Carcinoma in situ (CIS) of the testis

Francesco Ziglioli, Umberto Maestroni, Francesco Dinale, Matteo Ciuffreda, Pietro Cortellini
Unit of Urology - Surgical Department, University-Hospital of Parma, Parma, Italy

Abstract. Testicular cancer is the most common malignancy in 20- to 34-years-old males. It has been stated that testicular cancer derives from a precocious lesion, the carcinoma in situ of the testis, also known as Intratubular Germ Cell Neoplasia (IGCN) or Testicular Intraepithelial Neoplasia (TIN). This lesion deserves great attention, because the diagnosis of CIS may lead to a precocious diagnosis of testicular cancer. Generally, the diagnosis of CIS is incidental. Every physician should know the management of this precocious lesion, as the correct management of CIS can lead to a decrease of the incidence of overt testicular cancer (the most frequent malignancy in young men). Moreover, the correct diagnosis and management of CIS can shorten the hospital stay, reduce the cost, and improve the social impact of the testicular cancer. (www.actabiomedica.it)

Key words: Cis testis, testicular cancer, infertility, testicular biopsies

Search strategy and selection criteria: we reviewed the different aspects of carcinoma in situ of the testis, focusing on pathogenetic feature and management of the disease. We searched articles without any date limits between 1985 and 2009; we largely selected the articles published in English during the past 10 years without excluding older papers that we consider to be highly relevant to the topics discussed in the text below.

Introduction

Testicular cancer is the most common neoplastic malignancy in 20- to 34-years-old males. The majority of them derives from germ cell (TGCTs) and can be divided into seminomas and non-seminomas. The latter type may harbour one or several components, including embryonal carcinoma, teratoma, polyembrioma, chorioncarcinoma or yolk sac tumour. All these are believed to originate from a common precursor, the carcinoma in situ (CIS) of the testis (1), firstly described by Skakkebæk in 1972 (2).

The carcinoma in situ of the testis is also known as Intratubular Germ Cell Neoplasia (IGCN) (3) or Testicular Intraepithelial Neoplasia (TIN) (4).

It has been stated that CIS generally progresses to testicular cancer, although it is not well known how it happens. Moreover, the diagnosis of CIS is incidental, and it is not clear in which circumstances it should be suspected.

Epidemiology of the CIS testis

The carcinoma in situ (CIS) of the testis is usually asymptomatic and often it has no reason to be suspected, which is why information about its prevalence are not available and its epidemiology is not well known.

To date, infertility is considered as an important risk factor of the CIS testis and in retrospective studies on infertile men it has been demonstrated that the prevalence of the CIS testis is about 0,5-1% (5).

A lot of studies have been carried out about the prevalence of the CIS of the contralateral testis, because the diagnosis of CIS represents a risk factor of

the contralateral tumour develop: the prevalence of contralateral CIS is estimated to be around 5-8% (6).

Moreover, an increased prevalence in patients with cryptorchidism is reported: 2-4% of them are diagnosed with CIS. The risk of CIS developing does not depend on the age of surgical orchidopexy (7).

It has been stated that 50% of patient with CIS will develop an invasive germ cell cancer within five years (8, 9) if not treated.

Anyway, an early detection of testicular tumour is possible by diagnosing CIS.

Risk factors and pathogenesis

The carcinoma in situ of the testis can be considered as a part of the Testicular Dysgenesis Syndrome (TDS), made up of testicular germ cell tumour, genital malformation, cryptorchidism and decreased spermatogenesis, thus suggesting to have a prenatal origin.

Among the risk factors, the most important are infertility, familiarity for germ cell tumour of the testis, cryptorchidism, contralateral germ cell tumour and haermaphroditism (10, 12). Additional risk factors pointed in the last 10 years are the first birth, the low birth weight (1, 12, 13), and the delayed childbearing (14).

The genesis of CIS may be due to an anomaly in intrauterine or perinatal exposure to maternal hormones which delays the germ cell differentiation, thus determining an increased risk of malignant transformation (15).

So become more susceptible to malignant transformation, the germ cell may negatively respond to the endocrine stimulation during infancy or pubertal age (16). An observation that makes this hypothesis possible is the extremely rare occurrence of malignant transformation in patients with hypogonadotropic hypogonadism, in which the plasmatic hormonal level is very low.

Among the risk factors, some chemical molecules often present in cosmetics or components of pesticides are also reported in literature (1, 17). Moreover, lifestyle and occupational risk factors have demonstrated to play a role in CIS etiology (10).

The CIS derives from the gonocyte and the malignant process is believed to start during the embryonic life in primordial cells located in the midline of the embryo: in males, these primordial cells are able to differentiate in the cells of spermatogenesis. During this process, every disorder of differentiation could lead to the development of CIS.

In a precocious step of embryologic development, these cells migrate to the gonadal blastema, where they meet a perfect environment to differentiate in spermatocytes. As a consequence of the migration process, a misplacement could happen, resulting in the presence of gonadal cells in extragonadal tissue. This is the reason why in some patients it is possible to diagnose an extragonadal germ-cell tumour. Preferential extragonadal localizations are reported in literature: retroperitoneal space, anterior mediastinum and the midline of brain (18). It has been estimated that about 4% of patients diagnosed with extragonadal germ cell tumour (EGGCT) develop a testicular cancer in a 60-months follow-up period (19).

A study carried out by Hoei-Hansen et al., with the aim to seek for genes overexpressed in testes harbouring carcinoma in situ has taken an important contribute to the pathogenesis of CIS (20).

In testes containing carcinoma in situ, a lot of genes differentially expressed have been identified. The first genes recognized as fundamental in the pathogenesis of carcinoma in situ were found to be amplified and overexpressed in particular genomic ares, such ad 12p, 17q and Xq13 (21).

Traditionally, the genes responsible for the tumoral transformation in carcinoma in situ are c-KIT, AP-2γ and OCT3/4, which are not detectable in normal adult testis (1, 22). Particularly, KIT is a proto-oncogene involved in the differentiation and migration of primordial germ cells, AP-2γ is involved in self-renewal and survival of immature germ cell and OCT3/4, interestingly present in all CIS cells, plays a role in maintaining stem cells in a pluripotent stage thus preventing the differentiation process (1, 23, 24).

Many efforts are necessary to know more and to carry out other genes, whose role is important in CIS developing knowledge.

The kit receptor plays an important role in the pathogenesis of CIS. This gene encodes a cell membrane tyrosine kinase receptor for stem cell factor and it has been largely described in CIS (25, 26). C-kit is

a pro-survival factor for the germ cells, thus allowing the neoplastic proliferation.

To date, it is reported that in quick time the beginning of the transformation process lead to the overexpression of other genes, also. Particularly, there is an important up-regulation of collagen α2 type 1 (encoded by the gene COL1A2), presumably related to a remodeling capacity of the neoplastic cells (20) The modification of the cellular life is demonstrated by the increased mithocondrial activity, which is up-regulated thanks to the overexpression of the cytocrome b reductase 1 (CYBRD1) (20).

Histopathologic feature

Seminomas share histologic and morphologic characteristics with CIS (27).

CIS cells are located at the basement membrane of seminiferous tubules in a single row and are large cells with a large nucleus, large nucleoli and cytoplasm rich in glycogen, which makes the cytoplasm itself almost empty at optical microscopy (28, 29). CIS cells are found inside the seminiferous tubules (21). The basal lamina always remains intact (30, 31) (Fig. 1).

Despite the preserved integrity of the basal lamina, a loss of contractility usually occurs in the seminiferous tubules: in fact, myosin immunoreactivity reportedly disappears in the myofibroblasts with progressing CIS transformation (30). Since the contractility of seminiferous tubules is necessary for spermatozoa transport, the deficit of myosin may explain the sexual function impairment observed in testis harbouring CIS.

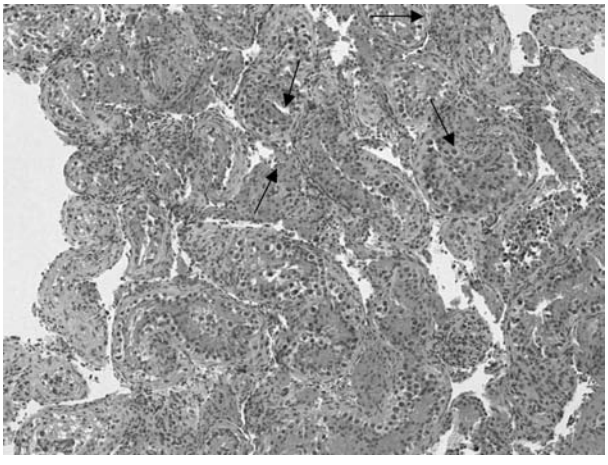

Figure 1. The cells of Carcinoma in situ may be observed at the basement membrane of the seminiferous tubules (hematossilin-eosin coloration). The black arrows hint at the CIS cells

As a consequence of the depletion of germ cells in seminiferous tubules harbouring CIS, tubules become little in diameter. Limphocytes very often infiltrate the testicular tissue and Leydig cells become hyperplastic (6).

In the context of CIS, calcifications are often found: in a study carried out by Kang et al, calcifications have been found in 38% of the samples (32).

At electronic microscopy, tight junction, adherence junction and gap junction are altered in number and distribution, with particular regard to zonulae occludentes (Zo), that show a lack of their blood-testis barrier function (33). This junction normally represents an effective blood-testis barrier.

In normal seminiferous epithelium, Zo-1 and Zo-2 form a continuous and strong belt strictly connecting cell to cell. Within CIS tissue, instead, zonulae occludentes are less strong and immunohistochemistry shows a lack of strength (34), resulting in a consistent decrease of the blood-testis barrier function (33).

It is also reported a change in the distribution pattern of Zo-1 and Zo-2, thus determining an increased permeability of tight junctions (35).

The distribution pattern of Zo-1 and Zo-2 is disrupted in a number of tumours and it is usually related to the degree of differentiation. The modification found within CIS tissue seems to confirm the process of dedifferentiation the gonocytes undergo as a consequence of the neoplastic transformation (33).

Clinical feature and diagnosis

Carcinoma in situ of the testis is a premalignant testicular germ cell lesion whose probability to develop in testicular cancer is 70% in 7 years and 5% of the patients diagnosed with testicular cancer harbour CIS within the contralateral testicle (36).

Clinically, carcinoma in situ of the testis is often asymptomatic, but the circumstances of the diagnosis could be an urologic evaluation made for other rea-

sons, the high level of α-fetoprotein (AFP) or β-human gonadotropin (β-hCG) or the detection of a contralateral mass (37).

The CIS cells normally remain quiescent during the childhood and progress into overt testicular germ cell tumour after puberty (38).

The majority of patients presents with primary tumour in the testis; in a minority of cases, the primary tumour is localized in an extragoandal site, such as retroperitoneum or mediastinum, as cited above (18, 36).

The diagnosis of CIS must be histologically confirmed, so that the biopsy is mandatory (Table 1). The biopsy is recommended in patients with unilateral testis cancer, with presumed extragonadal germ cell tumour, with somatosexual ambiguity and a Y chromosome, and in subfertile patients, especially if they present a history of cryptorchidism and a low semen quality or atrophic testicle (less 12 ml) (1, 36).

High level of suspicion should be the correct approach if these features are found in young men. With regard to patients affected by extragonadal germ cell tumour, the biopsy should not be routinely performed, since all patients diagnosed with extragonadal germ cell tumour receive platin-based chemotherapy, which is believed to eradicate CIS. It retains a significance, otherwise, in extragonadal germ cell tumour which are known to manifest a high risk of CIS, but it should be performed prior to chemotherapy (18, 36, 39).

A single open surgical biopsy of at least 3 mm in diameter, with more than 30 seminiferous tubules, is sufficient in order to obtain a correct diagnosis (40).

The limit of the biopsy is the possibility of false-negative. The cancer of false-negative are the very young age of the patient (below 18 years), because of the insufficient numbers of tubules yet populated by the tumour, and the interval between the diagnosis of germ cell tumour and the occurrence of CIS in contralateral testis (41, 42).

To date, it is generally accepted that the immunohistochemistry is mandatory for the diagnosis of CIS, because the use of hematossilin-eosin coloration could be not sufficient (43).

In 1983, PLAP was the choice marker for the detection of seminomas (Fig. 2). Successively, other markers have been used in the routine practice, such as c-KIT and AP2-γ to make the diagnosis of CIS more definitive (44) (Fig. 3).

Table 1. When the biopsy is recommendend

Which patient must be candidate to biopsy?
The biopsy is fundamental in case of: • unilateral testis cancer • presumed extragonadal germ cell cancer • somatosexual ambiguity • subfertile (or unfertile) patients, especially in case of low semen quality

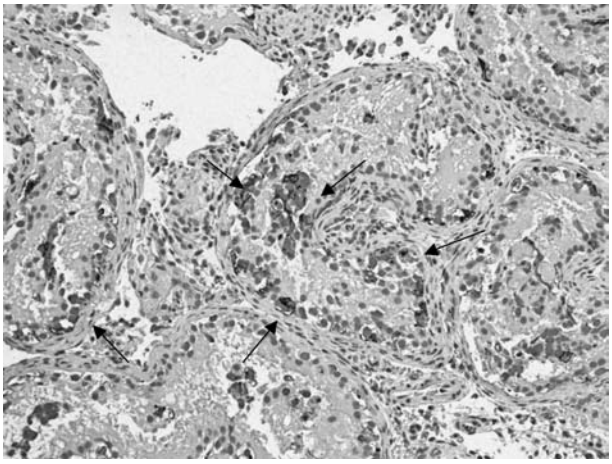

Figure 2. In the seminiferous tubules harbouring CIS, immunochemistry is positive for PLAP, one of the most important markers for the detection of seminomas. The black arrows hint at the PLAP staining areas

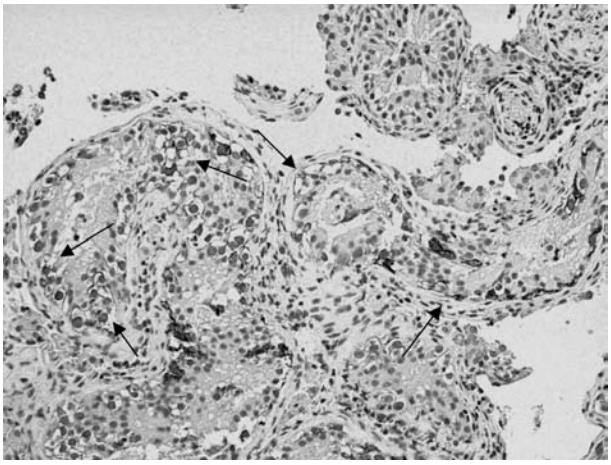

Figure 3. At the immunohistochemical study, c-kit staining demonstrates the proliferative potential of CIS cells. The black arrows hint at CIS areas positive to c-kit

Currently, OCT3/4 is the most robust marker in the diagnosis of germ cell tumour and CIS (45, 46). The research of OCT3/4 in the semen represents a non-invasive and reliable method in order to obtain a diagnosis of CIS. In fact, CIS is present in the seminiferous tubules and cancerous cells are supposed to exfoliate into the lumen, leaving their original location (47). OCT3/4 is not present in normal tubules, which is why the research of this nuclear marker is a method in order to obtain a precocious diagnosis of CIS, seminoma and embryonal carcinoma. OCT3/4 may be used in subfertile or unfertile men, in patients with diagnosis of contralateral germ cell cancer or already treated for germ cell cancer and in patients with microlithiasis (4, 47).

Another important aspect of the clinical feature of CIS is the impaired testicular function. It is well known that spermatogenesis is more poor in testes harbouring germ cell cancer (48). The hormonal onset is altered also: low testosterone levels and/or increased LH levels are commonly found (49). In fact, CIS cells probably inhibit Leydig cells function directly, or because of germ cells depletion. Leydig cell continuously interact with germ cell, thanks to the production of a plethora of growth factors and hormone-like factors and this makes possible a disruption of this interaction as a consequence of the neoplastic process (49). This is the reason why a correct management of the CIS includes an evaluation of the hormonal profile as well. Moreover, the high number of patients with spermatogenesis dysfunction makes necessary an andrologic follow-up.

CIS treatment and follow-up

A nomogram is reported in figure 4. However, the correct management of the carcinoma in situ of the testis needs to be formulated according to the single patient onset.

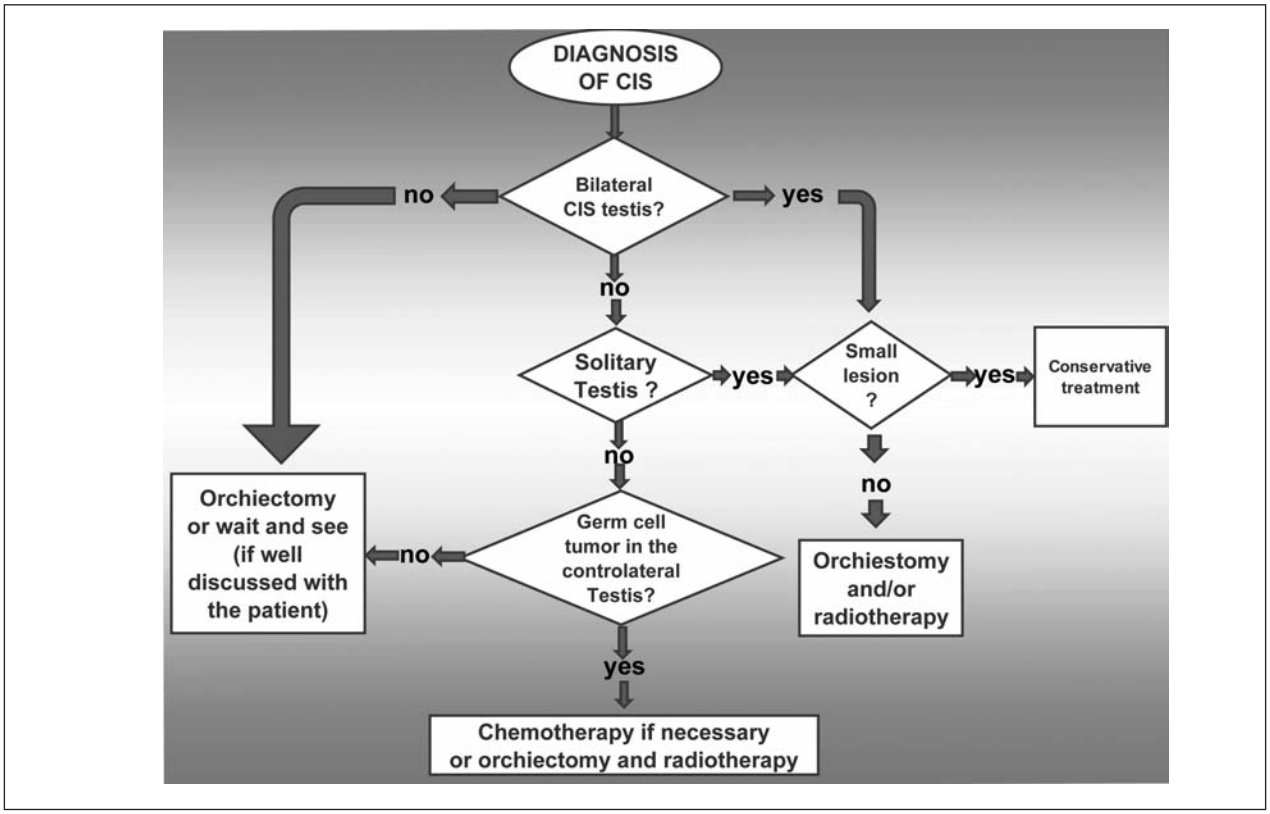

Figure 4. Treatment of CIS testis. Nomogram

First of all, wait and see is not recommended. In selected cases it is possible to perform a conservative management, it is to say tumorectomy (instead orchidectomy) followed by immediate radiotherapy. This treatment, however, is not the gold standard, because of the high incidence of recurrence in the same or contralateral testis. The conservative approach preserves the endocrine function and maintains the possibility of a natural childhood (50).

Nevertheless, the conservative management is not recommended in every patient diagnosed with CIS, because of the high percentage of recurrence and the high frequency of multifocality of the neoplasm. Definitively, tumorectomy and immediate radiotherapy is best indicate in patients with bilateral testis lesions or patients with a solitary testis; these patients should also present with a small tumour and no other risk factors (36, 50).

The usual management of CIS is made up of three approaches: surgery, chemotherapy and radiotherapy.

In patients diagnosed with unilateral CIS, the treatment of choice is orchidectomy, because it is definitive. Also radiotherapy is a definitive choice, but it may impair the fertility of the contralateral testis (50).

When a germinal germ cell tumour is diagnosed, a biopsy of the contralateral testis may be performed. If a CIS is found at the biopsy, orchiectomy + radiotherapy is considered the gold standard while the wait and see other should will be discussed with the patient. The same choices are possible if CIS is diagnosed in a solitary testis.

It must be considered that radiotherapy is likely to destroy every residual fertility chance. Radiotherapy consists of a total dose of 20 Gy, delivered in fractional doses of 2 Gy per week (51, 52).

The Leydig cells are very sensitive to radiotherapy: initially, the lack of Leydig function is well compensated by LH increasing level, but this effect does not persist going on with radiotherapy (53). With the aim to preserve a residual fertility (e. g. in patients who would like to father a child naturally) it is possible to perform a radiation dose of less than 20 Gy in order to avoid a strict impairment of Leydig cells function, but in this case it is not possible to be sure of the total effectiveness of the treatment (54, 55).

The role of chemotherapy (cisplatinum) in the treatment of CIS is confined to the case of patients who must receive chemotherapy anyway, i. e. in patients diagnosed with germ cell tumour in a testis and, in the meantime, with CIS in the contralateral testis. In about two-third of these patients chemotherapy is enough to eradicate CIS; otherwise, a low-dose radiotherapy treatment may be performed after chemotherapy (56). In conclusion, chemotherapy is able to eradicate CIS, but does not prevent the possibility to develop a testicular germ cell cancer (9).

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Correspondence: Francesco Ziglioli
Operative Unit of Urology, Surgical Department
University-Hospital of Parma
Via Gramsci, 14 - 43100 Parma, Italy
Tel. +39 (0)521 702182
E-mail: ziglioli@hotmail.it