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The diagnostic impact of testicular biopsies for intratubular germ cell neoplasia in cryptorchid boys and the subsequent risk of testicular cancer in men with prepubertal surgery for syndromic or non-syndromic cryptorchidism☆

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ABSTRACT

Introduction: Cryptorchidism is a risk factor for testicular cancer in adult life. It remains unclear how prepubertal surgery for cryptorchidism impacts later development of adult testicular cancer. The aim of study was to investigate tools to identify the cryptorchid boys who later develop testicular cancer.
Methods: The study cohort consisted of 1403 men operated prepubertally/pubertally for undescended testis between 1971 and 2003. At surgery testicular biopsies were taken from the cryptorchid testes. The boys were followed for occurrence of testicular cancer. The testicular cancer risk was compared to the risk in the Danish Population. Testicular biopsies from the boys who developed testicular cancer during follow-up underwent histological examination with specific diagnostic immunohistochemical markers for germ cell neoplasia.
Results: The cohort was followed for 33,627 person years at risk. We identified 16 cases with testicular cancer in adulthood. The standardized incidence ratio was 2.66 (95% CI: 1.52–4.32). At time of primary surgery in prepubertal/pubertal age Intratubular Germ Cell Neoplasia (ITGCN) was diagnosed in 5 cases and the boys were unilaterally orchiectomized. At follow-up new immunohistochemical staining indicated ITGCN in two of the 16 cancer cases at reevaluation of the original biopsies from time of prepubertal/pubertal surgery. One had syndromic cryptorchid and developed seminoma, and another showed nonsyndromic cryptorchidism and developed embryonic teratocarcinoma. Totally, ITGCN was diagnosed in 0.5% (7/1403) of prepubertal cryptorchid boys, whereof 57% (4/7) in syndromic-cryptorchidism.
Discussion: ITGCN is predominantly observed prepubertally in boys with syndromic-cryptorchidism. In nonsyndromic cryptorchidism testicular cancer develops postpubertally, generally not based on dormant germ cells of ITGCN caused by an early fetal maldevelopment.
Levels of Evidence: LEVEL I.

Cryptorchidism is the most well-characterized risk factor for malignancy of the testis [1,2] and 5%–10% of testicular cancers are associated with cryptorchidism [2,3]. It remains unclear how prepubertal surgery for cryptorchidism impacts later development of adult testicular cancer. For a boy with cryptorchidism the relative risk of developing a subsequent testicular malignancy was 2.2–3.8 times higher than the background population according to a recent meta-analysis [4]. However, in a cohort study from our region the relative risk was 4.7(95% CI: 1.7–10.2) [5]. Prepubertal Intratubular Germ Cell Neoplasia (ITGCN) is a precursor for testicular germ cell cancer and is characterized by large germ cells with large nuclei with a hyperchromatic, coarse chromatin pattern, large prominent nucleoli and abundant pale cytoplasm located both centrally and peripherally mixed with normal cells in the seminiferous tubules [2,6]. It has been hypothesized that understimulation or dysregulation of primordial germ cells or gonocytes during early fetal development is a key event leading to dormant germ cells of ITGCN [2,7,8].

Recently, it was shown that the most commonly used immunohistochemical markers for adult germ cell cancer, anti-Oct3/4 and anti-D2-

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40, failed to detect ITGCN in infant boys with congenital cryptorchidism, because positive staining is commonly seen below the age of two years, as the markers generally are related to early stages of fetal germ cell maturation which normally proceeds into early infancy [9,10]. After the age of 2 years Oct3/4 and D2-40 positive germ cells normally disappear, and ITGCN concomitantly with Oct3/4 and D2-40 positive germ cells has only been demonstrated in boys with syndromic cryptorchidism, and therefore at high risk of later invasive cancer [11,12].

The aim of this study was to identify the prevalence of testicular cancer among adult men who had surgery for cryptorchidism in prepubertal age and who all had a testicular biopsy taken from the undescended testis. Furthermore, we investigated whether use of immunohistochemical markers on these biopsies makes it possible to identify dormant cells of ITGCN and thereby to identify those cryptorchid boys who would develop testicular cancer in adult life.

1. Material and methods

The cohort consisted of 1403 consecutive boys operated on between 1971 and 2003 for cryptorchidism and with a perioperative testicular biopsy taken at orchidopexy. It was the routine of the department to take these biopsies in the whole period. In the Cancer Register the patients were followed for testicular cancer from 15 years of age or the age of orchiopexy plus one year until the date of diagnosis of testicular malignancy, emigration, death or December 31, 2013. Data from 897 of these patients were also included in previous (17–22 years old) publications [13–15].

The testicular cancer risk for the cohort was compared to the risk in the Danish male population as recorded in NORDCAN [16]. The calculation of person time at risk for the cohort by age and calendar year used a SAS-macro by Macaluso [17] and SAS 9.3 [18].

We estimated 95% confidence intervals (95% CIs) of the standardized incidences rates assuming a Poisson distribution [19]. The risk of death for the cohort was evaluated in a similar way. Statistical analyses for description of the cohort were performed in SAS, SPSS and Excel. Continuous data are presented as mean and medians with 25th–75th interquartile range (IQR). Categorical data are reported as proportions.

From the original patient files it was noted if the boys had associated congenital anomalies, chromosomal and/or genetic disorders and syndromes. These patients were classified as “other congenital malformations/syndromic cryptorchidism”. If no such anomalies were found and the boy had isolated cryptorchidism the term non-syndromic cryptorchidism was used. Patients who had unilateral orchiectomy at primary surgery or at close relation to the first surgery were also identified.

The original testicular biopsy specimens embedded in paraffin taken at time of surgery for cryptorchidism from the patients, who later developed testicular cancer, were analyzed. One H + E slide was produced. For immunohistochemistry and identifications of germ cells showing positive signals specimens were cut into 2 μm sections and mounted on coated slides for immunofluorescence analysis with Placental-like alkaline phosphatase (PLAP), Oct3/4, C-kit and D2-40 according to a method previously published [9,12]. For identification of germ cells showing a positive signal 5 to 10 cross-sections of seminiferous tubules were examined. It was classified as a positive result if positive signals were seen in any seminiferous tubules.

The study was conducted according to the Helsinki II Declaration and was approved by the ethics committee of Copenhagen (No: H-6-2014-061) and the Danish Data Protection Agency.

2. Results

The cohort included 1403 boys operated on for cryptorchidism between 1971 and 2003. Median age at orchiopexy was 11.8 years (IQR: 8.8–13.3 years), mean age 10.8 years (SD 3.6 years).

Table 1
Associated anomalies/syndromic cryptorchidism in 197 boys out of a cohort of 1403 patients.

Major Associated Anomaly	No. of patients
Adrenogenital syndrome	3
Nivergelt syndrome	1
Robinow syndrome	1
Rubinstein–Taybi syndrome	1
Arhrogyposis	1
Vertebral malformation or polydactyly	6
Myelomeningocele	11
Congenital hip dislocation	2
Crouzons syndrome	2
Klippel–Feil syndrome	2
Cerebral palsy and/or mental retardation	21
Cleft palate	2
Thyroglossal fistula	1
Congenital deafness	2
Congenital myxedema	1
Congenital Rubella syndrome (with or without congenital cardiac disease)	2
Congenital severe hypermetropia	1
Iris agenesia	1
Cystic fibrosis	3
Nanism	3
Diabetes (type 1)	4
Duodenal atresia	1
Esophageal atresia (with or without urological malformation)	3
Hemophilia	3
Hirschsprung's disease	4
Histiocytosis (Mb Letterer–Siwe)	2
Congenital cardiac disease	18
Imperforate anus (with or without hypospadias, sacral anomaly or chromosomal disorder)	18
Kallmann syndrome	4
Prader–Willi syndrome	3
Neurofibromatosis	1
Abdominal wall defects	3
Hypospadias	17
Epispadias/Bladder extrophia	3
Urethral valves	3
Other urological–congenital malformations (unilat agenesis, MCDK, VUR, megaureter, duplex system, PUJO, horseshoe)	23
Klinefelters syndrome	8
Other chromosomal disorder (unbalanced translocation 13/20, deletion 11)	2
Phenotypic male DSD (persistant Müllerian duct syndrome)	3
Phenotypic male DSD (including XYY, XY/XO, 45X/46XY)	7

The cohort was followed for a mean period of 28.9 (SD 8.1) years with a total of 33,627 person-years at risk. For 146 of the subjects follow-up ended before end of December 2013 owing to development of testicular cancer (n = 16), death (n = 76) or emigration (n = 54). In 197 cases the boys had other congenital malformations/syndromic cryptorchidism (Table 1).

We identified 16 cases with testicular cancer in the follow-up period (Table 2). The cancer cases were mean 12.6 years (SD 2.8 years), median 12.7 years (IQR: 11.4–14.5 years) at operation. The standardized incidence ratio of testicular cancer was 2.7 (95% CI, 1.5–4.3). Subjects with bilateral cryptorchidism had a standardized incidence ratio of testicular cancer at 4.1 (95% CI, 1.8–8.1), compared to 2.0 (95% CI, 0.8–3.9) in unilateral cases. We did not find any significant difference in risk between groups below or above 10 or 13 years at time of operation; neither did the risk of cancer development change in the subgroup with other congenital malformations/syndromic cryptorchidism. The overall mortality ratio for the cohort was 1.1 (95% CI, 0.9–1.4).

At time of orchidopexy for cryptorchidism six patients had testicular neoplasia diagnosed at first original evaluation of the histology slides at time of prepubertal/pubertal surgery. Five of these patients had ITGCN (whereof 3 boys had DSD, abnormal genitalia +/− abnormal karyotype and two boys had intra-abdominal testes) and one 18-year-old patient had a seminoma cancer. All had the affected side orchiectomized [15]. In all these original ITGCN cases the diagnosis was based on evaluation