Towards a non-invasive method for early detection of testicular neoplasia in semen samples by identification of fetal germ cell-specific markers

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BACKGROUND: Testicular germ cell tumours (TGCTs) originate from a common precursor, carcinoma in situ (CIS). Diagnosis at the CIS stage is desirable as it minimizes the necessary treatment. A detailed clinical evaluation of an approach to detect CIS cells in the ejaculate using primordial germ cell/gonocyte markers is presented. METHODS: Immunocytological staining for AP-2γ [and in some cases, OCT-3/4, NANOG or placental alkaline phosphatase (PLAP)] was performed in semen samples from 294 infertile patients and 209 patients with TGCTs or other diseases. RESULTS: Presence of AP-2γ-stained cells was detected in 50% of participants with CIS and in 33.9% of TGCT patients before treatment (non-seminomas: 56.6%, seminomas: 17.4%). OCT-3/4 results were similar to those of AP-2γ, whereas NANOG and PLAP stainings were unsuitable. Sensitivity was 54.5% for participants harbouring pre-invasive CIS but reduced in participants with overt TGCTs, perhaps because of obstruction. Assay specificity was 93.6%, positive predictive value (PPV) 83.3% and negative predictive value (NPV) 60.3%. CONCLUSIONS: Immunocytological semen analysis based on expression of fetal germ cell markers in exfoliated cells has auxiliary diagnostic value, as it detects some patients with CIS/incipient tumour, but a negative result does not exclude TGCT. Further effort is needed to improve this assay, for example, by employing a more sensitive biochemical method of detection.

Key words: AP-2γ/carcinoma in situ/intratubular germ cell neoplasia/OCT-3/4/testicular germ cell tumour

Introduction

The majority of patients with testicular germ cell tumours (TGCTs) survive with the current treatment regime, but regardless of recent improvements in the outcome of this disease, it is potentially lethal, especially in poor-prognosis patients with disseminated non-seminomas and in patients with relapsed testis cancer, which is often refractory to chemotherapy (Schmoll et al., 2004). The subset of patients that require chemotherapy and/or radiotherapy may experience side effects, severe psychological stress and reduction of their reproductive potential (Brydoy et al., 2005; Huddart et al., 2005). TGCTs of young adults originate from a common precursor, the carcinoma in situ (CIS) cell (Skakkebaek, 1972), also known as intratubular germ cell neoplasia. Because CIS can be cured by low-dose irradiation or unilateral orchidectomy alone, there is a great incentive for non-invasive or minimally invasive methods for detection of CIS. A surgical biopsy is at present the only reliable method for diagnosis of CIS, and unilateral or bilateral biopsies are performed in selected patients at risk of CIS, for example, those with a history of cryptorchidism or where clinical examination has revealed atrophic testes or ultrasonic microlithiasis (irregular echo pattern). Hence, very few patients are diagnosed at the CIS stage, particularly because most patients with CIS have no symptoms (Hoei-Hansen et al., 2005a). An additional incentive for obtaining diagnosis at the pre-invasive stage is the concern for the patients’ fertility. While the presence of an overt tumour requires surgery within a few days, a patient with CIS can be given some weeks, or even months, to fulfil his wish of fatherhood or to cryopreserve semen.

Seminiferous tubules with CIS are nearly always present in the vicinity of the tumour (Skakkebaek, 1975; Jacobsen et al., 1981; Oosterhuis et al., 2003). Abnormal cells in semen samples from patients with TGCTs were first described based on their cytological features (Czaplicki et al., 1987; Howard et al., 1989; Yu et al., 1990). Our group demonstrated cells with CIS characteristics in semen of TGCT patients, based on immunohistochemical detection of the oncofetal marker M2A (Giwercman et al., 1988a; Giwercman et al., 1988b) or based on aneuploidy of CIS cells (Giwercman et al., 1988c). The possible presence of cells with isochromosome 12p was explored in another study (Meng et al., 1998). However, owing
to a large overlap with control specimens, hyperdiploidy turned out not to be a parameter predictive of testis tumour (Salanova et al., 1999). An approach of flow cytometry, immunochemistry and enzymchemistry for alkaline phosphatase was reported, but it failed to discriminate CIS because of the high level of cross-reacting germ cell alkaline phosphatase (Brackenbury et al., 1993). RT-PCR-based detection has been attempted by our group (results not published) but showed a very low specificity. Thus, none of the described methods based on examination of semen samples have proved sufficiently reliable to be used for diagnostic purposes in the clinical setting, mainly due to frequent false-negative or false-positive results.

Nevertheless, we decided to re-visit the immunocytochemical approach after the discovery of novel transcription factors specific for neoplastic germ cells in the adult testis (Almstrup et al., 2004), and localized to the cell nucleus, which is usually well preserved in semen. We recently published an encouraging case report, where we diagnosed a young man with CIS testis, based on an immunocytochemical detection of the marker AP-2γ (TFAP2C) in CIS-like cells in his ejaculate (Hoei-Hansen et al., 2005b). AP-2γ is expressed in fetal gonocytes, but not in adult germ cells or structures in the ejaculatory pathway, and has been established as a marker of CIS and many types of overt TGCTs, including the most common classical seminoma and the embryonal carcinoma component of non-seminomas (Almstrup et al., 2004; Hoei-Hansen et al., 2004; Hong et al., 2005; Paula et al., 2005). Since then, we have tested this approach to detect CIS on more than 500 patients and controls in our andrology clinic. Here, we present our detailed experience with the putative method, which has also been expanded to analyse other stem cell and gonocyte marker proteins, including OCT-3/4, a transcription factor that is also not expressed in the adult testis and genital tract and is a marker of CIS, seminoma and embryonal carcinoma (Looijenga et al., 2003; Rajpert-De Meyts et al., 2004).

**Materials and methods**

**Characteristics of participants and semen samples**

A total of 503 men were included in the study. A trained doctor approached participants for inclusion in the project in May 2004–January 2006. Participants attended our department either for semen cryopreservation or for semen analysis as part of an andrological evaluation and were enrolled in this study if at least 200 μl of the sample was available after standard analysis. Inclusion was after written informed consent, and the local medical research ethics committee approved the study. Approximately 92% of the participants were Danish (based on their surnames). Data regarding referral and history were collected and TGCT patients where histology of the tumour was known (n = 151). This group included andrological patients who had testicular biopsies performed and TGCT patients where histology of the tumour was known and who all had a contralateral biopsy performed as is standard practice in Denmark.

**Immunocytochemistry**

Semen cytospin preparations were obtained for all included participants. Staining with AP-2γ was performed in all cases (n = 503), staining with OCT-3/4 in random samples where additional cytopsins were available (n = 84 cases) and staining with NANOG and placental alkaline phosphatase (PLAP) in a few test samples. A small aliquot of a fresh semen sample from each participant was diluted according to the following protocol: 0–25 × 10⁶ spermatozooa/ml non-diluted, 25–45 × 10⁶ diluted 1:2, 45–55 × 10⁶ diluted 1:3, 55–65 × 10⁶ diluted 1:4, 65–75 × 10⁶ diluted 1:5, 75–85 × 10⁶ diluted 1:6, 85–95 × 10⁶ diluted 1:7, 95–130 × 10⁶ diluted 1:8, 130–180 × 10⁶ diluted 1:9, 180–230 × 10⁶ diluted 1:10, 230 × 10⁶ and above diluted 1:15. For each cytopsin, 100 μl was used, and the amount of cells present in each cytopsin was approximately between 0 and 2.5 × 10⁶ per cytopsin. One hundred microlitres of the diluted portions was spun down on a microscope slide with 400 μl of PBS buffer, dried, fixed 10 min in formalin, washed, dried again and stored up to 2 weeks at room temperature. The following antibodies were used: monoclonal mouse anti-AP-2γ antibody (6E4/4; 1:12762, Santa Cruz Biotechnology Inc., CA, USA), monoclonal mouse anti-OCT-3/4 (C-10, sc-5279, Santa Cruz Biotechnology), polyclonal goat anti-human NANOG (AF1997, R&D Systems, MN, USA) and monoclonal mouse anti-PLAP, (BioGenex, San Ramon, CA, USA). A standard indirect peroxidase method was used for staining, as previously described (Hoei-Hansen et al., 2004; Rajpert-De Meyts et al., 2004; Hoei-Hansen et al., 2005c), except for small modifications especially regarding concentration of the antibodies, which were adjusted after analysis of control samples of testicular tissue containing AP-2γ-positive CIS or semen samples spiked with AP-2γ-positive seminoma cells (Figure 1E,F). Briefly, to unmask the antigen, the fixed cytopsins were heated in a microwave oven in a buffer (either TEG buffer = TRIS 1.21 g/l, EGTA 0.19 g/l, pH 9.0; 5% urea buffer, pH 8.5 or citrate buffer = 10 mmol/l, pH 6.0). Subsequently, the samples were incubated with 1.5% H₂O₂ to inhibit the endogenous
Table 1. Characteristics of the participants

<table>
<thead>
<tr>
<th>Participant category</th>
<th>n</th>
<th>Age (years)</th>
<th>Sperm concentration (×10⁶/ml)</th>
<th>Abstinence (days)</th>
<th>Number of analyses per participant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular germ cell tumour before orchidectomy⁶</td>
<td>65</td>
<td>31.4 (15.9–43.2)</td>
<td>23.8 (0–153)</td>
<td>3.1 (1–22)</td>
<td>2.1 (1–6)</td>
</tr>
<tr>
<td>Testicular germ cell tumour after orchidectomy</td>
<td>42</td>
<td>32.6 (15.9–51.1)</td>
<td>14.4 (0–119)</td>
<td>4.9 (1–42)</td>
<td>1.5 (1–9)</td>
</tr>
<tr>
<td>Carcinoma in situ-only</td>
<td>6</td>
<td>25.5 (20.3–30.1)</td>
<td>2.9 (0–8.2)</td>
<td>1.8 (1–3)</td>
<td>2.3 (1–4)</td>
</tr>
<tr>
<td>Other testicular tumours/lesions</td>
<td>8</td>
<td>33.1 (18.8–41.6)</td>
<td>71.6 (0–331)</td>
<td>3.9 (2–7)</td>
<td>2.1 (1–4)</td>
</tr>
<tr>
<td>Infertilityb</td>
<td>294</td>
<td>33.7 (18.8–53.6)</td>
<td>23.0 (0–244)</td>
<td>3.7 (1–30)</td>
<td>1.4 (1–9)</td>
</tr>
<tr>
<td>Other</td>
<td>88</td>
<td>32.1 (18.5–59.3)</td>
<td>53.3 (0–266)</td>
<td>3.1 (1–11)</td>
<td>1.3 (1–10)</td>
</tr>
<tr>
<td>Total</td>
<td>503</td>
<td>32.9 (15.9–59.3)</td>
<td>28.1 (0–331)</td>
<td>3.6 (1–42)</td>
<td>1.5 (1–10)</td>
</tr>
</tbody>
</table>

For each group, the number of participants, mean age, sperm concentration, abstinence time and number of analyses per participant are listed (95% confidence intervals are given in brackets).

⁶Three of these patients had had the other testis removed because of a testicular germ cell tumour. Three other participants had contralateral carcinoma in situ, all of whom had a borderline score.

⁷Including the participant diagnosed with carcinoma in situ in the course of the study (SC-008) and the infertile participant who had a false-negative AP-2γ staining (SC-371).

Figure 1. Examples of staining results according to the categories used for evaluation. (A) AP-2γ-stained cells. A1-4 classified as positive, A5-8 classified as borderline, A9-12 classified as negative. A1 from a participant with testicular CIS before irradiation, after orchidectomy of contralateral teratoma; A2 from participant SC-228, a control participant where biopsies have not been performed; A3 from a participant with a seminoma before orchidectomy, no contralateral CIS; A4 from a participant with unilateral CIS diagnosed in the course of the study; A5 from an infertile participant; A6 from a patient with an embryonal carcinoma and teratoma before orchidectomy, no contralateral CIS; A7 from an infertile participant, where bilateral biopsies were without CIS; A9-12 all from infertile participants. (B) Examples of staining with OCT-3/4; B1 and B3 from patients with a known testicular cancer; B2 and B4 from infertile participants. (C) Examples of staining with PLAP in a patient with CIS. (D) Examples of non-specific staining with NANOG in a control participant. (E) AP-2γ-stained semen sample with spiked seminoma cells. (F) AP-2γ-stained testicular tissue with a tubule containing CIS. CIS, carcinoma in situ; PLAP, placental alkaline phosphatase. Magnification is the same for all images; scale bar = 25 µm.
peroxidase, followed by diluted non-immune goat or human serum to block unspecific binding sites. Incubation with diluted primary antibodies (AP-2γ 1:30–1:50, OCT-3/4 1:200–1:250, NANOG 1:50 and PLAP 1:400) was carried out overnight at 4°C, followed by a secondary link antibody and the horse-radish peroxidase–streptavidin complex. Between all steps, the sections were thoroughly washed. The bound antibody was visualized using aminoethyl carbazole substrate (all reagents from Zymed, S. San Francisco, CA, USA). Sections were lightly counterstained with Mayer’s haematoxylin to mark unstained nuclei. For negative control, for each participant, another cytopsin was treated as above, except that it was only incubated with the dilution buffer. The sections were examined under a light microscope (Zeiss, Oberkochen, D) and scored systematically and independently by two investigators (C.H.H. and E.R.M) with no prior knowledge of the diagnosis. The time course from staining to analysis was in the range of 1–14 days. Stained cells were scored using an arbitrary scale based on the intensity and morphological resemblance of stained elements to CIS or tumour cells: ‘negative’ = no staining or only some unspecific particles stained, including trace reaction in small fragments of nucleus-resembling structures; ‘borderline’ = clear staining in a part of a nucleus or weak staining in a cell with correct morphology; and ‘positive’ = clear staining in one or several whole nuclei with correct, CIS-like morphology with a large nucleus and prominent nucleoles (see examples of the staining categories in Figure 1A). A final score was determined as the maximum score obtained in one of the analysed cytospins. The scoring was slightly modified from the categorization used in the previous publication (Hoei-Hansen et al., 2005b), as we in the present study applied a more strict definition of positive scoring and added the category ‘borderline’. Discrepancy in positive/negative scoring results (especially in the ‘borderline’ category) was detected in 7.2% of analyses, in which cases slides were re-evaluated by the observers together and a consensus score was reached. A tendency towards fading of stained elements with time was noted, perhaps especially in elements stained unspecifically. In two cases, positive-stained elements were detected in both AP-2γ-stained and negative control cytospins and were thus regarded as non-specific.

Statistics
Statistical analysis of the data was performed with the SPSS version 14.0 software. A significance level below 0.05 was considered significant. Correlations of score with various parameters were performed with non-parametric Kruskal–Wallis analyses. Confidence intervals for the sensitivity and specificity were calculated using the Confidence Interval Analysis (CIA) software, version 2.0 (Altman et al., 2000).

Results
Scoring results of AP-2γ and other markers
Examples of elements in semen samples stained with AP-2γ, OCT-3/4, PLAP and NANOG are presented in Figure 1. These markers were chosen because they were established markers of CIS, and AP-2γ was our focus because the antibody already in initial experiments showed a consistent staining of the majority of CIS cells in tissue samples (Hoei-Hansen et al., 2004). For AP-2γ, examples of elements categorized as ‘positive’, ‘borderline’ or ‘negative’ are shown. OCT-3/4 is a nuclear marker and the antibody is very specific; hence, stainings with OCT-3/4 were similar to the AP-2γ results. Stainings with the cytoplasmic marker PLAP were much less consistent with a substantial amount of unspecifically stained elements. The PLAP example shown is from a participant harbouring approximately 150 AP-2γ-stained CIS cells in another cytopsin (Figure 1C). NANOG is a nuclear CIS and GCT marker, but the example of NANOG staining is from an infertile participant where repeated AP-2γ-stained cytospins were negative, whereas the applied polyclonal NANOG antibody had scattered non-specifically stained elements (Figure 1D). The NANOG and PLAP antibodies were judged unsuitable for further use in this project and were abandoned after a few experiments.

The combined results of AP-2γ and OCT-3/4 stainings are shown in Figure 2. In participants with CIS, we detected positive or borderline-positive AP-2γ-stained nuclei in 50% of cases, and in patients with an overt TGCT before treatment, this figure was 33.9%. For six patients with TGCTs and for one patient with CIS-only, samples were available before and after treatment (orchidectomy/chemotherapy/radiotherapy). These cases were used for evaluation of whether positive elements disappeared from the semen with adequate treatment. Among these, four patients had positive or borderline-positive elements in cytospins before treatment, but after treatment all cytospins were judged as negative. For some TGCT patients, we only had a semen sample available after treatment for TGCT, and among a few of these cases, AP-2γ-stained elements were noted up to a few weeks after orchidectomy.

Characteristics of the putative diagnostic test
For determination of sensitivity, specificity and predictive values of the putative detection assay, we analysed the positive and borderline test results together in the subgroup of participants where bilateral testicular biopsies were available (n = 151). The sensitivity of the AP-2γ assay as a marker of neoplastic cells in semen analysis was 34.2% (95% confidence interval

![Figure 2](image-url). Summary and evaluation of the results of (A) AP-2γ and (B) OCT-3/4 staining of semen samples from the various participant categories. Depicted is the percentage of participants in each group that had semen cytospins assessed according to an arbitrary score of negative, borderline or positive. The evaluation was performed systematically and independently by two investigators, who had no prior knowledge of the diagnosis.
(95% CI = 24–45%) when assessing all patients with a positive/borderline score and 54.5% (95% CI = 28–79%) when assessing only testicular GCT patients with contralateral CIS, infertile and CIS-only patients. The specificity of the test was 93.6% (95% CI = 86–97%). The positive predictive value (PPV) was 83.3% (95% CI = 66–93%), and the negative predictive value (NPV) was 60.3% (95% CI = 51–69%).

There was no correlation between presence of AP-2γ or OCT-3/4-stained elements and semen concentration or abstinence time ($P = 0.9$, $P = 0.64$, respectively). The AP-2γ score was positively correlated with the number of analyses performed ($P < 0.001$); the OCT-3/4 score showed similar tendency, but without reaching statistical significance. We also found an increased discrepancy between the observers with an increased number of analyses ($P < 0.001$).

In 26 cases, infertile patients with negative AP-2γ staining had bilateral biopsies performed as a part of the andrological evaluation for reasons unrelated to the present project (mainly due to severe oligozoospermia with or without ultrasonic microlithiasis). In one case, the biopsies revealed bilateral CIS (participant ID SC-371). Despite performing a total of 10 separate cytospin analyses in this patient, we only detected ‘borderline’ AP-2γ-stained elements and no OCT-3/4-stained elements. In the remaining infertile participants, bilateral biopsies did not detect CIS, but varying abnormalities, including Sertoli cell-only, hypospermatogenesis and signs of atrophy. For the eight patients with other testicular tumours/lesions (see Table I) and a contralateral biopsy without CIS, no AP-2γ-positive cells were detected in semen samples.

We performed a sub-analysis of immunocytological score depending on the histology of the overt tumour in patients with TGCTs. Scoring was positive or borderline positive in 13/23 (56.6%) of non-seminomas but only 6/35 (17.4%) of seminomas, which was a significant difference ($P = 0.015$). A similar tendency was seen for OCT-3/4.

**Follow up of participants with a suspicion of CIS due to AP-2γ-positive cells in semen**

In the infertile and control groups, positive scoring results were unexpectedly detected in a few participants (<5%), and their follow up is described in Table II. In most cases, a second semen sample was requested from the participant. Because this putative test has not yet been approved as a diagnostic procedure, we have not yet implemented a consensus procedure. We chose to individualise the follow up for each patient after a discussion between the researchers (C.H.H. and E.R.M.) and attending andrologists (E.C., N.J. and N.E.S.), who informed the patients and obtained consent. As the only definite confirmation of the presence of testicular CIS, bilateral biopsies were performed in four patients with positive cells in semen, also because of the presence of other risk factors for CIS. In only one participant (SC-008), the biopsy revealed CIS in one testis, and so, he had an orchidectomy and required no further treatment [(this case story was described in Hœi-Hansen et al. (2005b)]. In three cases, biopsies failed to detect CIS, despite AP-2γ-positive or borderline elements present in semen. In four cases, biopsies were not performed, either because of control semen analysis showing no AP-2γ-positive cells or because of participant’s refusal. In one case, the patient had had bilateral biopsies showing no CIS performed a few years before the AP-2γ tests, and we decided not to repeat biopsies. Finally, one participant is still awaiting biopsies to be performed.

**Discussion**

In the present study, we analysed semen samples from a total of 503 participants and demonstrate that the immunocytological AP-2γ assay is able to detect CIS cells in semen. Particularly informative was a group of eight participants who had CIS without an overt tumour being present. Previous studies have only sporadically included patients at the stage of CIS; the majority were patients with a GCT and CIS in the vicinity. Tumour cells are usually not exfoliated into semen, because the tumour has no connection to the seminal ducts, unless it is present in the intratubular form. In one previous study, CIS cells were detected in 4/8 patients with CIS before tumour development (Giwercman et al., 1988c), and in a study applying immunohistochemistry to detect M2A-positive cells, 4/4 CIS patients (three in the vicinity of a TGCT) were positive, but 2/10 controls showed non-specific staining (Giwercman et al., 1988b). Our present study indicates that CIS/tumour cells perhaps are not always detectable in semen, at least not by a relatively insensitive immunocytological method. There are several possible reasons for this. Firstly, there may be very few CIS cells in semen, especially in testes with an overt tumour, where compression of tubules by the tumour may hinder the release of CIS cells into the ejaculate, which could account for the reduced sensitivity of our test in patients with an overt TGCT. Secondly, there may exist inherent properties of the CIS cells determining whether they detach from the basement tubular membrane and may be shed into the semen. Our observation of the higher proportion of AP-2γ-positive cells in semen samples of patients with non-seminomas supports these explanations. Firstly, seminomas do not metastasize as fast as non-seminomas and tend to grow larger within the testis, exerting mechanical pressure and sometimes destroying the entire remaining parenchyma. Secondly, testicular parenchyma adjacent to seminomas contains CIS less frequently than that adjacent to non-seminomas (85 versus 97%, respectively), and the number of CIS tubules is also significantly smaller (26 versus 32%), probably because of the immunological host response, which in some cases may eradicate CIS (Oosterhuis et al., 2003). On the contrary, intratubular tumour growth is found more frequently in seminomas. Thirdly, animal studies have suggested that seminiferous tubule fluid may be decreased in cryptorchid testes, testes containing Sertoli cell-only tubules, or in cases with reduced androgen action (Setchell, 1970). As this fluid is the vehicle via which exfoliated CIS cells would be transported into seminal plasma, it is possible that men with atrophic testis and/or history of cryptorchidism might thus shed fewer CIS cells. We do not have data in the present study regarding testicular size, but it is known that testes harbouring CIS have a reduced volume, and the presence of Sertoli cell-only tubules and partially undifferentiated Sertoli cells is also quite common, and therefore, a reduced flow of...
Table II. Summary of the follow up in the participants without overt testicular tumours who had AP-2γ- or OCT-3/4-positive stained elements in cytospin

<table>
<thead>
<tr>
<th>Participant ID</th>
<th>Diagnosis category</th>
<th>Number of analyses positive/total</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-008</td>
<td>Infertile</td>
<td>AP-2γ: 1/3</td>
<td>Biopsies with unilateral CIS</td>
</tr>
<tr>
<td>SC-228</td>
<td>Control from epilepsy research project</td>
<td>AP-2γ: 1/10 positive and 2/10 borderline</td>
<td>Awaiting participant’s decision, will perhaps have biopsies performed</td>
</tr>
<tr>
<td>SC-231</td>
<td>Infertile</td>
<td>AP-2γ: 2/6</td>
<td>Biopsies without CIS (right testis: normal; left testis: spermatogenic arrest and degenerated cells)</td>
</tr>
<tr>
<td>SC-237</td>
<td>Infertile</td>
<td>AP-2γ: 1/2</td>
<td>Participant does not want testicular biopsies</td>
</tr>
<tr>
<td>SC-238</td>
<td>Infertile</td>
<td>AP-2γ: 1/4, 1/4 positive and 1/4 borderline</td>
<td>Biopsies without CIS (right and left testis: reduced spermatogenesis and lymphocytic inflammation)</td>
</tr>
<tr>
<td>SC-341</td>
<td>Infertile</td>
<td>AP-2γ: 1/3</td>
<td>No biopsies, control semen analysis negative</td>
</tr>
<tr>
<td>SC-353</td>
<td>Infertile</td>
<td>AP-2γ: 1/4</td>
<td>Biopsies without CIS (right testis: reduced spermatogenesis; left testis: spermatogenic arrest)</td>
</tr>
<tr>
<td>SC-365</td>
<td>Cryopreservation before inguinal hernia operation</td>
<td>AP-2γ: 2/3, OCT-3/4: 0/3</td>
<td>No biopsies, control semen analysis negative</td>
</tr>
<tr>
<td>SC-506</td>
<td>Extragonadal GCT, no CIS bilaterally (2002)</td>
<td>AP-2γ: 1/2, OCT-3/4: 0/1</td>
<td>No follow up</td>
</tr>
<tr>
<td>SC-605</td>
<td>Infertile</td>
<td>AP-2γ: 0/6, OCT-3/4: 1/2</td>
<td>Control semen analysis negative, biopsies are planned for other reasons</td>
</tr>
</tbody>
</table>

CIS, carcinoma in situ; GCT, germ cell tumour.

The lifet ime risk of TGCT (and assumed prevalence of CIS) is about 1% in the male population in Denmark, and probably even higher among subfertile men, we anticipated at least one case of testicular CIS per 100 normal participants or subfertile participants analysed. In our study, 292 participants were included, because they were in an andrological evaluation for subfertility, and among these, two participants were identified with CIS. We do not have biopsies on all participants and therefore cannot rule out that other patients may harbour CIS. In addition, we cannot rule out that the participants we have stated as false positive (because of positive elements in semen, but no CIS in testicular biopsies) may later develop a TGCT, as false-negative testicular biopsies have been reported (Dieckmann and Loy, 2003). The outcome of our assay was, however, completely opposite in the two identified CIS cases. The first participant (SC-008) was a very encouraging example of early diagnosis, thanks to our AP-2γ assay (Hoei-Hansen et al., 2005b). The other infertile participant with testicular CIS (SC-371) was an example of low sensitivity of our assay. No AP-2γ-positive cells were detected in his semen sample when he was first examined, but the patient had bilateral biopsies performed due to other clinical findings raising the CIS suspicion (unilateral cryptorchidism, hypospadias, atrophic testes, severe oligozoospermia and ultrasonic microlithiasis). At histological evaluation, CIS was present in large areas of biopsies from both testes. Subsequently, we repeated the AP-2γ-analysis several times but only detected borderline-positive elements without being able to identify unequivocal CIS cells in semen.

As these two examples and the detailed evaluation of the assay presented here demonstrate, the present analysis does not yet meet criteria for a valid and sensitive diagnostic analysis that can be recommended for screening in routine clinical practice. However, the method does have some clinical value as it detects about half of patients harbouring CIS or incipient tumour in a non-invasive and simple way. Thus, this method can be used as an auxiliary diagnostic procedure in specialized andrology centres, where there is a possibility of repeated analysis and careful follow up of individual patients. The most important point for the attending clinician and the patient is to be aware that a negative result does not exclude the presence of germ cell neoplasia in the testis. There is some room for improvement in the immunocytological assay, and we are currently working on methods of enrichment of the fetal marker-positive cells in semen samples. However, in a subset of patients, especially where there are very few CIS cells or they are not exfoliated into ejaculate, another approach with much higher sensitivity has to be developed, for example, based on biochemical detection of a low concentration of CIS-specific markers.

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Conflict of interest statement

None of the authors have conflicts of interest, but a patent covering the use of newly identified markers for CIS, including AP-2γ, for diagnosis of germ cell neoplasia is pending.

References


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