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OCT 4 immunohistochemistry in postpubertal cryptorchidism

Research Article

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Abstract: Patients with cryptorchidism are at an increased risk for germ cell testicular cancer. OCT 4 has been shown to be a sensitive and specific marker for some types of germ cell testicular cancer. We undertook this study to establish whether OCT 4 immunohistochemistry is a useful tool in the pathohistologic evaluation of postpubertal patients with cryptorchidism. Seventeen postpubertal patients underwent orchidectomy for cryptorchidism at our center since 1997. Immunohistochemical staining with OCT 4 was performed on these samples. Characteristic OCT 4 nuclear staining was positive in two patients. One patient was correctly diagnosed on previous pathohistological evaluation, while OCT4 immunohistochemical staining revealed previously unidentified intratubular germ cell neoplasia in the other patient. OCT 4 immunohistochemistry can be useful in diagnosing a testicular germ cell tumor in patients with cryptorchidism. If we consider a low number of postpubertal patients with cryptorchidism a benefit of immunohistochemical staining with OCT4, this could favor the use of OCT 4 staining in work-up of cryptorchidism.

Keywords: *OCT4 • OCT 3/4 • POU5F1 • Cryptorchidism • Immunohistochemistry • Testis neoplasia*

1. Introduction

Testicular cancer is the most common malignancy in men between aged 15-34 years in most European populations [1]. In Croatia, age standardized incidence rate in 2006 of testicular cancer was 6,52 per 100,000 men [2]. Fortunately, due to a multimodal approach to treatment, 5-year survival rate is over 90% [3], with the greatest benefit from the introduction of cisplatin based chemotherapy in the mid 1970s [4].

There are few established risk determinants for testicular cancer (TC) one of which is cryptorchidism (undescended testis, UDT). Cryptorchidism is the best recognized risk factor for TC with relative risk of 4.8, according to recent meta-analysis [5]. Petersson et al. assessed in their cohort study involving 16,983 men who had orchiopexy for cryptorchidism at age of 13 or later relative risk factor of 5.4 [6]. Due to increased risk of TC current recommendation for management of postpubertal patients with unilateral cryptorchidism is orchidectomy [7,8].

Intratubular germ cell neoplasia (IGCNU) is a precursor lesion of malignant germ cell tumors of the adult testis [9]. It is seen with increased frequency in patients with cryptorchidism [10,11]. More than 95% of all testicular cancers are germ cell tumors (TGCT) [12]. TGCT appears in several histological forms, which are divided in approximately two equal groups: seminoma and nonseminoma.

Although lesions are often clearly discernable on hematoxylin and eosin stained sections a reliable and sensitive immunohistochemical marker would be useful. OCT4 (OCT 3/4, POU5F1) is a nuclear transcription factor that is expressed in early embryonic cells and germ cells [13,14]. OCT4 has been detected in neoplastic pluripotent germ cells, specifically those of testicular seminoma, embryonal carcinoma [15], and IGCNU [16]. Antibodies to OCT4 mark the nuclei of these pluripotent cells.

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Table 1. Clinical characteristics of patients.

Patients	17
Mean age	30,58 (14-43)
Cryptorchidism (right/left)	8/7
Bilateral cryptorchidism	2
Hospitalization	4,94 (1-19)
Palpable/impalpable undescended testis	14/3

The aim of this study was to determine the value of OCT 4 antibody in the pathohistologic evaluation for TGCT or IGCNU in postpubertal patients with cryptorchidism.

2. Material and Methods

2.1. Case selection

A retrospective analysis of health records revealed 17 postpubertal patients in whom orchidectomy of undescended testis was performed in the University hospital center Zagreb, Croatia since 1997 (Table 1). Fourteen patients presented with unilateral cryptorchidism and two with bilateral. Undescended testis were palpable in 82% of patients. Clinical examination was positive for testicular cancer in one patient. The average age was 31 years.

With all patients surgical options were discussed and orchidectomy was recommended. One patient with bilateral cryptorchidism had intraabdominaly placed testicles which were inaccessibly located for follow-up; the other patient had small atrophic testicles. To them orchidectomy was recommended and postoperatively replacement hormonal therapy initiated. Open surgical exploration and orchidectomy were performed in 13 patients and laparoscopic in 4 patients. In one patient orchidectomy of an undescended and impalpable testicle was performed during renal transplantation and in one patient during reposition of a ureter placed behind inferior vena cava.

We have assessed archive materials of cryptorchid testicular tissue. Initial patohistologic examination revealed TGCT- non seminoma in one patient and in material from two patients only fibrotic tissue was found without any testicular tissue. In the remaining 14 patients no signs of TGCT or IGCNU were found. All patients were included in the study.

2.2. Immunohistochemistry

Tissue sections ranging from 3–5 μm in thickness were cut from paraffin-embedded tissue blocks and placed on object slides (Menzel-Glaser, Germany). Slides with tissue sections were incubated for 20 min in a thermostat at 60°C. Sections were then deparaffinized and incubated for 3x5 min in Tris/EDTA pH9 in a Dako PT Link module. Subsequently, tissue slides were washed with TBS pH 7,4 and endogenous peroxidase activity was blocked by a 5 min treatment with hydrogen-peroxide (Dako-No.S2023). Slides were then washed with TBS-buffer and incubated for 60 min with monoclonal mouse antibody OCT 4 (clone C-10, SantaCruz Biotechnology, USA) diluted 1:50 at room temperature. After a wash in TBS-buffer, the secondary polymer conjugate antibody (Dako-No.K5007) was added for a 30-min incubation. Slides were then washed with TBS-buffer. Tissue sections were washed once more in TBS-buffer and then Chromogen (Dako-No.K5007) was added for 10 min. Slides were washed in distilled water, stained with hematoxylin (Dako-No.S2020) for 1 min, washed with water, dehydrated with alcohol (96%), cleared with xylene, and covered mechanically.

For each OCT 4 immunohistochemically stained slide, we visually estimated the percentage of tumor cells showing nuclear immunoreactivity for OCT 4. Cases with staining of more than 5% of the relevant nuclei were considered positive.

Table 2. Summary of histology results with and without OCT 4 immunostaining.

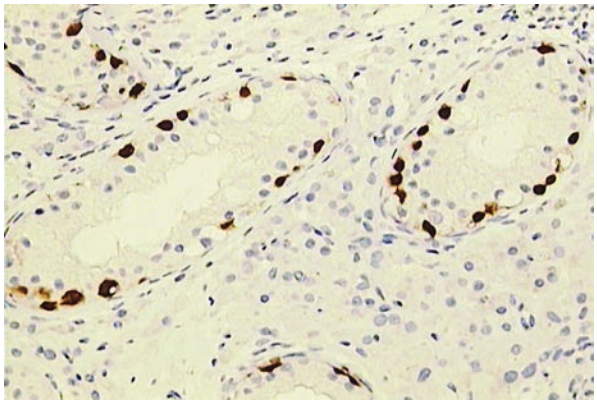
Histology	First evaluation	Evaluation with OCT 4
Testicular carcinoma	1 (non seminoma)	1 (non seminoma)
IGCNU*	0	1
Fibrosis	2	2
Without tumor	14	13

* IGCNU - Intratubular germ cell neoplasia

3. Results

OCT 4 in the patient with previously verified TGCT-non seminoma correctly stained nuclear region of cancer cells. In two patients with previously described fibrosis diagnosis was confirmed. Out of the remaining 14 patients in 13 patients new patohistologic evaluation as well as OCT 4 immunohistochemistry revealed no presence of testicular cancer or IGCNU (Table 2). In one patient new patohistologic evaluation revealed a presence of IGCNU with characteristic OCT 4 staining (Figure 1). Patient with newly diagnosed IGCNU was 14 years old at the time of operation. He was on hemodialysis for 22 months. Ipsilateral side of UDT was chosen for cadaveric kidney transplantation and during the operation orchidectomy was performed. The patient was on regular follow up with our team of nephrologists

Figure 1. Strong nuclear immunoreactivity of OCT 4 is limited to the neoplastic cells. (immunohistochemistry with OCT 4, 20x).



and urologists. He was without clinical signs of testicular cancer. Due to this new finding analysis of serum tumor markers was performed, and they are normal. Contralateral testis is without palpable tumor mass and ultrasound examination revealed no signs of tumor as well.

4. Discussion

For patients with undescended testis, there is a recommendation for performing orchiopexy preferably before the age of 2 and even as young as 6 months old [7].

However, in postpubertal patients with cryptorchidism treatment options remain orchiopexy, orchidectomy or close follow-up if an undescended testis is accessible to palpation and ultrasound examination. Previous studies showed for majority of adult patients with undescended testis to have very low fertility potential, impairment of endocrine function and increased risk of testicular cancer. Meta-analysis of 20 studies reported a relative risk factor of 4.8 for development of testicular cancer in UDT [5]. Furthermore, a recent study by Swedish authors reported relative risk for testicular cancer among the patients who underwent orchiopexy after age of 13 is 5.4 in comparison to patients before age 13 in who relative risk is 2.23 [6]. Decision for operation of UDT weighs a relative risk of death from germ cell tumor against relative risk of death from perioperative complications. Considering major improvements in therapy of TGCT and perioperative care, recently updated analysis recommends operation in all healthy males who present with postpubertal cryptorchidism until the age 50 [17]. Accounting all this, to patients with unilateral cryptorchidism and normal contralateral testicle orchidectomy is recommended.

Accurate histological assessment of TGCT or IGCNU is important. Therefore immunostain specific for these lesions, easily applied in surgical pathology practice would be helpful. Several immunomarkers have been proposed for testicular cancer of which PLAP is most widely used. However, studies showed OCT4 immunostaining has a greater staining intensity than PLAP immunostaining with less background staining artifacts [18] and to be easier for interpretation [19]. In addition to stain neoplastic germ cells, PLAP has been identified in non neoplastic germ cells while OCT4 is specific only for seminomas, embryonal carcinomas [20] and IGCNU in postpubertal testis [18]. Furthermore, this should not be disturbing as other components like yolk sac tumor, choricarcinoma and teratoma are rarely found as pure histologic types; they are most often found as a component of mixed germ cell tumor [21].

Undescended testes are developmentally defective from the start and/or dysgenetic usually showing delayed maturation. Furthermore, progressive histological damage to undescended testes means there is diversity of histological findings that can make proper diagnosis difficult. This may produce delayed expression of OCT4, especially in children [22]; therefore cautious interpretation of OCT4 staining is important. In those instances, using additional immunomarkers to assess proper diagnosis may be useful.

Our study includes small group of patients mainly due to a small number of postpubertal patients with UDT. Nevertheless, this study shows great value of OCT4 immunostaining as a diagnostic tool in patients who are at an increased risk of testicular cancer. We believe that confirmed specificity of OCT 4 should be used for doubtful cases if not for all cases where testicular cancer is in question. Similar studies on a larger number of patients are needed to confirm our results.

To conclude, OCT 4 immunohistochemistry can be useful in diagnosing a testicular germ cell tumor in patients with cryptorchidism. Considering a low number of postpubertal cryptorchidism a potential benefit of immunohistochemical staining with OCT4 we would favor a routine use of OCT 4 staining in work-up of cryptorchidism.

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