplasia in Carney complex patients. Genetic variation of the phosphodiesterase 11A gene can modify the development of the testicular tumors (223). Molecular genetic diagnosis is available to many of these patients. However, only a part of the mutations occur in the germ line, and therefore genetic analysis should be performed on affected tissues, such as the tumors, in cases of somatic cell line mutations.

In *Peutz-Jeghers syndrome* Sertoli cell tumors are similar in appearance to those found in Carney complex patients, but can be distinguished by typical intratubular proliferation of lightly eosinophilic cells with prominent basement membrane deposits, and occasionally, features of ovarian sex-cord tumors with annular tubules (178). Thus, the tumors are described as *intratubular large-cell hyalinizing Sertoli cell neoplasia*. These tumors may have strong aromatase activity and therefore be associated with gynecomastia and advanced bone age (224). No malignant testicular tumors have been reported in Peutz-Jeghers patients, but they have a highly increased risk of other neoplasms, especially colorectal, breast, pancreatic and ovarian cancers (214). Germline loss-of-function mutations in the *STK11*/LKB1 gene that encodes for a serine-threonine kinase causes Peutz-Jeghers syndrome in the majority of patients, allowing molecular genetic diagnostics (214, 224, 225).

Patients with the Sertoli cell tumors should be treated conservatively during childhood to give them a possibility for sperm banking before orchiectomy comes necessary (226). Autosomal dominant inheritance should be considered in genetic counselling. The patients should be frequently controlled with ultrasound examinations to follow changes in the tumors size, and reproductive hormone measurements. If precocious puberty and/or gynecomastia appear, aromatase inhibitors and antiandrogens can be used to prevent estrogen formation and androgen action (219, 226). There are currently no clear guidelines for the treatment of metastatic Sertoli cell cancers, but orchiectomy and retroperitoneal lymph node dissection are usually performed combined with individualized

chemotherapy and radiotherapy (227). Without surgery, the metastatic disease progresses and 20-month median survival time of patients was reported (IQR: 6–30 months) (227).

Granulosa Cell Tumors

Juvenile-type granulosa cell tumors are rare but are the most common somatic testicular tumors in infants and occur during the first 6 months after birth (2, 176, 178, 228). These tumors were described in patients with undescended testes with abnormal sex chromosomes and ambiguous genitalia (176). Prominent differentiation into follicles and immature nuclei distinguishes juvenile granulosa cell tumors from other Sertoli cell tumors that express tubular differentiation (178, 229). Most of the immunohistochemical markers in these tumor types are similar, e.g., inhibin, calretinin, but also distinct, e.g., expression of FOXL2 (229). Juvenile granulosa cell tumors always have a good prognosis (230). Testicular tumors do not show endocrine hyperactivity, in contrast to ovarian juvenile granulosa cell tumors. Aberrant WNT signaling (231) and stimulatory G-protein mediated signaling (232) have been linked with juvenile granulosa cell tumors. The patients should be managed comprehensively by a multidisciplinary DSD team, and the treatment of tumors may require orchiectomy.

Adult-type granulosa cell tumors are comparable to the ovarian tumors, but are extremely rare (2, 178, 233). These tumors occur in adults at an average age of 42 years. Twenty percent of the patients have shown gynecomastia due to the hormonal activity of the tumor. Most of the tumors are benign, but some malignant cases have also been reported (230). Mutations in *FOXL2* have been reported in adult ovarian granulosa cell tumors but only few secondary mutations have been found in testicular granulosa cell tumors (234, 235). The treatment is primarily surgical.

Fibroma-Thecoma Tumors

These exceedingly rare neoplasms are composed of fibroblastic cells of testicular stroma or tunica albuginea (2). These tumors are reported to be benign.

Mixed and Unclassified Sex Cord-Stromal Tumors

Tumors consisting of more than one stromal or tubular component or have indeterminate morphology are classified as mixed and unclassified sex cord-stromal tumors (2). Sex cord-stromal tumors can contain combinations of Leydig, Sertoli, granulosa, and theca cells, and are therefore called mixed tumors (178). Leydig cells can be difficult to recognize in these tumors. These tumors are rare and can occur at any age. Depending on the predominant cell type the tumors may behave differently. Gynecomastia, as a sign of endocrine activity, can be found in 10% of patients (2). These tumors are always benign in children, but in adults, malignancy can be found (232). Thus, most of the patients can be treated by orchiectomy, and lymph node dissection is indicated only in cases with overt malignant features on microscopic examination.

OTHER TESTICULAR TUMORS

Other tumors occurring in the testis are divided into miscellaneous and hematolymphoid tumors (2, 178). The first group includes ovarian epithelial-type tumors, serous or mucinous cystadenomas, adenocarcinomas, Brenner tumor, xantogranuloma, and hemangioma. The hematolymphoid tumors comprise malignant lymphomas (B-cell, NK/T-cell or follicular), plasmacytoma, myeloid sarcoma and Rosai-Dorfman disease (2, 178). In addition, testicular spread of malignant acute leukemia is common in young boys, and metastases from other solid tumors including the prostate gland, colon, kidney, stomach, pancreas, and malignant melanoma can be found in the testis of adults.

ENDOCRINE PROBLEMS AND LATE EFFECTS IN TESTICULAR CANCER PATIENTS

Testis Dysfunction and Fertility Issues

Relative imbalance of androgen signaling (excess or deficiency) causes the most pronounced secondary endocrine symptoms associated with testicular tumors. Testosterone is produced by tumors, such as Leydig cell tumors, or by normal Leydig cells stimulated by large amounts of hCG from some germ cell tumors. Excess of androgens would lead to precocious puberty in children (193, 194, 195). In addition, aromatization of androgens leads to a relative excess of estrogens, which causes impairment of spermatogenesis in adults and gynecomastia at any age (224, 236).

Testicular dysfunction in young adult patients with testicular cancer, who usually are in their best reproductive age, is a serious clinical problem. Patients with TGCT have poor spermatogenesis and decreased fertility even before the overt tumor has developed (67, 68, 69, 130) and before cytotoxic treatment (237, 238). Pathological features can include oligozoospermia or azoospermia, moderately decreased testosterone and elevated LH levels. If testicular biopsies are taken, a variable degree of testicular dysgenesis or atrophy is often seen, and in some cases further complicated by the presence of GCNIS. Examination of a contralateral biopsy in a patient with a unilateral tumor may show a similar picture; with at least 5-7% risk of the presence of GCNIS cells (71, 126).

Testicular function is further disturbed by treatment of malignancy. In recent years there is growing concern about adverse late effects of irradiation and chemotherapy, which induce severe impairment of spermatogenesis and DNA damage (1, 167, 168, 239, 240). Refinement of the dosage must be considered in each patient individually, to eradicate the neoplasm with least possible damage to the endocrine function. The eradication of GCNIS or a tumor by irradiation in bilateral cancer cases leads also invariably to the disappearance of all germ cells and sterility. This underlines the importance of semen cryopreservation before treatment, which will allow assisted reproduction treatment, if needed (238, 241).

All patients treated for testicular cancer require assessment of their reproductive hormones and spermatogenic capacity by semen analysis with respect to their future fertility. If semen banking or pre-operative sperm retrieval is not possible or if the patient has azoospermia or cryptozoospermia, testicular sperm extraction (TESE) at the time of orchiectomy ('onco-TESE') may be the only chance of fertility, and can be attempted in specialized centers (165, 166). TESE and subsequent intracytoplasmic sperm injection (ICSI) may be also an option for fertility treatment in some cases of post-chemotherapy azoospermia (242).

Fertility preservation is a serious challenge in children and young adolescents. In the adolescent boys who are unable to ejaculate, penile vibratory stimulation or electroejaculation can be considered (243). However, there is currently no treatment for prepubertal boys. There are ongoing studies attempting to optimize protocols of cryopreservation of immature testis tissue before cytotoxic treatment or orchiectomy (244). This approach would require *in vitro* or *in vivo* induction of spermatogenesis and gametogenesis, which has not yet been achieved satisfactorily in humans, but studies in experimental animals, including nonhuman primates are promising (245).

Long-Term Sequelae and Quality of Life

All patients treated for testicular cancer require monitoring of their reproductive hormone profiles not only with respect to their fertility, if paternity is desired, but also risk of testosterone deficiency and ensuing symptoms (246, 247, 248).

An increased risk of cardiovascular disease is present in TGCT survivors who have been treated with radio- or chemotherapy (249, 250) but a recent Danish study found that the risk decreases during

long-term follow-up, although it remains elevated (251). Additional problems in survivors of TGCT treated with radiotherapy or chemotherapy are increased risk of peripheral neuropathy or ototoxicity (after chemotherapy) and sexual dysfunction (after chemotherapy and radiotherapy) (239, 240, 250, 251, 252).

Survivors of metastatic TGCT have increased mortality rates when compared to the general male population. A study from Norway reported that most of the excess deaths occurred within a decade from diagnosis due to the testicular cancer, but during a longer follow-up, additional excess deaths appeared in patients treated with radiotherapy or cisplatin-based chemotherapy, mainly due to non-TGCT second cancers; primarily gastric, pancreatic or bladder tumors (253, 254). A population-based large study from the US found an increased risk of leukemia after chemotherapy and a marked excess of solid tumors in patients treated with radiotherapy, which can appear after long follow-up (255). This study confirmed previously published worrying reports from several centers, which called for reducing the use of radiotherapy and radiologic imaging procedures during the follow-up (256).

Of importance for the testicular cancer survivors are also quality of life issues related to prolonged anxiety, depression and psychological stress, and loss in socioeconomic status or unemployment, leading to excess of suicide among the patients (167, 252, 254, 257, 258, 255, 259, 260). Hence, a psychologist/social advisor should be added to the multidisciplinary team of experts caring for survivors of TGCT. Clearly, the focus of the current comprehensive care has moved from survival to survivorship of the patients after their recovery from testicular cancer.

CONCLUSION

Key take home points are shown in figure 7.

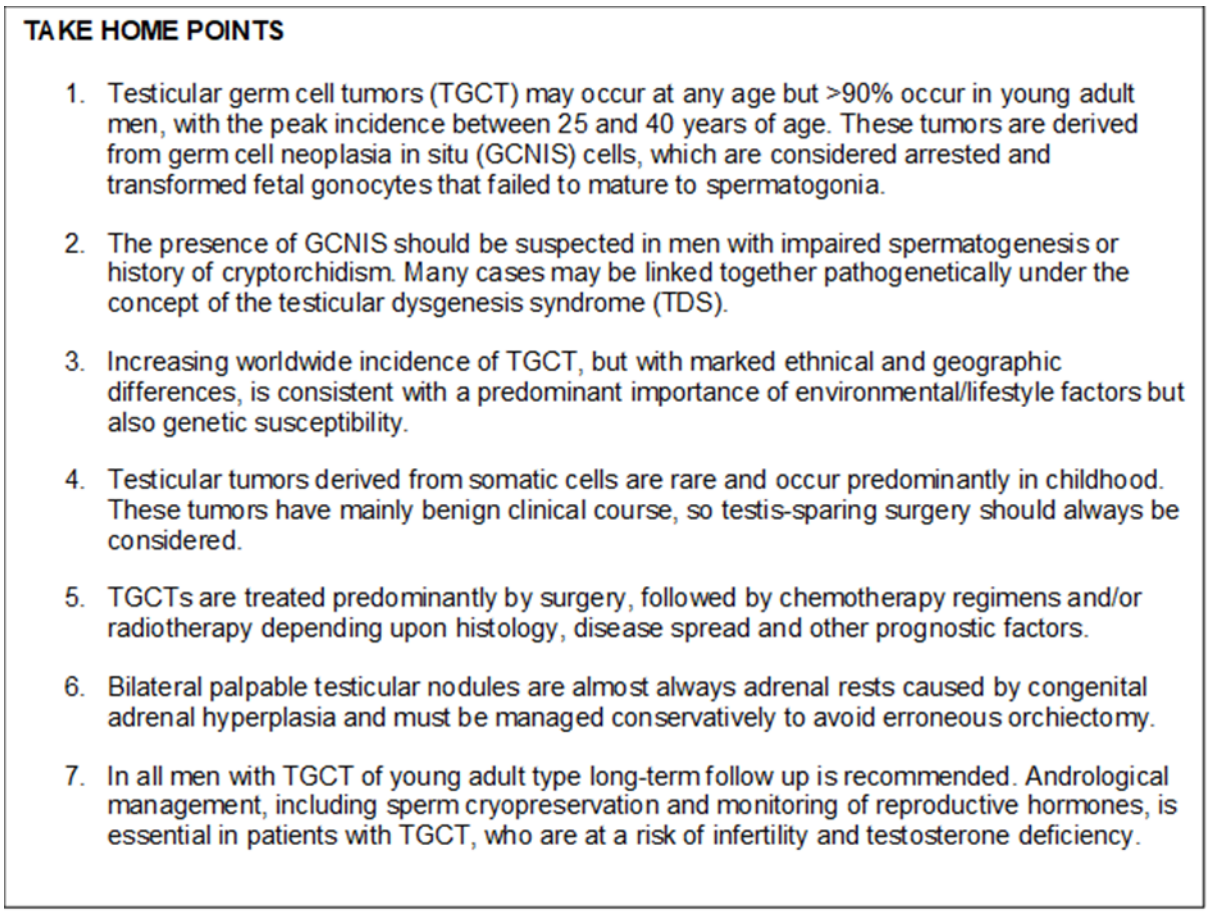

Figure 7. Key take home points.

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